

COMMUNICATIONS

BIOTECHNOLOGY

CRYOPRESERVATION PROCEDURE AFFECTS MORPHOLOGY, CHROMATIN INTEGRITY, ENERGY STATUS AND INTRACELLULAR ROS IN MOUSE EMBRYOS WITH GREATER DAMAGE AFTER SLOW FREEZING THAN VITRIFICATION

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KEY WORDS: Mouse embryo, Cryopreservation, Bioenergy/redox status.

ABSTRACT - The aim of the study was to compare the effects of slow freezing and vitrification on blastomere/chromatin integrity and energy/oxidative stress parameters of mouse preimplantation embryos. Mouse (4-cell to blastocyst stage) embryos were analyzed as fresh (controls) or after vitrification or slow freezing. Embryo collapsing was found after both slow freezing ($P<0.001$) and vitrification ($P<0.05$). Significantly higher rate of blastomere cytofragmentation was found after slow freezing ($P<0.001$) but it was not observed after vitrification. Chromatin damage and altered mitochondrial (mt) distribution pattern were observed, both after slow freezing ($P<0.05$) and vitrification ($P<0.05$). In embryos at the morula stage, mt activity was reduced by slow freezing ($P<0.05$) but it did not change by vitrification. In embryos at the blastocyst stage, mt activity was reduced by both slow freezing ($P<0.05$) and vitrification ($P<0.05$). Intracellular reactive oxygen species (ROS) level was significantly lower in slow-frozen ($P<0.05$) but higher in vitrified embryos ($P<0.05$) compared with controls. Mitochondria/ROS colocalization was significantly reduced after slow freezing ($P<0.05$) but it was not affected by vitrification. In conclusion, this study demonstrates that vitrification is a suitable method to preserve embryo bioenergy/redox parameters.

INTRODUCTION - Embryos cryopreservation (CP) has become an integral part of assisted reproduction both in human and veterinary medicine. Despite differences in size and physiological characteristics of embryos from various species, most of the embryos are frozen by either the traditional slow cooling method or by an ultrarapid procedure, namely vitrification¹. Appropriate mt distribution and activity within the embryo are important parameters for developmental potential, as being related to specific cell functions². However, little information is available about the effects of CP on chromatin integrity, mt dynamics/distribution and ROS production in mammalian embryos. Oxidative stress (OS) is an important mechanism underlying the toxic effects of CP procedures which then may trigger the apoptotic cascade leading to a decrease in the survival/developmental rate of gametes and embryos after thawing³. In this study, the effects of traditional controlled slow freezing versus vitrification on chromatin integrity, mt distribution, energy status and intracellular ROS levels in mouse embryos at different developmental stages, were compared.

MATERIALS AND METHODS - Embryos were produced as reported by Klambauer et al., 2009⁴. Until CP (either slow freezing or vitrification) or analysis (fresh control embryos), embryos were cultured at 37.5 °C under 6.5% CO₂ for further 20 to 96h⁴. Only morphologically normal cleaving embryos (4- to 8-cell, 8- to 16-cell, 16- to 32-cell), compact morulae and blastocysts were randomly destined to one of the two CP methods. Embryo slow programmable freezing was performed as previously reported⁵. Embryo vitrification was performed with the VitroLoop vitrification procedure⁴. Fresh and frozen-thawed or vitrified-warmed embryos underwent mt/ROS staining following

the procedure by Martino et al., 2012⁶. Hoechst 33258, Mitotracker Orange CMTM Ros and 2',7'-dichloro-dihydro-fluorescein diacetate (DCDHF DA) were used to label nuclear chromatin, mitochondria and ROS, respectively. Embryos were fixed with 3.7% paraformaldehyde solution until they were analyzed by confocal microscopy for mt/ROS distribution, intensity and colocalization. Embryo morphology (χ^2 -test), chromatin integrity (χ^2 -test) and energy/oxidative status (Student's t-test) were compared among groups.

RESULTS - Embryo collapsing was noticed in 100% (P<0.001) of frozen-thawed and in 93% (P<0.05) of vitrified-warmed embryos compared with controls. Blastomere cytofragmentation increased in both slow programmable freezing and vitrification groups compared with controls (P<0.05). Furthermore, this percentage was higher in frozen than in vitrified embryos (P<0.05). Both CP procedures induced nuclear chromatin damage (56% and 26% after slow freezing or vitrification compared with 5% in control embryos (P<0.05) and progressively increased with embryo development from 4/8-cell stage to the morula stage. The significance level was higher after slow freezing than vitrification (P<0.05). On the contrary, chromatin integrity of embryos at the blastocyst stage was not affected by both CP procedures. Mitochondrial distribution pattern was affected after both slow freezing (P<0.05) and vitrification (P<0.05). In embryos at the morula stage, mt activity did not change by vitrification but it was reduced by slow freezing (P<0.05). In embryos at the blastocyst stage, mt activity was reduced by both vitrification (P<0.05) and slow freezing (P<0.05). In morulae, ROS levels did not change after vitrification but were reduced after slow freezing (P<0.05). In blastocysts, ROS levels were increased after vitrification (P<0.001) but were reduced after slow freezing (P<0.05). Mitochondria/ROS colocalization was lower in slow frozen than in vitrified embryos (P<0.001).

DISCUSSION - The used CP procedures affected embryo collapsing and blastomere cytofragmentation at a higher extent after slow freezing than vitrification and could have triggered apoptosis and/or necrosis mechanism⁷. Both slow freezing and vitrification reduced the percentages of embryos showing nuclear chromatin integrity, in line with results of previous studies evidencing, even with different methods, damaging effects of CP procedures on embryo chromatin integrity and function^{5,8}. Observed altered mt distribution could be due to modifications of cytoskeletal microtubules involved in mt anchoring⁹. Increased OS in vitrified embryos in the present study could be a transient effect, as suggested by Tsang and Chow, (2010⁸) who reported that stress-related gene expression dropped down to normal levels within 7 hours after warming. On the other hand, slow freezing could have induced loss of embryo viability, as observed by reduced mt activity and ROS levels. Colocalization of mt/ROS has been reported as indicative of higher ATP turnover resulting from a more intense mt activity and thus indicative of healthy cell conditions⁶. Confocal 3D imaging, for embryo quality evaluation, can contribute to the improvement of used protocols, as well as to identify appropriate conditions to preserve integrity and nuclear and cytoplasmic maturity to ensure proper embryonic development.

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HOMING AND ENGRAFTMENT PROPERTIES OF EQUINE PLURIPOTENT AMNIOTIC-DERIVED CELLS FOR POSSIBLE USE IN CELL THERAPY

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KEY WORDS: equine amniotic-derived cells, homing, engraftment.

ABSTRACT - We reported that equine amniotic-derived cell (ACs) therapy was effective as local treatment in horse tendinopathies. We hypothesized that ACs could represent an innovative therapy also in other pathologies that require a different route of treatment. Before their use, for example in respiratory disease, it is very important to understand whether these cells have homing and engraftment properties. 1) In order to investigate the homing and the differences between the routes of administration, we induced an inflammatory reaction by intradermic administration of 10µl of *Candida albicans* antigen. After 48h, 1x10⁸ of ACs labeled by PKH26 were administered by intravenous or by endobronchial route. Our results showed that labeled ACs were detected in histological section after 48h by intravenous route and after 7-14 days by endobronchial route. 2) In order to verify the presence of ACs in airways, we treated a group of animals with 1x10⁸ labeled ACs into the right accessory lung lobe and the second group of animals with 20ml of PBS. Labeled cells were found at the rate of 10-15% in endobronchial biopsies performed after 4 weeks.

Our results demonstrate that ACs have better homing properties by intravenous route and better engraftment properties in the bronchial tissue.

INTRODUCTION - It is important that mesenchymal stem cells (MSCs), to be used in tissue regeneration and healing, are deprived of immunogenic properties to avoid rejection, have high of proliferation and differentiation target and have characteristics of homing and engraftment after transplantation. In our previous study¹ we demonstrated that ACs have high proliferation target, are pluripotent, lack *MHC-II* expression and are well-tolerated after allogenic local cell transplantation in horse tendinopathies. Many organs, instead, are not easily accessible and for this reason, it is necessary to know if these cells have properties of homing and engraftment to the site of injury after administration by systemic route. Horse may be an interesting model also in other pathologies like as respiratory diseases. Recurrent airway obstruction (RAO) is a spontaneous disease of horses similar to human asthma that could be treated by systemic transplantation of ACs. Aims of our work were to analyze the property of homing of ACs to the site of injury and to verify their engraftment in airways after bronchial instillation.

MATERIALS AND METHODS – In order to detect homing property, we performed an *in vivo* experiment inducing an inflammatory reaction by intradermic administration of 10µl of *Candida albicans* antigen suspension in the cervical region of 4 horses. After 48h, we labeled 1x10⁸ of ACs by PKH 26. These cells were administered in 2 horses by intravenous route and in the other 2 horses by endobronchial route. Punch biopsies of the swellings were taken at 48 h, 1 week and 2 weeks.

In order to verify the presence and the permanence of ACs in airway after bronchial instillation, we treated a group of animals with 1×10^8 labeled ACs into 20 ml of PBS solution into the right accessory lung lobe through a sterile Teflon[®] catheter introduced in the bronchoscope biopsy channel, and a second group of animals with 20 ml of PBS (CTR). We collected broncho-alveolar lavage fluid (BALF) of the right accessory lung lobe of both groups of animals after 48 hours, 1, 2 and 4 weeks. Moreover, multiple endobronchial biopsies were performed after 4 weeks.

RESULTS - Our results showed that labeled ACs were present in histological section of skin swelling after 48h by intravenous route and after 7-14 days by endobronchial route. Labeled cells were found in the BALF after 1 week at the rate of 30%, after 2 week at the rate of 10% and after 4 week at the rate of 5%. In the endobronchial biopsies we found labeled cells after 4 weeks at the rate of 10-15%.

DISCUSSION - Our results demonstrate that ACs have properties of homing and engraftment in the bronchial tissue assuming, then, their possible use in the RAO treatment or in other pathologies where a treatment by intravenous route is deemed necessary.

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MOLECULAR CHARACTERIZATION AND *IN VITRO* DIFFERENTIATION OF FELINE AMNIOTIC EPITHELIAL CELLS

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KEY WORDS: feline amniotic epithelial cells, molecular characterization, *in vitro* differentiation

ABSTRACT - Amniotic epithelial cells (AECs) are recently considered valid candidates in regenerative medicine and may be useful in cell therapies to treat trauma of tissues with loss of substance. In our study, we molecularly characterized and induced *in vitro* differentiation of feline AECs, obtained after enzymatic digestion of amnion. The mean doubling time value of AECs was 1,94±0,04 days. By RT-PCR, AECs expressed pluripotent (*Oct4*, *Nanog*) and some mesenchymal markers (*CD166*, *CD44*), but not *MHC-II* and *CD34*. After induction, AECs differentiated into mesodermic and ectodermic lineages, demonstrating high plasticity. In conclusion, feline AECs are pluripotent, weakly immunogenic and may be useful in feline cell therapies.

INTRODUCTION - Amniotic membrane (AM) represents an alternative source of stem cells (SCs) for use in cell-based therapies¹. While amniotic mesenchymal cells have been isolated and characterized in different species, including the cat^{1,2}, amniotic epithelial cells (AECs) have been found only in human³ and horse⁴, with reports that AECs express embryonic SCs surface markers, and the pluripotent markers *Sox2*, *Nanog* and *Oct4* (³); equine AECs also express mesenchymal SCs markers⁴. Moreover, AECs differentiate in the three germ layers cell lines^{3,4}. These findings confirm that AECs may be pluripotent. In addition, AECs have low MHC-I and not MHC-II antigens expression^{3,4}, supporting the possibility to use AECs in regenerative approaches. Basing on these evidences, in the feline species AECs may be useful in cell-based therapies, in order to treat tissue lesions especially with loss of substance. Therefore, in our work we isolated and characterized feline AECs.

MATERIALS AND METHODS - Feline AMs were digested with 2.4 U/ml dispase and 0.05% trypsin to obtain AECs. Cells were cultured in the medium reported by Lange Consiglio et al.⁴. Doubling time (DT) analysis was performed from passage (P)1 to P9. Gene expression analysis of mesenchymal (*CD166*, *CD44*, *CD29*, *CD90*, *CD73*), pluripotent (*Oct4*, *Nanog*), hematopoietic (*CD34*) and immunogenic (*MHC-I*, *MHC-II*) markers was assessed by RT-PCR analysis, using feline specific primers designed on *Felis catus* available sequences in NCBI database or on Mammal multi-aligned sequences. *In vitro* differentiation into osteogenic, chondrogenic and neurogenic lineages was evaluated with specific stains⁴ and with RT-PCR analysis, investigating the expression of tissue-specific markers⁴.

RESULTS - Cultured feline AECs exhibited a typical polygonal morphology with a mean DT value from P1 to P9 of 1,94±0,04 days. Cell expressed mRNA of *CD166*, *CD44*, *Oct4* and *Nanog*, but not *CD29*, *CD90* and *CD73*; AECs were positive for *MHC-I* and negative for *MHC-II* and *CD34* mRNAs. After *in vitro* differentiation, AECs expressed investigated genes and the differentiations were confirmed by positivity to lineage-specific stains.

DISCUSSION - Our results confirmed that feline AM could be considered a valid source of AECs. The mean DT value demonstrated the high proliferating capacity of AECs. The pluripotent capacity of AECs is proved by expression of pluripotent markers *Oct4* and *Nanog*, and confirmed by *in vitro*

differentiation competence. Furthermore AECs expressed some mesenchymal markers, suggesting that an epithelial-mesenchymal transition may occur in these cells⁵. The lack of *CD34* expression confirmed the not-hematopoietic origin of cells. Moreover, the possibility to use feline AECs in cell therapies resides in their low immunogenicity, due to the absence of MHC-II antigen expression. In conclusion, feline AM contains a population of pluripotent epithelial progenitor cells, that could be applied in feline therapeutic programs.

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EFFECTS OF GESTATIONAL AGE ON PROLIFERATIVE AND DIFFERENTIATION POTENCY OF MESENCHYMAL STEM CELLS ISOLATED FROM CANINE AMNION AND UMBILICAL CORD MATRIX

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KEY WORDS: dog, foetal adnexa, gestational age.

ABSTRACT - Amniotic membrane (AM) and umbilical cord matrix (UCM) mesenchymal stem cells (MSCs) have been isolated and characterized in humans and large animal models. In order to distinguish which cells retain the best features for different purposes, the effects of gestational age on proliferation and differentiation potency of canine AM-MSCs and UCM-MSCs was analyzed. Samples were recovered after elective ovariohysterectomy from bitches in early (35 to 40 days) and late (45 to 55 days) fetal stage of pregnancy. The proliferation study and the molecular analysis of embryonic, mesenchymal and hematopoietic markers were performed. Cell neurogenic and osteogenic differentiation were followed. No differences were noticed when comparing data obtained from cells isolated at different gestational ages. Doubling times, cell viability and Oct-4, CD29 and CD44 stemness markers expression were similar in cell isolated from bitches in early or late pregnancy. In both gestational ages, morphological features of neuronal and osteogenic differentiation were observed which need to be confirmed by molecular analysis. In conclusion, our data indicate the possibility to isolate MSCs from canine fetuses at early and late gestational ages with the same proliferative and differentiative capabilities.

INTRODUCTION - Mesenchymal stem cells (MSCs) are defined to be multipotent stem cells that can differentiate into various cell types in vitro and in vivo under controlled conditions¹. Important sources of MSCs could be foetal adnexa (e.g. amniotic fluid, AF; amniotic membrane, AM; umbilical cord matrix, UCM), as demonstrated in previous studies in humans², from which MSCs are easy to obtain and available in large supply³. In order to establish suitable criteria for selection of cells with most promising ability, the influence of various obstetric parameters on differentiation potency of human UCM-MSCs were analysed⁴. Human AF-MSCs recovered during the second or third trimester of pregnancy were isolated and characterized^{5,6}. Cells isolated from AM and UCM were also well characterized in several animal species⁷. Apart its obvious veterinary interest, the dog has been proved as a suitable research model to study human genetic diseases. More than 370 canine genetic disorders are reported, most of them similar to human diseases and dysfunctions⁸. The characterization and differentiation potency of MSCs from foetal adnexa in dogs were reported by several authors⁹. In the present study, we characterized and followed the in vitro differentiation of AM-MSCs and UCM-MSCs in dogs isolated from fetuses at early and late gestational ages.

MATERIALS AND METHODS - Cell isolation, proliferation and differentiation. Samples were recovered after elective ovariohysterectomy from four bitches in early (35 to 40 days) and four bitches in late fetal stage of pregnancy (45 to 55 days of gestational age). The proliferation study was performed as previously reported⁹. The Student's t-test was used and values with P<0.05 were considered as statistically different. Neurogenic and osteogenic differentiation were followed by Nissl and Von Kossa staining, respectively. **RT-PCR analysis.** The expression of embryonic (Nanog

and Oct-4), mesenchymal (CD29, CD44 and CD184), hematopoietic (CD34 and CD45) and major histocompatibility complexes class I and II (MHC-I: DLA-79 and DLA-88; MHC-II: DLA-DRA1 and DLA-DQA1) genes was assessed by RT-PCR. Canine HPRT1 was used as housekeeping gene. Subcutaneous fibroblasts and dog leucocytes were used as negative and positive controls, respectively. PCR products were visualized with ethidium bromide.

RESULTS - Proliferation study. All cell cultures (early and late AM and UCM) could be analyzed up to passage 10 (P10). No differences were observed when comparing cells obtained from early and late gestational age for all examined parameters. Proliferating cells typically showed fibroblast-like morphology. The DT of UCM cells isolated from fetuses at both early and late gestational age remained approximately at constant levels for four passages but, starting from P5, it significantly increased ($P < 0.001$). Similarly, AM cells isolated from fetuses at early and late gestational ages showed constant DT for initial three passages but, starting from P4, they underwent significant DT increase ($P < 0.05$). Early UCM-MSCs viability remained approximately constant for all passages whereas late UCM-MSCs viability decreased since P5 ($P < 0.001$). Early AM-MSCs showed viability decrease from P4 ($P < 0.001$) and the viability of late AM-MSCs remained constant for 4 passages and decreased from P5 ($P < 0.05$). **Differentiation potency.** When induced to neuronal differentiation, cells showed neuronal-like morphology and the presence of Nissl bodies. In cultures of cells induced to osteogenic differentiation, calcium mineralization was not observed nevertheless cells took a typical osteocyte-like morphology. **Stem cell and hematopoietic markers mRNA expression.** In all cells, expression of embryonic (Oct-4) and mesenchymal (CD29, CD44) markers was confirmed. CD184 expression was found in early UCM-MSCs, early AM-MSCs and late UCM-MSCs. Nanog expression was found only in late UCM-MSCs. Hematopoietic markers (CD34 and CD45) and MHC markers (DLA-DR A1, DLA-DQ A1, DLA-79 and DLA-88) were never found. Canine subcutaneous fibroblasts did not express any analyzed markers. Dog leucocytes expressed only hematopoietic and MHC markers.

DISCUSSION - Cells were spindle-shaped, plate-adherent and able to be cultured in vitro. The results of the proliferation study showed that cells, isolated at any gestational age, behaved similarly since DT values remained initially low (P1 to P4) and then increased with the passage number. Cell viability decreased with passage number, however it remained at high values for all passages. These data are in agreement with those reported in previous studies^{8,10}. The stemness of cells isolated from both early and late AM and UCM was confirmed by molecular expression of embryonic and MSC markers. Cells were able to differentiate in neuronal cell lines, as demonstrated by the presence of the intracellular Nissl bodies, whereas extracellular calcium mineralization was not found, possibly due to low cell density in culture during differentiation and subsequent staining. Differentiation in both lineages needs to be furtherly confirmed by molecular studies. In our study, no differences were noticed comparing data obtained from cells isolated at different gestational ages. This appears in contrast with a previous study⁷ in which it was reported that MSCs obtained from full term babies showed the greater differentiative ability. In conclusion, our data indicates the possibility to isolate MSCs, with the same proliferative and differentiative capabilities, from canine fetuses at early and late gestational age.

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VITRIFICATION OF GV AND IVM HORSE OOCYTES WITH TWO DIFFERENT EQUILIBRATION METHODS

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KEY WORDS: oocyte, horse, vitrification

ABSTRACT - A 3-steps equilibration method was compared to a single-step one in vitrification of cumulus-expanded (CE) and cumulus-compacted (CC) horse oocytes, both in GV and MII stage, obtained from ovaries of slaughtered mares. After thawing oocyte viability was assessed by nuclear maturation rate after *in vitro* culture for oocytes vitrified at the GV stage and by the presence of an intact zona pellucida and membrane after 2 h of *in vitro* culture for oocytes vitrified at the MII stage. The multi-step equilibration method allowed better survival rates after thawing for CE oocytes when vitrified at GV stage (61.3% vs 37.1%) and for CC oocytes when vitrified at MII stage (83.3% vs 61.4%).

INTRODUCTION - The development of a reliable method for cryopreservation of mammalian oocytes would be an important advance in the field of reproductive biology for the preservation of genetic resources. However, despite significant recent progress, the efficiency of oocyte cryopreservation is still very low. Successful cryopreservation of immature or matured oocytes has been reported in several mammalian species including cattle¹, rabbit² and sheep³.

In particular in the horse, this technique remains poorly studied; in fact, the lack of ovarian material limits the number of experiments needed to understand the optimal systems to avoid cryoinjuries and to maintain high viability after thawing. Until now few reports have been reported on the cryopreservation of equine oocytes and the experimental procedures are very different^{4,5}.

Aim of the present work was to compare two different equilibration methods in vitrification of GV (germinal vesicle) and IVM (*in vitro* matured) horse oocytes by assessing their viability after thawing.

MATERIALS AND METHODS - Oocytes were recovered from slaughterhouse ovaries and divided, on the basis of the morphology of cumulus cells, in cumulus-expanded (CE) and cumulus-compacted (CC) oocytes. Groups of CE and CC oocytes were vitrified immediately after recovery at the GV stage and after *in vitro* maturation at the MII stage according to 2 different equilibration methods as follow: E1) equilibration in 20 mM Hepes-buffered TCM-199 supplemented with 20% FCS (holding medium, HM) with 10% ethylene glycol (EG) + 10% dimethyl sulfoxide (DMSO) for 30 sec followed by incubation in HM with 20% EG + 20% DMSO + 0.25 M sucrose for 20 sec; E2) equilibration in 7.5 % (v/v) EG plus 7.5% DMSO (v/v) in HM by 3 multi-step methods for 15 min, incubation in HM containing 15% (v/v) EG, 15% (v/v) DMSO and 0.5 M sucrose for 1 min. In both systems oocytes were placed on the Cryotop strip and immediately submerged in liquid nitrogen. Thawing was performed at 38.5°C by washing the oocytes in HM with decreasing sucrose concentrations (1.25M, 0.62 M, 0.31M). Oocytes vitrified at GV stage were *in vitro* matured in TCM 199 + 10% FCS + FSH/LH (0.1 UI/ml) + cysteamine, fixed and stained with glycerol-Hoechst 33342 to

assess nuclear maturation. Oocytes vitrified at the MII stage were cultured for 2 h to evaluate their morphological survival on the basis of the presence of an intact zona pellucida and membrane. Non-vitrified oocytes undergoing the same maturation protocol were used as control.

RESULTS - Results on maturation and survival rates of GV and MII horse oocytes

Maturation stage		Vitrification system	
		E1	E2
		% MII after IVM (n)	
GV	CE	37.1 ^a (25/62)	61.3 ^{b#} (38/62)
	CC	41.5 ^a (22/53)	38.6 ^{a*} (22/57)
		% MII viable after thawing and 2h culture (n)	
MII	CE	69.4 ^a (25/36)	74.2 ^a (23/31)
	CC	61.4 ^a (27/44)	83.3 ^b (45/54)
		% MII after IVM	
CTR	CE	56.3 (67/119)	
	CC	53.3 (98/184)	

vitrified with the 2 equilibration methods are showed in the following table.

ab; #* Values with different letter/symbol within a raw/column differ significantly (P<0.05; χ^2 test)

DISCUSSION - Results indicate that *in vitro* maturation rate of CE oocytes vitrified at GV stage was significantly higher with E2 method (61.3%vs 37.1%), while no difference existed for CC oocytes. After cryopreservation in MII, a higher percentage of CC oocytes survived after 2h of culture with E2 (83.3%) method compared to E1 (61.4%). In conclusion, our results show that the multi-step equilibration method for vitrification of horse oocytes allows better survival rates after thawing for CE oocytes when vitrified at GV stage and for CC oocytes when vitrified at MII stage.

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EXPRESSION OF MU OPIOID RECEPTOR AND PROOPIOMELANOCORTIN ON SPERM AND FEMALE REPRODUCTIVE TRACT

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KEY WORDS: opioid precursor, opioid receptor, reproduction, swine.

ABSTRACT - The aim of this study was to demonstrate that POMC and the mu opioid receptor (MOR) were expressed in swine reproductive tract and swine spermatozoa. We found that the sow female reproductive tract expresses both POMC and MOR mRNA and MOR protein but, interestingly, the semen shows the highest expression level for both genes and also the highest MOR protein expression. The presence of POMC and MOR (opioid precursor/opioid receptor) transcripts suggests a local synthesis of opioids that could have paracrine and/or autocrine influence during some crucial points of reproduction such as fertilization and pregnancy.

INTRODUCTION - Proopiomelanocortin (POMC) is a precursor protein that contains the sequence for several bioactive peptides including adrenocorticotropin, beta-endorphin, and melanocyte-stimulating hormones¹. These POMC-derived peptides have been found in the central nervous system and in the pituitary, but it is becoming increasingly clear that the expression of these peptides occurs also in non-neuronal tissues. This is not surprising, considering the diverse physiological functions affected by the opioid peptides and the variety of biological effects they exert². Several reports demonstrate the presence of mu-opioid receptor (MOR) and beta-endorphin, its endogenous agonist, in germ cells and reproductive tract of many species³⁻⁶. Furthermore, the functional significance of opioids as modulators of the reproductive function would be better clarified if it could be demonstrated the local synthesis of POMC, thus suggesting a local paracrine and/or autocrine influences of opioids.

MATERIALS AND METHODS - Tissues from the reproductive tracts of n=3 sows, obtained from a local slaughterhouse, and swine frozen semen were processed for Western Blot analysis (WB), to check MOR protein, and for Real time PCR to check gene expression of MOR and POMC. As positive controls, the adrenal gland and the Buffy coat were used. WB was performed as previously described⁵. **Real Time PCR:** Tissues lysate were treated with DNase to degrade genomic DNA. Real Time PCR was performed by using Real Time TaqMan technology StepOne System (Applied Biosystems, Monza, Italy). The MOR and POMC primers and probe were obtained as TaqMan assays (Ss 03378066_u1 and Ss 03381950_u1 respectively) from Applied Biosystems; TaqMan swine Hypoxanthine phosphoribosyltransferase 1 (HPRT1), (Ss 03388273_m1), was used as endogenous control. Data were collected by using the OneStep Software and relative quantification, was performed. Differences in mRNA expression were calculated after normalization to HPRT1. Changes in gene expression were reported as percentage variations relative to controls.

RESULTS AND DISCUSSION - *WB:* As expected, the MOR protein was detected in each tested sample, as two bands of molecular masses of about 50 and 65 kDa; the depletion of the antibody by its immune peptide produced no immunoreactive signals. The densitometric analysis, whose results are expressed in arbitrary units (A.U.), revealed that the protein was expressed at highest level in the swine sperm cells.

The table reports the expression level of both POMC and MOR genes and also that of the MOR protein in each sample tested. We found that all samples express both genes and MOR protein. Among all samples tested, sperm cells express MOR and POMC genes and also MOR protein at

very high level compared to all the other samples, thus suggesting a possible primary and autonomous role of spermatozoa in managing the stressful events of reproduction.

Table 1 - Expression level of POMC and MOR genes (relative expression normalized to HPRT1) and protein (A.U.)

Tissues	Real Time MOR	Real Time POMC	WB MOR
Adrenal Gland	2368.9	46.85	1.32
Buffy Coat	20.39	0.36	2.34
Semen	190575.09	760.08	37.29
Granulosa	24.25	0.07	9.27
Follicle	18.25	21.56	1.29
I Stadium Corpus Luteum	0.43	4.92	1.46
II Stadium Corpus Luteum	21.41	2.6	1.29
Distal Salpinx	138.14	166.57	1.44
Midle Salpinx	3.66	0.98	1.61
Proximal Salpinx	1	1	2.05
Dx Myometrium	4.59	3.53	1.00
Dx Endometrium	5.78	1.06	1.17
Sx Myometrium	34.54	25.11	1.63
Sx Endometrium	8.69	3.46	1.61
Cervix	3.14	5.82	1.42
Pregnant Myometrium	282.09	2.01	3.41
Pregnant Endometrium	501.46	4.59	4.58

The swine female reproductive tract from the cervix, uterus (myometrium and endometrium) to the oviduct and ovary (follicle, granulosa, corpus luteum) expresses POMC and MOR at different levels; also the pregnant uterus shows a high expression of both genes. These data, together with the presence of MOR protein on pre-implantation embryos (data not shown), could be related with the management of all fine stressor events occurring in the embryo-maternal cross talking. Interestingly, the high expression level of both genes in uterine tube, especially in the distal salpinx suggests an active role of the opioid system in those events occurring during fertilization and embryo pre-implantation phase. Also interesting are data regarding genes expression in the follicle that let to suppose the involvement of the opioid system in oocyte maturation, confirming previously in vitro observations demonstrating the increase of oocytes maturation rate when an opioid antagonist is added to the in vitro maturation medium³. On the other hand, granulosa cells expressing the MOR gene and protein at high level but the POMC gene at very low level seems to have a passive role in the synthesis of opioid messengers, being able however, by MOR protein, to participate in the modulation of reproduction by the opioid system. We aim in the next future to check POMC and MOR quantitative gene expression on oocytes and embryos thus, trying to understand more in detail the paracrine/autocrine role of the opioid system.

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CUSTOM MICROARRAY ANALYSIS OF GENE EXPRESSION OF OVINE OOCYTES SUBJECTED TO VITRIFICATION.

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KEY WORDS: vitrification, oocyte, gene expression.

ABSTRACT - Aim of this work was to evaluate the effects of vitrification procedures on the transcriptome of ovine oocytes using a customized ovine cDNA microarray. The analysis was performed on vitrified-warmed in vitro matured oocytes (VTR) and on control oocytes (CTR) subjected only to in vitro maturation. Vitrification was performed with the minimum essential volume method using cryotop as device (Kuwayama et al., 2005).

We generated a medium density microarray based on Illumina DASL technology, comprising 1536 independent assays addressing 1039 transcripts relevant to several cell functions.

VTR and IVM oocytes were subjected to mRNA isolation and subsequently hybridized to the customized microarray. Comparison of mRNA content between VTR and CTR oocytes revealed 29 differentially expressed genes, mainly down-regulated (25 transcripts) after vitrification ($P < 0.01$). Microarray analysis results were validated by quantitative reverse transcription Real Time PCR of six selected transcripts.

This study highlights alterations in the transcriptome of ovine oocytes as a consequence of vitrification procedures. The identification of the molecular mechanisms involved in the oocyte reaction to cryopreservation is an important step for the improvement of the experimental procedures.

INTRODUCTION - Even if structural and morphological damages induced by cryopreservation in gametes and embryos have been extensively investigated, the information available on the molecular events is still limited.

Aim of this work was to evaluate the effects of vitrification procedures on the transcriptome (mRNA levels) of ovine oocytes using a customized ovine cDNA microarray.

MATERIALS AND METHODS - The analysis was performed on vitrified-warmed in vitro matured oocytes (VTR) and on control oocytes (CTR) subjected only to in vitro maturation. Abattoir-derived oocytes were matured in vitro in standard conditions. MII oocytes were randomly divided in two classes and 1) subjected to vitrification procedures, or 2) collected for gene expression analysis (added to 2 μ l diethylpyrocarbonate (DEPC) treated water, snap frozen in liquid nitrogen and stored at -80°C). Vitrification was performed with the minimum essential volume method using cryotop as device¹. Briefly, oocytes were cultured in holding medium (HM; 20 mM HEPES-buffered TCM-199 with 20% FCS) with growing concentration of cryoprotectants [10% ethylene glycol (EG) and 10% dimethyl sulfoxide (DMSO) for 30 sec and successively 20% EG and 20% DMSO for 20 sec], loaded on cryotop and immediately plunged into liquid nitrogen for storage. Warming was performed plunging the cryotops directly into HM supplemented with 1.25 M sucrose and then in solutions with decreasing sucrose concentration (0.62 M, 0.31 M) for 30 sec. Oocytes were washed in HM and collected for gene expression analysis after 2h culture.

We generated a medium density microarray based on Illumina DASL technology, comprising 1536 independent assays addressing 1039 transcripts. Sequences were selected in the OAGI (Ovis Aries Gene Index) database of the Dana-Farber Cancer Institute Gene Index Gene Ontology (GO) section (<http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=sheep>). The selected transcripts are relevant to several cell functions such as transcription, cell cycle regulation, signal transduction and immune response. VTR (9 groups of 20 oocytes) and IVM (3 groups of 20 oocytes) oocytes were subjected to mRNA isolation with Dynal microbeads kit (Oslo, Norway) and subsequently hybridized to the customized microarray.

RESULTS - Comparison of mRNA content between VTR and CTR oocytes revealed 27 differentially expressed genes, mainly down-regulated (24 transcripts) after vitrification ($P < 0.01$; Figure 1). Microarray analysis results were validated by quantitative reverse transcription Real Time PCR of six selected transcripts (VEGF, SGK1, TGFB3, PRP2, CDH2, AQP3).

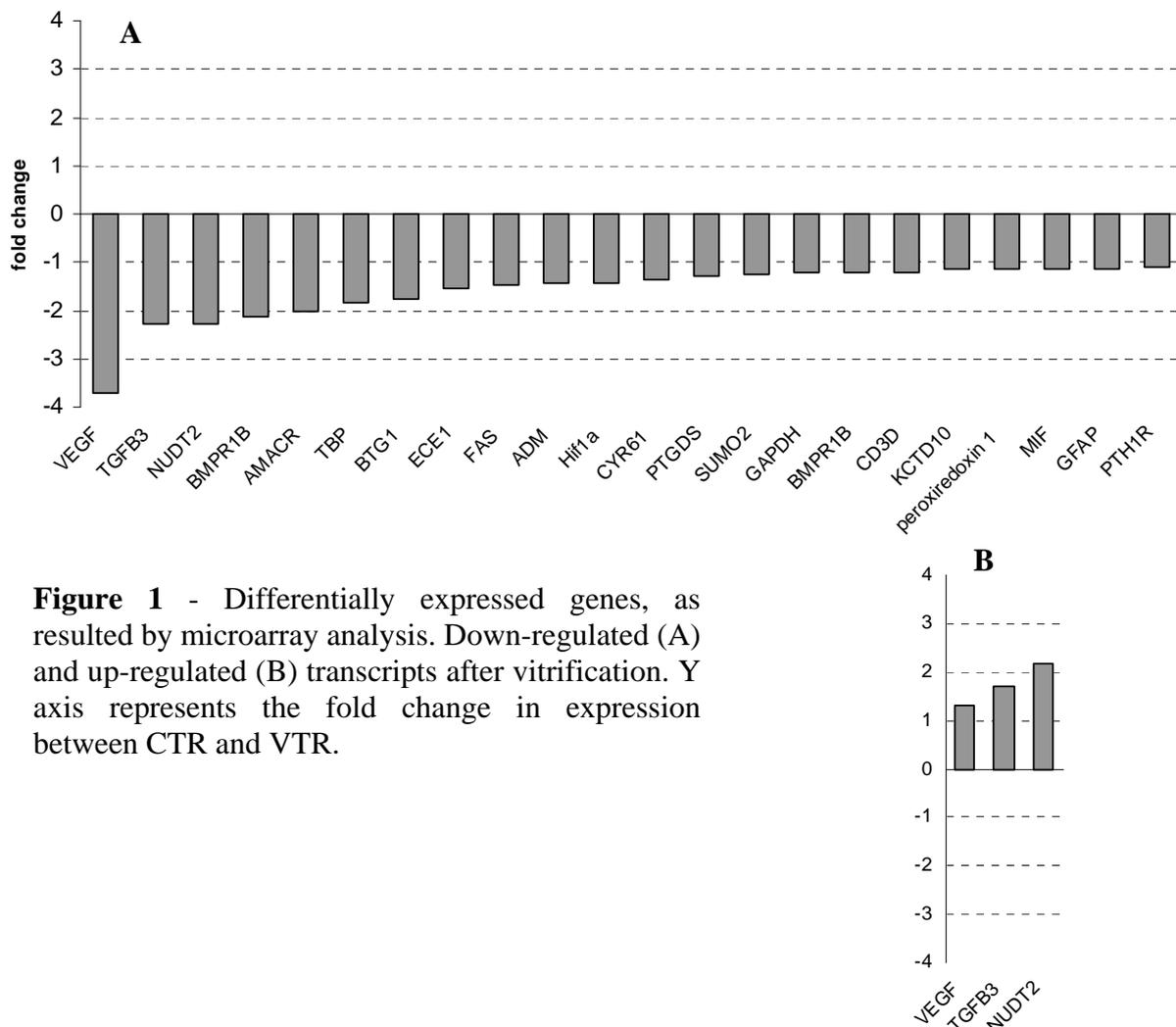


Figure 1 - Differentially expressed genes, as resulted by microarray analysis. Down-regulated (A) and up-regulated (B) transcripts after vitrification. Y axis represents the fold change in expression between CTR and VTR.

DISCUSSION - This study highlights alterations in the transcriptome of ovine oocytes as a consequence of vitrification procedures. The identification of the molecular mechanisms involved in the oocyte reaction to cryopreservation is an important step for the improvement of the experimental procedures.

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SUPEROVULATION STRONGLY AFFECTS GLYCOPROTEIN EXPRESSION IN OVINE ISTHMUS OVIDUCT

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KEY WORDS: glycoproteins; oviduct; sheep

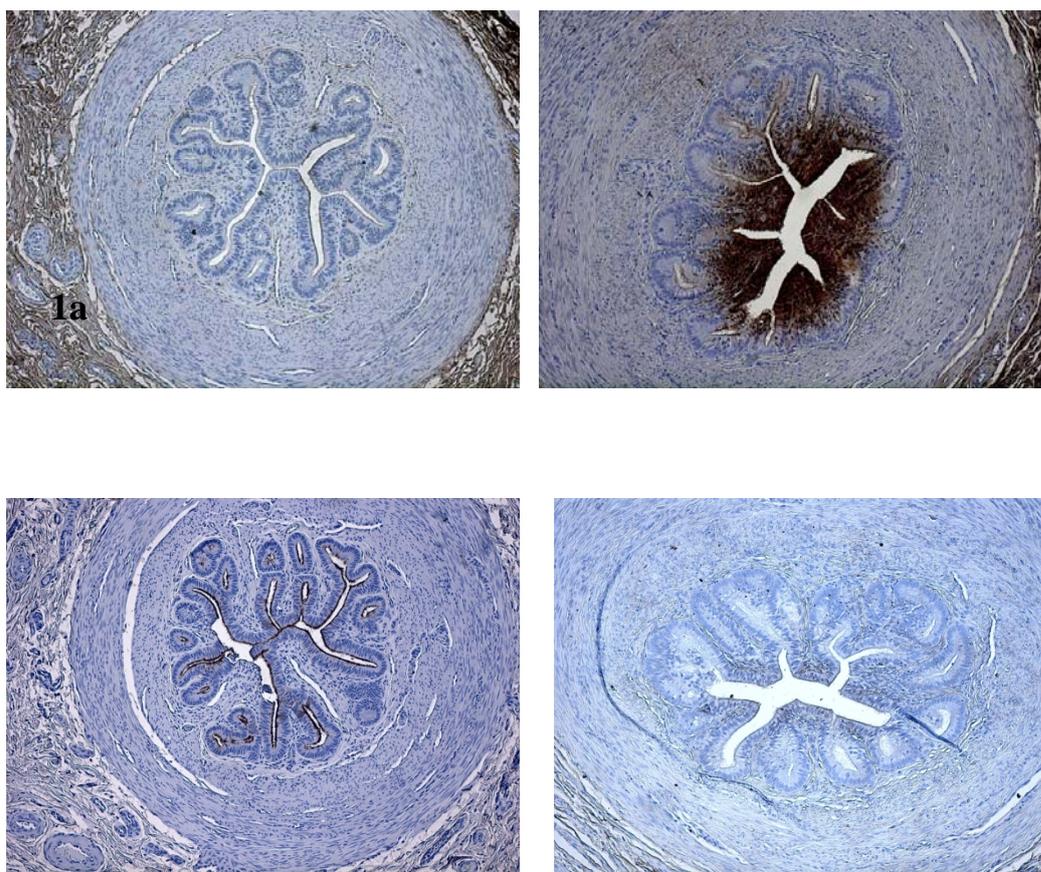
ABSTRACT - The oviduct isthmus is considered to be a sperm reservoir prior to ovulation in the reproductive tract of mammals. Ovarian steroids regulate the synthesis and secretion of specific molecules such as glycoproteins that are involved in the interactions between germ cells or embryos and oviductal epithelial cells. The objective of the present study was to investigate the effects on isthmus glycoprotein pattern from hormonally stimulated ewes by means of lectin histochemistry. Isthmus fragments were separated from oviducts immediately after laparotomy, and, after fixed in 4% (w/v) neutral formalin, they were embedded in paraffin wax. Then, the sections were stained with a panel of lectins which revealed: 1) an increase of reactivity with MAL II, SNA, RCA₁₂₀, SBA, GSA I-B₄, GSA II, UEA I and LTA in the whole cytoplasm of ciliated and non-ciliated cells of hormonally treated females, 2) a reduction of DBA affinity in the luminal surface, 3) no staining pattern modification with PNA, KOH-sialidase (s)-PNA, HPA, Con A and KOH-s-WGA. These results indicate that exogenous gonadotropin administration for superovulation may alter the oligosaccharidic composition of glycoproteins produced in the ovine isthmus.

INTRODUCTION - The mammalian oviduct is not a simple conduit of the female reproductive tract, but it plays an essential role in reproduction, since its epithelial cells create an unique environment for gamete transport and maturation, fertilization and early embryonic development. Particularly, the isthmus oviduct i) prevents polyspermic fertilization, ii) maintains sperm fertility, and iii) regulates capacitation and motility hyperactivation in order to ensure an effective sperm condition when ovulation occurs¹. Oviductal functions depend on the synthesis and secretion of oviduct-specific glycoproteins which are controlled by ovarian steroids². In this study, the effects of exogenous hormonal stimulation on the glycoprotein pattern of oviductal isthmi from superovulated ewes were analyzed by means of lectin histochemistry.

MATERIALS AND METHODS - Oviducts from two treated³ and two control sheep were collected by laparotomy, and immediately after collection the isthmus was separated from ampulla, fixed for 12h at room temperature in 4% (w/v) neutral formalin and embedded in paraffin wax. Serial sections were stained with Haematoxylin-Eosin for morphological studies and by means of a panel of 13 lectins for glicoconjugates characterization⁴.

RESULTS - The morphological analysis did not reveal differences between oviducts of normal and treated ewes except for the increase of the height of mucosal epithelium in treated ones (26.55±0.55 µm vs 22.21±0.72 µm). Lectin histochemistry revealed an increase of reactivity with MAL II, SNA, RCA₁₂₀, SBA, GSA I-B₄, GSA II, UEA I and LTA in the whole cytoplasm of ciliated and non-ciliated cells of hormonally treated animals (Figs.1a,b) which also displayed a reduction of DBA affinity in the luminal

surface (Figs. 2a,b). The lectins PNA, KOH-sialidase (s)-PNA, HPA, Con A and KOH-s-WGA did not show difference in their binding pattern due to hormonal treatment.



Light micrographs of ovine isthmus oviducts from normal 1a, 2a) and treated ewes (1b, 2b) showing LTA (1a, b) and DBA (2a, b) reactivity.

DISCUSSION - These results indicate that the superovulation treatment increases the expression of cytoplasmic glycoproteins containing terminal sialic acid (Neu5ac) Neu5ac α 2,3Gal β 1,4GlcNAc, Neu5Ac α 2,6Gal/GalNAc, GalNAc, α -Gal, GlcNAc, α -L-fucose, it decreases the expression of GalNAc α 1,3(LFuc α 1,2)Gal β 1,3/4GlcNAc β 1 terminating glycans, whereas it has no effect on oligosaccharides ending with Gal β 1,3GalNAc, Neu5ac Gal β 1,3GalNAc, α GalNAc as well as glycans with terminal/internal α Man and β GlcNAc. Since the effects of these changes are not known, further studies are needed to better understand whether the modified isthmus intraluminal microenvironment induced by superovulation treatment may improve any function of this oviductal region.

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EFFECTS OF GESTATIONAL AGE ON PROLIFERATIVE AND DIFFERENTIATION POTENCY OF MESENCHYMAL STEM CELLS ISOLATED FROM CANINE AMNION AND UMBILICAL CORD MATRIX

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KEY WORDS: dog, foetal adnexa, gestational age.

ABSTRACT - Amniotic membrane (AM) and umbilical cord matrix (UCM) mesenchymal stem cells (MSCs) have been isolated and characterized in humans and large animal models. In order to distinguish which cells retain the best features for different purposes, the effects of gestational age on proliferation and differentiation potency of canine AM-MSCs and UCM-MSCs was analyzed. Samples were recovered after elective ovariohysterectomy from bitches in early (35 to 40 days) and late (45 to 55 days) fetal stage of pregnancy. The proliferation study and the molecular analysis of embryonic, mesenchymal and hematopoietic markers were performed. Cell neurogenic and osteogenic differentiation were followed. No differences were noticed when comparing data obtained from cells isolated at different gestational ages. Doubling times, cell viability and Oct-4, CD29 and CD44 stemness markers expression were similar in cell isolated from bitches in early or late pregnancy. In both gestational ages, morphological features of neuronal and osteogenic differentiation were observed which need to be confirmed by molecular analysis. In conclusion, our data indicate the possibility to isolate MSCs from canine fetuses at early and late gestational ages with the same proliferative and differentiative capabilities.

INTRODUCTION - Mesenchymal stem cells (MSCs) are defined to be multipotent stem cells that can differentiate into various cell types in vitro and in vivo under controlled conditions¹. Important sources of MSCs could be foetal adnexa (e.g. amniotic fluid, AF; amniotic membrane, AM; umbilical cord matrix, UCM), as demonstrated in previous studies in humans², from which MSCs are easy to obtain and available in large supply³. In order to establish suitable criteria for selection of cells with most promising ability, the influence of various obstetric parameters on differentiation potency of human UCM-MSCs were analysed⁴. Human AF-MSCs recovered during the second or third trimester of pregnancy were isolated and characterized^{5,6}. Cells isolated from AM and UCM were also well characterized in several animal species⁷. Apart its obvious veterinary interest, the dog has been proved as a suitable research model to study human genetic diseases. More than 370 canine genetic disorders are reported, most of them similar to human diseases and dysfunctions⁸. The characterization and differentiation potency of MSCs from foetal adnexa in dogs were reported by several authors⁹. In the present study, we characterized and followed the in vitro differentiation of AM-MSCs and UCM-MSCs in dogs isolated from fetuses at early and late gestational ages.

MATERIALS AND METHODS - Cell isolation, proliferation and differentiation. Samples were recovered after elective ovariohysterectomy from four bitches in early (35 to 40 days) and four bitches in late fetal stage of pregnancy (45 to 55 days of gestational age). The proliferation study was performed as previously reported⁹. The Student's t-test was used and values with $P < 0.05$ were considered as statistically different. Neurogenic and osteogenic differentiation were followed by Nissl and Von Kossa staining, respectively. **RT-PCR analysis.** The expression of embryonic (Nanog

and Oct-4), mesenchymal (CD29, CD44 and CD184), hematopoietic (CD34 and CD45) and major histocompatibility complexes class I and II (MHC-I: DLA-79 and DLA-88; MHC-II: DLA-DRA1 and DLA-DQA1) genes was assessed by RT-PCR. Canine HPRT1 was used as housekeeping gene. Subcutaneous fibroblasts and dog leucocytes were used as negative and positive controls, respectively. PCR products were visualized with ethidium bromide.

RESULTS - Proliferation study. All cell cultures (early and late AM and UCM) could be analyzed up to passage 10 (P10). No differences were observed when comparing cells obtained from early and late gestational age for all examined parameters. Proliferating cells typically showed fibroblast-like morphology. The DT of UCM cells isolated from fetuses at both early and late gestational age remained approximately at constant levels for four passages but, starting from P5, it significantly increased ($P < 0.001$). Similarly, AM cells isolated from fetuses at early and late gestational ages showed constant DT for initial three passages but, starting from P4, they underwent significant DT increase ($P < 0.05$). Early UCM-MSCs viability remained approximately constant for all passages whereas late UCM-MSCs viability decreased since P5 ($P < 0.001$). Early AM-MSCs showed viability decrease from P4 ($P < 0.001$) and the viability of late AM-MSCs remained constant for 4 passages and decreased from P5 ($P < 0.05$). **Differentiation potency.** When induced to neuronal differentiation, cells showed neuronal-like morphology and the presence of Nissl bodies. In cultures of cells induced to osteogenic differentiation, calcium mineralization was not observed nevertheless cells took a typical osteocyte-like morphology. **Stem cell and hematopoietic markers mRNA expression.** In all cells, expression of embryonic (Oct-4) and mesenchymal (CD29, CD44) markers was confirmed. CD184 expression was found in early UCM-MSCs, early AM-MSCs and late UCM-MSCs. Nanog expression was found only in late UCM-MSCs. Hematopoietic markers (CD34 and CD45) and MHC markers (DLA-DR A1, DLA-DQ A1, DLA-79 and DLA-88) were never found. Canine subcutaneous fibroblasts did not express any analyzed markers. Dog leucocytes expressed only hematopoietic and MHC markers.

DISCUSSION - Cells were spindle-shaped, plate-adherent and able to be cultured in vitro. The results of the proliferation study showed that cells, isolated at any gestational age, behaved similarly since DT values remained initially low (P1 to P4) and then increased with the passage number. Cell viability decreased with passage number, however it remained at high values for all passages. These data are in agreement with those reported in previous studies^{8,10}. The stemness of cells isolated from both early and late AM and UCM was confirmed by molecular expression of embryonic and MSC markers. Cells were able to differentiate in neuronal cell lines, as demonstrated by the presence of the intracellular Nissl bodies, whereas extracellular calcium mineralization was not found, possibly due to low cell density in culture during differentiation and subsequent staining. Differentiation in both lineages needs to be furtherly confirmed by molecular studies. In our study, no differences were noticed comparing data obtained from cells isolated at different gestational ages. This appears in contrast with a previous study⁷ in which it was reported that MSCs obtained from full term babies showed the greater differentiative ability. In conclusion, our data indicates the possibility to isolate MSCs, with the same proliferative and differentiative capabilities, from canine fetuses at early and late gestational age.

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SMALL RUMINANTS

RESULTS OF AN ESTROUS SYNCHRONIZATION AND ARTIFICIAL INSEMINATION PROTOCOL BASED ON MALE EXPOSURE IN “NERA DI VERZASCA” GOATS

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KEY WORDS: Nera di Verzasca goats, estrous synchronization, artificial insemination

ABSTRACT - To preserve and develop “Nera di Verzasca” goat, we combined an estrous synchronization with sponges plus male exposure, and an artificial insemination (AI) protocol. This study was conducted on 53 goats and 5 bucks in two farms. At day 0, vaginal sponges were inserted in goats. At day 11, contemporaneously whit sponge extraction, bucks fitted with apron were introduced into the flock to detect goats in estrus. At day 12, three buck tests were carried out at 28, 34, 40 hours after sponge removal. The morning following the buck test (day 13) an artificial vagina was used to collect semen and at 52 hours from the extraction of sponges AI with fresh semen was performed. Only goats in heat were inseminated (38/53; 71.7%). The rate of goats in estrus in relation to the buck tests was 68.75% in the farm A and 85% in the farm B with a concentration of heats at the first two buck tests of 56.3% and 76%, respectively. Ultrasonography showed that 26 goats were pregnant at day35, resulting in an overall pregnancy rate of 68.42%.

INTRODUCTION – “Nera di Verzasca” is a native Alpine endangered goat breed¹. For this reason, we aimed to preserve and develop the breed using selected males by means of AI protocol. Such approach is supposed to enable the farmer to select the period of deliveries to combine the period of milk production and the request of cheese by the customer (economic advantage); to group parturitions facilitating the management practices (adult feeding and replacement); lastly, to guarantee a sure paternity to the animals, allowing genetic progress and variability due to the planned matings. The experimental protocol used was based on estrus synchronization^{2,3} using vaginal sponges combined with male effect and AI with fresh semen after 52 hours from the sponges extraction.

MATERIALS AND METHODS – The study was conducted in two farms at the start of the reproductive season: in farm A 21goats were selected whereas in farm B the number of goats was 32 (n=53 goats). 4 bucks were prepared in farm A and only 1 in farm B. Males were kept away from females for two months and they were sexually stimulated by contact with goats in heat fourteen days prior to their reintroduction into the flock. The females selected for the study were prepared combining the use of the sponges (Cronogest 45mg) with the subsequent exposure to the males. At day 0, vaginal sponges were inserted in goats. At day 11, contemporaneously with the sponge extraction, the bucks fitted with an apron were brought in. To check whether the goat was in heat, or if the synchronization protocol produced any effect, on day 12 three buck tests were carried out at 28, 34, 40 hours after the sponge removal. This procedure allowed to identify animals in heat. The morning following the buck test (day 13) the artificial vagina was used to collect semen. Semen was diluted with a skimmed milk extender to obtain 0.2 ml paillettes containing 100x10⁶ sperm/ml, which is the fertilizing dose. At 52 hours from sponge extraction, AIs with fresh semen were performed. Twenty days later, blood samples were collected to determine progesterone level by enzyme linked fluorescent assay (ELFA) to detect non-pregnant goats, whereas the pregnancy diagnosis was performed at day 35 by transabdominal ultrasonography.

RESULTS – The rate of goats in heat in relation to the buck tests performed was 68.75% in farm A and 85% in farm B with a concentration of heat at the first two buck tests of 56.3% and 76%, respectively. Only goats in heat were inseminated (38/53; 71.7%). Ultrasonography showed that 26 goats were pregnant at day 35, resulting in an overall pregnancy rate of 68.42%. Data obtained from progesterone evaluation showed that 6 of 38 (16%) goats with less than 2ng/ml of progesterone were not pregnant, whereas ultrasound at 35 days revealed that 12 goats were non-pregnant (32%).

DISCUSSION – The results obtained from this study, even if it has been conducted on a limited number of subjects, allow a positive evaluation of the reproductive protocol employed, with a satisfactory pregnancy rate (68.42%). The male exposure has an effect on females by acting on the hypothalamus-pituitary complex resulting in a delayed response and inducing short and infertile cycles. The combination with the vaginal sponges allows to suppress such short cycles and to make the goats fertile at the first induced ovulation. Early embryonic mortality could be responsible of the different pregnancy rate between day 21 and 35 after AI.

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EFFECT OF NUTRITIONAL FLUSHING ON REPRODUCTIVE PERFORMANCES OF AWASSI EWES

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KEY WORDS: Awassi ewes, flushing, estrous cycle, lambing, minerals

ABSTRACT - The present study aimed to improve the reproductive performances and lamb crop of Awassi ewes by a new regime of nutritional flushing. At the beginning of the breeding season (autumn and spring) the ewes were flushed by balanced mixture of crushed yellow corn 58%, soy bean pulps 41%, mineralized trace element 0.01% and sodium chloride salt 1% for one month before breeding. This regime of flushing increased the incidence of estrus (85 vs 66.1%), shortened the estrous cycle length (16.35 vs 19.85 days) and increased the lambing percentage (120.84 vs 106.25%) and increased the lamb birth weight (4.04 vs 3.43 Kg) significantly than the non flushed group. It also, improved the blood concentrations of calcium, phosphorus, magnesium, total protein, albumin and globulins. It was concluded that application of flushing for Awassi ewes can overcome the nutritional deficiencies and increase lamb crop.

INTRODUCTION - The Awassi evolved as a nomadic sheep breed through centuries. Awassi is a fat-tailed sheep breed that is indigenous to many near eastern countries¹.

Reproduction in sheep had received much attention in Albania owing to its high value as a source of animal protein⁶. However, some of the evidences presented in the literature shows that proteins and energy supplementations of grazing animals improve fertility³. Fat supplementation improved ovulation rate and embryo production⁴ and reduced post lambing period of ewes³.

The present investigation aimed to improve the reproductive performances of Awassi ewes, which will be positively reflected, especially the lamb crop.

MATERIALS AND METHODS - The present investigation was carried out on 20 healthy, mature Awassi ewes of 3-4 years old raised under Korça Region conditions, for one year. Ewes were fed on maintenance ration of concentrated mixture (400 g/ewe daily) beside wheat straw (ad Lib) during summer and hay (alfalfa,) during winter. Ewes were dividing into 2 similar groups. One group was flushed for one month at the beginning of the breeding season (August) by additional 200 g/ewe daily of balanced supplement mixture composed of crushed yellow corn, 58%; soy bean pulps, 41%; mineralized trace elements, 0.1% (iron, manganese, copper, iodine, selenium and zinc) and sodium chloride salt 1% (18.5% total protein). The other group was kept without supplementation to serve as control.

The incidence of estrous was detected by using 2 rams fitted on the brisket with marking material and allowed to run with ewes all-over the day (one ram for each group)³. Statistical analysis was carried out to evaluate the difference in results between experimented and control animals using student "t" test⁵.

RESULTS AND DISCUSSION - Table 1 reveals that flushing of Awassi ewes with 200g/ewe daily of a balanced mixture composed of 58% crushed yellow corn, 41% soy

bean pulps, 0.1% traces elements and 1% sodium chloride salt for one month pre breeding season increase estrous incidence, shortened the estrous cycle length and increased the lambing percentage and lamb birth weight significantly.

Table 1 - Effect of flushing on some reproductive performances of Awassi ewes.

Parameter	Non flushed ewes	Flushed ewes
Incidence of estrous (%)	66.1	85
Estrous cycle length (days)	19.85±1.87	16.35±1.50**
Gestation length (days)	150.46±2.44	149.14±3.13
Lambing rate (%)	106.25	120.84
Lamb birth weight (kg)	3.43±0.23	4.04±0.25*

*P<0.05 **P<0.01

It could be concluded that application of flushing for Awassi sheep is essential particularly for free grazing ewes flocks of Albanian private farmers, in order to increase the reproductive activity and lamb crop and to overcome the poor nutritional and poor body condition score during the shortening of grazing period. Flushing with oil bearing grains (soy bean pulps and crushed yellow corn) is available and has economic values.

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REPRODUCTIVE MANAGEMENT OF THE AWASSI EWE AND THE RAM FLOCK

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KEY WORDS: ewe; ram; reproduction management.

ABSTRACT - Productivity of the Awassi ewe flock is a direct reflection of reproductive efficiency. The goals and objectives we have for our next lamb crop are determined before and during the breeding season. Reproduction in sheep is influenced by numerous factors. These include: genetic potential, nutritional status, environmental factors, day length or photoperiod effects, health status and other factors. These factors are important in both ram and ewe. If there are nutritional deficiencies they will have poor body condition, low fertility and decreased lamb crop.

INTRODUCTION - The Awassi sheep breed in Albania has been improved 26 years ago. Awassi breed ewes have great potentials for the harsh socio-economic conditions, depending on free grazing in pastures¹; they would be used to improve the milk production through crossbreeding⁴. The Awassi producers require increasing their production efficiency with the use of improved technologies². Many of these technologies are used routinely by producers outside Albania⁵. Typically, farmers may expose their ewes to rams during the beginning of the fall breeding season for the equivalent of two (35 days) or three estrous cycles (51 days)². However, lambing occurs in a shorter and more concentrated lambing periods of 10 days². The Awassi reproductive performance has to be improved through the good management of ewe and ram flocks³.

REPRODUCTION IN THE EWE - Female lambs born early in the season come into heat in October at the age of nine or ten months, and are served or inseminated at the first heat, provided that they weigh not less than 45 kg. About 60-70 percent of the young ewes lamb as yearlings, the rest at the age of two years. Peak fertility for the ewe is from late September. Ewes will cycle every 16 to 17 days until they are bred or they reach the anestrous period. They are normally receptive to the ram for 24 to 48 hours. The ewes give birth from 144 to 152 days after mating.

REPRODUCTION AND MANAGEMENT OF THE RAM - Well-fed male Awassi lambs show first signs of sexual desire at the age of 3 or 4 months when they weigh 40-50 kg. To determine fertility and reproductive capacity in rams, a breeding soundness exam can be conducted, which means visual appraisal of general health and condition, as well as a soundness check on feet, legs, eyes, teeth, jaws, etc. Testicle mass should be firm, but not hard, with no indication of abscesses, injuries. The penis should be examined to determine if there are indications of adhesion, pizzle rot or injury. A semen sample can be collected from rams by using an artificial vagina. Semen collected would then be microscopically examined for percent live cells, percent motility and percent abnormal sperm cells. The use of brisket paint or other tools to monitor breeding

activity of the ram. Rams should be sheared, treated for internal parasites, have their feet trimmed and started on the diet 2 to 4 weeks before turning in with the ewes.

EFFECT OF TEMPERATURE ON REPRODUCTION - Increases in body temperature can lower reproductive rate in ewes by decreasing ovulation rate, delaying heat cycles or by increasing embryonic mortality. Rams that are heat stressed can be temporarily sterile for 6 to 10 weeks. Common sense should be used to prevent heat stress.

NUTRITION AND MANAGEMENT - Two to four weeks before breeding, ewes can be flushed. Usually, this will result in an increased lambing rate, a shorter breeding season and a decreased number of open ewes. To flush ewes, they can be fed either with grain or by grazing on excellent quality grass pasture, containing high amounts of legumes (alfalfa, trefoil, clover) two to four weeks before breeding. The ewe flock should have access to a free choice salt and mineral mixture. The mineral mix should be designed for sheep to prevent potential copper toxicity problems. Special attention should be given to feet, for foot rot or other problems. Rams and ewes should be wormed prior to breeding. If there have been specific health's problems, vaccinations can also be administered at this time.

SELECTION - Heritability of reproductive traits is considered low (15 to 20%). Select replacements from multiple births that are born early in the lambing season. Select from multiple births from young ewes. Keep triplet ewe lambs. Select replacements from dams that have high lifetime productivity. Select replacements from ewes that are less seasonal in their breeding ability. Don't keep replacements from rams that are infertile during hot weather. Save ewe lambs from rams that have large, well developed, problem free testicles.

BREEDING EWE LAMBS - The ewes bred to lamb at 12 to 14 months of age have higher lifetime productivity than ewe lambs not bred. Ewes that lamb as yearlings don't deposit as much internal fat, fat in tail and the udder as ewes held over until two years of age. This contributes to the increased lifetime productivity. Also, selection decisions within that age group of ewes can be made a year earlier, based on those individual's performance. If genetic progress is being made, those ewe lambs should be some of the best ewes in the flock. The two major disadvantages are: increased management requirements during lambing and, increased nutrient requirements before and after lambing. The ewe lambs should be separated into their own breeding group so older ewes do not dominate them. The young ewes can be bred to a smaller breed ram, and don't breed ewe lambs to rams known to have large lambs at birth.

USING RAM LAMBS - A well developed ram lamb can be bred to 15 to 25 ewes, if managed well. Ram lambs will need to be monitored closely during the breeding season. Attention should be given to breeding technique and any other important breeding details. Ram Lambs often need extra nutrition during breeding. One method that can be successfully used is to turn them in with the ewes only in the evening and at night.

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REPRODUCTIVE PERFORMANCE OF ALPINE GOAT BREED IN ALBANIA

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KEY WORDS: Alpine, genetic group, lactation, yield, gain

ABSTRACT - Effects of genetic groups of RL, FA, F₁, F₂ and F₃ on prolificacy were statistically proved (P<0.05) and on fertility (p<0.01) to F₁. There was significant effects (P<0.001) of genetic groups for total milk yield/lactation and length of lactation and average daily yield (P<0.05). Effect of non genetic factor “year” was significant (p<0.05) on lactation length and total milk yield and not significant on average daily milk yield. The effects “year” and “genetic groups” were significant (p<0.05) on live weights at birth, live weights at weaning and daily gain of kids. Effect of sex on live weight at birth was statistically proved (p<0.05). The effect of the type of rearing was significant on birth and weaning live weights as well as average daily gain from birth to weaning. Genetic groups (RL, FA, F₁, F₂ and F₃) provide better performance in lowland compared to hilly mountain area.

INTRODUCTION - Local goat population amounts to 620 000 heads in the region where the study was carried out^{5,7}. Local goats are distinguished to high sustainability toward harsh environment condition, resistance to disease and very good ability for the utilization of the meadows². Nevertheless, it should be accepted that local goats are characterized by low genetic capacity, low yield, short lactation and relatively low fertility⁵. Alpine goat breed is distinguished to high productive and reproductive performance^{2,6}. French Alpine goat breed has served to improve local goat performance⁴. The management of pure bred population of French Alpine breed and the use of this breed to improve local goat performance by means of crossbreeding have been the main ways. Actually, Alpine goat population amounts to 1500 heads. Population size of crossbred individuals accounts for 5000 heads, which are raised on hilly-mountainous areas and lowland in Albania⁵.

MATERIALS AND METHODS - 200 heads of French Alpine (FA) goats, 350 heads of local goats (RL), 400 heads of F₁(n=200), F₂ (n=100), F₃ (n=100) crossbreds (Alpine♂ x local goats♀) were used in study. The data of live weight at birth and weaning, daily gain from birth to weaning, fertility, prolificacy were used to study the performance of FA and goats ecotypes “Korça”, “Librazhd” and “Gramsh” as well as crossbreeding between them(FA♂ x RL♀) for both, lowland and hilly-mountain areas. Number of animals comprised in genetic groups was equal to both areas- lowland and hilly-mountain ones.

RESULTS AND DISCUSSION - Tables 1 and 2 show FA, RL, F₁, F₂ and F₃ performance, achieved under conditions of Albania for both areas (lowland and hilly-mountain). French Alpine pure breed goats presented approximate performance with origin one.

Effects of factors affecting fertility and prolificacy at birth are shown in Table2.

Reproductive performance is one of the most important criteria to be considered in planning for goat improvement. There are some environmental causes of variation in

ESTRUS SYNCHRONIZATION IN EWES OUT OF THE SEASON

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KEY WORDS: Synchronization, oestrus detection, lambing rate, seasonality

ABSTRACT - The ewes follow seasonal reproductive pattern what in the climate conditions of Albania means that the sexual activity is manifested from May to August. We assessed the efficiency of two hormonal protocols for the oestrus synchronization in ewes out of sexual season (during October 2011). For this reason two experimental groups, each comprised of 25 Lacon breed ewes, are formed. The both groups had the same breeding and physiological status (all are about 9 months) as well as management conditions. The treatment for the first group was comprised of progestagen vaginal sponge insertion (Fluor Geston Acetate, 40 mg) for 14 days and the 500 IU of eCG (equine Chorionic Gonadotrophin) i/m were applied at the time of vaginal sponge removal. The second group followed the similar protocol, but were additionally treated with 0.1 mg/ewe gonadotrophin releasing hormone (GnRH; Fertagyl) 36h after the eCG application. The oestrus was detected by presentation of the ewes to ram 48h after vaginal device was removed. The presented ewes were mated twice in the interval of 12 hours. The oestrus detection and lambing rate were determined for each group. The oestrus was detected in 24 out of 25 ewes in each group (96 %). The lambing rate in the first group was 2.1 ±0.12 and 2.3±0.2 for the second group (P<0.05).

INTRODUCTION - The ewes follow seasonal sexual activity. This activity changes according to geographic attitude and photoperiod. In our country, the sheep show their sexual activity during the period from May to August. During the period from December to March, they show sexual inactivity (deep anoestrus). According to the advanced studies, the sheep's sexual activity can be manipulated by hormonal treatments during and out of reproductive season. The rate of anoestrus depends on many factors such as healthy status, nutrition, photoperiod, nursing, male factor, the level of milk production etc.^{1,6,9,10}. The gravidity corpus luteum (CL) regresses before parturition^{2,4,8}. This means that the ovary doesn't have either CL as source of progesterone, neither antral follicle as source of estrogens. These hormones stimulate the hypothalamus to produce GnRH^{3,2,5}. As both of those hormones are absent in the circulation of sheep, there is no GnRH, what inhibit hypofysis to produce follicle stimulating hormone (FSH) and luteinising hormone (LH). The external hormones protocols (progestagen, equine chorionic gonadotrophin and GnRH), could stimulate the hypothalamic-pituitary-ovary axis and activate reproduction processes during and out of sexual season. The hormonal treatment of sheep out of season consists on ability of vaginal sponge to release the progesterone and to stimulate the hypothalamus and hypofysis to release FSH and LH^{6,7}.

MATERIALS AND METHODS - The study was realised in Kruja region during anoestrus season. Two experimental groups, each comprised of 25 Lacon breed ewes in the age of 2-4 were formed.

The treatment for the first group was comprised of progestagen vaginal device insertion for 14 days and the 500 IU of eCG i/m were applied at the time of vaginal sponge removal. The second group followed the similar protocol, but were additionally treated with 0.1 mg/ewe GnRH 36h after the eCG application. The oestrus was detected by presentation of the ewes to ram 48h after vaginal device was removed. The presented ewes were mated twice in the interval of 12 hours. The oestrus detection and lambing rate were determined for each group.

RESULTS AND DISCUSSION - The oestrus was detected in 24 out of 25 ewes in each group (96 %) after external use of synthetic hormones. Oestrus detection and mating in both groups indicate the high effectiveness of the hormonal protocol. The performed protocols stimulated a natural fertile oestrus.

Table 1. Date of oestrus respond from hormonal treatment.

No. Ewes	Hormonal treatment	Ewes in oestrus	%
25 (G1)	P ₄ + PMSG	24	96
25 (G2)	P ₄ + PMSG + GnRH	24	96

The lambing rate in the first group was 2.1±0.12 and 2.3±0.2 for the second group respectively (P<0.05). The second group of animal was additionally treated with GnRH to synchronize the ovulation what explain the higher lambing rate in the second group. It seems that GnRH increased the number of matured follicles and their ovulation as well. It is described the role of GnRH on lambing rate in sheep and in other species^{2,6,9}.

CONCLUSIONS - In our country conditions, the ewes exhibit ovarian activity even during anoestrus season (during October) what is manifested with follicles 2-4 mm by ultrasound exam. The measured concentration of glucose in blood (P<0.05) could be an indicator of sheep's healthy status before hormonal treatment. The hormonal treatment of sheep in anoestrus effectively stimulates the oestrus in treated sheep. The hormonal treatment of ewes during anoestrus is efficient and economically reasonable particularly when GnRH was included in the protocol what significantly increase the lambing rate.

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INFLUENCE OF SEX ON SOME QUALITY ASPECTS OF LAMB MEAT

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KEY WORDS: lambs, sex, meat quality

ABSTRACT - Effect of sex on carcass characteristics and lamb meat quality of crossbred lambs (Gentile di Puglia Val di Belice), were analysed. Eighteen lambs distributed in two groups (male and female), after weaned at 30 days, were slaughtered at 70 days of age. Dressing proportion and physical characteristics of carcass were significantly different ($P < 0.05$), between males and females. Sex affected pH 24h in the muscles while color parameters were similar in both sexes.

INTRODUCTION - Some rustic breeds have been selected in the southern regions of the Italy due to very hard feed and environmental conditions. Gentile di Puglia breed has been originated, by Merinos breed substitution on autochthonous breeds and is known for its adaptation to the local environment (hills and mountains) hard conditions. Another typical native local breed from the South Italy is Val di Belice. The breed is also adapted to live on arid soils and produce a lot of milk that is employed for the production of traditional fresh cheese. In Italy lambs are slaughtered very young, and less frequently, after a short fattening period, generally under intensive husbandry conditions. Many factors influence lamb meat quality such as breed (Purchas et al., 2002;) and sex (Dransfield, et al. 1990;). Meat quality and acceptability is determined by its physicochemical characteristics. Our aim is to use the effect that meat-type rams (Gentile di Puglia) produce on a milk-type sheep (Valle del Belice) in order to improve lamb meat quality.

MATERIALS AND METHODS - The study was conducted with eighteen crossbred lambs (Gentile di Puglia x Val di Belice), distributed in two groups (male and female), after weaned at 30 days, received commercial concentrate and cereal straw ad libitum. All lambs were slaughtered at 70 days of age. After slaughter, carcass weight was determined and the carcasses were then chilled at 4°C for 24h in a conventional chiller, dressing (D) and shrink losses (SL) were evaluated. The pH values have been revealed, using a glass electrode, on *Semimembranosus* (Sm), *Longissimus dorsi* (Ld) and *Biceps femoris* (Bf) muscles at slaughtering (pH₁), after refrigeration to +4°C for 24 (pH₂₄), 48 (pH₄₈) and 72 hours (pH₇₂). On a sample of Ld muscle the color was estimated by the Hunter Lab system using a colorimeter Minolta CR200 (illuminant D 65), which measured the values of Lightness (L), Redness (a) and Yellowness (b) by making 5 readings for each meat sample, approximately 2.5 cm thick, after refrigeration to +4°C for 24, 48 and 72 hours. Tenderness was measured using a Warner Bratzler shear device applied to an Instron 5544 and expressed as the cutting force (kg/cm²). In order to evaluate losses due to cooking, samples were weighed before and after cooking, in a convector oven at an inside temperature of the sample of 60° C over 30 minutes, measured by a diving (sinking) probe thermometer. Cooking loss was considered as a percent of the initial weight. Data were analyzed for variance using the GLM procedure of SAS.

RESULTS AND DISCUSSION - Table 1 shows effect of sex on carcass characteristics and some quality aspects. Significant differences ($P < 0.05$) in dressing, cooking loss, raw and cooked meat tenderness were found between males and females. Our findings did not agree with previous report and meat of females was more tender (1.56 vs 1.74; $P < 0.05$) in raw and cooked meat (2.03 vs 2.12; $P < 0.05$). Sex did not influence the carcass weight. Females showed less capacity to release water than males with a lower cool loss % ($P < 0.01$). These results agree with those reported by Vergara et al. (1999). Results for pH and color are presented in Tables 2 and 3. Sex influence significantly the

pH values 24 h after slaughter ($P < 0.05$) with values ranged from 5.44 to 5.77 in the three muscles (Ld, Sm and Bf) indicating that the animals were not stressed at time of slaughter. These values agree with the findings of Devine et al. (1993), within the normal pH range, assuming that an ultimate pH greater than 5.8 is regarded as undesirable. The CIE colour (lightness, redness, yellowness) are also listed in Table 2. No significant effect of sex was observed for these traits in Ld muscle. Color parameters were similar in both sexes, although females had slightly lower a^* values than males, but differences were not significant. However, Teixeira et al. (2005) found an effect of sex on lightness, with males having significantly higher values than females, which, as suggested by Thompson et al. (1979), could be that females are darker than males at equal slaughter weight due to their greater precociousness. The current study has shown that the sex in Merino lambs modify few characteristics of the meat quality.

Table 1 - Productive performance and physical traits

	Male	Female	Significance
Carcass weight (g)	11,916.67	13,283.33	NS
Dressing (%)	61.22	62.83	*
Cool loss (%)	2.45	1.95	**
Cooking loss (%)	36.19	34.59	*
Raw meat tenderness (kg/cm²)	1.745	1.567	*
Cooked meat ten.ss (kg/cm²)	2.123	2.035	*

Table 2 – pH

pH	Male	Female	Significance
pH₁ Sm	6.34	6.46	NS
Ld	6.35	6.46	NS
Bf	6.39	6.30	NS
pH₂₄ Sm	5.67	5.77	**
Ld	5.44	5.64	*
Bf	5.71	5.52	*
pH₄₈ Sm	5.74	5.61	NS
Ld	5.48	5.57	NS
Bf	5.61	5.51	NS
pH₇₂ Sm	5.34	5.61	*
Ld	5.64	5.52	NS
Bf	5.48	5.41	NS

Table 3 - Ld muscle color

	Male	Female	Significance
Color₂₄ L	38.54	42.35	NS
a	15.28	15.08	NS
b	13.46	14.49	NS
Color₄₈ L	35.59	38.72	NS
a	12.17	11.23	NS
b	14.52	15.28	NS
Color₇₂ L	39.61	39.40	NS
a	12.35	11.65	NS
b	16.42	16.52	NS

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REPRODUCTIVE PERFORMANCE OF LOCAL ALBANIAN SHEEP

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KEY WORDS: Sheep, breeding, programs, reproductive performance

ABSTRACT - Different populations of local breeds and those crossbred in the past by imported breeds such as Merino and Tsigiaia, compose sheep of Albania. The breeding plan was focused in the genetic improvement of the yield and quality of wool. The small farms with 1-30 animals are the characteristic ones. The average farm size is 1.5-2 hectares of cultivated land and the system of production is extensive. The animals are kept based on the traditional methods. In this study the reproductive performance (n=1545) and growing performance (n=1350), were analyzed. The data were collected in each season from 2007-2010 in the Experimental Domain QTTB-FK.

INTRODUCTION - For centuries, the Albanian farmer has considered the sheep and goats as traditional animals². Even nowadays, the growing of these animals is one of the important directions in animal production³. The small ruminants provide 30% and 13% of the total national meat and milk production, respectively³. During the years 1991-1994, when political and economical transformations towards the market economy took place, the populations of these two species significantly increased².

The groups of farmers keeping the sheep of Rude Bardhoke, and Shkodrane breeds, in flocks of more than 50 animals, have extended their cooperation in milk collection and marketing². Such organizations, however, remain at the level of fulfilling needs of the moment. Those are not yet institutionalized in the level which could undertake and solve, at the required technical level². There are the complex problems of animal production¹. Taking into consideration the farmers' low co-operation level and being aware of the importance of the technical information⁴, we must provide the technical advice and technologytransfer to the private farms.

MATERIALS AND METODS - The study on analyzing 1545 reproductive performance and 1350 performance of growth, of the local breeds Ruda, Bardhoka, Shkodrane. Data were collected during seven periods, between 2007-2010. The study was carried out on a farms located in the north of Albania. This location features a continental climate. The mating period was chosen to be during August and September (spring season), when average temperatures were $25.7 \pm 1.4^{\circ}\text{C}$ (range 24.5 to 27°C) and humidity was recorded as $65.5 \pm 5.7\%$ (range 60-70.5%). The method of zonal estimation was used. This method has its priorities compared to other ones under conditions of information infrastructure that exist in our country. Data was statistically processed by ANOVA.

RESULTS - The obtained data are reported in tables 1 and 2. The ewes showed a fertility average rate of 98%, a gestation length of 151 ± 4 days, at birth and at weaning (65 days) the sizes litter were 1.28 and 1.22, the weight 2.81 ± 0.45 kg and 15.25 ± 2.15 kg, respectively.

The growth rate average was 215 gr/d from birth to 30 days and 175gr/d from 30 to 90 days. The average mortality between birth and 90 days was 5%. The average age at slaughtering was 175 days and the carcass yield 50%.

Table 1 - The average data for the sheep of the local breed

Breed	Heads Nr	Live weight (kg)	Production (kg)		Fertility (%)	Birth l. weight (kg)		Weaning l. weight (kg)	
			Milk	Wool		M	F	M	F
Ruda	500	45 ±2.75	95 ±15	2 ±0.25	110	2.4 ±0.15	2.2 ±0.2	13.5 ±0.3	12 ±0.35
Bardhoka	545	48 ±3.15	105 ±17	2.2 ±0.16	120	2.5 ±0.15	2.15 ±0.15	13.8 ±0.25	12.5 ±0.3
Shkodrane	500	35 ±2.45	55 ±14	3 ±0.22	115	2.2 ±0.2	2 ±0.16	11.5 ±0.2	10.5 ±0.2

Table 2 - Growing of lambs of the local breed, estimated by Gompertz's model in the different production systems.

Indicators	Unit	Production system	
		Farm with over 50 ewes	Farm with no more than 5 ewes
Live weight at birth	kg	2.25	2.5
Live weight at 6 months	kg	31	35
Age at inflection point	day	47	40
Weight at inflection point	kg	9.04	10.55
Live weight;			
at 1 month	kg	6.5	7.55
at weaning	kg	13.5	16.5
at 4 month	kg	25	30
Average daily gain;			
Birth to 1 month	gr	150	166
Birth to weaning	gr	183	233
Weaning to 6 month	gr	170	194
Birth to 6 month	gr	180	200

CONCLUSION - Referring the information of table 2 that could interpret as the lambs response in different production systems, it must be formulated the following. In general, lambs local breed; differently respond to different conditions of environment.

Based on the above data, shows that local breeds of sheep have a greater potential reproductive and productive in extensive conditions in which they are held.

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PRELIMINARY STUDY OF EFFECTS OF ANTI GnRH VACCINE ON THE GERM LINE IN 8 MONTHS SARDA RAMS

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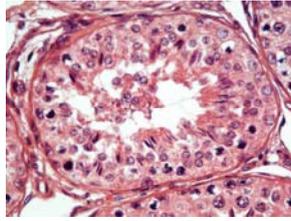
KEY WORDS: Anti GnRH vaccine, testis, ram

ABSTRACT - Several treatments can change reversibly (high and low temperatures and alteration of testosterone levels) or irreversibly (x-ray and chemotherapy) the structural morphology of the testis with alteration of spermatogenesis. The aim of this study was to evaluate the effects of an anti GnRH vaccine on germinal line and seminiferous epithelium in 8 months Sarda rams. The study was carried out using 10 Sarda rams divided into treated and control group. In both groups scrotal circumference and testosterone levels were measured weekly for 12 weeks and histological samples of the testes were collected. The results showed that scrotal circumferences and testosterone levels decreased in the treated group and the seminiferous tubules appeared depleted of the germinal line compared to the control group. The present study showed that the anti GNRH vaccine can be successfully used in rams to inhibit spermatogenesis.

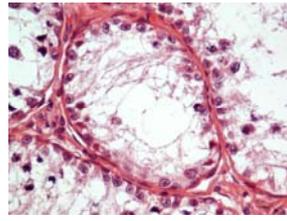
INTRODUCTION - Several treatments can change reversibly (high and low temperatures and alteration of testosterone levels) or irreversibly (x-ray and chemotherapy) the structural morphology of the testis with alteration of spermatogenesis. An anti GnRH vaccine (Improvac®, Pfizer Animal Health) has been already tested on other species obtaining a reversible castration of the animals^{1,2,3}. The aim of this study was to evaluate the effects of an anti GnRH vaccine on germinal line and seminiferous epithelium in 8 months Sarda rams.

MATERIALS AND METHODS - The study was carried out using 10 Sarda rams aged 8 months, randomly divided into two groups: untreated group (CG, control group; n=5), and anti-GnRH immunized group (TG, treated group; n=5). The TG received sub-cutaneously 1 ml of Improvac® and the treatment was repeated 4 weeks later according to company datasheet. The scrotal circumferences and the blood testosterone concentration were measured weekly for 12 weeks in both groups. On the 12th week, 4 rams (2 TG and 2 CG) were castrated and the testis samples were submitted to histological analysis. To test the reversibility of the treatment on the 30th week the semen of 3 TG rams was collected by artificial vagina and evaluated for volume, motility and concentration.

RESULTS - In TG rams, the scrotal circumferences showed a constant decrease, while the CG group carried on with a normal physiological testicular growth. Similarly, the levels of testosterone in blood samples collected from TG rams decreased constantly until reaching 0ng/ml on the 9th week and gradually raising although remaining low. In the testis from CG rams the histology of seminiferous tubules appeared morphologically normal with Sertoli cells, a complete germinal line (from spermatogonia to spermatozoa) and Leydig cells in the inter-tubular space (Pic.1).



Pict. 1: Appearance of a section of seminiferous tubules in the control group



Pic. 2: Appearance of a section of seminiferous tubules in the treated group

In samples of testis from TG rams showed few Leydig cells with undefined margins. Sertoli cells showed normal morphology but lacked almost completely of the germinal line (spermatogonia and spermatocytes, Pic. 2). However after 30 weeks from the treatment the quality of semen of the 3 TG rams presented average characteristics of volume motility and concentration.

DISCUSSION - The present study showed that the anti GNRH vaccine can be successfully used in rams to inhibit spermatogenesis. In fact the immunization treatment led to almost complete depletion of the seminiferous tubules, accompanied by a decrease in testosterone levels and testicular development. The effects of the treatment are completely reversible as showed by the resumption of a normal spermatogenesis after 30 weeks.

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ESTRUS SYNCHRONIZATION IN GOATS OF SANA BREED

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KEY WORDS: Estrus detection, Synchronization, season of reproduction

ABSTRACT - The aim of this study is to evaluate the role of the hormonal treatment of goats into of the copulation season in stimulation and the synchronization of estrus. The goats follow seasonal reproductive pattern what in the climate conditions of Albania means that the sexual activity is manifested from August to November. This study was performed during the year 2011 in Kruja district. 40 does of Saanen breed have been selected to realize this study. All of kid goats had the same breeding condition (space, food and water). The hormonal treatment is based on the combination of the Progesterone and eCG (equine Chorionic Gonadotropin) according the below scheme:

Day 0: Vaginal sponge with 45 mg fluorogestone acetate (FGA) for 15 days.

Day 15: All goats received 400 UI eCG and sponge removal.

Estrus was detected with the aid of buck warning an apron. The occurrence of estrus was controlled every 4 h from 24 to 60 h after sponge removal. The number of goats that has reacted positively after the hormonal treatment is 37 animals or 92.5%. The estrus has begun to exhibit on the average 26±2 h after sponge removal. The animals in estrus have been mated twice with buck of the same breed enter intervals of 12 hours. At the end of the pregnancy period the parturition was about 94.5% of animals with average parturition 2.3 kids per goats. In conclusion the hormonal treatment during season of reproduction is efficient on induction and synchronization of estrus in goats.

INTRODUCTION - The goats are species with high reproductive seasonal activity. In our country conditions the reproductive season varies from August till November according as the geographical position. Hormonal treatments for induction and synchronization of estrus have been used as an aid to artificial insemination (IA) and to reduce seasonal effects on reproduction in goats. The most widely used method FGA (fluoregestone acetate) vaginal sponges combined with injections of eCG^{3,4,6}. The use of this treatment has resulted infertility rates exceeding 60 % following a single cervical AI with frozen-thawed semen at a predetermined time after the end of treatment. However, in some cases, poor fertility rates are recorded and it has been recently observed that this may be due to a delay in the occurrence of estrus^{2,4,5,7,8}. In some goats this delay is associated with the appearance of anti-eCG antibodies subsequent to several hormonal treatments^{1,5,9}. In the goats, efficiency of estrus induction and synchronization treatments depends on factors such as nature, dose and rout of progestagen priming. The purpose of this study was, therefore, to evaluate the effectiveness of the hormonal treatments on estrus induction and synchronization, fertility and prolificacy in season period of reproduction in Sana bread kid goats.

MATERIALS AND METHODS - The experiment was performed on 40 Sana kid goats 9 months of the age during the reproduction season (October, 2011). The breeding conditions were the same for all the animals (stable, food, water, etc).

The goats received hormonal treatments as following.

Day 0: one vaginal sponge impregnated with 45 mg of fluorogestone acetate (FGA), (Chrono-gest, Intervet S.A) for 15 days.

Day 15: all goats received 400 of eCG (Chrono-gest, Intervet S.A) and sponge removal. The goats were mounted by bucks the same bred. All goats were mounted two times with 12 h interval.

RESULTS - The present study was focused in two principal directions.

- a. In the rating of the estrus synchronization.
- b. In range of parturition and number of kids per goats.

Table 1 - Respond of kid goats to hormonal treatment

Treated Animals	Animal in estrus	%
40	37	92.5

In kid goats the hormonal treatment resulted positive. Our records are in accord with many studies of foreign authors who have experimented in this area of study^{1,4,6,10}. The intervals between sponge removal and beginning estrus were 26±2 h.

The data of the pregnancy are evaluated through the experiment with buck and ultrasound examination 21 and 45 days after the copulation. The pregnancy is normally developed in all the inseminated animals. From 37 goats was borne 85 kids or 2.3 kids-goats.

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THE APPLICATION OF ARTIFICIAL INSEMINATION IN SHEEP WITH FROZEN SEMEN OF THE WHITE NORWEGIAN BREED FOR MEAT

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KEY WORDS: Artificial Insemination (AI), sheep, frozen semen, lambs

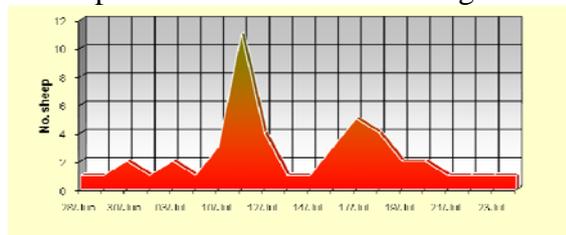
ABSTRACT - The study was initiated to test the efficacy of AI with frozen semen of sheep. Research was conducted in Fier region involved 60 sheep heads. No hormonal treatment was applied but the sheep were subject to stimulated effect of ram. The frozen semen packed in straw was conserve in liquid nitrogen in the deep freezing (-196°C)³. Was applied AI, the vaginal method approach 15-20 hours after beginning of heat. During the period was monitored several key indicators: natural period coming in heat that lasted about 28 days, came into heat and inseminated about 80% of herd and 60.4% conceived. Estrus cycle length range 16±1 days and 60.4% of the sheep were calved. The normal range of gestation in sheep is 148±3 days. There was no damage during calving process and resulted normal. The lambs born were vital and weight in birth resulted in 4.5±0.6 kg breed standards.

INTRODUCTION - Actually in Albania, the breeding situation of the sheep was not satisfactory. Widely used today had no control of rams in view of fertility and the breed. Application of AI is one of the effective ways to improve the situation, especially in medium and large flocks of sheep. Encouraging the application of AI in sheep we increase breed and economic performances through using semen from male reproducer with high genetic quality. As in other species AI provides a genetic improvement and carries a genetic control of male fertilizing capacity used for reproduction². These advantages derive by applying AI with frozen semen, was applied for the first time in our country.

MATERIALS AND METHODS - We selected the farm conditions and included 60 heads which weight was greater than average, to avoid difficult at lambing. The flock of sheep were treated normal conditions. AI was carried out without hormonal treatment for estrus synchronization, usually applies in such studies. The group was stimulated by ram effect¹. Frozen semen (-196°C), was the straw packed. The appropriate time for AI was 15-20 hours after the beginning the heat and was performed by an expert technician and vaginal insemination method was applied. We took all possible measures for ID of animals: number of sheep coming in heat, number of sheep inseminated, % of returns, gestation period, abortions, difficult births, period of calving, lamb's weight at birth and weaning period.

RESULTS AND DISCUSSION - Results of application of AI in sheep with frozen semen within the natural breeding season, are presented through analysis of the occurrence of heat, % of fecundity, % of fertility, calving and the number of lambs born.

In Graphic 1 the results of coming in heat of the sheep and lasted of breeding period, are reported.



The natural period coming in heat lasted about 28 days, with the peak reached on July 10 to

13. During this period 48 sheep (80%) came on heat and were inseminated. Results of fecundity

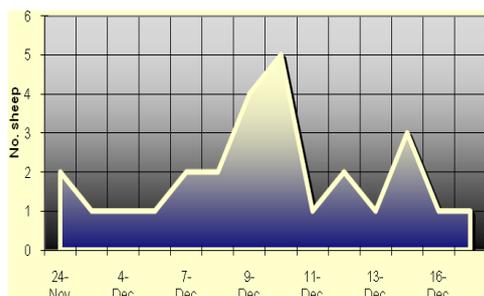
Table 1 - Fertility parameters

No	Indicators	Sheep	%
1	Total number	60	100
2	Coming Heat	48	80
3	Returns	5	8
4	Insemination rate	48	80
5	Conception rate	29	60.4
6	Calving rate	29	60.4
7	Lambs/sheep	30	1.03

are shown in Table 1. Coming in heat Index, about 80% of heard is the satisfactory level but this indicator can be improved by applying the scheme of hormonal treatment⁴ for induction of heat especially in conditions of high environ. Temp. The extension of the breeding period that has come as a result of sheep condition score was below average. The extension of the estrous cycle is 16 ± 1 day. If the indicator compared insemination

rate 80% of head and conception rate 60.4% showing satisfactory the fertility levels, the optimal level is 65-70% for specific features of the itself reproductive tract in sheep⁵. However the main factors that have affected the conception rate and coming in heat were the high environmental temperature above 38°C in summer season (especially until 10 July) and the lack of supplement of diet, before and during the breeding season, with mineral/vitamin. The results are given in Graph. and Table 2.

Graph. No. 2 – Calving period



Statist. Indices	Gestation period
Mean	148.5
Standard deviation	2.75
Minimum	143
Maximum	154
Sum	4158
Confidence	1.06

Calving rate 60.4%, calving period lasted from 24/11 - 20/12. Gestation period lasted 148 ± 3 days with an interval Min. 143 and Max.154 days. During the birth process there was no damage and resulted normal with few intervention. In table 3 are reported the weights of lambs at birth and at 55 days.

Table 3 – Statist. Parameters for weight ((kg) of lambs at birth and at 55 days of age

Statist. Indices	Weight at birth	Weight at 55 days
Mean	4.5	20.75
Standard Deviation	0.67	3.24
Minimum	3	15
Maximum	5	28
Sum	99	415
Confidence	0.2	1.5

Weight of lambs at birth 4.5 ± 0.67 is standard index breed. Lambs 290 gr growth rate/day and weight at age 55 days on average, 20.7 ± 3.24 kg, very pleasing and indicative levels for optimum breed performances.

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SHORT SYNCHRONIZATION PROTOCOL: LAMBING RATE AFTER AI IN SARDA EWES

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KEY WORDS: synchronization, lambing rate, ewes

ABSTRACT - At the moment, the only protocol for oestrus synchronization used in AI campaigns in Sarda ewes is the conventional one of 14 days. The aim of the study was to test the efficiency of a 7 days protocol of synchronization on lambing rates. For the present experiment, carried out during the breeding season, 180 adult ewes were randomly split into 2 groups: a “conventional protocol” group (n=90); and a “short protocol” group (n=90) treated with intravaginal sponges of medroxyprogesterone acetate 60 mg respectively for 14 and 7 days. Both groups were inseminated with fresh semen from rams of proved fertility 55 hours after the removal of the sponges. Pregnancy rates were recorded at 42 days through trans-abdominal scan and confirmed at lambing 145±5 days after insemination. The results showed that at 42 days after insemination in the “conventional protocol” group the pregnancy rate was 41/90 (45.5%) while in the “short protocol” group it was 39/90 (43.3%). In both groups data were confirmed at lambing 145±5 days after insemination. In conclusion, the present study showed that shortening the time of exposure to progestagen in synchronization protocols does not affect pregnancy rates in ewes.

INTRODUCTION - Conventional synchronization protocols in small ruminants are based on a 14 days exposure to progestagens and a subsequent stimulation of follicular development and ovulation through the action of PMSG¹. Shortening the time of exposure to progesterone might facilitate the management of an AI program and reduce the incidence of infections of the reproductive system (i.e. vaginitis)² meeting animal welfare requirements. The aim of the study was to test the efficiency of a 7 days protocol of synchronization based on the use of a combination of progestagens, Cloprostenol and PMSG, on lambing rates in Sarda ewes during the breeding season.

MATERIALS AND METHODS - The experiment was carried out on 180 Sarda adult ewes during the breeding season. The flock was randomly split into 2 groups. The control group (n=90; “conventional protocol” group) was treated with intravaginal sponges of medroxyprogesterone acetate 60 mg (Ovigest[®], Hipra Italia SRL, Rovato, Italy) for 14 days and injected with 400IU of PMSG (Ciclogonina, Fort Dodge Animal Health SPA, Bologna, Italy) at the time of sponge removal. The remaining group (n=90, “short protocol” group) was treated with intravaginal sponges of medroxyprogesterone acetate 60mg for 7 days, 125 µg of Cloprostenol (Estrotek, ATI, Azienda Terapeutica Italiana SRL, Ozzano Emilia, Italy) on the day of sponge insertion and 400 IU of PMSG on the day of sponge removal. At 55 hours after the removal of the sponges the ewes of both groups were artificially inseminated with fresh semen of rams of proved fertility with a dose of 400x10⁶ spermatozoa/straw. Pregnancy rates were recorded at 42 days through trans-abdominal scan and confirmed at lambing 145±5 days after insemination.

RESULTS - At 42 days after insemination pregnancy rates were 41/90 (45.5%) and 39/90 (43.3%) respectively for the “conventional protocol” group and the “short protocol” group. In

both groups data were confirmed at 145 ± 5 days with lambing rates of 41/90 (45.5%) in the “conventional protocol” group and 42/90 (46.6%) in the “short protocol” group.

DISCUSSIONS - The present study showed that there was no difference in pregnancy rates between the conventional 14 days synchronization protocol and the short 7 days one. Shortening the time of exposure to progestagen providing luteolysis at the moment of sponge insertion does not affect pregnancy rates in ewes during the breeding season and could be effectively used as a valid alternative in AI programs. Moreover this protocol might reduce the incidence of vaginitis overcoming the concerns about animal welfare.

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UNILATERAL ANORCHIA IN A CRYPTORCHID RAM

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KEY WORDS: ram, testis, anorchia

ABSTRACT – Among ovine abnormalities of the male reproductive tract, cryptorchidism is a common defect. This study described a case of bilateral cryptorchid ram in which unilateral anorchia was found. Caudally to the left kidney, an abnormal epididymis without testis and spermatic cord, confirmed also histologically, was recognized. The retained contralateral testis showed some vascular abnormalities and, histologically, hypoplastic seminiferous tubules and some foci of atypical Sertoli cells. Other genital organs were normally developed. A defect in the blood supply occurring in the early fetal period with complete testicular disappearance was hypothesized, as already reported in humans.

INTRODUCTION – Ovine testicular diseases have been poorly reported. Among congenital disorders, cryptorchidism represents a common genital defect with a overall prevalence from 4 to 7 per cent^{1,2}. It can affect one or both testes. Bilateral cryptorchid rams are generally sterile, although libido may be normal. Unilateral cryptorchid rams may be fertile but they should be eliminated from the flock for the hereditary nature of the disorder. This study described a case of bilateral cryptorchid ram in which unilateral anorchia was found.

MATERIALS AND METHODS – A 1 year old crossbreed ram was studied at the slaughterhouse for no scrotal palpable testes. Gross evaluation of the abdominal cavity was performed. Samples from the genital tract were collected and processed for routine histological examination, including 10% buffered formalin fixation and paraffin wax embedding. Sections of 5 micron were stained with haematoxylin-eosin.

RESULTS – The ram had a hypoplastic scrotum with no testes and normal male external genitalia. At the exploration of the abdominal cavity, the right testis was found under the caudal pole of the right kidney. It was small and soft; on sections, numerous blood-filled cavities (2-3 mm in diameter) were observed. The epididymis was normally attached to the testis and connected with the vas deferens. The spermatic cord was short, enlarged and laterally folded. An elongated mass (6x2.8x1.5 cm), resembling an abnormal epididymis without testis and spermatic vessels was found caudally to the left kidney. It was connected caudally to the left vas deferens. Normal accessory sex glands were found. No other malformation of the urogenital system was found. Histologically, the right testis showed seminiferous tubules of reduced diameter, lined by Sertoli cells with few germ cells and normal interstitial cells. Numerous atypically enlarged vessels were seen scattered in the testicular parenchyma. In a small area (2 mm), tubular structures containing pleomorphic Sertoli cells with atypical nuclei but not visible mitoses and small foci of intratubular mineralization were recognized. The right epididymal duct, containing no spermatozoa, and the spermatic cord were well differentiated. The left elongated mass was an abnormal epididymis, which showed cranially a pseudostratified epithelium with different degrees of degeneration, with a thin muscular layer and surrounded by abundant dense fibrous connective tissue. Caudally the epididymal duct, surrounded by loose fibrous connective tissue, resembles that of the epididymal tail. Remnants of left testis were also excluded microscopically.

DISCUSSION – The case reported showed different genital abnormalities related to a bilateral cryptorchidism. Anorchia refers to the absence of one or both testes. Unilateral anorchia occurs in about 4.5% of human cryptorchid testes³ and the left testis is more frequently absent than the right⁴. It is extremely rare in domestic animals and it is generally associated to ipsilateral absence of epididymis⁵. The defect has been reported in 1 on 2281 Merino rams⁶, but no case has been identified on 2750 studied Algerian Rembi rams including 108 cryptorchid animals². The term anorchia was used in this work because there is a strong suspicion that the left testis underwent to a total degeneration during fetal period, although the congenital absence of the testicular sketch could not be excluded. The presence of an abnormal epididymis, the lack of the spermatic vessels and the detection of vascular abnormalities in the controlateral testis are suggestive of a defect in the blood supply occurring in the early fetal period with consequent degeneration of the testicular sketch up to the complete testicular disappearance. On the other hand, a vascular deficit is considered the most frequent mechanism involved in human anorchia⁷. The retained right testis presented different degenerative changes, despite its endocrine activity was sufficient to differentiate the internal and external genitalia. The presence of hyperplastic foci of atypical Sertoli cells, in agreement with Foster e Ladds⁸, may be interpreted as a preneoplastic change of the retained testes. Testicular neoplasia is associated with cryptorchidism in several species⁸, but, this relationship is unknown in the ram where only few testicular tumours have been reported^{5,9}. Testicular microlithiasis, seen in the right testis, is another degenerative change, already reported in human undescended testes⁴.

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A NEW METHOD FOR THE TREATMENT OF NECROBACILLOSIS ON THE HOOVES OF SHEEP

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KEY WORDS: Sheep, Necrobacillosis, Lincospectin SC injection, Lincospectin IM injection.

ABSTRACT - For control of Necrobacillosis infection in sheep were experimented three treatment schemes, in animals with varying degrees of gravity for lameness clinic. For comparison with the preparation regimen Topoxy i/m, were used Lincospectin i/m and Lincospectin s/cutaneous injection in the crown area of the damaged hooves. After 5 days of treatment with clinically are improved preparation Topoxy 54 leaders from 82 of the total treated or 65.8%. With using the i/m Lincospectin preparation were improved clinically 80 heads of 101 of total treated or 79.2% (regardless of the degree of severity of lameness). Final results in the treatment of sheeps with a Necrobacillosis Lincospectin preparation s/c injection in the crown area of the damaged hooves (regardless of forms of performance clinical lameness) showed clinical improvement in 99 of 73 rulers of total treated or 73.73%. In our conditions can still be used the Lincospectin preparation for with half dose of s/c injected; it provides approximately the same result as to injection into the muscle.

INTRODUCTION - Necrobacillosis on the hooves or limp is a disease with a strong impact on productive and reproductive capacity of ruminants, especially of sheep^{1,3,7,8}. Necrobacillosis as infection is widespread in cattle herds in the plains and hilly areas and continues to be problematic especially for small ruminants^{1,4,7}. From this infection depending on weather conditions and precipitation may be affected by 20 to 90% of heads. Necrobacillosis on the hooves or limp is a costly disease in the sheep breeding farms. Many manufacturers lose time and money every year to try to control it in their flocks⁵. Necrobacillosis on the hooves as polyfactorial disease is derived in the form of severe and accompanied by painful flaws^{2,6}. The Necrobacillosis disease is seen in every season but the highest density occurs in the season of spring and autumn because the humidity is higher. Efforts are now directed at finding effective ways to control the infection with minimal risk of antibiotic residues in milk and meat.

MATERIALS AND METHODS - During the year 2011 the Necrobacillosis infection the heel was studied in four batches with 830 head of sheep. Sealed with a clinical control the number of animals with lameness syndrome, degree of lameness and vulnerable age. For each batch, in study groups of animals were raised to experiment treatment schemes. Groups of animals were composed of 3 experimental subgroups with lambs and sheep (with not less than 50 heads), with individuals in the degree of mild, moderate and severe lameness. In experiment was applied 3 regimen on an equal breeding, age and race. Treated animals were kept under schemes under the same conditions and were marked with paint to follow consistently. In the first group was used Topoxy preparation 10%, 1 ml dose for 10 kg live body weight, i/m for 5 consecutive days. In the second group was used Lincospectin preparation, 1ml dose for 25kg live body weight, i/m for 5 consecutive days. In the third group was used a half dose preparation Lincospectin, 1ml dose for 12 kg live body weight, s/c area of the damaged hooves crown. Animals were re controlled

clinically after treatment with the three schemes and the results obtained were calculated in percentage.

RESULTS - Throughout the study period of lameness in sheep syndrome was observed in 282 out of 830 control heads or 33.19%. Density Index for Necrobacillosis in sheep it seems relatively high, but it is similar to other authors reporting on this problem. Wassink et al., (2003) reported that Necrobacillosis on the sheep have encountered in 8-10% of sheep controlled (with fluctuations that ranges from 3.32% to 74.52% in the different seasons of the year). While Green et al., (2005) conclude that Necrobacillosis have encountered in sheep in 27.38% of heads. Our data obtained at the end of the experiment showed that a total of 82 chapters deal with the preparation Topoxy (regardless of the form of lameness and degree of damage to the heel) clinical cure was achieved in 54 heads or 65.85%. Treatment of sheep with parenteral preparation Lincospectin IM gave better results. After treatment were clinically cured 80 heads of 101 heads of treated or 96.82 %. Treatment with a preparation scheme Lincospectin SC in the crown area of the damaged hooves (regardless of the form of clinical performance) clinically improved 73 of 99 of treated heads or 73.73%.

DISCUSSION - Final results in the treatment of sheep with different schemes Necrobacillosis with antibiotics showed that best results were obtained with the use of the preparation of Lincospectin i/m. Results of using the preparation Lincospectin s/c injection in the crown area of the damaged hooves were lower than during use of the preparation of i/m. The difference of 5.47% in treatment efficacy between the preparation schemes Lincospectin think that fully justifies the use of s/c Lincospectin preparation in crown area of the damaged hooves and this is not only for the low dose but to use the lowest level of waste the antibiotic in the milk and sheep meat treat. Justifying the use of s/c Lincospectin preparation becomes more visible when analyzing the cost of treatment schemes used. Treatment of sheep by s/c Lincospectin Necrobacillosis a preparation in the crown area cost is about 0.82 Euros for animals treated, against 2.31 euro from the cost of treatment with this medicine injected i/m.

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fertility such as genetic groups, parity and year. The age of the does is the most important factors affecting fertility and prolificacy in goats.

Table 1 - FA, RL, F1, F2 and F3 performance in lowland

Item	Genotypes				
	FA	RL	F ₁	F ₂	F ₃
Fertility (%)	ns 100	ns 94	** 95	ns 93	ns 93
Prolificacy (%)	* 130	* 110	* 125	* 130	* 135

*(P<0.05) ** (P<0.01) *** (P<0.001) ns= no significant

The changes are explained as being a consequence of the development of neural and endocrine mechanisms of regulation and the control of ovaries and genital tract with age. This physiological maturation is expressed as an increase of fertility and prolificacy with advanced age. These results were similar to those reported by FAO³.

Table 2 - Influence of different factors upon growth performance

Item	Live weight At birth (kg)	Live weight at weaning (kg)	Average daily gain Birth to Weaning (g)
Sex	*(P<0.05)	ns	ns
Male	3.2±0.55	13.5± 0.84	137±6.5
Female	2.85±0.35	11.65±0.88	117±8.5
Year of Birth	*(P<0.05)	*(P<0.05)	*(P<0.05)
2005	2.65±0.85	12.65±0.7	133±5.5
2006	2.3±0.7	11.6±0.4	124±3.6
2007	2±0.45	10.6±1.1	115±4.7

*(P<0.05) ** (P<0.01) *** (P<0.001) ns= not significant

CONCLUSIONS - French Alpine goats show approximate productive and reproductive performance to origin ones, under conditions of Albania. Crossing French Alpine breed on local goat leads to the improvement of local goat performance. F₂, F₃ crossbred progenies provide better performance in lowland when compared to hilly mountain area. As, F₁ crossbred progenies provided better performance in hilly- mountainous area. Therefore, under conditions of Albania, F₁ crossbred progenies are recommended to be managed in hilly mountainous area. More advanced crossbreds are associated with declining performance and constitutional weakness of crossbred progenies due to hard environment conditions.

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EQUINE

DENSITY GRADIENT CENTRIFUGATION OF FROZEN-THAWED EQUINE SEMEN WITH LOW MOTILITY AND FERTILITY

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KEY WORDS: Equine frozen sperm, density gradient centrifugation, low dose-low volume deep horn uterine insemination.

ABSTRACT - Stallions are not selected for fertility, and breeders can demand the use of frozen semen of high valuable stallions despite a poor quality after thawing. The aim of this study was to verify if density gradient centrifugation of low quality frozen-thawed semen can improve sperm quality and fertility rates after deep horn uterine insemination. Low quality frozen-thawed semen, recovered from one stallion with a breeding report of no pregnancy, was centrifuged on a discontinuous density gradient. Sperm recovery rate, motility and membrane integrity were assessed. Four mares were inseminated for two consecutive cycles with low dose-low volume deep horn technique. Sperm quality and viability was improved after gradient centrifugation, but any of the mares became pregnant after insemination.

INTRODUCTION - Logistical limitations on cooled-shipped semen and the added benefits of frozen semen have led steadily increasing number of mares bred with frozen semen¹. Major impediments to the widespread commercial application of frozen semen are lower fertility, compared with fresh or cooled-shipped semen, and no selection of stallions for fertility¹. Four basic procedures can be used for selecting morphologically and functionally high quality sperm: dilution and washing (centrifugation and re-suspension), sperm migration (swim-up), selective washing of subpopulations (density gradient centrifugation) and techniques with adhesive substances to eliminate dead sperm and debris (glass wool-GW, glass beads, Sephadex and LeucosorbTM)². Continuous and discontinuous density gradient centrifugation, using different concentrations of colloidal silica particles, have been applied in clinical situations such as poor quality semen (oligospermic sub fertile stallions)³, for selecting sperm before freezing⁴ and for enhancing sperm quality in cryopreserved-thawed semen⁵. One study reports no differences in pregnancy rate using low number of frozen/thawed sperm non selected or selected by Percoll gradient inseminated in the uterine body or onto the papilla by video-endoscopic technique; in this study semen from one fertile stallion with 40%-20% total motility and progressive motility respectively, was used⁶. The aim of the present study was to evaluate if density gradient centrifugation of frozen semen with low motility after thawing can improve sperm quality and fertility after low dose-low volume deep horn uterine insemination.

MATERIALS AND METHODS - Frozen semen from a 22 years old high valuable stallion, no more available for breeding because the age, was used. Semen was frozen in a commercial freezing center as previously described⁷. During one breeding season semen was used for artificial insemination in five mares (unknown number of cycles) with no pregnancy. In the present study, after thawing at 37°C for 37 sec, motility was evaluated by a computer assisted sperm analysis (CASA, HTM IVOS Version 12, standard equine setup) and HOS test⁸ was performed. For selecting sperm, 1 ML (2 straws) of thawed semen was layered on each of four 10ML tube containing 2ML+2ML

of Redigrad™ 45%-90%, with higher density gradient at the bottom. Semen was centrifuged at 400g for 40 min; after centrifugation, recovery rate of motile spermatozoa was calculated, and motility and sperm membrane integrity, were evaluated. Based on sperm quality and recovery rate, a pellet containing 40×10^6 progressively motile sperm was re-suspended with 1 ml of extender and deposited deep in the uterine horn near the utero-tubal junction (UTJ) ipsilateral to the preovulatory follicle by manipulation per rectum of a 65 cm flexible, smooth tipped pipette fitted with a smaller diameter, inner catheter for delivery of a low volume (MiniTube). Four mares were inseminated for two consecutive cycles between 32-40 hrs after induction of ovulation with hCG 2500 IU (Vetecor®). Pregnancy diagnosis by ultrasonography was performed 14 days following ovulation.

RESULTS - Mean sperm concentration post-thawing was 216.7 ± 41.1 million/ml and after selection 107.2 ± 48.2 million/ml, with a mean recovery rate of $47.5 \pm 18.2\%$ (range 23.7%-67.7%). Table 1 reports the results from CASA analysis and HOS test of semen samples after thawing and after density gradient centrifugation.

Any mare became pregnant after artificial insemination.

Table 1 - Sperm motility and membrane integrity post-thawing and after separation by density gradient centrifugation.

Treatment	TMOT	PMOT	VAP	VCL	VSL	LIN	STR	ALH	HOS test
Post-thawing	15.3 $\pm 3.1^a$	6.6 $\pm 2.8^a$	39.1 $\pm 7.3^a$	47.9 $\pm 9.2^a$	35.5 $\pm 11.5^a$	75.2 $\pm 6.8^a$	91.5 ± 3.5	2.4 $\pm 0.3^a$	36.2 $\pm 11.0^a$
Post-separation	36.8 $\pm 3.0^b$	24.1 $\pm 2.8^b$	65.2 $\pm 4.6^b$	39.1 $\pm 7.3^b$	66.5 $\pm 3.9^b$	81.3 $\pm 4.5^b$	92.6 ± 2.2	2.6 $\pm 0.4^b$	53.2 $\pm 3.4^b$

a vs b $P < 0.05$ (Paired t-Student test, Statistics for Windows)

DISCUSSION - The results obtained in the present study show that, despite an improvement of motility and membrane integrity after Redigrad separation, spermatozoa inseminated deep in the uterine horn were not capable of establishing pregnancy in the bred mares. Using low number of frozen spermatozoa (14×10^6 PMS) has been proved to achieve commercially acceptable pregnancy rates⁹, and no differences were found when mares were inseminated with 10×10^6 selected or 10×10^6 non selected spermatozoa with 35% motility after thawing⁵. The stallion used in the present study showed sperm motility $< 35\%$ after thawing, and selection of better quality spermatozoa through density gradient centrifugation, coupled with deep horn insemination, was likely not able to compensate for sperm damages occurred during cryopreservation and not evaluated by the Authors. The results obtained support the conclusion that in “bad freezer” stallions density gradient separation does not improve fertility, even if more data from different stallions are needed.

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HYPERACTIVATION OF EQUINE SPERMATOZOA BY LEPTIN OR PROGESTERONE FOR IN VITRO FERTILIZATION

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KEY WORDS: equine spermatozoa, leptin, progesterone

ABSTRACT - The aims of this study were to detect the immunolocalization and the expression of leptin (Ob) and leptin receptor (Ob-R) in equine spermatozoa and to hyperactivate stallion sperm using follicular fluid (FF) or leptin (10 ng/ml) to test whether hyperactivation allows equine *in vitro* fertilization (IVF). The immunolocalization of Ob and Ob-R was weak but well localized in post-acrosomal region and in the tail of equine spermatozoa, while there was no expression of Ob and Ob-R mRNA. Treatment of sperm with FF and leptin resulted in the decrease ($P < 0.05$) of three motility parameters (straight line velocity (VSL), straightness (STR) and linearity (LIN) indicative of hyperactivation and in an increase of acrosome reaction rate in respect to control (48% and 35% vs 15% in follicular fluid, leptin and control, respectively; $P < 0.05$). In our IVF setting, reproducible percentages of 8 cells stage embryo (18.56%) were obtained by coupling capacitating conditions with the induction of hyperactivation using 10% FF, but not by leptin addition.

INTRODUCTION –The main limiting factor for equine IVF is the inefficient sperm *in vitro* capacitation. Progesterone and leptin appear to affect both capacitation and AR in different species: leptin would increase protein tyrosine phosphorylation¹ while progesterone would mainly lead to an increase in intracellular calcium concentration. Progesterone receptors were detected on the surface of spermatozoa in different species including the equine but no data are available about leptin on equine spermatozoa. In this context, we investigated the immunocytochemical detection and the expression of Ob and Ob-R in equine spermatozoa and hyperactivated stallion sperm by coupling capacitating conditions with the induction of hyperactivation using FF or leptin (10 ng/ml) to test whether hyperactivation allows equine IVF.

MATERIALS AND METHODS - Pooled follicular fluid from preovulatory follicles of estrous mares was used and progesterone concentration was determined by immune enzymatic assay. Immunocytochemical detection of Ob and Ob-R was performed on spermatozoa from fertile stallions, selected by a swim-up procedure, using polyclonal rabbit anti-human Ob and Ob-R antibodies and a goat-anti-rabbit FITC-conjugated secondary antibody. Molecular expression of Ob and Ob-R was assessed by RT-PCR analysis. For IVF, 10×10^6 sperm/ml were pre-incubated for 6h in capacitating medium² (CTR), then diluted to 1×10^6 sperm/ml with 10% FF or leptin (10ng/ml) or capacitating WM and incubated for other 6h at 37°C. For each condition, sperm motility was assayed by CASA, rates of AR by FITC-PNA staining and rate of apoptosis by annexin V test. Five mature mare oocytes were transferred into droplets of 100µl of sperm suspensions and incubated for 18h at 38.5°C in 5% CO₂. After that, oocytes were

transferred in DMEM/F-12 to evaluate the rate of embryos and of parthenogenesis by Hoechst staining. Data were analyzed by ANOVA.

RESULTS - The average concentration of progesterone in FF was 2000ng/ml. The immunolocalization of Ob and Ob-R was weak but well localized in post-acrosomal region and in the tail in equine spermatozoa while there was no expression of Ob and Ob-R mRNA. Treatment of sperm with FF and leptin resulted in the decrease ($P<0.05$) of VSL, STR and LIN indicative of hyperactivation and in the increase of AR rate in respect to the control (48% and 35% vs 15% in FF, leptin and CTR, respectively; $P<0.05$). FF and leptin do not affect sperm apoptotic rate and viability in respect to the CTR. In our IVF setting, reproducible percentages of 8 cells stage embryo (18.56%) were obtained by coupling capacitating conditions with the induction of hyperactivation using 10% of FF, but not by leptin addition.

DISCUSSION – We detected the presence of Ob and Ob-R but not their molecular expression, so it is probable that the transcription occurred during early spermatogenesis. Leptin and progesterone had effect on capacitation and AR but only progesterone allowed sperm penetration by standard IVF. The rate of IVF was low but very encouraging in this species. The immunocytochemical presence of Ob and Ob-R on equine spermatozoa, however, do not preclude the possible action of leptin on other sperm functions such as sperm-zona pellucida binding and/or zona pellucida-induced AR.

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BLOOD GAS ANALYSIS IN DONKEY FOALS IN THE FIRST 24 HOURS OF AGE

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KEY WORDS: donkey, newborn foal, blood gas analysis

ABSTRACT - The study was performed on 9 Martina Franca donkey foals. Venous blood samples were collected at 5 minutes and at 2, 6, 12 and 24 hours of age. Blood gas analysis was performed measuring total carbon dioxide (TCO₂), carbon dioxide partial pressure (PCO₂), oxygen partial pressure (PO₂), oxygen saturation (SO₂), bicarbonate (HCO₃), bases excess (BE), pH, and lactate (LT). Significant differences were found for all parameters especially from 5 mins-2 hours compared to 12-24 hours of age.

INTRODUCTION - At birth the foal undergoes to a series of physiological changes to adapt to independent life. Among these changes, the adaptation of the cardiovascular and respiratory systems are chiefly. Equids are uniparous, so that the foal death is a dramatic event because of the economic and genetic loss for the owner. Blood gas analysis is regarded in equine neonatology as a diagnostic tool to study the neonatal adaptation period and to assess respiratory function in neonates. Modern portable clinical analyzer allow the contemporary analysis of both blood gases and pH, but also some biochemical parameters and electrolytes. The blood gas analysis has been widely studied in both healthy and diseased newborn horse foals, but the authors are not aware of studies conducted on newborn donkeys. For this reason, the aim of the present study was to provide information on blood gas parameters of normal and healthy donkey foals during the first 24 h of age.

MATERIAL AND METHODS - The study was performed on 9 Martina Franca donkey foals, born at term by surveilled spontaneous vaginal delivery, without human interference. Immediately after birth foals were clinically evaluated for maturity¹ and physical defects absence and submitted to APGAR score index within 5 minutes after birth². The time to stand up (TSU) and to first suck (TFS) were also recorded for each foal. Venous blood samples were collected from the jugular vein by venipuncture using a heparinized syringe. Samples were scheduled as follow: at 5 minutes (T1), at 2 hours (T2), at 6 hours (T3), at 12 hours (T4) and at 24 hours after birth (T5). Blood gas analysis was performed by portable analyzer³ (i-STAT System, Abbott Laboratories, Chicago, USA) using CG4+ cartridges, measuring the following parameters: total carbon dioxide (TCO₂, mmol/l), carbon dioxide partial pressure (PCO₂, mmHg), oxygen partial pressure (PO₂, mmHg), oxygen saturation (SO₂, %), bicarbonate (HCO₃, mmol/l), bases excess (BE, mmol/l), pH, and lactate (LT, mmol/l). The univariate ANOVA followed by a Tukey's post-hoc test was used to evaluate possible differences for each parameter among sampling times. Statistical significance was set for $p < 0.05$.

RESULTS - All the donkey foals, 3 males and 6 females, were born at term (362 ± 7.10 days of pregnancy), after spontaneous vaginal delivery, were mature, without gross physical defects, with normal birth weight (29.6 ± 3.71 kg) and viable as assessed by APGAR score (9.4 ± 0.71), TSU (64.8 ± 27.1 mins) and TFS (98 ± 31.25 mins). The mean

±SD values of TCO₂, PCO₂, PO₂, SO₂, HCO₃, BE, pH, and LT at each sampling time in the 9 donkey foals are reported in table 1.

Table 1. Mean (±SD) values of TCO₂, PCO₂, PO₂, SO₂, HCO₃, BE, pH, and LT at each sampling time in the 9 donkey foals. Different superscript letters within columns refer to significant differences (p<0.05)

Sample	TCO ₂ (mmol/l)	PCO ₂ (mmHg)	PO ₂ (mmHg)	SO ₂ (%)	HCO ₃ (mmol/l)	BE (mmol/l)	pH	LT (mmol/l)
T1	27.0±1.1 ^a	46.1±3.1 ^a	32.4±4.9 ^a	59.0±14.6 ^a	25.7±0.7 ^{ab}	-0.4±1.0 ^a	7.35±0.05 ^a	5.5±1.4 ^a
T2	26.8±1.7 ^a	43.6±3.2 ^a	34.0±3.6 ^{ab}	61.1±5.7 ^a	25.9±1.3 ^{ab}	0.2±1.5 ^{ab}	7.39±0.08 ^{ab}	4.6±1.3 ^a
T3	27.0±1.7 ^a	39.1±1.9 ^b	35.3±5.9 ^{ab}	68.8±10.2 ^{ab}	26.1±1.9 ^{ab}	0.7±1.4 ^{ab}	7.41±0.02 ^{ab}	3.9±1.4 ^{ab}
T4	27.6±1.3 ^{ab}	37.1±2.9 ^b	39.9±4.5 ^{bc}	78.0±9.2 ^b	26.6±1.1 ^{ab}	2.2±1.6 ^b	7.47±0.05 ^b	2.5±0.4 ^{bc}
T5	29.6±1.1 ^b	39.0±2.8 ^b	38.8±5.9 ^{bc}	76.0±11.9 ^b	27.4±1.3 ^a	2.7±2.7 ^b	7.44±0.04 ^b	1.5±0.4 ^c

DISCUSSION - The 9 donkey foals enrolled in the study were mature, normal and healthy both at birth and the in the following 7 days of age. Looking at the blood gas analysis, TCO₂ significant increases, observed only at 24 hours of age, suggests an increase of the peripheral tissues metabolism. PCO₂ significant lower values are detected from 6 to 24 hours compared to 5 mins-2 hours of age, while PO₂ increases from 5 mins to 12-24 hours; the value of PO₂ at 24 hours is similar to that reported in horse foals⁵. SO₂ values increased significantly from the sampling times 5 mins-2 hours compared to 12-24, reflecting the tissues oxygenation improvement. The value at 24 hours is in agreement with data reported in horse foals⁵. The values of bicarbonate did not show any significant difference along the time of sampling, despite an apparent trend of increases. This trend of increases from birth to 24 hours was previously reported in the horse foal⁶, although with lower values at 5 mins and at 24 hours. Although BE values significantly increases only from 5 mins after birth compared to 12-24 hours of age, it is important to underline the change from negative value at 5 mins after birth to positive values at 2 hours, with a continuous trend of increases in the following sampling times. This finding, associated to the similar trend in blood pH (with significant increases only from 5 mins to 12-24 hours), seems to be related to the adaptive changes in respiratory, renal and tissue functions, that becomes fully efficient in the foal only at 12-24 hours. In the adult the blood pH is very stable, thanks to several systems (e.g. hemoglobin, respiratory rate, renal filtration) that compensate any metabolic unbalance. The mean values of pH in donkey foals were slightly higher than the horse foal^{5,7} and also higher respect to the arterial values reported in the same sampling times in horse foals⁸. When lactate was considered, a significant decreases was detected from 5 mins-2 hours compared to 12-24 hours of age, but also from 6 to 24 hours. This trend of decrease is in agreement with data previously reported in normal foals⁹. The higher value detected at birth could be explained by the release of catecholamines and cortisol during the delivery. The estimation of the blood lactate in the foal is regarded as useful to detect some neonatal diseases⁹. In conclusion, this study reported the blood gas analysis values observed in 9 mature, viable and healthy donkey foals from birth to 24 hours of age. Since the early recognition of abnormalities in the respiratory tract is mandatory for the successful management of critically ill foals, these data could be useful to recognize any possible abnormality in blood gas parameters.

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INDUCTION OF OVULATION WITH BUSERELIN ACETATE IN JENNIES

(*Equus Asinus*)

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KEY WORDS: Donkey, GnRH, Induction of ovulation

ABSTRACT - The aim of this study was to evaluate the use of buserelin acetate for the induction of ovulation in the jenny. Animals were treated by subcutaneous administration of 3.15 mg of buserelin acetate in 10 cycles while 13 cycles were used as control. Ovulation occurred between 24 and 48 hours in 8/10 (buserelin) and 4/13 (untreated) jennies ($P < 0.05$). According to the results of this study it seems possible to induce ovulation with buserelin acetate in jennies.

INTRODUCTION - The GnRH agonist buserelin acetate administered every 12 hours for 4 days was able to induce ovulation in the cyclic mare^{1,2}, but this practice is unlikely to be used in the field. Single administration of higher doses of the GnRH agonists Deslorelin acetate (2.1 mg, implant, sc)³ and Buserelin acetate (6.3 mg, injectable, sc)⁴ resulted in ovulation rates between 24 and 48 hours analogue to hCG in the mare⁵. Carluccio et al.⁶ successfully induced ovulation using the GnRH agonist Lecirelin acetate (100 µg, ev) and hCG in the jenny. Aim of this study was to determine if subcutaneous administration of 3.15 mg of buserelin acetate was able to induce ovulations in jennies in estrus, as described in the mare.

MATERIALS AND METHODS - Twenty-three estrus cycles of 10 Amiata jennies (age: 2-16; weight: 265-298 kg), maintained in dry lots and fed with hay *ad libitum* at the Clinical Sciences Veterinary Department of the University of Pisa, were monitored daily by ultrasound. At the evidence of a follicle of 33 ± 1 mm (hour 0), the jennies in estrus were randomly treated with 3.15 mg, sc (n=10 cycles) of buserelin acetate (Suprefact[®], Sanofi-Aventis, Milan, Italy) or left untreated (n=13 cycles). Mann-Whitney test was used to compare follicle diameters (\emptyset) at hour 0 and before ovulation. Fisher's Exact test was used to compare the time of ovulation among groups.

RESULTS - Follicle \emptyset at induction and before ovulation are shown in Table 1.

Table 1. Follicle \emptyset at induction and before ovulation in jennies after treatment with 3.15 of buserelin acetate or untreated.

Treatment	Follicle \emptyset at induction (mean \pm sd)	Follicle \emptyset at ovulation (mean \pm sd)
3.15 mg buserelin acetate, sc	33.3 ± 0.8	35.6 ± 3.9
Untreated	33.0 ± 0.9	36.8 ± 4.1

($P > 0.05$)

Ovulation rates of jennies treated with 3.15 of buserelin acetate or left untreated are described in Table 2.

Table 2 - Ovulation rates after induction with 3.15 of buserelin acetate or in untreated jennies in estrus

Treatment \ Range	0-48 hours	24-48 hours	>48 hours
3.15 mg Buserelin	9/10 (90.0%) ^A	8/10 (80.0%) ^A	1/10 (10.0%) ^A
Untreated	5/13 (38.5%) ^B	4/13 (30.8%) ^B	8/13 (61.5%) ^B

A≠B: Values within columns with different superscript are significantly different (P<0.05)

DISCUSSION - Treatment with 3.15 mg of buserelin acetate resulted in higher ovulation rates between 24 and 48 hours than control (P<0.05). This result is analogue to what observed in the mare^{3,4,5} and with the only report of induction in the jenny with a single administration of GnRH agonist or hCG⁶. Follicle Ø at induction and before ovulation were not different between the two groups (P>0.05). Carluccio et al.⁶ reported smaller follicle Ø at ovulation in jennies treated with Lecirelin than in controls. In a previous work, spontaneous ovulation of the same jennies of this study occurred at a follicle Ø between 30 and 50 mm (average±sd: 40.3±7.8)⁷. According to this great variability at ovulation⁷, the small number of cycles followed in this work could have influenced the results. This study shows that is possible to induce ovulation in the jenny with buserelin acetate, at a dose of 3.15 mg, sc. Larger scale studies are needed to determine its applicability in the field.

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DO ENVIRONMENTAL FACTORS INFLUENCE HAIR CORTISOL IN FOALING SEASON?

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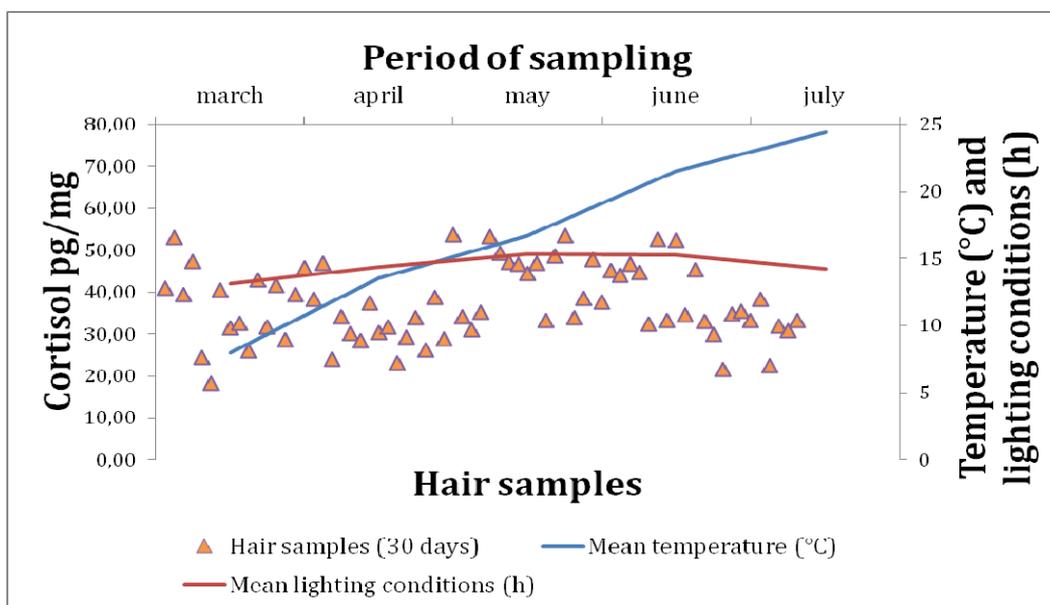
ABSTRACT - Plasma cortisol (C) in adult horse is influenced by several environmental factors, such as temperature and lighting conditions but to date it is not clear if also hair cortisol is subjected to the same factors. The aim of this study was to investigate the influence of these environmental factors on foal hair cortisol levels at 30 days of age during the breeding season. Hair samples were collected from 69 foals at 30 days of age from March to July and analyzed for C by RIA. For each sample were considered the averages of environmental factors (lighting conditions and temperature) during the 30 days preceding the sampling. Hair cortisol levels were 37.32 ± 8.87 pg/mg (mean value \pm SD). None of the climate variables was related to cortisol hair concentrations. In conclusion these data suggest that hair cortisol in 30 days old foals is not affected by the influence of temperature and lighting conditions and cortisol variations are due to an individual HPA axis activation and not to environmental factors.

INTRODUCTION - Cortisol (C) concentrations in plasma depend on different factors, such as lighting conditions¹, temperature, diet, age, pregnancy and period of lactation². Plasma C in adult horse has a well-established circadian rhythm which is synchronised with the light - dark and sleep - wake cycles³. Moreover it is unclear the presence of a seasonal cycle of this hormone in horse^{2,4}. However in neonatal foal the establishment of this circadian rhythmicity begin in the second month of life⁵. These studies have been all performed on plasma samples but never on hair, in which it is not clear whether hair cortisol levels are influenced by environmental factors and if show seasonal variation⁶. For these reasons the aim of this study was to investigate if environmental factors (temperature and lighting conditions) during the breeding season can influence hair C levels in foals of 30 days of age.

MATERIALS AND METHODS - The study was carried out in accordance with EC Directive 86/609/EEC for animal experiments. The study was performed from March to July, in a single Standardbred farm, in north east Italy. The study enrolled 69 foals, 33 females and 36 males. Environmental data have been recorded in collaboration with OSMER (Osservatorio meteorologico regionale dell'ARPA FVG). For each foal has been considered data relating to 30 days before sampling. At 30 days of age, hair was carefully collected with clippers from the withers shaved to the level of the skin, from an area previously shaved at birth. Samples were stored in dry tubes at room temperature until analysis. Cortisol was determined using the RIA method⁷. Hair cortisol concentrations were analyzed by ANCOVA. The mean temperature and photoperiod of the 30 preceding days were considered as covariates. Statistical significance was set at $\alpha \leq 0.05$.

RESULTS - The distribution of hair C levels during the period of observation, in relation to environmental factors, in the 69 foals is reported in Figure1.

Figure 1 - Distribution of hair C levels during the period of observation, in relation to environmental factors, in the 69 foals.



The average hair C concentration of all foals was 37.32 ± 8.87 pg/mg (mean \pm SD). Hair C levels did not change along the period of sampling despite changes in environmental temperature. In addition, hair C levels did not differ between females and males foals.

DISCUSSION - Hair cortisol measurement reflects hormone accumulation over months and can be used to assess cortisol variations in the long term⁷. To date it is not clear whether hair cortisol levels are influenced by environmental factors and if show seasonal variation⁶. Since the period March to July in north east Italy is characterized by increasing temperatures and photoperiod, the study was aimed to verify whether hair C levels could be influenced by these factors. Hair cortisol concentrations are unaffected by circadian hormonal variations in young foals⁵, so that it allows the study of long-term changes without circadian variations interference. Foal hair cortisol at 30 days of age is the result of a cumulative picture of HPA axis stimulation during the most critical period after birth, characterized by the neonatal adaptation to the new extrauterine life. The results of the present study show that lighting conditions and temperature do not influence significantly foal hair cortisol at 30 days of age. In conclusion, hair cortisol in 30 days old foals is not affected by the influence of temperature and lighting conditions and the cortisol variations are due to an individual HPA axis activation and not to environmental factors.

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EFFECTS OF COMMON MALLOW (*MALVA SILVESTRIS L.*) ON ANTIOXIDANT ACTIVITY OF JENNY MILK

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KEY WORDS: jenny milk, *Malva sylvestris L.*, antioxidant activity

ABSTRACT - Dehydrated common mallow (*Malva sylvestris L.*) capsules have been fed to jennies in lactation as a supplementary food to evaluate their effect on the antioxidant activity of milk. Common mallow is widespread in Mediterranean pastures and its nutraceutical properties are known. Jennies were divided into 2 equal groups, one group receiving the additional dietary supplement of mallow capsules. Milk was collected at day 30 post foaling and every 30 days for 4 times. In this period we have also surveyed the flora of the pastures near the farm. The chemical composition and the antioxidant characteristics of milk from two groups were determined. No differences were observed in chemical composition. The antioxidant activity (ABTS essay) resulted significantly higher ($P < 0.05$) in the treated group in the last control only (79.54 vs 55.52%).

INTRODUCTION - Donkeys can graze a relatively high number of plant species, in this revealing their characteristic as rural animals adaptable to marginal lands. In the natural pastures of Basilicata where this specie is reared is present also the common mallow (*Malva sylvestris L.*), a biennial-perennial herbaceous plant native of Europe, northern Africa and Asia, now naturalized in the Americas. It is known for its medicinal properties and consumed by human as a natural remedy, as a nutraceutical and is commonly used in cosmetics, because of its emollient and anti-free radicals properties. Some of the biological activity of this plant may be due to antioxidants, such as polyphenols, vitamin C, vitamin E, and β -carotene^{1,2}. The antioxidant capacity is higher in flowers, leaves and leafy flowered stems than in fruits. Because of its properties and its widespread occurrence in the rangelands of southern Italy as well as its high palatability we have assessed a possible antioxidant effect on jenny milk. To assure the ingestion of the plant by the animals dehydrated capsules of leaves and flowers were given.

MATERIALS AND METHODS - Eight pluriparous jennies which foaled in July 2010 were investigated postpartum during four months. The study was carried out in a rearing farm situated near Avigliano in Basilicata at the altitude of 700 m a.s.l.. The jennies were kept in box stalls with paddocks without access to pasture, and divided into 2 groups. Each group was fed with a *ad libitum* diet of 3.0 kg/d of a concentrate mixture and oat hay. In the experimental group were administered 9 common mallow capsules/day (*Malva* - Erboristeria Angelini - ABTS test, 80.26%). Milk samples were collected monthly, starting from the 2nd post-foaling month; in the period August-November individual samples were collected and refrigerated at 4 C. The following parameters were measured: protein, fat, and lactose by Milkoscan FT 6000; dry matter and ash content³. In addition, the antioxidant activity was measured in percentage by ABTS and DPPH test⁴. Differences between means were tested by Student's *t*-test.

vs 0.29%; lactose, 6.37 vs 5.98%; ashes, 0.40 vs 0.45% (in the control and in the treated group, respectively) (fig.1). The antioxidant activity (ABTS test) resulted significantly higher ($P < 0.05$) in the treated group in the last control only (79.54 vs 55.52 %) (fig.2). Regarding the rangeland flora in the areas interested to donkey rearing, a few data are available from a survey in areas near Avigliano carried out in the Fall, and grazed by donkeys. At the time of the visit, *Agrimonia eupatoria* L., *Dorycnium pentaphyllum* Scop., *Prunus spinosa* L. *Prunus* spp., *Quercus cerris* L. and some grasses: *Dactylis hispanica* Roth. *Phleum pratense* L., *Holcus lanatus* L., and *Malva sylvestris* L, were recorded.

Fig.1 - Jenny milk: chemical parameters within sampling (1-4), (mean \pm s.e.).

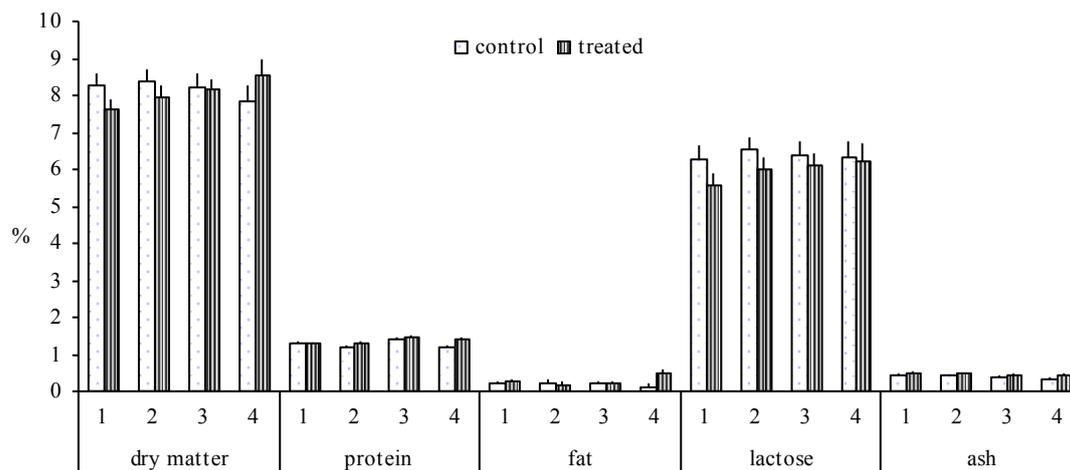
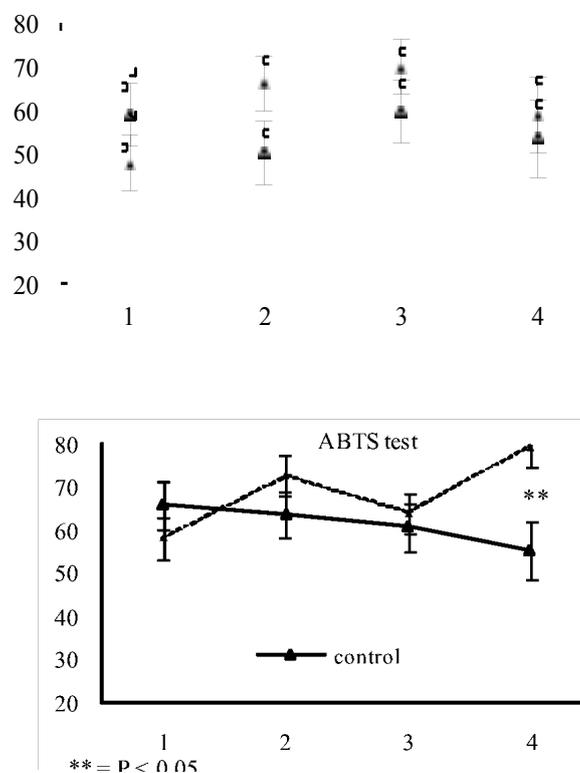


Fig. 2 – Antioxidant activity, % (mean \pm s.e.).



DISCUSSION – The antioxidant activity has shown significant values in the experimental group as compared to the control group only in the last test. Our

preliminary conclusion is that the presence of the common mallow in natural pastures might contribute to increasing the antioxidant activity of jenny milk. Of course, more work is necessary to assess if this is actually the case, and the next step will be the use of the actual plants from the grassland.

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CONGENITAL ABNORMALITY OF THE CERVIX IN A JENNET

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KEY WORDS: jennet, cervix, congenital abnormalities

ABSTRACT - Congenital abnormalities of uterus and cervix are rare in horses, often associated with infertility, despite normal ovarian activity and sexual behaviour. An abnormality of the cervix in 5-year-old jennet was described. Clinical and ultrasound examination of the genital tract showed a normal development of the uterus with regular cyclic activity in both ovaries. Vaginoscopy revealed a normal development of the vagina and a malformation of the cervix which appeared adherent to the vaginal wall and left deflected; the cervical os was surmounted by a dorsoventral band. A stenosis of the cervix was demonstrated during oestrus by inoculating fluid and revealing it in uterus by ultrasound. Reproductive history, clinical and histological examinations allowed a diagnosis of congenital abnormality of the cervix.

INTRODUCTION - Congenital abnormalities of the uterus and/or cervix are rare in horses, often associated with infertility, with normal or abnormal ovarian activity and sexual behaviour¹. These subjects are often mated for repeated cycles or for several reproductive seasons, before the defect is diagnosed. Genital abnormalities reported in the mares are: segmental aplasia of the paramesonephric duct²; hypoplasia of the cervix with normal ovarian activity³; double cervix caused by incomplete fusion of Müllerian ducts⁴; cervical hyperplasia with prolapse⁵; multiple recurring uterocervical leiomyomas⁶; extreme tortuosity of the cervix with fluid accumulation in the uterine body during estrous¹. In the jennet, only two cases of congenital hypoplasia of the cervix have been reported in the Portuguese breed⁷. The aim of this study was to report a multiple congenital abnormality of the cervix in a jennet.

CASE REPORT – A nulliparous, 5-year-old, mixed breed jennet was admitted to the Center of Animal Reproduction and Assisted Reproduction of the Faculty of Veterinary Medicine of Messina. Reproductive history revealed that the jennet, in the previous reproductive seasons, received multiple natural services with jackasses of proven fertility, without pregnancy. In addition, after each mount a noticeable leakage of semen was seen to come out the vagina. On clinical examination, the vulva was normally-shaped and almost vertical; rectal examination revealed a normally developed genital tract and a regular activity in both ovaries. Ultrasound examination, performed by a portable scanner (Esaote-Eagle-PieMedical) with a linear multifrequency endocavitary probe (from 6-0 to 8-0 MHz), showed a normal development of the left (4.8 x 3.0 cm) and right (5.0 x 3.2 cm) ovary, and the presence of growing follicles and a corpus luteum on the right. Vaginoscopic examination, using a speculum, allowed to see a normal vagina, while the cervix was not in a central position but deviated and adherent to the left wall of the vagina. The cervical os was surmounted by a dorsoventral band. To improve visualization, a flexible 10 mm fiberscope, equipped with a light source system and a blowing/suction Olympus CLK-532 was employed. The cervical os was recognized diverting laterally the fold, which appeared to be partially adherent to the ostium. Catheterization of the cervix with a 5 mm catheter was not feasible. Some biopsies were performed on the dorsoventral band and on the cervical os. Histological

examination showed that the band was made up of vaginal epithelium and the cervical os was constituted by a single layer of low epithelium resembling that of the cervix. No granulation tissue or inflammatory infiltrates were seen. The jennet was re-evaluated during oestrus. The vaginal portion of the cervix appeared much more prominent, swollen and globular-shaped. Catheterization of the cervical channel was possible for just a few centimetres but allowed to inoculate a small amount of sterile saline solution which mostly flowed back into the vagina and partially reached the uterine body, as demonstrated by concomitant ultrasound examination.

DISCUSSION AND CONCLUSION - The acquired origin of the described lesion was unlikely. Postpartum lacerations were excluded by the clinical history. Iatrogenic lacerations or adhesions due to uterine treatments with caustic or irritant substances⁹ were excluded by the absence of granulation tissue or inflammation in biopsies. On the other hand, congenital abnormality of the genital tract may be associated to sex chromosome defects. Although karyotype was not available, the normal size of the ovaries, the presence of growing follicles and corpora lutea allowed excluding such defects, especially the Turner's syndrome⁸. The presence of the dorsoventral band looks like the lesion observed in a mare affected by incomplete fusion of Müllerian duct⁴. Clinical and histological observations of the cervix were in agreement with a condition of hypoplasia, as recently described in donkey species⁷, resulting in a congenital stenosis, which was certainly responsible of the infertility of the animal. The clinical and histological examinations confirmed that the jennet was considered not suitable for breeding.

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OTHER SPECIES

MORPHOLOGY PARAMETERS OF CRYOPRESERVED BOAR SPERMATOZOA

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KEY WORDS: CASA, cryopreserved boar semen, morphology parameters

ABSTRACT - Ejaculates obtained by 3 different breeds of boars, were cryopreserved using 2 different cryopreservation protocols. The samples were assessed by CASA before freezing, and after thawing. The objective of the study was to estimate the difference of the morphology parameters (elongation, and area the spermatozoa head) of the in liquid semen, and the spermatozoa cryopreserved by two different protocols. The samples taken by glowed hand technique, were extended to ratio 1:1 and taken to laboratory for CASA analysis. The first group of ejaculates was criopreserved according the procedure described by Westendorf et al.¹ modified by Thurnston et al.². In the second group, only P1 of SRF was cryopreserved according the procedure by Rodriguez-Martinez and Wallgren³. The analysis was performed on CASA, using the standard set-up for boar semen. The elongation values ranged from 45.35±0.13%, 44.92±0.17%, and 44.39±0.23%, in liquid semen, TCP semen, and SCP semen, respectively. The area was on levels of 9.83±0.05 µm²; 9.71±0.12 µm² and 8.74±0.16 µm² respectively in the mentioned groups. The results showed significant difference in elongation between the first and the third group (p<0.05), and highly significant difference in area in the third group (p<0.001).

INTRODUCTION - Despite the widespread of use of cryopreserved semen in cattle, its utilization in swine is limited, mostly as a result of the poor cryo-survival of boar spermatozoa. In order to overcome the mentioned problem, most of the work so far was focused on designing a suitable cryo-protocol for boar semen.

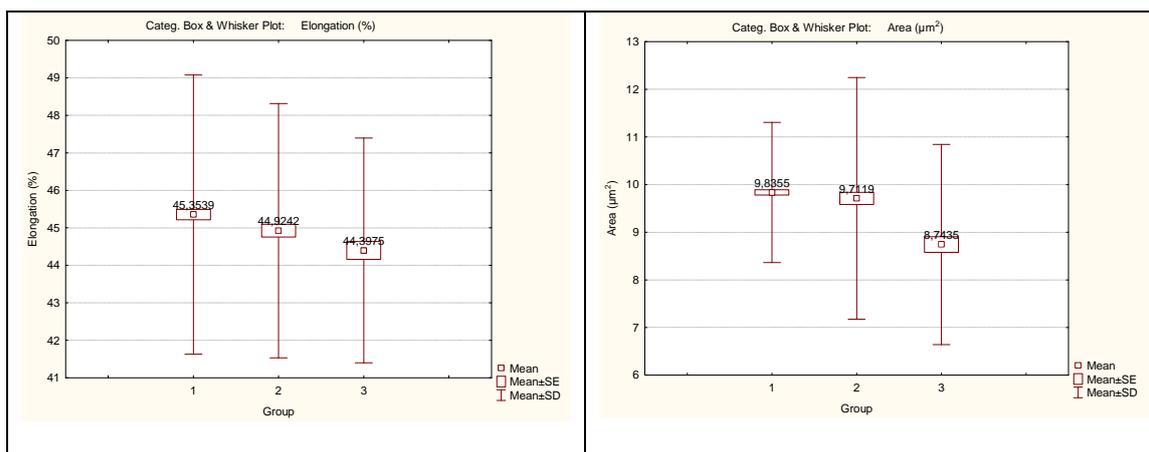
The shape of spermatozoa's head is a significant feature, which gives information about the changes of the hydration status of the cell during the process of cryopreservation. The objective of this study was to determine the significance of the alteration of the elongation and area of sperm's head during two different protocols of cryopreservation.

MATERIALS AND METHODS - Total of 713 ejaculates, obtained from 19 boars of 3 different breeds (Landrace, Yorkshire and Durock) were used in our investigation. The ejaculates were obtained traditionally, by 'glowed hand' technique, in field conditions. The first, contaminated, fraction of the ejaculate was separated, while the rest of the P1 or full SRF of the ejaculate was collected in a plastic bag, which is filtering the gelatin fraction from the spermatozoa rich fraction. After the routine examination of the quantitative parameters of the obtained ejaculates, by phase-contrast microscopy, the semen was diluted (Androhep®, Minitub, Tiefenbach, Germany) in ratio 1:1 and transferred in glass containers. Chilled on 17°C were transported to the laboratory, where were assessed by CASA (HTM-IVOS V.12.0, Hamilton Thorne Research, MN, USA), and cryopreserved.

The first group of ejaculates (389) consisted by the complete SRF, were cryopreserved according the protocol described by Westendorf et al.¹ modified by Thurnston et al.²

and the second group (171), consisted of P1 of SRF, was frozen according to the protocol described by Rodriguez-Martinez and Wallgren³ while the group of 713 evaluated ejaculates of liquid semen, were considered as a control group. The thawing was performed in a water-bath preheated on 39°C, and all the samples were submitted for CASA evaluation of the elongation and area of the spermatozoa's head. Statistical analysis was carried out in Statistica 7.0 (StatSoft Inc. Tulsa, OK, USA).

RESULTS AND DISCUSSION - The preview of the obtained results regarding the morphological parameters of liquid and cryopreserved spermatozoa, along with the descriptive statistic analysis, and analysis of variance is given on following figure.



As the graphs are showing, the levels of Elongation (transversal/longitudinal axis of the spermatozoa's head ratio) were $45.35 \pm 0.13\%$, $44.92 \pm 0.17\%$, and $44.39 \pm 0.23\%$, in liquid semen, TCP semen, and SCP semen, respectively. The area (area of the spermatozoa's head in μm^2) was on levels of $9.83 \pm 0.05 \mu\text{m}^2$, $9.71 \pm 0.12 \mu\text{m}^2$ and $8.74 \pm 0.16 \mu\text{m}^2$ respectively in the mentioned groups. The results showed significant decrease in elongation between the first and the third group ($p < 0.05$), and highly significant decrease in area in the third group ($p < 0.001$).

The conclusion is that the cryopreservation affects the morphology of the boar spermatozoa that could be explained with the evacuation of the intracellular water during the cooling, and with an insufficient rehydration during the thawing.

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THE INFLUENCE OF INSEMINATION NUMBERS DURING THE SAME ESTRUS ON SOME REPRODUCTIVE PARAMETRES AFTER FERTILIZATION OF SOWS WITH ARTIFICIAL INSEMINATION

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KEY WORDS: Sow, Artificial insemination, reproductive parameters.

ABSTRACT - In this paper some main reproductive parameters were studied in a pig farm, after the application of different numbers of artificial insemination in 83 sows during the same estrus. The age of the sows and the conditions were the same for the three groups of the study. In each respective group, during the same estrus, one artificial insemination (group A, 28 sows), two artificial inseminations (group B, 31 sows) and three artificial inseminations (group C, 24 sows), was applied. There were no significant differences between group B and group C in the conception rate of the sows during the 1st estrus after weaning 83,87% and 83.33%, respectively and in the total number of piglets born 10.8 ± 0.2 and 10.6 ± 0.3 piglets, respectively. Comparing the group A, 28 sows in which we applied one artificial insemination during the same estrus, a difference in the conception rate (78.57%) and in the total number of piglets born (10.22 piglets per sow) between groups B and C, was observed but not statistically significant.

INTRODUCTION - The artificial insemination wended in some pig farms in Albania last year. The purpose of this study was the observation of possible differences in the reproductive parameters of sows in the application of artificial insemination methodology, one two or three times during the some estrus. Those data could be useful for the implementation of artificial insemination in sows in most effective and economic way.

MATERIALS AND METHODS - In a pig farm in central Albania, a total number of 83 sows has been used, which shows estrus during the first half part of the year 2011. From those sows (Large white x Landrace), we inseminated artificially 28, 31 and 24 sows respectively, one, two and three artificial inseminations during the same estrus. The age of the sows that participated in the study had no significant differences between the groups. The calving period of piglets per sow in the three groups was the same. We used the same boar for ejaculation (Landrace x Large white), the semen diluted with BTS (Beltsville Thaw Solution), and for the artificial insemination that was made by sperm preserved up to 24 hours with 3 billion spermatozoa per dose². The detection of the estrus of the sows was made with the use of detective boars. For the first group the only insemination took place 12 hours after the detection of estrus. For the second and the third group the first insemination took place immediately after the detection of estrus and the second or third inseminations were with 12 hours difference. The conditions of breeding, nutrition, prophylactic program etc, were the same for both groups of sows.

RESULTS - On this table we have the conception rate and the total piglets born for the group of sows with different number of Artificial Inseminations (AI) during the same estrus.

Groups	Nr of sows	Insemination number during the estrus	Sows that conceived at 1 st estrus N - %	Total Piglets born X±DS
Group A	28	1	22 - 78.50	225 10.22±0.3
Group B	31	2	26 - 83.87	281 10.8±0.2
Group C	24	3	20 - 83.33	212 10.6±0.3

DISCUSSION - As we can see on the table above, the percentages of sows that were fertilized in the first estrus after the weaning of the piglets with artificial insemination were 78.57%, 83.64 and 83.33% for the three groups respectively. There were no differences between groups B and C in the conception rate of the sows during 1st estrus after weaning (83,87% and 83.33%, respectively and in the total number of piglets born (10.8±0.2 and 10.6±0.3 piglets, respectively). But in group A, in which there was applied one artificial insemination during the same estrus, we observed a difference of about 4.76-5.3% in the conception rate (78.57%) and one difference of about 0.38-0.58 piglet per sow which is lower than groups B and C. According to some researchers, the percentage of fertility in the artificial insemination technique influenced¹ or not² by the number of inseminations in the same estrus. Influenced by the factors that come from the number of spermatozoa per dose³ and other factors like, breeding, conditions etc⁴. The reproduction parameters of the artificial insemination can get influenced by the detection time of estrus as well⁵. From the conclusions of this work and of other authors as well, it is shown that the percentage of fertility and the total number of piglets born, in artificially inseminated sows with different numbers of inseminations in the same estrus, is the same between 2 and 3 inseminations or a little higher of 2 and 3 insemination than 1 insemination during the same estrus. It is recommended to the pig farms that they use 2 artificial inseminations during the same estrus. Using it one or three times will bring practically lower results (group A, 1 insemination), or more expenses comparing to 3 inseminations (group C, 3 inseminations).

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EFFECTS OF METHOD OF FORMULATION DIETS ON BROILER BREEDER REPRODUCTIVE PERFORMANCE

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KEY WORDS: broiler breeder, digestible amino acids, reproductive performance.

ABSTRACT - A study was conducted to determine the effects of method of formulation diet based on type of amino acid (digestible and total) and nitrogen corrected - metabolizable Energy (True and apparent) on reproductive performance of broiler breeder from 50 to 64 wk of age. 168 female and 24 male breeders in 4 treatments with 6 replicates (7 females: 1 male) were used in randomized factorial 2×2 design. Four experimental diets were formulated based on two factors: two levels of Apparent and True Metabolizable Energy corrected for Nitrogen (AMEn and TMEn) and two levels of Total (TAAF) and Digestible Amino Acids (DAAF) of Feedstuffs. The results showed that egg weight, fertility, hatch ability and No. Chickens were significantly different and affected by diet formulation based on energy. Hens on treatment 3 (TMEn+TAAF) was at the lowest level significantly different in egg weight 65.73 (g), fertility 61.06%, hatchability 56.36 %, No. Chickens 28.01 and chicken weight 45.4 g. This experiment showed that the diet formulation based on AMEn+ TAAF had highest significant effect on egg weight 65.73 (g), fertility 61.06 %, hatchability 56.36 %, chickens No: 28.01 and chicken weight 45.4 g.

INTRODUCTION - Energy and amino acids are the most important factors in broiler breeder hen's diet. Any changes in the daily nutrients intake in broiler breeder hens must be done base on their requirements¹. Therefore, knowing the requirements of metabolizable energy (ME) in broiler breeder in any age and phase of production and metabolizable energy value of feedstuffs in the diet is essential for their optimal production (NRC, 1994). The objective of this experiment was to determine the effects of types of broiler breeder formulation diets on reproductive performance. Diets formulated based on two levels of energy (AMEn, TMEn) and two levels of amino acids of feedstuffs (total and digestible).

MATERIALS AND METHODS - At 50 weeks of age, Arian broiler breeders were weighted, and allocated to treatment groups on the basis of mean body weight (g), female (3550±25) and male (4390±30). Birds were housed in 24 floor pens of 1.2 × 2 m with 1 bell-type drinker. Light program provided for 16 hours of light per day from 7 am to 23 pm (50 to 64 weeks). Diets were adjusted based on the requirements of broiler breeder (NRC, 1994)² at level of the total amino acids requirements. Feeds provided were in mash form and were milled with a 3 mm screen to obtain a similar particle size in all diets. Both males and females broiler breeder received the same diets at 8 am. Diets provided 410 Kcal metabolism energy and 21/2 g protein daily. The basis only differences between the treatments were the type of metabolizable energy, amino.

RESULTS AND DISCUSSION - The egg weight was significantly heavier on treatment fed diets formulation based on AMEn to 68.69 than TMEn to 68.19 (g) (P<0.05). These results agree with Leeson et al.¹ showed that increased energy intake had significantly

positive effects on egg weight. Metabolizable energy levels in the diet of broiler breeders are very important. A deficiency may lead to a poor performance, as well as an increase of body fat due to excess in energy levels. The main effects of diets formulation based on amino acids of feedstuffs had no effect on hatchability and chicken weight and No. Attia et al.³ observed the broiler breeder hens (21 to 61 weeks) had a significant positive correlation between energy intake (396, 423 and 450) and fertility and hatchability. In this experiment the treatment fed diets formulation based on AMEn was better in egg weight, fertility and hatchability than treatment fed diets formulation based on TMEn. This difference was statistically significant ($P<0.05$). No reports describe the effects of diet formulation based on MEN and amino acids of feedstuffs on fertility and hatchability.

Table 1 - Main effects of diets formulation based on Metabolizable Energy, Amino Acids of Feed on reproduction traits of broiler breeder (50 to 64 weeks)

MEn	Amino Acids (Feedstuffs)	Egg weight (g)	Settable Eggs(%)	Fertility (%)	Hatchability (%)	Chick (No/hen)	Chicken Weight(g)
Apparent		69.69 ^a	97.29	85.26 ^a	73.91 ^a	38.8 ^a	50.18
True		67.05 ^b	96.54	75.63 ^b	63.97 ^b	32.1 ^b	47.40
<i>P-Value</i>		0.041	0.542	0.001	0.001	0.015	0.169
	Total	67.03 ^b	99.14 ^a	80.87	68.14 ^a	35.2	47.83
	Digestible	69.17 ^a	94.7 ^b	79.23	65.69 ^b	35.6	49.75
	<i>P-Value</i>	0.046	0.005	0.12	0.007	0.409	0.328
Apparent	Total	68.33 ^b	98.79 ^a	85.69 ^b	69.97 ^{ab}	37.2 ^{ab}	50.1 ^{ab}
Apparent	Digestible	69.97 ^a	95.79 ^{ab}	84.23 ^a	78.03 ^a	40.3 ^a	50.27 ^a
True	Total	65.73 ^c	99.48 ^a	76.05 ^b	66.32 ^b	33.2 ^b	45.4 ^c
True	Digestible	68.37 ^b	93.6 ^b	75.22 ^b	61.59 ^c	30.8 ^b	49.4 ^b
<i>P-Value</i>		0.011	0.025	0.015	0.046	0.023	0.046
SEM		0.805	1.174	1.438	2.198	2.855	1.114

a-c Means within the same column not sharing a common superscript differ significantly ($P<0.05$).

The egg size and internal quality of eggs are important for hatching eggs. Fertility and hatchability are the major economical traits in broiler breeder reproductive performance. Hatchability was significant in diet formulation based on energy and amino acids of feeds. Main Effects of energy was significant in fertility ($P<0.05$). The fertility and hatchability were significantly heavier on treatment fed diets formulation based on AMEn to 80.46 and 67.36% than TMEn to 66.63 and 56.15% respectively ($P<0.05$). The hatchability was significantly heavier on treatment fed diets formulation based on TAAF to 65.13 than DAAF to 58.39 ($P<0.05$). Feeding broiler breeder digestible amino acids of feedstuffs significantly increased egg weight by 2.14g more than broiler breeders fed total amino acids of feedstuffs density diets. Formulating broiler diets based on digestible amino acids of feedstuffs gives a better prediction of dietary protein quality and broiler breeder performance than total amino acids.

CONCLUSION - Feeding broiler breeder AMEn diet significantly improved fertility, hatchability and No, chicks by 14.85%, 12.06% and 8.8 (No/hen) more than broiler breeder fed TMEn diets respectively. This study showed that diets formulation for AMEn, TMEn, TAAF and DAAF have significant effects on broiler breeder reproductive performance.

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RAPTOR AND PSITTACINE BIRD GYNECOLOGICAL SCREENING USING A MININVASIVE DIAGNOSTIC TECHNIQUE

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KEY WORDS: conservation program, raptors, parrots, sex endoscopic evaluation.

ABSTRACT - Although the availability of proven bio-molecular tests for sex determination, additional information provided by a direct view of the reproductive system performed with an endoscope are unique. In this work were studied about 2500 subjects of several species of birds (raptors and parrots), in order to identify functional status of reproductive system and, eventually, possible semen donors. During the tests it was also possible to diagnose several diseases, sometimes in a subclinical form, that allowed to identify better clinical therapy or discard some birds from reproductive chain, cutting down the economic impact, in private collections.

INTRODUCTION - According to recent study¹ of about 8700 species of known birds specie, more than 500 are threatened or endangered, and despite the difficulties due to poor knowledge of reproductive physiology and “details” of these animals (the studies in bibliography are very preliminary), it could promote both the maintenance of biodiversity and the recovery of some of these species, through specific assisted reproduction programs in captivity. The coelioscopy in birds is the largest utilized endoscopic technique, because there is no other zoological group in which this minimally invasive technique is much facilitated by the anatomical situation. In addition the absence of diaphragm allows to examine all the organs of coelomic cavity and the air sacs makes unnecessary to insufflate the abdomen and lets, at the same time, a good quality observation of viscera. The determination of sex in monomorphic species was the first application of endoscopy proposed in birds^{2,3,4} and actually it remains one of the most popular endoscopic techniques. Endoscope is placed on patient's left side since only the left ovary and oviduct are generally developed in almost all species of birds

MATERIALS AND METHODS - Raptors were wild animals sheltered at the CANC (Centro Animali Non Convenzionali) Center of Animal Pathology Department while parrots belonged to private collections whose owners required to known sex for commercial purposes; a total of 2497 subjects was studied (30 raptors and 2467 parrots). All birds were submitted to coelioscopy in isoflurane anesthesia in order to determinate their sex. The endoscopies were carried out using left lateral access, and particularly the post-femoral variant², which allows to move the endoscopic optic in all directions, without any alteration of the respiratory dynamics. The access was identified by palpation of the triangular area bounded by muscle, last rib and pubic bones. The endoscopic probe was introduced in a “hole” obtained by a 3 mm skin incision and a subsequent blunt dissection of semimembranosus muscles, performed with an Adson and a hemostat forceps. In each subject were finally collected multiple image to compare the possible similarities and/or intraspecific differences, considering also the gonads dimensional changes, in relation to the reproductive activity of tested bird.

RESULTS - In this study were tested animals belonging to 53, of the 350 existing psittacine species. As regards birds of prey only 30, of 135 sheltered at the CANC, subjects were evaluated; in fact the severe clinical conditions of a lot of animals did not allow to carry out the coelioscopy. The 30 evaluated raptors belonged to three nocturnal species (*Athene noctua*, *Strix aluco* and *Asio otus*) and five to diurnal species (*Buteo buteo*, *Accipiter nisus*, *A. gentilis*, *Milvus migrans* and *Pernis*

apivorus). In some cases it was also possible to identify some subclinic pathological processes involving reproductive tract (3 hermaphroditism, 5 ovarian cysts, 1 ovarian granulomas, 152 atrophy of one or both testicles) and/or other anatomical districts (3 aspergillosis, 4 respiratory parasites, 27 inflammatory reactions). Many of these 195 pathological cases (7.8%) were promptly and correctly treated. This clinical approach allowed to reintroduce or permanently removed these subjects from to reproductive chain.

DISCUSSION - Results obtained in this preliminary work show that this technique has proved to be essential in selection of potential semen donors (sometimes of very rare species) and at the same time to understand healthy conditions and sexual maturity. It is also remarkable the rapid recovery time and the absence of side effects: in fact few hours later all animals normally ate and surgical lesion healed in about ten days. The possibility of a direct view of bird gonads bypasses excessive manipulations and long waiting timing that are often critical in working with high stress susceptibility animals.

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POST PARTUM DISEASE IN PUP AND ADULT PET CHINCHILLA (*Chinchilla lanigera*)

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KEY WORDS: pet chinchilla, post partum disease, pups disease

ABSTRACT - In this report we want to describe the diseases more common in female chinchillas during the postpartum period and diseases more common in pups. Fundamental knowledge of the needs of the species and the management of a large brood, intended as being composed of more than 3 puppies.

INTRODUCTION - In recent years, the chinchilla has become more usual as a pet since it is a docile animal, very clean, odourless, and is very appreciated for the wonderful and soft fur. In Italy many fur farms turned to the production of pet animals and the Veterinary Doctor is called to follow the breeding and welfare of this Hystricomorph rodent originating from the cordillera of the Andean.

MATERIALS AND METHODS - Chinchilla at the Italian latitudes has a polyestrous reproductive season cycle from November to May the estrous cycle lasts 28-50 days with a flair of 24-48 hours. The female has two uterine horns and two cervixes, three pairs of mammary glands: one inguinal pair and two lateral thoracic pairs¹. The chinchilla has a very long pregnancy during 105-115 days (usually exactly 111 days) and the pups, usually 1-5 per litter, are born with early fur and eyes already open.² Weaning takes about 8 weeks³.

The reproductive disorders that frequently occur in these Hystricomorph are hypocalcemia post-partum, and postpartum constipation; to remember then genetic abnormalities caused by the coupling between certain phenotypes.

During and after gestation, the chinchilla has paraphysiological constipation due to the size of the uterus, this condition may be exacerbated during the first week of post-partum and can be confirmed by X-ray. Usually this pathology resolves spontaneously but it is good practice to advise the owner to control the appearance of the feces of the animal, its weight, his appetite and vivacity after parturition⁴.

The post-partum hypocalcemia may occur 2-3 weeks after parturition. It is a severe condition, the chinchilla lies in lateral recumbency characterized by depression and gastrointestinal tympanism. Usually the disorder affects debilitated animals fed with inappropriate food (especially low in calcium) or animals with a large litter; it is necessary a supportive therapy and the administration of calcium gluconate.

As for the proper management of the breeding it is important to remember that some phenotypes are not recommend for pairing. Some genes do not allow the survival of the fetus in homozygous condition (lethal factor). The two lethal genes involved in the chinchilla coat colors are white and the TOV (Touch Of Velvet). If there is a condition of homozygosis for alleles White + White or TOV + TOV, there is a 25% chance that the embryo does not develop and that it is reabsorbed by the mother (but sometimes you can have stillbirths or puppies that die a few hours after birth).

Breeding of these animals are also relatively frequent phenomena of aggression among pups, sometimes severe as to cause death. As mentioned previously, the female chinchillas have 3 pairs of breasts, 2 chest and 1 inguinal. Usually the groin are those that develop and are the richest in milk. Often with numerous litters (3-5 pups) there are problems of lactation and small chinchillas can fight furiously to grab the breast richer in milk. At this stage they become very aggressive to each other and sometimes injure themselves so seriously, especially the ears and muzzle, can also create very painful injuries at the breasts of the mother who refuses nursing. To overcome these difficulties some farmers and some authors¹ recommended cutting the upper and lower incisors of the pups a few hours after parturition, the teeth will grow back completely within 7-10 days. It 's important to monitor their weight daily and write it down, usually in the first week of life, they usually increase 1-2 grams per day except for the first 1-3 days in which the weight may remain stable or may slightly decline physiologically. At birth the cubs weigh about 30-50 grams and at the age of 7-10 days are able to eat small amounts of solid food.

If problems arise during lactation action is needed by artificial feeding but it is important still to leave the pups with the mother to be heated properly and learn the normal ethogram of the species. Personally in the last years I had good results by doing the 'rounds' with the mother and leaving 1 or 2 cubs at a time, trying to find the cubs more aggressive. The time spent with the mother varies according to age of the puppy and based on weight, in principle, for the first few days will be about 3-4 hours, taking care to keep the puppies that are not with parents, with the increasing age these periods can be longer shifts of 6-8 hours. To accelerate the transition from a digestive enzyme (fed milk) to a fermentative (with herbivorous feeding) and stimulate the spontaneous power can be given small amounts of very dilute mixed formula for herbivores (eg. Critical Care – Oxbow) with fecal pellets from healthy adults.

If the puppies do not grow and the suspect is a problem of agalactia or the mother dies can be groped to adopt the pups from another chinchilla and usually have good nannies and accept children of other females. Obviously close monitor the behavior of animals and readily separate the puppy if the new mother shows signs of aggression.

The composition of milk is chinchilla: protein 7.2%, fat 12.3%, 1.7% lactose.

Proper nutrition during pregnancy is estimated to has 16-20% protein, 2-5% fat, 15-35% fiber, 1% calcium, 0,5% phosphorus.

RESULTS AND DISCUSSION - This study wants to underline the importance of profound knowledge of the specie, in particular the reproductive physiology, the diseases during the pregnancy and of the post partum period; also the aim of this work is to describe the correct approach to large brood illustrating the opportunities to maintain in life all pups: by turning to mother or by artificial nursing.

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THE IMPORTANCE OF NEUTERING IN *RATTUS NORVEGICUS* FOR THE PREVENTION OF REPRODUCTIVE DISEASES

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KEY WORDS: *Rattus norvegicus*, reproductive disease, neoplasia

ABSTRACT - This case report want to demonstrate the need to support to the clinical visit by laboratory exams in order to arrive at a certainty diagnoses. This is the case of a female rat of 3 years, entire, brought to visit for the presence of a mass at the level of the axillary region. The popularity of the rat and the extensive knowledge about anatomy, physiology, health and disease are largely derived from its use as laboratory animals in scientific research. Given the high prevalence of disorders of the reproductive system in young patients should be considered routine sterilization, which will be the best form of prevention; the ovariectomy is indicated for the control of reproduction, prevention and treatment of ovarian cysts and uterine diseases, reducing the incidence of mammary and pituitary neoplasia¹. Moreover, in both sexes, performed at any age, may be beneficial behaviour, such as reducing the marking of territory.

INTRODUCTION - Neoplasia development can affect both sexes. The neoplasia which has higher prevalence in rats is mammary gland fibroadenoma^{2,3}. Mammary tissue is very extensive and neoplasia may develop from the neck to the groin region, reaching a considerable size quickly. The treatment of choice is represented by the surgery, which still presents difficulties related to the location and size. The incidence is significantly reduced in neutered rats. The cause of death is not associated with the development of metastases, but most often secondary to ulceration and subsequent infection of the mass. Ultrasonography is the diagnostic tool of choice to determine origin and characteristics of the mass.

MATERIALS AND METHODS - Clinical examination is shown the presence of a mass, 3 cm in length and 2 cm in diameter, mobile, well-defined. We proceed with the direct removal of the mass because the owner does not consent to carry out further investigations regarding the nature of the mass, like cytology. Given the location and age of the animal is presumed to be a mammary glands neoplasia. The mass was removed by radio surgery unit; when the operation is performed to shortening of the incisors to prevent self-trauma. Antibiotic and analgesic therapy was performed in the days following the operation.

In literature, mammary gland neoplasia are usually benign fibroadenomas⁴; they have higher incidence in animals over one year of age⁵. Less than 10% appears to be malignant⁶. They are usually single, large, solid, non-adherent to deep structures and well tolerated by the animal.

These neoplasia appear to be sensitive to hormonal stimuli³. It was shown that estrogens are not the main factor in the development of these cancers⁵; it has been suggested the involvement of prolactin produced by pituitary neoplasia as a major stimulus to the development of mammary neoplasia. However, estrogens may contribute to the development of pituitary neoplasia, which in turn release higher levels of prolactin, which stimulates the development of mammary tumors⁷. The ovariectomy in association with the removal of mammary neoplasia helps to prevent relapses. Before the surgery will be important to determine the nature of the mass to be able to plan the surgery and provide a prognosis. For this purpose it must carry out an echo guided needle aspiration for obtaining a sample of the

tissue to be examined microscopically. The use of oestrogen antagonists did not demonstrate efficacy, resulting also hepatotoxic⁶.

RESULTS - Two months later the animal showed to be well, to eat and to haven't self-trauma injuries and recurrences. In the coming months will be useful to conduct periodic checks to highlight the possible development of new masses.

DISCUSSION - For the author ovariectomy and ovariohysterectomy involve many risks to the safety of the animal, but are the best preventing procedures in females to reduce the incidence of mammary and pituitary neoplasia, increasing life expectancy.

Since mammary gland fibroadenoma may also develop in males⁵, but as mentioned earlier, that estrogens contribute to the development of pituitary neoplasia contributing to the development of mammary neoplasia, castration should have no effect on the incidence of neoplasia. Will require studies aimed at evaluating the effects of castration on the development of mammary glands masses.

An alternative could be represented by the use of hormonal implants: studies on this should be conducted to determine the duration and effects in this species, with differences also between the two sexes.

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THE COMPARATIVE STUDY OF CONCEPTION RATE, TOTAL NUMBER OF PIGLETS BORN AND WEANING, AND THE INTERVAL FROM FARROWING TO CONCEPTION, AFTER FERTILIZATION OF SOWS WITH NATURAL SERVICE OR ARTIFICIAL INSEMINATION

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KEY WORDS: Artificial insemination, sows, conception rate

ABSTRACT - The artificial insemination in sows in Albania had started on 1982¹, but it was stopped for a period of time, from 1990 to 2005. The five last years the artificial insemination in sows has been restarted in some pork farms and it is gradually growing. In this paper certain reproductive parameters were studied in a swine unit, after the application of natural service (group A, 75 sows) and artificial insemination (group B, 88 sows). The allocation of the sows was chosen randomly. There were no significant differences between the two groups in the conception rate of the sows during 1st estrus after weaning (78.6% and 80.7%, respectively), the mean 1st to 2nd oestrus interval in the sows returning to estrus (24.3±1.3 and 25.6±1.7 days, respectively), the mean number of piglets born alive (10.3±0.4 and 10.7±0.3 piglets, respectively), and the mean number of alive piglets at weaning (8.9±0.3 and 9.1±0.2 piglets, respectively). Comparing the total number of the sows fertilized with artificial insemination to the total number of the sows naturally mated, we observed significantly shorter farrowing to the conception interval (40.1±2.6 and 51.2±2.9 days, respectively), as well as significantly shorter weaning to the conception interval (16.1±1.7 and 25.6±1.8 days, respectively) in the former group.

INTRODUCTION - The pig production in Albania is relatively low, fact that has been related to the husbandry conditions, the low fertility and the low average number of piglets born per sow. In this context, time dictates the assessment of natural mating method, in terms of economic, genetic, sanitary-veterinary and its gradual replacement by artificial insemination technology. The purpose of this study was the application of artificial insemination methodology and the comparison of reproductive parameters obtained by the technique of artificial insemination with natural service mating to obtain data that could be used to stimulate the implementation of artificial insemination in sows in different regions of Albania.

MATERIALS AND METHODS - In a pig farm in a region of Middle Albania, a total of 163 sows has been used, which shows estrus during the last 6 months of 2010. 75 and 88 from those sows (Large white x Landrace), were respectively mating naturally and inseminating artificially. The age of the sows that participated in the study regarding the number of calving had no significant changes in both groups. There was also no significant difference between the average calving period of piglets per sow in both groups. Out of all the sows there were made 2 mating within a 12 hours interval (with Belgian Landrace x Large white boars). The detection of the estrus of the sows in the second group (AI) was made with the use of detective boars. For the dilution of the semen, has been used BTS (Beltsville Thaw Solution), and for the artificial insemination was made by sperm

preserved up to 24 hours with 3 billion spermatozoa per dose². The conditions of breeding, nutrition, prophylactic program etc, were the same for both groups of sows.

RESULTS - On the following table we have some of the main reproductive performance values for the group of sows with natural services (NS) and for the group of Artificial Inseminations (AI).

Type of insemination	Conception rate at 1st estrus (%)	Total Piglets born	Piglets born alive	Piglets at weaning	Interval farrowing to conception (days)	Interval weaning to conception (days)
Natural (NS)	78.6	10.8±0.4	10.3±0.4	8.9±0.3	40.1±2.6	16.1±1.7
Artificial (AI)	80.7	11.2±0.4	10.7±0.3	9.1±0.2	51.2±2.9	25.6±1.8

DISCUSSION - From the table above, we observe that the percentage of sows that were fertilized in the first estrus after the weaning of the piglets with natural service and artificial insemination was respectively 78.6% and 80.7% without any statistically significant difference. These findings correspond to other studies of this kind carried out by other researchers with the use of artificial insemination by fresh semen³. According to Uhlen⁴, the percentage of fertility obtained after the mating of the sows by the artificial insemination technique significantly varies in different countries of the world (by 70-86%). This oscillation is dedicated to other factors that directly affect the fertility of the sows, such as the genetic material, race, the management of the sows⁵ etc, and less to factors that come from the sperm diluters. Based on the results of this study, the average period from farrowing to conception, significantly reduces in sows that were artificially inseminated. From the conclusions of this work and of other authors as well, it is shown that the percentage of fertility in artificially inseminated sows is the same or higher in comparison to that of naturally service mated of sows. It should be noted that artificial mating allows us to use boars with the highest breeding value, influencing the increase of meat production and other economic indicators⁶. It is recommended to Albanian farmers to aim at the implementation of the technique of artificial insemination to sows, which may result to the improvements of the indicators of reproduction and the possibility of further economic profits.

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AN UNCOMMON CASE OF ADENOCARCINOMA IN A DWARF RABBIT

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KEY WORDS: adenocarcinoma; rabbit; uterus

ABSTRACT - Epithelial tumors of female genital apparatus are rare in domestic animals. Uterine adenocarcinoma generally appear as discrete enlargements of the uterus composed by firm, dense, white to yellow tissue and metastases can often occur. These tumors tend to be solitary and develop in the uterine horn. We describe an uncommon case of uterine adenocarcinoma associated with contemporary presence of mammary adenocarcinoma in a 5 years old dwarf rabbit accidentally diagnosed in an animal without symptomatic disease and conduct to clinical examination due to behavioral changes characterized essentially by extreme aggressiveness. With this case we like to describe an unusual and rare case of adenocarcinoma to try also to underline the efficiency of preventive controls in the health management of domesticated rabbits¹.

INTROCUCTION - Primary epithelial tumors of the cervix are extremely uncommon in domestic animals. The primary ones that do occur are carcinoma in cow and leiomyoma in the bitch. Carcinoma of the endometrium is rare too as a clinical problem but in many species, like in rabbits, is a well known as a lesion found at the time of necropsy. This tumor often is found in the depths of the endometrial glands of the horns and less often in the body of the uterus. The tumor can be small and annular or involve a large area of the uterine wall. Microscopically the neoplasm is readily distinguished from normal endometrium by the increased size, pleomorphism and disarray of the glandular epithelial cells. Metastases occur to the regional lymph nodes (iliosacral) and lungs and they seed the serosal surface of the abdomen².

MATERIALS AND METHODS - A female dwarf rabbit, 5 years old, “properly feed” from a private owner was presented to veterinarian after a sudden behavioral changes characterized essentially by extreme aggressiveness. Clinical evaluations revealed the presence of a small mass at level of abdominal mammary gland. The nodular mass appeared well circumscribed, firm, mobile and not infiltrative to the underlying muscles fascia and with size of 7,0x12,0x5,0 mm. A needle aspiration was performed and cytological samples was collected and stained with routinary methods. During the clinical examinations of the animal, also supported by instrumental investigations (ultrasound scan of the abdomen and radiological scan of the thorax) a severe enlargement of the left uterine horn, was identify. According with the owner the rabbit was anesthetized and undergo to surgical remove of both mass. The uterine neoplasia was localized to intermediate portion of the left uterine horn. Samples of both tumors were immediately fixed in neutral buffered 10% formalin and collected for histopathological investigations. No cytological sample was collected from uterine mass.

RESULTS - Cytologically the samples collected from mammary gland mass revealed the presence of big pleomorphic epithelial cells organized in small and medium groups, with acinar and papillary pattern, with anisokaryosis and anisocytosis with modification of N/C ratio and prominent nucleolus. A strong suspect of malignant epithelial mammary tumor, was made.

No metastases were detected at the radiological scan of the thorax as well as in other apparatus. Histologically mammary tumor appeared well demarcated and separated by connective septa from adjacent mammary tissue and regional lymph node.

Histopathological investigations of neoplastic tissues collected from either mammary and uterine tumors revealed the same histopathological neoplastic pattern essentially characterized by papillary, sometimes cauliflower-like structures, well covered by epithelial mature cells organized in a cuboidal to columnar malignant epithelium. Marked signs of pleomorphism characterize these big epithelial cells with abundant eosinophilic cytoplasm and frequent mitotic figures. No signs of invasion of veins and lymphatics was founded at histopathological investigations.

Uterine and mammary tumors were classified as uterine and mammary papillary adenocarcinoma.

DISCUSSION - Uterine adenocarcinomas is the most common tumor in rabbits. It is to referred with an incidence of 50-80% in rabbits older than 3 years³ and it is consider a slow growing infiltrative neoplasia that can affect both uterine horns⁴. Metastases can occur even after a couple of years after onset neoplasia⁵ to regional lymph nodes, lungs, liver, bone and brain⁴. Clinical signs are characterized by reduced fertility, increase of fetal retention, hematuria, vaginal discharge, depression, anorexia and dyspnea⁴. This case represents an uncommon case of tumor growth with the same type of tumor diagnosed at the same time either in uterus and mammary gland (primitive or metastases?). No other similar lesion was detected in other tissues and/or apparatus of the rabbit. Actually, after 5 months from surgery, the animal is still alive and it appear in good condition of health without signs of recidiva or metastases, without application of specific antitumoral treatments therapy. The animal was only treated with traditional post-surgical antibiomatic and analgesical therapy. Further investigations must be performed consequently to next clinical controls.

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AMPHIBIANS IN CAPTIVITY: INDOOR BREEDING TECHNIQUES AND RELATED PATHOLOGIES

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KEY WORDS: amphibians breeding; urodeles; hormones.

ABSTRACT - The main task of the amphibian's veterinary surgeon consists in controlling and inducing both the natural and pharmacological reproduction and arranging a correct breeding plan.

DISCUSSION - The amphibians captive breeding involves both research institutes and private breeders, giving the opportunity to the veterinary surgeon to deal with problems related to reproductive disorders. Among the three orders of amphibians, only two have a good spread: the *anurans* and *urodeles*. Both orders have mostly oviparous reproduction with a larval stage. An overview of the main indoor, natural and hormonal, reproductive technologies is provided. The courtship and reproduction methods are very different between urodeles and anurans and usually they takes place in water. In urodeles, fertilization is internal, by the male *spermatophore* collected by the female in the *cloaca*, while in the anurans fertilization is external. Normally the eggs are laid and than abandoned, while some anuran species show complexes parental cares. Some urodeles can be ovoviviparous and sometimes they can be neotenic as well. All the amphibians need latency period followed by favorable climatic conditions before the breeding season. The species of the temperate climates require a period of hibernation; this period is spent in the aquarium or buried underground. The spring triggers hormonal mechanisms that induce the maturation of the gonads, major somatic changes, migration to breeding areas, beginning of courtship and reproduction. In order to enable the reproduction it is determinant to set a proper enclosure: water bath and suitable substrates to stick or hide eggs. After spawning and fertilization, the eggs can be left into the terrarium/aquarium to the parental cares or can be bred artificially. The unfertilized eggs are rapidly contaminated by mold, which can also affects the fertilized eggs and all the remaining healthy ones disinfected with a solution of methylene blue^{4,5}. The amphibian reproductive disorders are linked to infertility, to genetically determined reduction of embryo developing (as in *Triturus* genus) and consequent dead^{1, 2, 3}, to lack of right environmental conditions. As for the eggs, the tadpoles and urodeles larvae the disinfection with solutions of methylene blue is generally enough^{4,5}. Besides to the natural systems, is it possible to breed the amphibians by hormonal stimulation, as often is done in many others species. Many several methods are described to induce mating, release of sperm, ovulation. The most used hormone is GnRH 0.1 mg/Kg subcutaneous (in the dorsal lymph sac of anurans) or intramuscular (the males should be injected 8-12 hours later the females)⁷. The second one used is the hCG (SC or IM) that can induce the mating or the release of the sperm (hCG should be followed by GnRH in 8-24h)^{8,6} or just induce the ovulation (followed by MSG or progesterone)⁸; the LRH mixed with DMSO via transdermal administration can induce spermiaton in

some Bufonidae⁹ or in intracelomatic administration induces oviposition in some Plethodontidae¹⁰. The PMSG (pregnant mare's serum gonadotropin) causes both in *Xenopus sp.* and in *Ambystoma mexicanum* the induction of the ovulation⁸, followed by hCG 72 h later⁶; the last hormone is progesterone that it is used in conjunction with PMSG or hCG for induction of ovulations among *Xenopus sp.* and *Ambystoma mexicanum*⁸.

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REPRODUCTIVE PHYSIOLOGY AND RELATED DISEASES IN PET CHINCHILLA (*Chinchilla lanigera*): A CASE REPORT

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KEY WORDS: pet chinchilla, reproductive physiology, reproductive disease

ABSTRACT - We describe a case of dystocia in a pet chinchilla of six years of age presented with abdominal contractions associated with signs of anorexia and moderate depression. In this report we want to describe the reproductive physiology of this specie and the main related diseases. A program of correct husbandry should be discussed to the owner to reduce the possibility of disease during pregnancy and post-partum. Excellent nutrition, good environment and accurate investigations in last period of pregnancy (eg X-ray, ultrasound) minimize the risks of diseases in pet chinchilla.

INTRODUCTION - In recent years, the chinchilla has become more usual as a pet since it is a docile animal, very clean, odorless, and is very appreciated for the wonderful and soft fur. In Italy many fur farms turned to the production of pet animals and the Veterinary Doctor is called to follow the breeding and welfare of this Hystricomorph rodent originating from the cordillera of the Andean.

MATERIAL AND METHODS - Clinical case concerns about a pregnant female chinchilla, pluriparous, six years old properly fed with polyphyte hay and specifically formulated food for chinchillas of excellent quality; brought to visit because totally anorexic, depressed, with no effective abdominal contractions. The symptomatology was present since 8 hours.

RESULTS - The alterations observed in the clinic visit were moderately depressed sensorium, dehydration around 7%, blood in the vulvar region, abdominal palpation revealed the presence of fetuses. Abdominal X-rays in double lateral and dorsal ventral position showed the presence of 3 fetuses. At the same time it was also made an ultrasonography examination to assess the viability of the pups. For two of them the heartbeat was absent while in the third cardiac activity was present. As described, medical therapy was attempted by administration of calcium gluconate intravenously and subsequently oxytocin at a dose of 1 IU/animal. After 30 minutes without any response to therapy it was administered oxytocin again. Due to the failure to expel the fetus cesarean section was decided. The chinchilla had a good recovery and nursed her baby since a few hours after surgery.

DISCUSSION - Reproductive physiology: the chinchilla at the Italian latitudes has a polyestrous reproductive season cycle from November to May, even though in farming activity is observed continuous polyestrous cycle with interestrous interval variable depending on the season (personal experiences 2010). The estrous cycle lasts 28-50 days with a flair of 24-48 hours; the most typical manifestation of the female in estrus is the vaginal opening of the slit with the vulva which is slightly swollen and changed from a pink color to a deep red. The female has two uterine horns and two cervixes, three pairs of mammary glands: one inguinal pair and two lateral thoracic pairs¹. The

post-partum estrus, very fertile, occurs after about 2 hours after parturition and can last up to 6 days (Petrini D., unpublished data). After mating the sperm into the vagina undergoes coagulation, forming the so-called "cap" that prevents the release of seminal fluid. A few hours after mating the cap is expelled by the contractions of the vagina and can be found in the cage, but it can happen that the animal ingests it and then it can not be found. The shape resembles that of a chrysalis, measuring about 2.5 cm long and 6-7 mm in diameter; the color is white at the moment of expulsion and after hours it turns to yellow. The chinchilla has a very long pregnancy (about 111 days) and the pups, usually 1-5 per litter, are born with early fur and eyes already open². Weaning takes about 8 weeks.³ The reproductive disorders that frequently occur in these Hystricomorph are hypocalcemia post-partum, and postpartum constipation; to remember then genetic abnormalities caused by the coupling between certain phenotypes. The premature loss of fetus can occur at any stage of pregnancy and may be caused by poor nutrition or poor quality, concurrent illness during pregnancy, stress or trauma. Another cause of iatrogenic abortion is represented by not adequate palpation when breeders seek to identify a possible early pregnancy⁴. During gestation, the chinchilla has paraphysiological constipation due to the size of the uterus, this condition may be exacerbated during the first week of post-partum and can be confirmed by X-ray. Usually this pathology resolves spontaneously but it is good practice to advise the owner to control the appearance of the feces of the animal, its weight, his appetite and vivacity.⁴ The post-partum hypocalcemia may occur 2-3 weeks after parturition. It is a severe condition, the chinchilla lies in lateral recumbency characterized by depression and tympanism. Usually the disorder affects debilitated animals or animals with a large litter; it is necessary a supportive therapy and the administration of calcium gluconate. As for the proper management of the breeding it is important to remember that some phenotypes are not recommend for pairing. Some genes do not allow the survival of the fetus in homozygous condition (lethal factor). The two lethal genes involved in the chinchilla coat colors are white and the TOV (Touch Of Velvet). If there is a condition of homozygosis for alleles White + White or TOV + TOV, there is a 25% chance that the embryo does not develop and that it is reabsorbed by the mother (but sometimes you can have stillbirths or puppies that die a few hours after birth). The dystocia are not very frequent and can occur due to maternal causes (such as uterine inertia, changes in the anatomy of birth canal, uterine torsion) or fetal causes (such as malpositioning, too large size, abnormal morphology, death). Radiographic examination is important for assessing fetal position and an ultrasonography allows to evaluate fetal conditions. If the chinchilla is in labor for more than 4 hours and supportive care has not given results it is advisable to perform a cesarean section, the prognosis in these cases is usually good.² The aim of this report emphasizes the importance of knowledge of reproductive physiology of chinchillas and the correct approach to any disease. It is important for a developing embryo in breeding provide proper nutrition (protein 16-20%, 2-5% fat, 15-35% fiber, 1% calcium, phosphorus 0.5%)¹ for prevention of diseases related to pregnancy such as hypocalcemia or constipation in the post-partum, stress is another cause of abortion and then is important to keep animals in a more peaceful as possible. To ensure correct management of the couplings is important not to cross white phenotypes each other or TOV phenotypes each other; it is desirable to record the day of mating or finding of the mucous plug to estimate the day of parturition (assuming that the majority of animals has a pregnancy that lasts exactly 111 days). In this way about a week before you can perform a radiographic examination to count the number of unborn children and prevent a possible dystocia.

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NUTRITION AND CHICKS MORTALITY IN *AGAPORNIS ROSEICOLLIS*

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KEY WORDS: *Agapornis roseicollis*, nutrition, chicks mortality

ABSTRACT - Diet is quantitatively and qualitatively of great importance, especially for a good reproductive success. These two case reports describe problems of high chick mortality in two *Agapornis roseicollis* breeding. In the first case problem was due to improper food storage, in the second case mortality was due to excessive protein level diet. Parasitological examinations, histology and Gram stain allowed to determine significant elements for the diagnosis. Essentially adjusting nutrition management solved chick mortality problem.

INTRODUCTION - In recent decades, breeding has been increased the detection of many species of parrots, especially since its importation was prohibited from countries outside the EU. Veterinarians are frequently involved to follow its management both under the standpoint of health, than the other related to lead a good breeding season. The nutrition thus becomes a fundamental health point to manage these kind of animals and their detention. These two case reports of neonatal mortality revealed an incorrect feeding and management of animals that can severely affect the survival of the offspring.

MATERIALS AND METHODS

Case 1

An *Agapornis roseicollis* breeder reports an high chick mortality in the first week of life (15/40) in its own breeding. There was no detected other specific health problems in its breeding. The clinical signs were characterized by regular growth for the first 5-6 days after birth, follow by a stop of the growth for about one day, and sudden die, with red and very swollen abdomen. Parasitological examination of faeces was made, and a faecal sample was investigated also with Gram stain to evaluate the presence of bacterial overgrowth. Two death chicks from different clutches was fixed in 10% neutral buffered formalin solution and sent to the laboratories for histopathological investigations. The parasitological examinations performed by faecal flocculation, were always negative. The gram stain from cloacal swab revealed a very high total bacterial contamination (cft 582)¹. The histopathological investigations revealed lesions referred to severe enterocolitis, with presence of diffuse edema and lymphomonocitary infiltrations of the intestinal wall, associate with polymorphonuclear and plasma cells infiltration. Mucosal epithelium showed hydropic degeneration, with diffuse microerosions of the intestinal mucosa. Mucosal capillary thrombosis was also detected in different areas. Liver show diffuse lesions of suppurative-necrotic widespread phenomena associated to cholestatic and vacuolar degeneration of hepatocytes. Kidneys was involved by extensive tubular necrosis, due to the widespread collemical nephrosis and glomerular degeneration. This phenomena was referred to a condition of toxicosis secondary to severe acute generalized enterotoxigenic bacteria. The disease process, was correlated with the ingestion of food contaminated with faecal material and/or fermented for the high temperature, or even to transmission by the parents of a microbial flora altered. Breeder decided to not analyzed seeds and softfood because it takes too much time and cost. It was decided to establish an probiotics integration (*Streptococcus thermophilus*, *Bifidobacterium spp*, *Lactobacillus spp*) new seeds and new soft foods was put in closed containers (were stored in open bags). Two chicks died after 2 days, one chick in the successive week, then the problem of mortality disappeared.

Case 2 - An *Agapornis roseicollis* breeder reported a high mortality in 10 days old chicks (13/54), which died without apparent symptoms or signs. There were no detected other specific health problems in his breeding. Two death chicks from different clutches were fixed in 10% neutral buffered formalin solution and sent to the laboratories for histopathological investigations. Parasitological examination (performed by flocculation) was negative, faecal sample examined by Gram stain was normal. Histopathological investigations revealed: a widespread interstitial nephritis, a moderate tubular degeneration and extensive points of ectopic calcifications, a serious glomerular hyaline degeneration. More than 70% of the nephrons was involved by lesions. In pulmonary tissues were present uric crystallization in many septa. All these lesions represent a severe framework of chronic nephritis and active uremic pneumonitis resulting from a state of terminal renal failure.

It was assumed a diet too high in protein². It has optimized the balance of the adjusted diet by decreasing the protein, changing the soft food, by reducing the amount of protein in soaked pulse diet (legumes like soybean, peas, etc and cereals kept in water for 24-48 hours), and was increased the amount of fiber and also of probiotics³. In the second hatching, mortality was significantly decreased (4/56).

RESULTS AND DISCUSSION - In both cases, the severe chicks mortality was due to improper nutrition. In the first, there was a dismicrobism, and by adjusting it the problem was solved. In the second case the problem was referred to an high and sudden increase of protein level in diet, as usually breeders do to start reproductive season (parrots are sensible to protein and fresh food increase). In both cases, at second clutch, chick mortality was not present. **First case:** it confirms the importance of well preserved food, the veterinarian supporting breeders have to give them aid to prevent diseases but also for the healthy management of animals. **Second case:** the case reported is in accordance with those described in literature^{2,3,4}, which indicate kidney damage due to an excess of proteins or resulting from a sudden change from a low in protein diet to a high level protein diet, as breeders do usually before the breeding season.

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THE SNOW LEOPARD (*PANTHERA UNCIA*): MONITORING REPRODUCTIVE ACTIVITY IN A CONTROLLED ENVIRONMENT

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KEY WORDS: wild felines, reproduction, sexual hormones

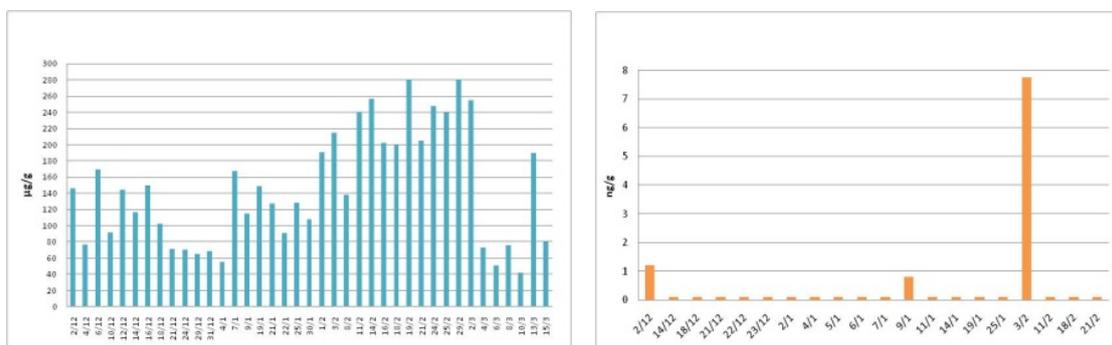
ABSTRACT - Conservation programs for endangered wild felines in controlled environments are subordinated to the objective of ensuring proper management of these animals. The aim of this study is to monitor the reproductive activity of snow leopards (*Panthera Uncia*) in a controlled environment -Natura Viva Park in Bussolengo (VR)- using a non-invasive method for collecting samples. The snow leopards, object of this study, haven't produced offspring despite the manifestations of oestrous in the female. The reproductive patterns were assessed observing the animals' behaviour and using radioimmunoassay for the determination of faecal steroid concentrations (progesterone and testosterone). The female showed a cyclic alternation of follicular and luteal phases: the seasonal pattern therefore appears to be normal. The progressive rise in progesterone levels following ovulation could have been either induced by coupling (not observed) or could have been spontaneous since the subjects live together. In the male, the testosterone concentrations remained low during the week of maximum female progesterone concentration. Therefore it can be assumed that the reproductive failure observed is linked to poor and erratic production of testosterone, promoter of spermatogenesis and sperm maturation.

INTRODUCTION - Of the 37 existing feline species, all except the domestic cat are threatened with extinction¹. Most felines housed in controlled environments reproduce poorly because of behavioural incompatibility, captivity stress, inappropriate husbandry and progressive loss of genetic variability¹. It is therefore essential to recreate a habitat compatible with the animal's needs and to guarantee conservation through efficient breeding programs. To monitor the reproductive activity of wild felines using a non-invasive method of sample collection is a approach preferred in non-tractable and stress-susceptible species as it is more practical compared to other procedures which require the capture, the containment and the sedation of animals². The objective of this study is to detect the reproductive activity and the causes of infertility in a couple of snow leopards.

MATERIALS AND METHODS - Two adult felines, a male and a female housed in the park "Natura viva" in Bussolengo (VR), NE Italy, haven't produced offspring despite the evident estral manifestations of the female. Faecal progesterone and testosterone concentrations were measured every couple of days and correlated with behavioural observations. The trend of steroid metabolites was determined using techniques validated on wild animals³.

RESULTS - The concentrations of progesterone increased progressively reaching the maximum values in the spring time (280 µg/g). The trend then decreased rapidly during autumn reaching the minimum values in winter (50 µg/g) (Graph 1). The concentrations of faecal testosterone, low at the start of the winter, increased in January and in the first half of February (8 ng/g) but then fell to minimum values again (Graph 1). Most of the

values were close to 0 ng/g especially during the breeding season, when the female exhibited sexual behaviour (rolling, lordosis, growls, marking, urinating and calling).



Graph 1 - Trend of Progesterone (µg/g) and Testosterone (ng/g) in faecal samples in the snow leopard.

DISCUSSION - Progesterone trends are more accurate than oestrogen trends in detecting malfunctions and pathologies connected with infertile mating⁴. The results show that the female exhibits both the follicular and luteal phases. The hormone trend is normal since the concentration of progesterone increases during the winter months reaching its peak in February⁵. The secretion of progesterone could be induced by mating or could follow spontaneous ovulation as the animals are kept together⁶. The testosterone concentrations measured do not follow the trend defined by Johnson et al.⁷ Further invasive analysis will therefore be necessary in order to establish the causes of infertility. These measures include the capture and the sedation of the animal, followed by an exam of the quality and quantity of semen produced and a specific ultrasound scan of the reproductive tract. Future studies, using a non-invasive method of monitoring, aimed at increasing knowledge on reproductive physiology (behaviour and endocrine information) in both sexes should enable us to strengthen management programs⁸. As well as studying reproductive aspects, it is also essential to offer an adequate habitat (environmental enrichment) in which the animal can adapt, grow and reproduce without developing anomalies or stereotypies. Only by doing so it will be possible to improve the biological functions and guarantee the well-being of these animals⁹.

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EFFECTS OF RESTRICTED FEEDING DURING PREGNANCY ON HORMONAL AND METABOLIC CONCENTRATIONS AND PRODUCTIVE PERFORMANCE IN DOES

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KEY WORDS: rabbit, pregnancy, feed restriction

ABSTRACT - This study aimed to evaluate the effects of food restriction at different stages of pregnancy on maternal hormones and metabolites and on productive performance. New White Zealand does (n=30) were randomly assigned to three groups according to their nutritional treatments. Control group (C) was provided with 130 g/d of commercial feed. The other groups were supplied with a reduced amount of food (30% restriction, 90 g/d) in early pregnancy, from day 0 to 10 day (R1), and in late pregnancy, from day 19 to 26 of gestation (R2). During the restriction period, plasma insulin, leptin, and T3 levels were lower (P<0.05) in R1 and R2 than in control. Furthermore, NEFA concentrations were higher (P<0.05) in R2 compared to the other groups. R2 showed lower litter size (P<0.01), live born (P<0.01), weight at kindling (P<0.05), litter weight at weaning (P<0.05), milk production (P <0.05) and higher perinatal (16.1 vs 7.9%) and pre-weaning mortality (26.7 vs 16.8%) than control and R1. In summary, nutritional status of pregnant does, modified by food restriction, greatly influenced metabolic hormones, metabolites and productive performance.

INTRODUCTION - Nutritional disorders are known to reduce reproductive and productive performance¹. Diets with inadequate energy contents can induce both excessive fatness than weight loss of does with subsequent infertility, high culling rate and reduction in the number and growth of newborns². In commercial rabbit farm, body condition and energy balance of female are particularly critical, but the effect of feed restriction on the regulatory mechanisms of metabolism during pregnancy is poorly known. Furthermore, food restriction during pregnancy in rabbits could be an useful experimental model to elucidate the effects of anorexia on fetal development. The aim of the present study was to examine the effects of food restriction during different periods of gestation on leptin, insulin, T3, cortisol, glucose, and NEFA concentrations as indicators of hormonal and metabolic adaptation to underfeeding and productive performance of does.

MATERIALS AND METHODS - A total of 30 primiparous New White Zealand does were randomly assigned into three groups (n=10) according to their nutritional treatments. Control group (C) was fed with a standard ration of commercial food (130 g/d) from the day of IA until parturition. The other two groups were fed with a restricted amount of food (30% restriction, 90 g/d) from day 0 to 10 (R1) and from day 19 to 26 of pregnancy (R2). At the end of the restriction period the does returned to eat standard ration. Housing and experimental procedures were carried out according to recommendations of the IRRG³. At AI and on days 4, 8, 10, 14, 18, 22, and 26 of pregnancy, the does were subjected to: assessment of body weight and blood sampling for hormones and metabolites assays. Twenty-four hours after birth, the number of suckling kits was adjusted to 8 per litter, and the pups were weaned at 26 d. Were also

assessed productive performance and the milk production by weighting the doe immediately before and after suckling. Insulin, leptin, T3 and cortisol concentration were determined by RIA, as reported elsewhere⁴. NEFA and glucose concentrations were analyzed by enzymatic-based colorimetric assays as reported by Brecchia *et al.*¹. Data were scored and statistically analyzed by ANOVA and t-test.

RESULTS - Rabbits R1 showed a rapid compensatory increase in weight after the re-feeding with standard ration that is not evident in R2. Hormonal and metabolites concentration during pregnancy as shown in Table 1. R1 group showed lower average leptin concentrations during pregnancy ($P < 0.001$). R2 does had higher NEFA concentrations as a consequence of the strongly negative energy balance. Feed restriction in early gestation (R1) not affects the productive performance, while in late pregnancy (R2) had significant effects on litter size ($P < 0.01$), weight at kindling ($P < 0.05$), live born ($P < 0.01$), litter weight at weaning ($P < 0.05$), perinatal (7.94 vs 16.13%) and pre-weaning mortality (16.85 vs 26.67%), respect to the control. Milk production in R2 group is lower compared to control ($P < 0.05$).

Table 1. Hormonal and metabolites concentration during pregnancy

	Day of pregnancy							
	0	4	10	14	18	22	26	
C	Cortisol (µg/dl)	3.04	2.45	2.67	4.32	3.63	3.38	6.00
	Leptin (ng/ml)	1.89	2.40	2.99	3.17	1.76	1.77	2.17
	T3 (ng/dl)	209.25	235.40	188.70	187.15	193.90	203.65	166.15
	NEFA (mmol/L)	0.66	0.52	0.95	0.89	1.11	0.86	0.95
	Insulin (µU/ml)	26.42	30.25	46.81	53.65	38.10	63.35	51.15
	Glucose (mg/dl)	110.46	131.12	112.42	120.75	118.11	124.21	111.47
	Cortisol (µg/dl)	3.15	3.51**	3.41	2.46*	2.55	3.03	4.66
R1	Leptin (ng/ml)	1.66	0.93*	1.13*	1.19	1.35	1.17**	1.33***
	T3 (ng/dl)	206.20	164.10**	150.75**	165.95	176.15	175.68	120.62
	NEFA (mmol/L)	0.60	0.51	0.78	0.85	0.78	0.83	0.87
	Insulin (µU/ml)	29.60	26.20*	27.90**	42.51	48.53	58.30	46.38
	Glucose (mg/dl)	137.17	134.17	116.22	138.34	141.91	157.97	123.52
	Cortisol (µg/dl)	3.41	2.98	3.58	3.58	3.85	3.98*	4.54
	Leptin (ng/ml)	1.85	2.20	2.26	1.90	1.94	1.17**	1.42***
R2	T3 (ng/dl)	207.27	193.22	178.53	178.29	167.87	144.79**	146.42*
	NEFA (mmol/L)	0.60	0.62	0.87	0.74	1.21	1.28*	1.45**
	Insulin (µU/ml)	27.02	34.93	46.90	51.59	41.31	42.27*	40.13*
	Glucose (mg/dl)	110.75	103.57	105.20	106.24	94.22	93.05	90.58

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ significance compared to control group (C) by t-test

DISCUSSION - The effects of restricted feeding on pregnancy depend on the gestational period in which the privation occurs. The rabbit, if energy demands are not excessive, can recover the weight lost, probably as a consequent of the anabolic effect due to the low concentrations of leptin. Food restriction in early pregnancy does not seem to determine significant differences in productivity, as reported also by Rommers⁵, while a restriction in the last third of pregnancy, a critical period for energy homeostasis, induce a reduction of productive performance and an impaired milk production.

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CARETTA CARETTA: RELATIONSHIP BETWEEN BODY SIZE AND SEXUAL HORMONES

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KEY WORDS: *caretta caretta*, sexual hormones, body size

ABSTRACT - Sex determination in juvenile *Caretta caretta* is difficult because of the lack of heteromorphic sex chromosome and the absence of dimorphic secondary traits. Up till now the most reliable method of gender determination is laparoscopy, that is an invasive and time consuming surgical technique. Testosterone or vitellogenin concentration are proposed as alternative methods but are still not satisfactory. We tried to predict sex of juvenile loggerhead during winter combining the evaluation of body size with the concentration of sexual steroid hormones and vitellogenin. Our results didn't evidence any correlation between the variables analyzed confirming the general idea of laparoscopy as essential technique in juvenile *Caretta caretta* sex determination during winter.

INTRODUCTION - Sex determination in juvenile *Caretta caretta* is difficult because of the lack of heteromorphic sex chromosome and the absence of dimorphic secondary traits. Sex is determined by the temperature at which eggs are incubated¹. When loggerheads are juveniles sex differences begin to emerge. Males produce increasing levels of testosterone (T) as they approach maturity, which triggers tail growth and plastron softening. Females produce estrogens (E) and small amounts of T, but externally grow larger. Maturity occurs at age of 10 and 30 years. Adult females reach a carapace length of 70-100 cm and an average weight of 70 kg, while adult males 80-110 cm and 80 Kg respectively². Breeding may occur year-round, but it peaks between May and July. The most reliable method of gender determination is laparoscopy, that is an invasive and time consuming surgical technique³; in contrast plasma T concentration [T] as sex indicator has been suggested⁴ as well as the presence of vitellogenin (VTG) in the blood of females. VTG is an egg yolk protein present only in female blood because linked to egg formation. Hepatic genes encoding for VTG are activated by E produced by the ovaries and remain inactive in males and immature animals⁵. The aim of this work was to find a new approach for sex determination in *Caretta caretta* combining the measure of body size with blood VTG and sexual hormones concentrations (T and 17- β -estradiol (E₂)) thus highlighting a possible correlation between morphological and endocrine parameters in subjects with a length greater than 60 cm.

MATERIALS AND METHODS - Samples were collected in October/November 2011 from 21 animals recovered at Wild Animals Recovery Centre (CRAS) of the WWF (Molfetta, BA, Italy). Each animal was measured (curved carapace length and width) and weighed. Blood was drawn from the jugular vein and collected in not heparinized tubes. Serum was obtained after centrifugation and employed for VTG and sex hormones determination. ELISA kits were employed for [T] (Radim, Italy) and [E₂] (DiaMetra, Italy). The presence of VTG in the blood was revealed by a qualitative test observing agglutination after the addition of rabbit polyclonal VTG antibody.

RESULTS - Obtained results are summarized in the table below

Subject	E ₂ (pg/ml)	T (ng/ml)	VTG	Weight	Carapace length
1	170.0	0	P	22.4	57
2	222.3	0	N	34	68
3	260.3	0.09	N	33.2	65.5
4	178.0	0	P	32.5	65.5
5	167.7	0	P	51.4	71.5
6	182.6	0	P	13.8	47
7	197.0	0.07	P	28.1	62
8	193.6	0	P	25.5	61
9	96.3	0	N	7.9	40.5
10	264.9	0	N	24.7	60
11	180.9	0	P	18.5	55
12	166.5	0	N	22.1	58
13	162.5	0	N	30.9	63
14	159.6	0	N	29.4	63
15	182.1	0	N	12.7	48
16	111.3	0	N	33.3	65
17	210.2	0,29	N	38.5	69
18	170.0	0	P	34.4	68
19	203.9	0	P	35.1	68
20	94.8	0	P	62.6	79
21	183.2	0,07	N	31.2	67

P= positive; N=negative

DISCUSSION AND CONCLUSIONS - We analyzed juvenile subjects whose weight was below the average weight for adult subjects of both sexes with the aim to predict their sex correlating body size and sexual hormone concentrations. We found that [T] was very low in 4 out of 21 turtles. In the remaining subjects T was undetectable. Because previous studies have documented that immature males have significantly elevated [T] compared to females⁴ we can hypothesize that the subjects analyzed were probably females. The fact that [T] was very low or undetectable could be related to capture stress as reported in green sea turtle⁶ or to the depressant effects of photoperiod on [T] during winter time⁷. The presence of VTG was not correlated with [E₂] and didn't add any information in the discrimination between mature or immature females. Taken together these results confirm the general idea of laparoscopy as essential technique in juvenile *Caretta caretta* sex determination during wintertime.

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AQUACULTURE

MODIFICATION OF GLYCAN PATTERN IN CULTURED SENEGAL SOLE, *SOLEA SENEGALENSIS* (KAUP) OVARIAN FOLLICLES

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ABSTRACT - The effect of culture-rearing conditions on glycoprotein pattern of Senegalese sole *Solea senegalensis* developing ovarian follicles was investigated by means of the lectin histochemistry. Ovaries from cultured specimens stopped the oocyte development in the late vitellogenic stage, whereas they reached the hydration stage in wild specimens. Compared to ovaries from wild specimens, in cultured *S. senegalensis*¹ the follicular layer showed the appearance of sialic acid (Neu5Ac) linked to Gal β 1,3GalNAc in perinucleolus stage and the lack of Neu5Aca2,6Gal/GalNAc residues², the zona radiata expressed mannosylated glycans in cortical alveoli stage³, the cortical alveoli, during the late vitellogenic phase, revealed the presence of Neu5AcGal β 1,3GalNAc, Neu5Aca2,6Gal/GalNAc as well as asialogalactosides⁴, yolk-globules expressed mannosylated oligosaccharides only in the late vitellogenic phase⁵, the oocyte nucleoplasm did not show sialylated and fucosylated glycans. These findings demonstrate that the glycan pattern of fish ovarian follicles is affected by culture-rearing conditions.

INTRODUCTION - Glycoconjugates such as glycoproteins play a key role in the reproduction of the fishes because they are constituents of zona radiata (chorion), cortical alveoli, yolk granules of the fish vitellogenic oocytes as well as they can be involved in hormones binding, transport of metabolites and ion across the plasmalemma, sperm-egg binding, polyspermy blockade and in the regulation of the early stages of embryo development^{1,2,3}.

Since the Senegalese sole *S. senegalensis* is a target species for the aquaculture industry in Southern European and its F1 generation raised in captivity often fail to reproduce naturally. In this study we investigated by means of the lectin histochemistry if the glycan pattern of developing ovarian follicles is affected in cultured fish.

MATERIALS AND METHODS - Ovaries fragments from cultured (total body weights ranging from 1.37 to 1.82 kg and gonadosomatic indexes -GI- between 5.8 and 10.7%) and wild *S. senegalensis* females (total body weights from 0.95 to 1.05 kg and GI between 4.1 and 12.1%) were fixed in 4% (w/v) neutral formalin, and embedded in paraffin wax. Sections were stained with a panel of lectins (MAL II, SNA, PNA, DBA, Con A, GSA I-B₄, GSA-II, LTA) before or after sialidase treatment according to⁴.

RESULTS - The ovaries of wild *S. senegalensis* consisted of developing oocytes from chromatin nucleolar stage to hydration phase⁵, whereas those of cultured fish contained follicles from chromatin nucleolar stage to late vitellogenic stage as well as post-vitellogenic oocytes which entered in atresia without embarking into maturation. Although no morphological difference was observed in the ovarian follicles of the same developing stage from wild and cultured fish, the lectin histochemistry displayed the following binding changes in the cultured *S. Senegalensis* (Table 1): 1) the follicular

cells showed the appearance of K-s-PNA reactivity in perinucleolus stage and the lack of SNA binding sites, 2) the zona radiata expressed Con A reactivity in cortical alveoli stage, 3) the cortical alveoli, during the late vitellogenic phase, bound also SNA, PNA, K-s-PNA, and GSA I-B₄, 4) yolk-globules did not react with GSA I-B₄, and 5) the oocyte nucleoplasm lacked of MAL II and LTA reactivity, whereas SNA affinity lacked during the perinucleolus stage.

Table 1 - Comparison of lectin binding pattern of wild and cultured *Solea senegalensis* ovarian follicles

Sample	Follicular cells	Zona radiata	Cortical alveoli	Yolk granules	Oocyte nucleus
Wild	MAL II, SNA ^v , s-PNA ^{c,v} , DBA ^{c,v} , Con A ^{c,v} , GSA I-B ₄ ^v	DBA ^v , Con A ^v , GSA I-B ₄ ^v	SNA ^v , DBA ^{c,v}	Con A, GSA I-B ₄	MAL II ^c , SNA ^{pn,c} , Con A, GSAI-B ₄ , GSAL, LTA
Cultured	MAL II, s-PNA, DBA ^{c,v} , Con A ^{c,v} , GSAI-B ₄ ^v	DBA ^v , Con A ^{c,v} , GSA I-B ₄ ^v	SNA ^{c,v} , PNA ^{c,v} , s-PNA ^{c,v} , DBA ^c , GSAI-B ₄ ^v	Con A	SNA ^c , Con A, GSAI-B ₄ , GSAL ^{pn,v}

c, cortical alveoli stage; pn, perinucleolus stage; s, KOH-sialidase treatment; v, vitellogenesis. When not specified, the reactivity refers from perinucleolus to vitellogenesis stages, since chromatin nucleolar stage did not show reactivity with the lectins used.

DISCUSSION - These results demonstrate that glycosylation pattern changes occur in the ovary of cultured *S. senegalensis* and they suggest that the damage of the glycan pattern could be among the factors affecting the reproduction in captivity rearing conditions.

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PARENTAGE ALLOCATION BY DIFFERENT MOLECULAR MARKERS FOR GENETICS SELECTION IN FARMED SEA BREAM

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KEY WORDS: farmed sea bream, genetics markers, parentage allocation

ABSTRACT - The management of modern selective breeding programmes in aquaculture requires the use of pedigree information to carry out sound and efficient genetics evaluations for multi-traits improvement but also for a better control of inbreeding. Molecular markers represent a powerful tool to assist breeding programs, in particular for parentage inference of selection candidates reared in communal tanks. In REPROSEL project, a large STRs multiplex (13 loci) and a panel of 59 SNPs, selected from a 128 SNPs array format, have been developed to infer complex pedigree structures in aquaculture raised seabream in order to assess the spawning kinetics and the hormonal vs control spawning synchronization in this species. For the SNP markers, a starting database of 49.000 ESTs (10.000 contigs) has been screened and processed (SNPs mining, annotation and mapping) reaching to a database of 488 SNPs from which a set of 128 SNPs were selected and spotted on the array. The STRs multiplex allowed to unambiguously allocate more than 95% of the progeny whereas the SNP panel allowed to allocate unambiguously the 97.5% the offspring to their specific pair of parents. The comparison between SNP array and STRs multiplex opens the discussion on the limits and advantages of the use of these two different type of markers in parentage allocation analysis.

INTRODUCTION - Selective breeding schemes are widely recognized as efficient means to improve aquaculture production. Compared to the individual selection, family-based selection allows a better control of inbreeding and represents the only alternative to improve disease resistance and slaughter traits. However, fish farmers are reluctant to invest in such programs due to the high costs. This is a limitation for the emergence of breeding programs for multiple-spawning species such as the seabream. However, molecular markers could be valid tools to enhance breeding programs, in particular for parentage inference of selection candidates reared in communal tanks.

Two efficient and powerful multiplex sets (STRs multiplex and a SNPs array) have been developed for farmed gilthead seabream, in order to obtain higher rates of unambiguous parentage allocations at the lower cost and to assess the spawning kinetics and the hormonal vs control spawning synchronization.

MATERIAL AND METHODS - STR screening was carried out from a database of about 100 microsatellite (www.ncbi.nlm.nih.gov/nuccore/) for which we already had the data of informativeness as already tested in previous work^{1,2,3}. In order to select the STR candidates for the development of multiplexes, priority choice was given to the highly 'normal' polymorphic microsatellite markers, with different sizes and known linkage on the gilthead sea bream linkage map¹.

For the SNP markers, a database of 49.000 ESTs (10.000 contigs) has been screened. SNP mining on the contigs was performed using GigaBayes⁴, PolyFreq⁵ and ssahaSNP⁶.

Contigs containing SNPs were functionally annotated and mapped against the stickleback (*Gasterosteus aculeatus*) genome ENSEMBL. A first batch of 488 SNPs was identified and further processed in order to select the final set of SNPs spotted on a 128 SNPs array format and analysed by OpenArray technology.

RESULTS - The STRs multiplex proved to be functional and efficient for parentage assignment and allowed to unambiguously allocate more than 95% of the progeny to a specific couple of parents. All the loci were in Hardy-Weinberg equilibrium.

The number of SNPs positively evaluated for parentage assignment was 59 (~50% of the total number spotted on the array). Their informativness was tested on 173 “wild” and “selected” broodfish. The SNP panel allowed to allocate unambiguously the 97.5% the offspring to their specific pair of parents.

The genetic variability of the STRs multiplex and SNPs panel are displayed in following table:

	STRs	SNPs
Alleles (n)	12	2
He	0.73	0.44
Ho	0.76	0.41
PE	0.99973119	0.9999943
PE(2)	0.99999982	1.000000
PI	2,370104E-24	1.060928E-30

He and Ho= Expected and observed Heterozygosity;
 PE and PE(2) = Exclusion Probability given 1 or both parents; PI = Identity Probability.

DISCUSSION - The preliminary data of parentage allocation obtained utilizing the two different multiplex sets (SNPs array and STRs multiplex) opens the discussion on the advantage and the limits of the use of the two different type of markers in parentage allocation analysis.

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THE IMPORTANCE OF USING DISEASE-FREE BROODFISH IN FISH FARMING

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KEY WORDS: broodfish, ovarian fluid, fish disease

ABSTRACT - The effective method for controlling viral disease outbreaks lies in the capability to utilize virus-free broodfish, and to rear fry and fingerlings in disease-free water. The research is carried out for the detection of viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and infectious pancreatic necrosis (IPN) viruses in farmed rainbow trout broodfish. Samples of ovarian fluids are collected from broodfish on reproduction phase in years from 2006 to 2009. Samples taken from 247 broodfish are pooled in 90 samples (pools), taken in 6 trout farms. Samples are analyzed with RT-PCR and Nested-PCR method. The IPN virus was detected in 7 from 90 pools (7.78%) and in 4 from 6 fish farms (66.67%). No VHS virus and IHN virus were detected. This research highlights the role and importance of latent infected broodfish on spread of viral diseases in fish farming.

INTRODUCTION - The viral disease are continues threat for fish farming because after outbreaks no medications is available, serious losses can occur and difficult to eradicate them, especially when we have to deal with asymptomatic virus carriers and vertical disease transmission. Viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis (IHNV) are viruses of the *Rhabdoviridae* family¹, both viruses may infect salmonide fish of all ages and can cause 80% to 100% mortality². Transmission of VHSV and IHNV viruses occurs horizontally, however vertical transmission has been evidenced for IHNV³. Infectious pancreatic necrosis virus (IPNV) belongs to the *Birnaviridae* family⁴. The disease can be spread horizontally as well as vertically through ovarian and seminal fluids⁵. As reported from different authors infection with IPNV can result in clinical disease or subclinical infection. In both cases, survivors could become life-long carriers without showing any evidence of disease^{6,7}. In carriers approaching sexual maturity, variable levels of virus are found in the ovarian and seminal fluids. The main purpose of this study was to highlight importance of latent infected broodfish on fish farming on spread of diseases and viral disease outbreaks.

MATERIALS AND METHODS - Samples were collected from broodfish ovarian fluid and saved frozen in the period 2006 to 2008. Samples taken from 247 broodfish are pooled in 90 samples (pools), taken in 6 trout farms. Samples are analyzed with RT-PCR (IPNV and IHNV) and Nested-PCR (VHSV) method. Total RNA was extracted from pooled ovarian fluids using RNasy Mini Handbook, (Qiagen). RT-PCR and Nested-PCR mixture was from Abgenecompany. Primers (Abgene) were selected on the basis of published sequences of the cDNA of virus genome. PCR products were analyzed on a 2% agarose gel by electrophoresis and a UV transilluminator (Biorad) was used to visualize the bands, and

results were recorded by photography. Laboratory analyses were performed at the animal health service laboratory in Munich in Germany.

RESULTS - All samples were analysed for detection of VHSV, IHNV and IPNV. From the 90 pools of collected ovarian fluid, seven pools (7.78%) were IPNV positive, and 4 from 6 sampled fish farms (66.67%) were IPNV positive. No VHSV and IHNV were detected. At the time of sampling, no visible clinical signs of disease are seen in IPNV-positive broodfish.

DISCUSSION - The study highlighted importance of controlling broodfish for the presence of VHSV, IHNV and IPNV in farmed rainbow trout. All the IPNV positive samples (7.78%) were from subclinically infected rainbow trout with a significant distribution among the fish farms (66.67%). The results indicate the importance of broodfish on spreading the viral diseases and the importance of using disease-free broodfish on fish farming in order to prevent disease outbreaks. Keeping the asymptomatic virus carriers for as future parents for next generation could be a serious mistake, causing considerable losses. Therefore, results from this research indicate that it is ultimately time to perform regular health checks and routine inspection of broodfish in order to insure that fish are free from certain important diseases. These broodfish could either be acquired through a tedious selection process or by direct transfer from a virus-free station. Special care should be with IPNV positive eggs, where vertical transmission has been shown to occur despite comprehensive surface disinfection of eggs, as the virus may be carried internally in eggs^{6,8}. The presence of asymptomatic virus carriers among broodfish populations requires sensitive and reliable methods for detection and control. The general advices to prevent spread of diseases from broodfish are: a) Avoid introduction of infectious pathogens into the brood fish populations and optimize management in order to prevent stress-induced diseases; b) Test for infectious pathogens in broodfish; c) Eliminate infected populations/individuals; d) Effective infection barriers between egg producer and egg customer.

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**ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) DOMESTICATION:
THE APULIAN PROJECT ALLOTUNA**

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KEY WORDS: bluefin tuna, spawning induction, larval rearing

ABSTRACT - Atlantic bluefin tuna adult specimens reared in captivity in the South Tyrrhenian Sea were administrated GnRH α implants during the reproductive season of three consecutive years in order to obtain fertilized eggs and set up a rearing protocol for fry production. A total of approximately 20, 40 and 130 million fertilized eggs were collected in 2008, 2009 and 2010, respectively. The eggs were transferred to a commercial hatchery where different rearing protocols for bluefin tuna larvae were tested.

INTRODUCTION - The Atlantic bluefin tuna *Thunnus thynnus* (L.) is a high valued fish threatened by a strong fishing pressure. In the last decade, intense research has been carried out in an effort to convert the present capture-based rearing activity in a real self-sustained aquaculture system. This conversion would consent to safeguard wild bluefin tuna stocks and increase aquaculture production. Since fish reared in captivity are often unable to reproduce due to reproductive dysfunctions, one of the main steps towards the domestication of a new fish species is to achieve the control of reproduction. In the framework of the Apulian Regional project ALLOTUNA, three years (2008-2010) of experiments on spawning induction of captive bluefin tuna were carried out, followed by larval rearing trials.

MATERIALS AND METHODS - About 90 bluefin tuna (body mass 50-60 kg) were caught in the fishing season 2007 in the spawning grounds around the Aeolian Islands and confined in captivity for 1 to 3 years in a cage located 0.5 miles off the coast of Vibo Marina (South Tyrrhenian Sea, Italy). From 2008 to 2010, during the natural reproductive season of bluefin tuna (June-July), when the sea surface temperature was above 24°C, fish were administrated a sustained-release delivery system loaded with gonadotropin releasing hormone agonist (GnRH α)¹. In 2008, prior to implantation, 10 fish reared in a different cage of the same farm were sacrificed and their gonads analysed to evaluate the reproductive state. The administration of the GnRH α implants was carried out underwater through a spear gun. Implantation took place under appropriate conditions to avoid panic frenzy that can cause an extensive ovarian atresia and prevent oocyte maturation. Fertilized eggs were collected with plankton hand nets and transferred to a commercial hatchery Panittica Pugliese (Torre Canne, Brindisi, Italy). Various larval rearing protocols were tested, using different light intensities, photoperiods, water renewal rates, tank volumes, aerations and diets.

RESULTS - The histological analysis of the gonads showed that, at the end of June 2008, females were in full vitellogenesis and then ready to undergo to the hormonal treatment. After GnRHa implantation, approximately 20 million eggs in 2008, 40 million in 2009 and 130 million in 2012, were collected. Tuna eggs had hatching rates of about 80% in 2008, and 98% in 2009 and 2010. Improvements in larval survival and juvenile growth performances were recorded year after year: in 2008 the last juvenile died 65 days post-hatch (dph) (total length, TL = 87 mm); in 2009 a longevity of 110 dph was reached (TL = 169 mm); in 2010 the last juvenile died 148 dph (TL = 245 mm).

CONCLUSIONS - The ALLOTUNA project demonstrated that wild-caught adult bluefin tuna are able to carry out gametogenesis, at least for females, until the completion of vitellogenesis after spending one year in captivity. GnRHa administration through a sustained-release delivery system was highly reliable in inducing final oocyte maturation and spawning. Egg collection devices were efficient with enough tuna fertilised eggs collected for hatching and larval rearing trials. Further research is required to improve tuna larval rearing protocols and reduce the high mortality, which is likely due to sinking behaviour of newly hatched larvae, cannibalism and nutrition.

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REPRODUCTION OF WILD AND CAPTIVE-REARED ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*)

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KEY WORDS: Atlantic bluefin tuna, reproduction

ABSTRACT - The Atlantic bluefin tuna is an overexploited fish species. The present review summarizes the knowledge achieved in Europe in 10 years of research on bluefin tuna reproduction aimed at assisting fishery regulation and laying the basis for the conversion of current tuna farming to a real aquaculture industry.

The Atlantic bluefin tuna *Thunnus thynnus* (ABFT) is a species of great interest both for fishery and aquaculture¹. During the reproductive season it is captured alive by modern fishing vessels that surround entire schools made of thousands individuals. Once captured, ABFT are transported to farms where they are fattened for a few months before slaughtering. The overfishing, which occurred in the last years, caused a decline of the ABFT population, so that a recovery plan, based on severe fishery limitations, was adopted by the International Commission for the Conservation of Atlantic Tuna. In 2010, the Convention on International Trade in Endangered Species of Wild Fauna and Flora examined, but did not approve, the proposal of Monaco to include ABFT in the list of species threatened with extinction¹. The knowledge of the reproductive biology of ABFT is important for both fishery management and aquaculture. For fishery management, information on size/age at puberty, spawning areas and period, spawning frequency and fecundity are essential. For aquaculture, it is necessary to understand if captivity affects the reproductive activity and if possible dysfunctions can be overcome with hormonal therapies. This review summarizes the studies carried out in the last 10 years in Europe on ABFT reproduction.

In the Mediterranean Sea, ABFT reproduce in May-June according to a quite well definite, temperature-guided, spatial-temporal pattern². During the reproductive season, all the adults aggregate in large schools and migrate towards the spawning grounds swimming in surface waters³. Puberty is reached between 3-5 years of life⁴. Spawning occurs every day for 2-3 weeks and the batch fecundity, i.e. the number of eggs released during each spawning event, is around 100 g⁻¹ body weight⁵. After 3 years of residence in an experimental cage in the western Mediterranean Sea, about 20% of the females showed post-ovulatory follicles⁶. The administration of GnRH α through sustained-release implants induced pituitary release of luteinizing hormone and spawning in almost the totality of the treated fish^{7,8,9}. A prolonged residence of adult ABFT in sea cages resulted in a massive spontaneous spawning (F. de la Gandara, unpublished data).

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ESTIMATION OF GROWTH DYNAMICS OF MICRO-ALGAE *Nannochloropsisgaditana* IN MESOCOSMOS SYSTEM

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KEY WORDS: nannochloropsis, growth, turbidity

ABSTRACT - *Nannochloropsisgaditana* is one of the most appreciated micro-algae in aquaculture industry. Its use is more common in hatcheries of marine fishes because in the first food chain of rotifers and fish larvae. The present study examines the growth dynamics of this micro-algae using biomass estimation by counting of cells/ml or by spectrophotometry. Also, this study explains the procedure of culturing this species of algae and the right volume of sea water, mother culture and nutrients needed. According to the results the sigmoid phase lasts 6 first days; the exponential phase lasts the next 3 days and the lag phase is in the day 9. In this phase should be the best to pass the harvest on higher volumes of bag. After this the culture reaches in the senescence phase (day 10). The maximal turbidity is obtained at the day 9 (1282 NTU) and after this the culture begins to change color from green to brown. This coincides with the senescence phase. From this study results that need more research to enlarge the time of the culture growing phase paying more attention on finding the most appropriate nutrients for this important micro-alga.

INTRODUCTION - *Nannochloropsis* is a well-known genus of microalgae in marine aquaculture. In the hatcheries the importance of this genus is large, because they start the food chain of marine larvae. The nutritional value makes *Nannochloropsis* well appreciated for the accumulation of high levels of polyunsaturated fatty acids and the buildup of a high concentration of pigments such as astaxanthin, zeaxanthin and canthaxanthin¹. Also, it is considered as an energy-rich food. One of the most appreciated species of these algae is *Nannochloropsisgaditana*. The culture of this species faces several restrictions. First, it is difficult to prevent bacterial contamination, competition with other microalgae and predation by protozoa². Because of this needs severe culturing conditions regarding hygiene.

MATERIALS AND METHODS - This experiment was conducted in the mesocosms of Instituto Canario de Ciencias Marinas GC (Spain). For counting was used Neubauer camera and light microscope with 40X. Method of spectrophotometer used was "Nanocolor". The compression aeration system was 150 to 300 mbar. Was used pre-treated filtrated water using a sand filter and then treated with UV. For the culture was used artificial light (tungsten lamp) 100 W in distance 20 cm.

RESULTS - The data are reported in graph 1 and in the table 1.

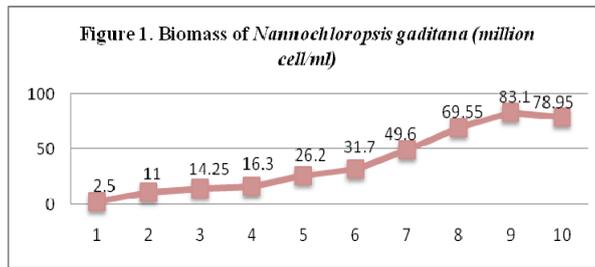


Table 1 - Turbidity of the population of *Nannochloropsis gaditana* (NTU)

Days	1	2	3	4	5	6	7	8	9	10
Turbidity	33	113.8	147.5	275	354	451	560	652	1282	1253

DISCUSSION - Explaining the figure 1, at the beginning of the culture there is a sigmoid shape phase that represents the adaptation phase of our culture (day 1 to day 6). Normally this is a phase where our culture adapts to new conditions. The duration of this phase is closely related to the mother culture conditions. If the stock culture is in exponential growth phase of the adaptation phase takes very little compared to a stock culture in the stationary phases or senescence that takes much time.

Then we have an exponential growth phase (day 6 through 9) that culminates in the lag phase. In the exponential growth of microalgae our population grows during the time in the same quantity. The growth rate is an important element to estimate how our culture grows. This phase lasted very little because it is related to the size of the bag and we have a bag of only 55 liters. Also this depends on the conditions of our culture and environmental capacity and can influence many other factors.

The lag phase (day 9) where our culture is in a very high level of growth but lack of food the population grows more. In this phase should be better to harvest a crop or pass on higher volume, but for lack of space in the laboratory we could not do it. After this, comes a very small stationary phase. In this phase the level of growth is zero. At the end we arrive at the senescence phase (day 10) when vegetative cell metabolism cannot be maintained at the appropriate level is the stage of senescence. At this stage we have seen that our culture has slowly begun to change color. In this phase begins to increase the population of bacteria because there is enough food (dead cells of microalgae). This is the cause of the change of culture color (from green to brown).

Regarding the turbidity curve (figure 2) is quite identical the biomass curve. The turbidity starts to drop at the day 9, which coincides with the lag phase of the biomass curve. This means that this form of measurement will be a good alternative to a more time involved method such as cell counting.

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CONGENITAL ANOMALIES AND TECHNOPATHIES IN TELEOSTS

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KEYWORDS: skeletal deformities, radiology, teleosts.

ABSTRACT - Congenital abnormalities and technopathies in fish have rarely been detected and only sporadically studied. This report describes different congenital defects and some technopathies observed in farmed, wild and laboratory fish. In particular disorders of zebrafish embryos are evaluated in order to provide a valuable model for comparative pathology. In this paper anomalies of the skeletal have been described. Causes and pathogenesis are discussed with the aim to give a tribute to the knowledge of such disorders in farmed fish as well as in human beings.

INTRODUCTION - Fish farming and in particular controlled fish reproduction are consolidate practices since many years and today can be named using the term “industry”. In both aquaculture and aquarium-fish culture, the main objective is not to assure the perfect conditions to obtain spontaneous reproduction of fish, but to determine and optimize protocols useful to control and synchronize spawners, to obtain the highest percentage of fertilization, highest grade of eggs hatching and, thus, the highest number of juveniles survived. For these reasons the human action is central and indispensable in all the phases of the reproductive cycle, although, sometimes artificial environmental conditions, as well as the failure of complete control on these last, could have severe influences on fish health, also evoking the appearance of developmental anomalies and technopathies. Body malformations may be caused by environmental factors^{1,2} and are often determined in the earliest stage of development³. In these last years, frequency of congenital defects has increased in percentage as a cause of infantile mortality/morbidity and of severe handicap, in contrast to the marked decrease of acquired disorders. The aim of this paper is to describe some congenital defects in farmed, wild, as well as laboratory fish and to discuss their possible etiology and pathogenesis.

MATERIAL AND METHODS - Over a twelve years long period (1998/2010), the research activities and the diagnostic service of fish pathology of the University of Messina have permitted to show and study some developmental disorders and technopathies in teleosts. Fish studied were belonging to some farmed teleost species, such as sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), sharp snout sea bream (*Diplodus puntazzo*), bream (*Boop boops*), as well as aquarium/laboratory teleosts, such as zebrafish (*Danio rerio*). In this study, some rare and frequent malformations observed in different specimens of *wild-type* zebrafish (*Danio rerio*) embryos born and reared in a fish facility of the Centre for Experimental Fish Pathology of Sicily (CISS) were also taken in consideration. Some fish were examined alive. Anesthesia was performed using tricaine methanesulfonate (MS-222) at the concentration of 100 mg/l of water, generally for no more than 5 minutes. Radiography was performed by a Univet LX 160, Supply 230 VAC, 50 Hz, 6 kVolts, Power 99 kVolts, 160 mA. Because most specimens were less than ten centimeters thick, a grid was not considered necessary. High definition intensifying screens and detail film were used to optimize radiographic detail. Kodak T-MAT G/RA film in a X-

Ray cassette was used. Fish were radiographed in two projections: dorsoventral and right lateral. To clarify the nature of tissue changes, where it was considered useful, organ and tissue samples were routinely processed for histopathological exam, after fixation in 10% buffered formalin solution and paraffin wax embedding. Sections were stained with Haematoxylin eosin, Masson's trichrome, Van Kossa, PAS and Giemsa.

RESULTS - Deformities of the vertebral column have been frequently registered in both sea bass and sea bream. Fish showed different forms of lordosis, kiphosis, scoliosis, as well as mixed forms. Due to the frequency of such disorders further details are not provided. In farmed gilthead sea bream, apart skeletal deformities, the only frequently detected congenital anomaly was the absence of the gills operculum. Also sharp-snout sea bream juveniles, even if with a lower frequency than in gilthead sea bream, were often operculum-less. In a wild specimens belonging to the species *B. boops* agnatia was described. The lower jaw was clearly reduced in size and both lateral margins of the sketch were turned dorsally and medially towards the median line, probably as effect of tissue regeneration aimed at guaranteeing the negative/positive pressure necessary within the oro-pharyngeal cavity during respiration. Morphological examination performed on *wild-type* zebrafish embryos (48 hpf) showed a normal development. Some weeks later showed several skeletal malformations.

DISCUSSION - In this study the observations carried out on different cases of congenital anomalies and technopaties in wild, laboratory and farmed fish, collected during twelve years of diagnostic activity, are showed. The interest to study such disorders is justified by the exiguity of data available in literature. In this paper, several skeletal malformations have been described. As regards the skeletal deformations, showed in sea bass and gilthead sea bream specimens, they could not be related to the quality of the commercial food, being these species the most consolidate ones in Mediterranean aquaculture, eating the same good quality food from several years. The cause must be found in farming conditions, especially population density with following exacerbation of hierarchy, as well as concurrent diseases. Only a single case of agnatia was reported in a wild bream. Such deformity of the jaw has been studied in Atlantic salmon⁴. The choose of studying congenital abnormalities of zebrafish is also supported by the fact that genes responsible of congenital defects in human beings, in mutant zebrafish can cause similar defects to those mutations happening in human, because of the strict biological similarity between these two organisms⁵. Because many causes of congenital malformations are not to date completely known, such as isolate deformities (limbs, facial, head malformations) and multiple malformations of the same individual, probably due to the contemporaneous action of predisposing genetic factors, the possibility of studying zebrafish as a prologue for future experimental studies aimed at confirming the possible involvement of genes as causative entities of such abnormalities is at least desirable.

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GOLDFISH PNST IS NOT CLONALLY TRANSMISSIBLE

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KEY WORDS: goldfish tumour, experimental transmission, microsatellites

ABSTRACT - Peripheral nerve sheet tumour (PNST) is the most common tumour in goldfish, observed with high prevalence in some colonies. The aim of this study was to investigate if goldfish PNST is so contagious as suspected and if it is a clonally transmissible cancer or not. Twelve PSNT-affected goldfish were enrolled in three different experiments (natural contact, artificial intimate contact, inoculation of tumour cell) but no tumour spread was observed in unaffected goldfish. In the fourth experiment comparative analysis of microsatellites between tumours and host tissues allowed to exclude a clonal origin of the tumour. A virus or a genetic involvement may explain the epidemiology of this tumour, which, in any case, represents a good model to increase the knowledge of neural tumours in animals and humans.

INTRODUCTION - Peripheral nerve sheet tumour (PNST) is the most common tumour in goldfish^{1,3}. It is usually benign and arises as soft subcutaneous nodules in different parts of the body⁴. The peripheral nerve sheet origin of the tumour has been demonstrated by means of immunohistochemical expression of calretinin and S-100 antigens⁴. The epidemiology of the disease suggests that it may have infectious origin, as the tumour has a high prevalence in some fish colonies, whereas it is absent in other colonies. A viral aetiology, has been hypothesized for damselfish's schwannoma, in which experimentally transplanted tumour cells are capable to cause new growths in unaffected fish⁵. Finally, it has been recently demonstrated that a Tasmanian devil's schwannoma is clonally transmissible, without any virus involvement⁶. The aim of this study was to investigate if goldfish PNST is so contagious as suspected and if it is a clonally transmissible cancer or not.

MATERIALS AND METHODS - To investigate our hypotheses, twelve PSNT-affected goldfish were collected from the same tank containing only another unaffected goldfish (prevalence 92%). The first tumour was recorded 3 years before and during the intervening time other tumours became recognizable in the other 11 fishes. The affected fish were moved to the Sicilian Centre for Experimental Fish Pathology, which was authorized by the Italian Ministry of Health for the following experiments. In the first experiment, five PNST-affected goldfish (three females and two males) were reared in the same tank with five healthy goldfishes (two females and three males). Social manifestations were allowed including spawning behaviour which need repetitive and intimate skin contacts between fish. Fish were observed at weekly intervals for the presence of externally visible lesions. After 1 year, the five control group fish were euthanized to perform necropsy. In the second experiment, five female PNST-affected goldfish and five female healthy goldfish were anaesthetized with 120 mg ml⁻¹ MS 222 at pH 8. Using scalpels, 50 mm² of skin were scarified in the each of the control group fish and about 50 mm³ incisional biopsies were taken from the tumours in the PSNT-affected group. Biopsies were rubbed on the scarified areas. Then, fish were placed in fresh water to obtain recovery. Fish were observed at weekly intervals. After 1 year, the

five control group fish were euthanized to realize necropsy. In the third experiment, five female PNSST-affected goldfish and five female healthy goldfish were anaesthetized, as described, and submitted to another experiment. Using a 20 G needle connected to a 2,5 ml syringe tumours were gently aspirated in order to collect a narrow cylinder of live tumoural tissue, which was quickly inoculated under the dorsal fin skin of the healthy fish. Then, fish were placed in fresh water to obtain recovery. Fish were observed at weekly intervals. After 1 year, the five control group fish were euthanized to realize necropsy. In the fourth experiment, under anaesthesia, incisional biopsies were taken from the tumour, from four female goldfish. Blood samples were taken from the caudal vein to obtain host tissue for genetic comparison. The tumoral tissues were microdissected and DNA was extracted from each tumoral sample and blood, for analysis and comparison of microsatellites.

RESULTS - In experiment 1, 2 and 3 no post-challenge complication was recorded and no tumour transmission was observed. In the fourth experiment, genotyping of 3 polymorphic goldfish microsatellite loci, the same genetic makeup was demonstrated between tumours and hosts.

DISCUSSION - This study did not find any evidence of horizontal transmission of PSNT in goldfish, especially in a clonal way. Although a virus transmission cannot be excluded, the tumour is not highly contagious, as suspected. The high prevalence observed only in specific colonies might be caused by an unknown environmental factor, such as a chemical pollutant or a toxin, which may directly act on DNA or indirectly interfere with the target genes. Commercial goldfish, as other ornamental fish, generally originate from a low number of progenitors. Then, inbreeding is expected to be very common in goldfish. Inbreeding is a condition that may facilitate the spreading of transmissible cancers⁶, but it is also able to reveal genetic disorders. If a genetic pattern had to be verified, a comparison between goldfish PSNT and human neurofibromatosis might be started. Neurofibromatosis is a human genetic disorder in which the peripheral nerve tissue grows tumours, in which NF gene inactivation also through a second hit seems to be of crucial importance⁷.

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LARGE RUMINANTS

MANAGEMENT OF FUNCTIONAL OVARIAN DISORDERS IN HIGH YIELDING DAIRY COWS

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KEY WORDS: cow, ovary, dysfunction

ABSTRACT - Various health, nutrition and management issues can impact reproductive performance in dairy cattle and reduced fertility, observed in modern high yielding dairy cows nowadays. Functional ovarian disorders are recognized as an important reproductive pathology and can interfere seriously the production leading to significant economic losses on dairy farms. Managing regular ovarian cyclicity postpartum is demanding function in total farm management. The use of ultrasound diagnostics, as a clinical method for examination of reproductive organs, enables better understanding of the essence of these disorders. Ultrasonography offers opportunity to visualize cyclic ovarian structures and monitoring of the dynamic changes during regular estrous cycle, as well as during pathological conditions. Purpose of this article is to review recent scientific information about the most common forms of the functional ovarian disorders e.g. Cystic ovarian follicles, Static ovaries, Ovulatory defects, Luteal deficiency, True anoestrus, etc. In addition, the current scientific findings and experiences of Institute for Reproduction on the Faculty of Veterinary Medicine in Skopje, regarding adequate hormonal treatment of these diseases are discussed.

INTRODUCTION - Alarming trend of decreasing fertility in dairy cows has been reported recently by several researchers worldwide¹⁻³. There are numerous factors influencing this fertility, including milk production, poor herd health management, especially nutritional management, stress, metabolic disorders, as well as other diseases (infections, mastitis, lameness etc.) which have indirect impact on reproductive performance of dairy cows. The inadequate herd management results with a high percentage of infertility, has been considered as a main causes of functional infertility as follow: cystic ovarian disease (37.1%), suboestrus (20.9%), anoestrus (13.5%) and anovulation (5.6%)⁴.

In this article we will present review of articles published by our Institute, based on more than 20 years "on farm" experience in the field of reproduction management in dairy cows.

CYSTIC OVARIAN FOLLICLES - Several experiments conducted by Dovenski et al. (2002)⁵ have shown that the cysts could be classified according to the thickness of the wall as follows: luteal cyst with thickness of the wall ≥ 3 mm and appearance as gray echogenic layer of the inner wall; follicular cyst with thickness of the wall < 3 mm which shows uninterrupted anehogenic antrum, with a relatively smooth and thin wall with occasional presence of echogenic threads on the wall or in the antrum. Investigating fate of spontaneous generated ovarian cysts by ultrasound, we have found that ovarian cysts are dynamic structures and should not be considered always as a severe pathological condition. Single ovarian cysts have partial capability for spontaneous self-recovery in short period of time, probably undergoing to the process of luteinization. Multiple follicular cysts have tendency for persistency for more than three weeks, and in this cases hormonal therapy is recommended. Observing by ultrasound the fate of the ovarian cystic formations after application of

different concentrations of GnRH Atanasov et al. (2011)⁶, have concluded that independently of the GnRH dose, the cows responded individually presenting two pathways of reaction. First, ovulating or luteinization of one of the subordinated follicles and forming functional corpus luteum; and Second, increase of luteal tissue within the cyst and reduction of antrum space, leading to full luteinisation of the cyst.

STATIC OVARIES, OVULATORY DEFECTS - Norgestomet treatment has been successfully applied for resumption of reproductive function in postpartum non-cyclic dairy cows⁷ as well as low dose GnRH and eCG in the beginning of the transition feeding period¹⁰. Treatment with single dose of GnRH or eCG, in many cows with true postpartum anoestrus, caused resumption of follicular growth and ovulation (87% and 75%, respectively) in comparison to control 20%. However, eCG treatment resulted in a quicker response, but higher ovulation rate compared to GnRH treatment. Similar results for improvement of pregnancy rate after the β -Carotene treatment (51.7% vs 35.6%, treated and non-treated animals, respectively; (p<0.01), has been reported⁸.

Recent investigations have been shown that dietary fat is beneficial to the reproductive system in dairy cows. It has been shown that fatty acids composition is different in follicular fluid and blood serum in cows with static ovaries and cystic ovarian follicles and interferes with other reproductive failures⁹. Studying influence of negative energy balance (NEB) on reproductive performance of dairy cows during summer heat stress by measuring serum concentration of NEFA, BHB, glucose & triglycerides Dovenski et al. (2010)¹⁰ reported deteriorating effect on the reproductive efficiency of dairy cows by decreasing dry matter intake and consecutive negative energy balance.

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EFFECT OF eCG ON ESTRUS RESPONSE AND FERTILITY IN NELORE (*BOS INDICUS*) HEIFERS SYNCHRONIZED WITH CIDR[®] AND PgF_{2α}

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KEY WORDS: Zebu, Heifers, Estrus, eCG, Fertility

ABSTRACT - Effect of eCG on Nelore heifers treated with CIDR[®] and PGF_{2α} on: estrous behavior, estrous distribution and fertility. A lot of 367 heifers were randomly distributed in two groups (C=Control and T=Treatment), maintained on the same pasture, integrated with mineral salts and free water. A three step synchronization protocol was applied: on day 0, CIDR[®] 1.9 g administration, on day 6, withdrawal of CIDR[®], injection of D-cloprostenol 0.15 mg and - only on T group - injection of eCG 300 IU. Heifers who failed to exhibit estrus within 5 days after first PGF_{2α} were re-injected 11 days after. Statistical analysis were performed by SAS[®] 9.2. (significance $P < 0.05$; trend between 0.05 - 0.10). T heifers, showed a significant ($P = 0.04$) estrus behaviour than control (68.29% vs 55.44%) after first protocol. On second PGF_{2α} group ($P = 0.17$), any significant difference was recorded. The distribution of estrous signs presented a trend ($P = 0.08$), (20.6 h) and (25.3 h) respectively. Following 1 and 2 PGF_{2α} injection, no difference was found for CR 1 ($P = 0.89$) neither for CR2 ($P = 0.39$). No difference was detected in PR1 although in PR2 a trend ($P < 0.09$) was evidenced (T group = 30.77%; C group = 16.53%).

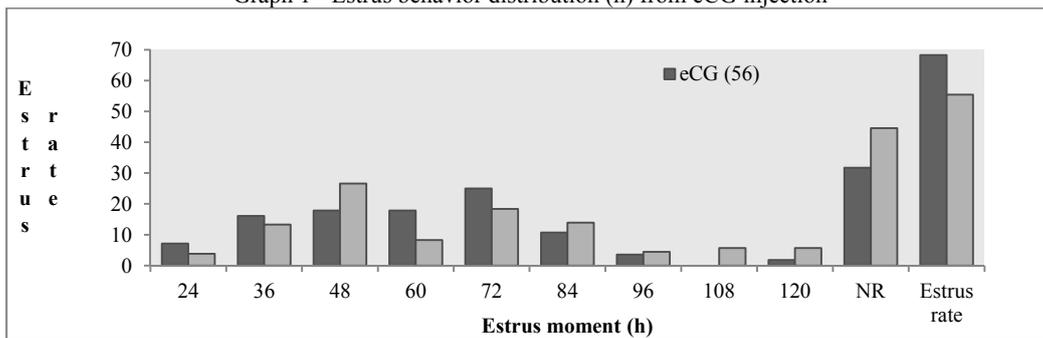
INTRODUCTION - Synchronization of estrus is the only option to apply AI in large wild farms. The proposed protocol is a simplified scheme with three points of animal trapping in order to reduce manipulation and stress. The AI can be performed on fixed time² but choice to operate on evident estrus reduce the semen expenses and improve results. Concentration in estrous behavior is desirable to reduce time of observation and hours of work. The administration of eCG has demonstrated to be beneficial in stressed and underfed cows to determine better follicular development and better fertility^{2,4}. The work tended to test the hypothesis that injection of eCG can stimulate also a more fertile return in heat in heifer negative at first AI.

MATERIAL AND METHODS - The study was conducted in commercial beef farm located at Mato Grosso, Brasil. An homogeneous group of 367 Nelore (*Bos Indicus*) heifers was enrolled. All cows were maintained on pasture (*Brachiaria brizantha*) integrated with mineral salts and free access to water. The animals were randomly distributed in 2 groups (C=Control, 285) and (T=Treatment eCG, 82). All heifers on day 0 received a CIDR[®] (1.9 g, Pfizer, Brasil), on day 6, CIDR[®] was removed and 0.15 mg de D-cloprostenol was injected (PGF_{2α} Tortuga[®]) and - only T heifers - received 300 IU im eCG (Novormon[®], Coopers Saúde Animal, Brasil). Heifers, that failed to exhibit estrus within 5 days after the first injection of PGF_{2α} were re-injected 11 days after. All heifers were observed continuously for detection of estrus behavior and inseminated 12 h after. Statistical analysis was performed using the Statistical Analysis System software for Windows[®] SAS (SAS, 2000). Following this results, the GLM procedure and Tukey's test were used for analysis of variance and to determine differences

between treatments. The binary variables (rates of estrus, conception rate and pregnancy rate) were analyzed by the GLIMMIX procedure of SAS. It was considered as significant difference of the tested variables $P < 0.05$ and trend between 0.05 and 0.10. The parametric dependent variables were expressed as mean and standard error (mean \pm SEM) and binary percentage.

RESULTS AND CONCLUSION - eCG injected cows, show a significant ($P=0.04$) increase of estrus rate than the control (68.29% vs 55.44%) but no significant difference was recorded on second $\text{PGF}_{2\alpha}$ ($P=0.17$). The mean estrus moment between eCG group (59.4 \pm 2.8) and control group (65.5 \pm 2.0) did not differ ($P=0.15$), but the distribution presented a trend ($P=0.08$), (20.6 h) and (25.3 h) respectively^{3,4}. When pregnancy and conception rates were analyzed, after both $\text{PGF}_{2\alpha}$ injections, no difference was found for CR 1 between T and C ($P=0.89$) neither for CR2 ($P=0.39$). No difference was detected in PR1 although in PR2 a trend ($P < 0.09$) was shown between eCG group (30.77%) and control group (16.53%). Although a higher CR and PR in eCG injected cows was generally notable in the two groups. The eCG administration seem to improve the number of cows that shows estrus within 5 days from $\text{PGF}_{2\alpha}$, but not the estrus distribution^{2,3,4}. Even if eCG seems to be effective to improve the fertility performance in stressed and low body condition score cows, in good condition ones the eCG does not further improve significantly reproductive performances.

Graph 1 - Estrus behavior distribution (h) from eCG injection



Graph 2 - Estrus rate (%) and distribution (%) between the two groups

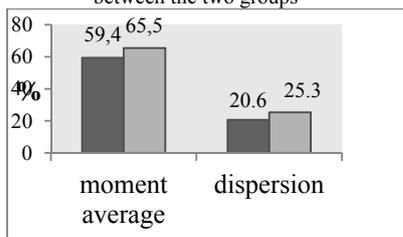
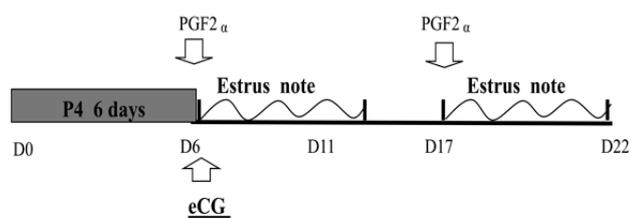


Fig 1 - protocol used in the study with eCG (case) and without eCG (controls)



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STUDY OF A.I. EFFECTS IN HEIFERS WITHIN TECHNICAL CRITERIA (FOR BREED) IN PRODUCTION AND REPRODUCTION

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KEY WORDS: Heifer, Artificial Insemination, Production

ABSTRACT - One of the limiting factors of production in our country is AI of heifers at early age and low weight for breed. The effect of optimal age and weight AI of heifers, in milk production and reproduction performances was studied. The research was carried out in Duress district, Ishem Shkafan over the period 3 years. Holstein Heifers were included in two groups. In the group of experiment, heifers were inseminated at the age of 15.5 months and live weight 402.5±41.12 kg whereas control group at the age of 13.4 months and live weight 304.2±20.58 kg. There are not significant differences (P<0.054) for the age, as for the weight, a significant difference (P<0.05). For milk production (305 days lactation) and reproductive performances the differences were significant (P<0.05).

MATERIAL AND METHODS - Holstein heifers were divided in two groups: experimental group: 4 heads; control group 7. During the study all heifers were managed as usual, by the farmer condition. A.I. was applied weight, age standards of breed. In control group the heifers were inseminated as usual at early age, low weight. For both groups was applied AI with frozen semen by the same technician. In both groups body weight, insemination, returns on heat, gestation period, health status, difficulty at calving, the quantity of milk produced at the first lactation, were monitored.

RESULTS AND DISCUSSION - Age and body weight at first service and conception:

Table 1 - Age and weight at first insemination and conception

Groups	Breed	First insemination		Conception		A.I. Index
		Age (days)	Weight (Kg)	Age (days)	Weight (Kg)	
Exp. group X±DS	Holstein	471.25±55.73	402.5±41.12	496.2±43.08	417.5±21.65	1.5±0.57
Contr. group X±DS	Holstein	410.10±52.43	304.2±20.58	410.1±52.43	304.2±20.58	1.0

The exp. group was inseminated for the first time 402.5±41.12kg by breed criteria and on conception the weight averaged 417.5±21.65kg. The control group was inseminated for the first time with 304.2±20.58kg. The heifers in the exp. group age weight of 15.5 months while the control group age 13.4 average. So the difference in age was 2.1 months earlier and 113.3 kg less. Difference between the two groups regarding age not verified statistically (t Stat < t Critical, 1.82 versus 2.26) for P<0.05. Difference between the two groups in terms of weight verified statistically (t Stat > t Critical, 4.46 vs 2.77) for P<0.05. The difference in weight, at the time of AI reflected body weight during/after calving.

Table 2 – Milk (l) production in first lactation

Groups	Months						Months average l/head	Average 6 months l/head	Average 305 day lact. l/head
	I	II	III	IV	V	VI			
Experimental	660	700	620	540	500	400	570±11.53	3420	4993±438
Control	400	395	540	380	305	255	379.1±97.43	2125	3102±519.5
Differences	260	305	80	160	195	145	190.9	1295	1891

There are significant differences in terms of average milk production during the first 6 months of lactation, and 305 days lactation. (T stat.> T Critical, 3 versus 2.28) for P<0.05. The average daily production of milk, 6 months and 305 days lactation, is more in the exp. than contr. group inseminated later and they have greater body weight after calving. At calving, heifers of exp. group 66.7% were born normally, 33.3% with assistance. Any genital injury was observed. Heifers contr. group 43% were born normally, 43% with difficulty and only in one (14%) a severe damage in the genital tract, was observed. Extension of the gestation period is different for both groups but not statist. proven (t stat<t Critical) for P<0.05 well not affect the performance of the period after calving.

Table 3 - First calving performances

Groups	Calving Cows	In Calving		Calf weight	Loss Calf
		Age/days	Weight/kg		
Experimental	3	755±8.66	563.3±5.7	42±12	/
Control	7	687.7±58.7	458±67.9	28.4±4.9	1

Differences between the two groups for age at first partum in months 24.8 in the exp. group versus 22.6 in the control group proved statis. (t stat. >T Critical, 2.95, versus 2.36) for P<0.05. A significant difference in weight of newborn calves was observed. The weight in the exp. group was 42±12 kg while in the control group was 28.4±4.9 kg (P<0.05). Health status of cattle after calving appeared normal. Uterine involution monitored by manual rectal individual control for each heifer completed within data rate (average about 40 days after calving)¹. Heifers exp. group ended in 39.6±1.2 days, wherein heifers control group in 36.5±2.1 days after calving. Regarding uterine involution any statistical difference was observed between the two groups.

Table 4 – Resumption of ovarian activity

Groups	Ovarian activity after calving (estrus) days			Interval Calving-First AI X±DS
	40–50 days%	50–70 days %	Mbi 70 days%	
Exp. Group	2 66.6	1 33.4	-	50.3±10.2
Contr. group	1 16.6	-	5 83.4	90.8±24.1

There are evident differences regarding the first estrus after calving. The optimal rate, compared with literature data, 40-50 days after calving, estrus presented 66.6% in the exp. group and 16.6% in the contr. group. Most of the cattle in the contr. group 83.4%, occurred first estrus >70 days after calving. There are stat. diff. between the two groups for interval calving-first insemination period (P<0.05). In heifers of the exp. group, first heat appears relatively within the standards breed, 50.3±10.2 days after calving and AI index was 1.5. In control group heifers presented the first heat later after calving, 90.8±24.1 days and service AI index was 1. The analysis of blood selective metabolites, measured during lactation (20-80 days after calving) only in the experimental group, are reported in table 5.

Table 5 - Metabolic profile in blood

Indicators	Metabolite Level	Normal Range			
Glucose nmol/l	3.44 (2.86÷3.86)	3.50-4.00	Albumine g/l	46.5 (44.7÷48.06)	34-40
Urea nmol/l	4.43 (3.77÷5.46)	4.50-5.70	Globuline g/l	34.39 (32.4÷36.8)	40-50
Protein (total) g/l	80.9 (78.72÷84.87)	77-85	Ca nmol/l	1.96 (1.63÷2.38)	2.50-2.70
			P. Inorg nmol/l	2.65 (2.41÷2.88)	1.30-2.0

The average value of Glucose, which indicates the energy level of the feed ration, is below the normal. Incorrectly energy from feed-stuff are among the most common causes of a low fertility². Urea as indicator protein level of ration shows deviation from the normal limit. Protein deficiency is associated with extended interval calving-conception. While Ca and PI vary significantly from the normal range marking a deficiency of Ca and a significant excess of PI disordering by Ca:P. Not only the low level of P but also the increased quantities are associated with reduced reproductive

efficiency. It is reported that the increased levels of P cause deterioration of reproductive parameters².

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INDUCTION OF OESTRUS WITH OVSYNCH AND PRID PROTOCOLS IN ACYCLIC HOLSTEIN HEIFERS

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KEY WORDS: Acyclic heifers, ovsynch, PRID

ABSTRACT - The objective of this study was to determine the effects of two protocols on induction of oestrus in acyclic Holstein heifers. In Group 1 (n: 25), acyclic Holstein heifers treated with PRID protocols. In Group 2 (n: 25), acyclic Holstein heifers treated ovsynch protocols. Heifers were inseminated after the detection of oestrus. 2.43 ± 0.48 and 1.35 ± 0.39 days were found the time of behavioural oestrus in group 1 and 2, respectively. The percentages of oestrus detection were 92% (23/25) in group 1 and 64% (16/25) in group 2 ($P < 0.05$). The pregnancy rates were 78.26% (18/23) and 43.75% (7/16) in group 1 and 2, respectively ($P < 0.05$). In Group, a significant difference was observed for the oestrus and pregnancy rates. As a result, it is concluded that PRID protocols are useful for the treatment of acyclic Holstein heifers.

INTRODUCTION - Anoestrus was first recognized as a problem over 60 years ago¹. True anoestrus (inactive ovary) is a condition, in which the ovaries are quiescent without signs of cyclicity or cycle related ovarian structures². Anoestrus cause a delay in puberty or infertility because of non active ovaries². The cow or heifer would not have shown any sign of oestrus and rectal palpation reveals small ovaries, which are either flat and smooth or sometimes rounded³. Researchers have treated inactive ovaries with estradiol, Gonadotropins Releasing Hormone (GnRH), Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), progesterone and concurrent GnRH followed by Prostaglandin F_{2α} (PGF_{2α}).

MATERIAL AND METHODS - This study was conducted on 50 acyclic Holstein heifers with inactive ovaries which aged 16-22 months in two commercial dairy herds, located in Famagusta provinces in Cyprus from November 2011 to March 2012. 50 acyclic Holstein heifers, which did not show oestrus cycle, were controlled by rectal palpation at 10 day intervals for determining of activities of ovaries. In group 1, acyclic Holstein heifers received PRID® (1.55 gr Progesterone; Sanofi Dogu Ilac, Ankara, Turkey) on Day 0 with oestradiol benzoate (10 mg, vaginal capsule) and PGF_{2α} (Iliren, Farma Intervet) injection on Day 8 and GnRH (Receptal® inj., 0.0042 mg busserelin asetat/ml, Intervet Ltd., Istanbul, Turkey) injection on the Day 9. The PRID was removed on Day 9 and heifers were inseminated after the detection of oestrus. In Group 2 (n: 25), acyclic Holstein heifers received GnRH on Day 0, PGF_{2α} on Day 7 and GnRH on Day 9. Heifers were inseminated after the detection of oestrus. In both groups, heifers which did not show oestrus after the treatment were not included to the statistical analysis. The uterus of heifers that could not be observed in oestrus was palpated per rectum 45-50 days after insemination to determine pregnancy status. The differences in oestrus rates and pregnancy rates between two protocols were analyzed by using Chi-square Test and SPSS 14.01.

RESULTS - Consummately, 50 heifers were used in the study. 25 of them were allocated in group 1 and 25 in group 2. Oestrus rate and timing of oestrus in group 1 and

2 are presented in Table 1. In group 1, 23 heifers were detected in oestrus and artificially inseminated. 45-50 days after AI, 18 heifers resulted pregnant at diagnosis made by rectal palpation. In group 2, seven of 16 heifers became pregnant (Table 2).

Table 1 - Oestrus rate and timing of oestrus in group 1 and group 2.

PROTOCOLS	Estrus Rate	Timing of Estrus (Day)
Group 1 (PRID + PGF _{2α} + GnRH)	92% (23/25)	2.43± 0.48
Group 2 (GnRH + PGF _{2α} + GnRH)	64% (16/25)	1.35 ± 0.39
P	(P<0.05)	

Table2 - Pregnancy Rates at Day 45-50 in group 1 and group 2.

PROTOCOLS	Pregnancy Rates at Day 45-50
Group 1 (PRID + PGF _{2α} + GnRH)	78,26 % (18/23)
Group 2 (GnRH + PGF _{2α} + GnRH)	43,75 % (7/16)
P	(P<0.05)

DISCUSSION - The treatments of acyclic heifers has been evaluated for oestrus rates, timing of oestrus and pregnancy rates. Timing of oestrus was detected after 2.43±0.48 day in group 1 and 1.35±0.39 day in group 2 in this study. Researchers reported 52.0±5.8 hours and 3.22±0.97³ days as a time of oestrus in their study. Aral and Colak⁴ reported 62.6 hours after PGF_{2α} injection as time of oestrus in ovsynch protocol. Ozyurtlu et al.³ reported that oestrus induction rate following PRID removal was 75% in heifers with inactive ovaries. Cetin et al.⁵ have found that Holstein heifers treated with norgestomet showed an oestrus rate of 86%. Nak et al.⁶ reported similar recovery rate of 72% after PRID treatments in heifers. In this study, oestrus rates were found 92% in PRID groups and results were similar to the other study groups. In the treatments of acyclic heifers with progesterone, pregnancy rates were variable. In many studies of PRID, pregnancy rates reported between 14.28% and 73% after first inseminations⁷. In this study acceptable pregnancy rates were achieved which are 78,26% for group 1. After ovsynch protocols oestrus rates reported 15.4 %⁴ and 78%⁸. Oestrus rates were found 64% in our study. Pregnancy rates were observed in different rates between 32% and 76.92% in many studies of ovsynch protocols^{4,9,10}. In this study, pregnancy rates were found 43.75% in group 2.

CONCLUSION - It is suggested that PRID + PGF_{2α} + GnRH protocols are important approaches in the treatment of acyclic Holstein heifers because of statistically significant results.

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EFFECT OF pH ON SPERM KINETIC IN THE BULL

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KEY WORDS: bull, sperm motility, pH

ABSTRACT - Fresh semen diluted in vitro remains viable for a limited period of time. Much research has been done to understand the influence of different factors and to design a useful storage medium to enhance sperm survival in a non-frozen state. Some paper suggested an influence of pH on sperm kinetic characteristics, but there is no study that specifically describe how motility change in a different environmental pH. In this study we evaluate the effect of different environmental pH (5.5, 6, 6.5, 7, 7.5, 8, 8.5) on motility parameters, sperm viability and mitochondrial activity of bull semen after dilution. The higher seminal parameters were recorded at pH 6.5, 7 and 7.5 while environmental pH lower (6 and 5.5) or higher (8 and 8.5) affected significantly both motility parameters and sperm function. At higher pH, a reduction in sperm motility and mitochondrial activity but not sperm viability was recorded, which resulted in the immobilization of spermatozoa due to a reduction in metabolic function.

INTRODUCTION - Since the mammalian spermatozoa become capable of motion, during the epididymal transit, the spermatozoon swim in a liquid medium. The cells go through testicular and epididymal secretions, after ejaculation the spermatozoa are suspended in the fluid medium, called seminal plasma (SP) secreted by rete testis, epididymis and accessory sex glands (AGs) of the male reproductive tract¹. Then the semen swim in the female tract in the uterine secretion, and in the uterine tube to ampulla site of fertilization that allows the milieu of male and female gametes. All of these physiological environment modulate differently the function and the motility of spermatozoa^{2,3}. Thus sperm function could be affected by environmental fluid characteristic such as pH, osmolarity, and temperature. Some paper suggested an influence of pH on sperm kinetic characteristics^{4,5}, but no studies in any species specifically describe how motility changes in a different environmental pH.

The aim of the present study was to evaluate whether different pH conditions affect bull sperm function and characteristics as well as determine the possible alteration and if these modifications are affected by the incubation time before the routinely semen processing.

MATERIALS AND METHODS - This study was performed on 10 Swiss Brown bulls (2-7 yr old) used in regular artificial insemination (AI) service and housed in the Alpenseme AI Center of the Provincial Breeders Federation of Trento (Ton, Trento, Italy). After collection, semen was assessed for volume and concentration (Accucell photometer, IMV Technologie, L'Aigle, France). A modified Tyrodes medium (TALP) was adjusted at different pH: 5.5, 6, 6.5, 7, 7.5, 8, 8.5 by the addition of HCl or NaOH soon before the start of the trial. All samples were divided in seven aliquots and each one was diluted in TALP with a specific pH at 30×10^6 sperm/ml. After dilution each sample was evaluated for motility parameters using a computer assisted sperm analyzer (CASA) IVOS 12.3 (Hamilton-Thorne Bioscience, Beverly, MA, USA). An aliquot of each sample was rewarmed at 37°C for 10 minutes and analyzed in a Makler chamber (Sefi Medical Instruments, Haifa, Israel). The following parameters were considered: total motility (TM, %), progressive motility (PM, %), average path velocity (VAP, $\mu\text{m/s}$), amplitude of lateral head displacement (ALH, μm), and straightness (STR, as VSL/VAP , %). Sperm viability (SV, %), assessed by Calcein-AM (Molecular Probes Inc., Eugene, OR, USA - final concentration, 1 μM), and mitochondrial activity (MA, %), evaluated using Mitotracker® deep red (Molecular Probes Inc., Eugene, OR, USA - final concentration of 100 nM) were performed by a flow cytometer

(EPICS XL; Becman COULTER) on a 1-ml sample after 10 min of dark incubation. The flow cytometric analysis was performed on 30,000 events.

Data were analyzed statistically by a General Linear Model using a univariate ANOVA followed by a Tukey post-hoc test. Values were considered different with $p \leq 0.05$.

RESULTS - The mean volume of the ejaculates used in this study was 7.2 ± 1.3 ml, with a concentration of $857.1 \pm 427.3 \times 10^6$ sperm/ml. Parameters recorded in spermatozoa incubated at different pH were summarized in the Table 1.

Table 1 - Sperm parameters at different pH. Different letters in the same row differ significantly $p \leq 0.05$.

Parameters	pH 5.5	pH 6	pH 6.5	pH 7	pH 7.5	pH 8	pH 8.5
TM (%)	73.3±7.2 ^a	82.4±17.8 ^b	83.1±15.6 ^b	86.3±10.3 ^b	88.1±8.5 ^b	65.3±13.6 ^a	36.3±8.4 ^c
PM (%)	39.5±7.6 ^a	57.2±16.5 ^b	60.3±17.4 ^{bc}	67.1±10.8 ^c	70.6±11.7 ^c	31.7±15.3 ^a	24.3±13.7 ^a
VAP (µm/s)	79.9±9.8 ^a	94.3±18 ^a	99.6±17 ^{ab}	120±15 ^b	113.6±16.6 ^b	92.5±16.7 ^a	77.2±14.5 ^a
ALH (µm)	7.6±0.9 ^a	7.1±1.4 ^{ab}	6.9±0.9 ^{ab}	6.8±0.5 ^{bc}	6.1±0.7 ^c	6.3±1.3 ^c	6±1.1 ^c
STR (%)	73.7±4.9 ^a	79.1±8.5 ^b	81±4.8 ^b	83.7±4 ^{bc}	86±5.8 ^c	82±2 ^{bc}	80±3.2 ^b
SV (%)	48.2±7.8 ^a	60.7±13.7 ^b	76.3±6.8 ^{bc}	80.3±6.2 ^{cd}	84.6±7.1 ^d	71.2±3.7 ^{cd}	65.7±5.6 ^b
MA (%)	36.9±5.3 ^a	53.3±7.1 ^b	59.1±4.9 ^b	71.3±5.2 ^c	74.5±4.6 ^c	40.1±6.6 ^a	28.4±5.7 ^d

DISCUSSION - In this study a significant effect of the environmental pH was demonstrated on both sperm motility, sperm viability and mitochondrial function. An effect of the pH on sperm motility was previously suggested in human and other mammals. Carr and Acott⁵ suggested the involvement of the acidic pH in the sperm immobilization in the bull epididymis. De Pauw et al.⁶ reported an higher survival of spermatozoa stored in a medium setted at pH 6 then 7 or 8. Human sperm exhibits a greater reduction in motility in response to acidic conditions than to alkaline conditions. Spermatozoa immobilized by acidic conditions are able to regain motility after the pH is returned to normal, but the effects of alkaline conditions are not reversible⁷.

Interestingly, in a medium with alkaline pH a drop in the progressive motility, but not sperm viability was recorded. This reduction seemed due to the contextual reduction in mitochondrial activity of these spermatozoa. This finding suggests that the increase in the intracellular pH resulted in the immobilization of spermatozoa via the inhibition of the activity of the mitochondria. The mitochondrial dysfunction could result in the alteration of other metabolic pathways related with sperm function. These results could explain the effect of some pathological conditions on seminal quality. The increase of the environmental pH is generally recorded in urospermia or infection of the male genital tract. Thus the elevation of the pH could partially explain the reduced motility recorded in these conditions. Similarly, an increase in the uterine pH was reported in cows with cytologic endometritis⁸. On the basis of the data reported in this study, the increase of the pH in the endometrial environment could result in a immobilization of the most spermatozoa leading, in turn, to subfertility or infertility.

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POSTPARTUM OF THE DAIRY COW: IMPACT OF THE ADMINISTRATION OF VITAMIN A AND E ON SERUM THYROID HORMONE CONCENTRATION

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ABSTRACT - Postpartum is a critical period for the dairy cow, during which the high metabolic demands and the increased metabolism may lead to oxidative stress and to the development of many dysfunctions. Thyroid hormones are known to contribute to energetic balance, lipid metabolism and to the modulation of ovarian activity. Given these premises, the effect of an association of β -caroten and α -tocopherol (Dalmavital®, Fatro, Italy) parenterally administered within 24 hours after calving on circulating free thyroid hormone (biologically active hormone) concentrations between the 10th and 16th day after A.I., as well as on the main reproductive parameters, was tested. Circulating fT3 and fT4 concentrations were statistically higher in the group administered Dalmavital (group D), compared to the group Control (group C). Moreover, reproductive parameters were improved in the Group D, compared with Group C. The results of this study encourage the parenteral administration of antioxidant vitamins in the delicate phase of the early postpartum, to counteract the ubiquitous damages exerted by oxidative stress and the related dysfunctions, likely to develop in such a period.

INTRODUCTION - Negative Energy Balance (NEBAL) is a critical condition for the transition cow, during which an over production of Reactive Oxygen Species (ROS) is likely to develop¹. This makes the transition cow prone to the development of many dysfunctions leading, directly or indirectly, to reduced fertility^{1,2}. This is related to ubiquitous damages exerted by ROS on DNA, lipids and proteins, with subsequent alteration of different structures (receptors, channels, enzymes, cell membranes) and, consequently, of their biologic activities³. Thyroid function may be a potential target of oxidative stress. Free thyroid hormones (biologically active thyroid hormones) improve metabolic conditions⁴. Moreover, they play an important role in modulating ovarian functions and steroidogenesis⁵. Given these premises, aim of this study is to evaluate the impact of parenteral vitamin (β -caroten and α -tocopherol) i.m. administration within 24 hours after calving, on circulating free thyroid hormone concentrations and on reproductive parameters (calving-first oestrus, calving-conception, pregnancy rate at first and second A.I.), in dairy cows, from the 10th to the 16th day after calving.

MATERIALS AND METHODS - Twenty postpartum Friesian healthy dairy cows, between the third and fifth lactation, with a mean daily milk production of 27 kg and a BCS ranging between 3 and 3.5 were enrolled in this study. The cows were kept under a semi-intensive breeding system and were fed a mixture of pasture and a ration consisting of fodder, concentrates and minerals. The cows were randomly divided in two groups (of 10 cows each), as follows: **Group D** (Dalmavital): administered Dalmavital® (Fatro, Italy) at a dose of 7 ml/100 kg B.W. (corresponding to 1 mg/kg of β -carotene and 1.3 mg/kg of dl- α -tocopherol), within 24 ore after calving and **Group C** (Control), administered, at the same time point, an equal volume of sterile saline solution (NaCl 0.9%). All the cows underwent blood collection, from coccygeal vein, in

refrigerated vacutainer serum tubes: T10, T12 T14, T16 (10, 12, 14 and 16 days after calving, respectively), before evening milking, for fT3 and fT4 assay. Once serum was obtained, sera underwent analysis by means of Immulite 1000 Free T₃ and Immulite 1000 free T₄ (Immulite® Immunoassay systems, Siemens, USA) a competitive and quantitative immune dosing system (sensitivity: 1 pg/mL and 0,3 ng/mL for fT3 and fT4, respectively). Besides recording the abovementioned reproductive parameters, transrectal ultrasonographic pregnancy diagnosis was performed 40 days after A.I. (SonoSite, MicroMaxx Bothell, WA, USA, linear probe 5-10 MHz, set at 7.5 MHz). The data obtained underwent statistical analysis (PASW Statistics 18, IBM, Italy).

RESULTS - The results are expressed in the following tables:

Table 1 - fT3 concentrations (pg/mL) (mean± d.s.) in Groups C and D at 10, 12, 14, 16 days post-partum (T10, T12, T14, T16 respectively) In the column: A,B: p<0,05; C,D: p< 0,01; E,F: p<0,001.

fT3 (pg/mL)	T10	T12	T14	T16
Group C	1,68±0,52 ^A	1,54±0,4 ^C	1,67±0,4 ^A	1,41±0,27 ^E
Group D	2,16±0,35 ^B	2,19±0,61 ^D	2,14±0,73 ^B	2,16±0,57 ^F

Table 2: fT4 concentrations (ng/dL) (mean± d.s.) in Groups C and D at 10, 12, 14, 16 days post-partum (T10, T12, T14, T16 respectively). In the row: a,b: p<0,05. In the column: A,B: p< 0,05; C,D: p<0,01

fT4 (ng/dL)	T10	T12	T14	T16
Group C	0,88±0,17 ^a	0,75±0,17 ^{Cb}	0,85±0,24	0,76±0,15 ^{Ab}
Group D	1±0,11	0,96±0,18 ^D	1±0,17	0,95±0,27 ^B

Table 3: Reproductive parameters in the two groups C and D. In the column: A,B: p<0.05

Group	Calving-first oestrus (days)	Calving-conception (days)	Pregnancy rate (1° A.I.)	Pregnancy rate (2° A.I.)
C	78,6±14,65 ^A	99,6±14,65	20%	62%
D	47,6±16,1 ^B	76,8±19,9	30%	71%

DISCUSSION: The increase in fT3 and fT4 observed in Group D compared to group C suggests that antioxidant vitamins are able to exert a stimulating effect on thyroid activity which mirrors in the shortened calving first oestrus and calving conception intervals, as well as in the higher pregnancy rates observed in the group D. These results let us infer that vitamin administration in postpartum, besides restoring the physiological oxidative status, is able to promote thyroid activity with subsequent positive reproductive outcomes. These data indirectly confirm the strong relationship between thyroid hormones and reproduction⁵, thyroid activity and metabolism⁴ and encourage vitamin administration in the delicate early postpartum in dairy cows¹.

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AN ECO-DOPPLER EVALUATION OF THE MORFO-FUNCTIONAL LUTEAL PARAMETERS IN CYCLIC AND PREGNANT DAIRY COWS.

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KEY WORDS: Corpus luteum, pregnancy, power doppler

ABSTRACT - The aim of this study is to evaluate luteal morfo-functional parameters at 11 (T11) and 13 (T13) days after artificial insemination in pregnant and non-pregnant dairy cow. Ultrasonography was carried out to evaluate luteal area, vascular luteal area and the % of the luteal area occupied by blood vessels. Progesterone concentrations were also assessed. This study shows the positive relation among the CL dimension, luteal perfusion and P4 production and highlights the evolution of this parameters from T11 to T13, in the pregnant cows.

INTRODUCTION - The transition of the corpus luteum (CL) from non-pregnant to pregnant status is a crucial moment for embryo survival¹. Physiologically, the luteolysis occurs around the 18th day after ovulation², however, previous researches^{3,4}, showed that progesterone circulating concentrations decrease already around the 12th day after artificial insemination (A.I.), in case of precocious embryo resorption. Given these premises, aim of this study is to evaluate luteal morfo-functional parameters at 11 and 13 days after A.I., in pregnant and non-pregnant dairy cows.

MATERIAL AND METHODS - The study was carried out on 20 Friesian dairy cows, between the third and fourth lactation, with a BCS ranging between 3 and 3.5. The cows were kept under as semi-intensive breeding system and were fed a mixture of pasture and a ration consisting of fodder, concentrates and minerals. The cows underwent a clinical exam aimed at assessing their healthy status and excluding those with a repeat breeder anamnesis.

On the 11th (T11) and 13th (T13) days after A.I., blood samples were performed for progesterone (P4) determination (Vidas® Progesterone-Bio Meriaux SA, Lyon, France; sensibility 0.05 ng/mL; specificity: 100%). At the same moment, a transrectal ultrasonographic exam with multifrequency linear probe (5-10 MHz) (SonoSite, MicroMaxx Bothell, WA, USA), was performed to evaluate the area and perfusion of the corpus luteum. Three B-mode longitudinal scan of the CL were performed. On the same scans, Power Doppler was used to evaluate CL perfusion. All images were recorded and analyzed by Adobe® Photoshop® CS4 (2008 Adobe Systems Incorporated), to quantify the area of the corpus luteum (pixel) and the one occupied by blood vessels (pixel). As to CLs with cavity, the cross-sectional area of the cavity was assessed separately and subtracted from the total luteal area. For each CL, the mean of these parameters obtained from the three single images was calculated. Finally, the mean luteal area, the luteal vascular area and the % area of CL occupied by blood vessels were calculated. Pregnancy diagnosis was performed 40 days after A.I. and then the cows were divided in two groups: pregnant cows (**PC**) (12 cows) and non-pregnant cows (**NPC**) (8 cows).

All values were evaluated by a statistical program [PAWS® Statistics 18 (Chicago, USA)]. A value of $p < 0.05$ was set as significance level.

RESULTS - The results were expressed in table 1.

Table 1 - Luteal area, vascular luteal area, % luteal area occupied by blood vessels, progesterone concentration (Mean±s.d.) in the groups of the pregnant (PC) and non-pregnant cows (NPC) at 11 (T11) and 13 (T13) days after I.A.

Groups\ Times	LUTEAL AREA (pixel) (Mean ±s.d.)		VASCULAR LUTEAL AREA (pixel) (Mean ±s.d.)		% VASCULAR LUTEAL AREA (Mean ±s.d.)		PROGESTERONE (ng/mL) (Mean ±s.d.)	
	T11	T13	T11	T13	T11	T13	T11	T13
PC	33284 ±9876.05	36246.6 ±7190.7 ^A	1601.2 ±1088.9 ^a	1641.8 ±678.9 ^{bA}	4.9 ±2.9	5.0 ±2.5	4.3 ±1.02	4.55 ±0.77
NPC	31692.6 ±9388.5	25866.2 ±5040.2 ^B	1692.2 ±821.3	729.2 ±430 ^B	5.4 ±2.55 ^a	2.27 ±1.178 ^b	3.7 ±1.31	3.438 ±1.65

In the row: a,b: p<0,05. In the column: A,B: p<0,05.

As shown in the table, all the values of the parameters in the PC group were generally higher than in the NPC group. All the values were similar at T11 in the two groups, to increase at T13 in PC cows and to decrease in NPC cows.

The comparison between the two groups showed a statistically significant augment at T13 in the PC group compared to NPC group, as CL area and vascular luteal area (p<0.05). As to the correlations among the tested parameters, they were positive in both groups, even if not in a statistically significant way. A significant positive correlation (R=0.937; p<0.05) was found at T13 in NPC group, between the luteal area and the luteal area occupied by blood vessels.

DISCUSSION - This study shows the positive relation among the area, luteal perfusion and P4 production. Furthermore this experimentation highlights the positive evolution of these parameters from T11 to T13 after A.I., in pregnant cows. These data confirm the results of the study of Utt et al.⁵ in which the luteal blood flow reflects the functional status of the CL. Moreover the decrease in all these parameters observed in the NPC group validate the tight connection existing among luteal area, perfusion and steroidogenesis.

These are preliminary data, because of the small number of the cows, but widening it to a larger number of animals could let obtain more objective results, which could be useful to early diagnose (11-13 days) at risk pregnancies or a non-pregnancies. As a consequence, this could let choose the best therapeutic approach, orienting between a luteotropic or a luteolytic precocious intervention.

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RELATIONSHIP BETWEEN SPERM PENETRATION SCORE AND CERVICAL MUCUS QUALITY DURING ESTRUS IN COW

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KEY WORDS: Bovine, Sperm, Cervical mucus penetration test.

ABSTRACT - Generally cervical mucus penetration test (CMPT) have been used for assessment of semen quality. In this study, a simplified CMPT was applied to evaluate quality of cervical mucus of cows in estrus using semen of proven fertility. Viscosity, pH, spinnbarkeit, cellularity, crystallization and penetration, were measured. A significant positive linear correlation was found between sperm concentration [Sp] detected in mucus after CMPT and the quality of each mucus samples according to physical characteristics. Comparing [Sp] values of cyclic cows with those of pathologic ones, we obtained higher [Sp] for cyclic cows respect to the pathologic cows. The evaluation of [Sp] could be useful to improve the diagnostic framework of bovine fertility.

INTRODUCTION - Cervical mucus controls sperm transport in female genital tract and it may block abnormal sperm from entering uterus and/or may serve to preserve the normal sperm for long time after ejaculation¹. It's clear that cervical mucus has much more meaning than just a lubricant secretion, its characteristics can facilitate or prevent outcome of fertilization. An evaluation of capacity of cervical mucus to be penetrated by sperm can be obtained by in vitro mucus penetration tests. Mucus penetration test is generally used to assess the fertilizing ability of sperm that is strongly correlated with the capacity to penetrate cervical mucus². Considering all these knowledge our study aimed to perform a cervical mucus penetration test (CMPT) with bull semen of proven fertility to have information about quality of bovine cervical mucus.

MATERIALS AND METHODS - Detection of estrus - Estrus was detected by direct observation of cows behavior and by observation of external genitals. Uterus and ovaries were examined by rectal palpation. Estrus was confirmed by quantitative determination of 17 β -estradiol in serum (DiaMetra, Milano). **Cervical mucus** - Cervical mucus was collected from cows at estrus and stored at -80°C. Bovine cervical mucus can be frozen with only minimal changes in rheological properties³. For each mucus samples volume, viscosity, crystallization, spinnbarkeit, cellularity, pH were recorded as previously described by Insler et al.⁴. **Semen** - We used frozen semen of two bulls (Fibrax and Silve), which quality was evaluated by Computer Assisted Sperm Analyser (CASA, IVOS Ver.12, Hamilton Thorne, USA). **Mucus penetration test** - Basing on a protocol developed by Clarke⁵ in human, this procedure was adapted to the bovine model. Cervical mucus was aspirated into a capillary using positive displacement. Capillary end was immersed 5 mm below the surface of semen. Incubation was conducted for 60 min at 38°C. Capillary was removed from semen and mucus end was mixed for 5 sec in bromelain in Tyrode's solution to remove loosely adherent spermatozoa. Mucus was expelled into bromelain and the mixture was incubated at 37°C for 30 min to dissolve mucus. Sperm count was performed by Thoma chamber.

RESULTS - The computer assisted sperm analysis for Fibrax showed 55% of overall sperm motility and 31% of sperm with progressive motility while for Silve showed 22% and 14%, respectively. According to Insler et al.⁴, the score of physical characteristics of each mucus samples ranged between 6-13. The [Sp] of Fibrax and Silve ranged from 80 to 438 cells/ μ l and from 26.7 to 240 cells/ μ l, respectively. The data shown a strongly correlation between quality of the mucus and [Sp] after CMPT: $r=0.967$, $r=0.972$. Animals were divided into 2 groups: 38 healthy cyclic cows and 16 cows with reproductive pathologies. The latter group includes repeat breeders, ovarian cysts, cervical-uterine adhesions. The average value of [Sp] obtained with both samples of semen was higher in cyclic cows than pathologic with [Sp] of 241.3 cells/ μ l versus 206.08 cells/ μ l for Fibrax and 152.34 cells/ μ l and 143.54 cells/ μ l for Silve.

DISCUSSION - We try to develop a CMPT that could be used to obtain information about mucus quality. Sperm viability and conception rate are influenced by physio-chemical properties. Correlation of fertility with viscosity, ferning, pH and spinnbarkeit of estrus cervical mucus has been documented⁶. There was a significant linear correlation between mucus quality and [Sp] in mucus column. According to these evidences, [Sp] could give information on animal's fertility. Would be interesting to test by Western blot the level of mucins expression in both cyclic and pathologic bovine cervical mucus.

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INFECTION AND ABORTION OF COWS FROM NEOSPORA CANINUM DUE TO EVEN THE ENVIRONMENTAL CONTAMINATION FROM DOGS THAT CARRIERS THIS PARASITE

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KEY WORDS: *Neospora caninum*, neosporosis, cow

ABSTRACT - During 2011, blood sera of 141 dairy cows were tested by ELISA against *Neospora caninum* that were part of the 12 farms: 4 in Saranda, 4 in Vlora and 4 in Lushnja. 47 cows at each district were tested. In total, resulted seropositive 23 cows or 16.3%: Saranda 7 heads or 14.9%, in Vlora 11 or 23.4% heads and 5 heads in Lushnja or 16.3%. From 12 farms resulted seropositive nine (75%), of which: 3 (60%) in Saranda, 2 (50%) in Vlora and 4 (100%) in Lushnja. Of 60 samples with the feces of dogs resulted positive for *Neospora caninum* oocysts 17 of them (28.3%), of which: 6 (30%) of Saranda, 4 (20%) from Vlora and seven (35%) of Lushnja. So 28.3% of dogs that move near farms with cows, pollute the environment with *Neospora caninum* oocysts enabling infecting cows through.

INTRODUCTION - In Albania until now there has been no study on *Neospora caninum*, although it is known that it is one of the leading causes for the failures in cows and other animals^{2,3,6}. To highlight the *N. caninum* seroprevalence level in dairy cows at three southern districts and environmental impact contaminated by feces of dogs in the prevalence of this parasitosis, we undertook this study.

MATERIALS AND METHODS - During 2011, blood sera of 141 dairy cows were tested by ELISA against *Neospora caninum*, they were part of the 12 farms: 4 in Saranda, 4 in Vlora and 4 in Lushnja. 47 cows at each district were tested. Ten farms were selected at random, while in Vlora 2 farms were chosen away from the residential areas, without the presence of dogs and good breeding conditions. Moreover, 60 samples of feces of dogs (5 samples at each farm, i.e. 20 in each district) were collected, and analyzed with coproscopioocisteve for the presence of *N. caninum*. It was considered *N. caninum* oocysts with length less than 10.7 μ^4 .

RESULTS AND DISCUSSION - From the 141 cows tested, resulted infected by *N. caninum* 23 heads (16.3%) and suspicious 9 (6.4%) heads. So the neosporosis seroprevalence average of cows in the three districts was 16.3% with a range from 10.63% in Lushnja, 14.9% in Saranda and to 23.4% in Vlora. The literature reports that in some cases proved to *N. caninum* 90% cattle tested resulted seropositive². Of the 12 farms that were included in the study 9 of them (75%) resulted seropositive for *N. caninum*. In Saranda 3 farms (75%) resulted seropositive and 1 (25%) suspicious. In Vlora resulted seropositive 2 farms (50%); in Lushnja of 4 farms tested (100%) resulted seropositive. Our results can be compared with those reported in literature. An article published in 2007³

indicates that were seropositive for *N. caninum*: in France 26% of tested farms, in Canada 57%, in Germany 6.8%, etc. The coproscopy analysis shows that 17 (28.3%) of 60 dogs were carriers of *N. caninum*, so those with their feces can pollute the environment of farm with oocysts of this coccidie. So increasing the chances through the contaminated food and water these oocysts will be transmitted to the cows, which by their own will transmit the infection to subsequent generations in transplacentally ways^{2,3,5,6}.

Neosporosis seroprevalence of cattle farms is related to the presence of the dog on the farm¹, this in a way is also observed in our study. The cows in two farms district of Vlora, which had no dogs, where implemented biosecurity measures and were away from the inhabited area, resulted seropositive for *N. caninum*. Neosporosis seroprevalence level of bovine is determined by many factors, more important of which are: 1) the presence of the dog in the farms, 2) the proximity of farms to residential areas, 3) implementation of biosecurity measures at farm, 4) the age of cows, etc.^{2,3,6}. Most farms included in this study were guarded by dogs, 10 of them were near residential areas, and almost have the absence of biosecurity measures. All three of these factors constitute the premise of biological pollution of the environment where the animals stay with oocysts of *N. caninum* and the horizontal transmission of the parasite from definitive hosts (dog) to the intermediate hosts (cows)^{1,3,5}. We don't have information about the age of cows, but this factor has to do with the fact that much older to be an animal, the more numerous are the chances of being exposed to dogs carriers of *N. caninum*. *N. caninum* caused considerable economic loss because of it can fail up 42% of cows³. Therefore the monitoring of the epidemiological situation and the implementation of measures that reduce significantly the level of infection and prevent its spread to cattle farms, should be considered seriously by the veterinary service of our country.

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HYGIENIC CONDITION MONITORING OF RAW MILK IN REGION OF KORÇA

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KEY WORDS: raw milk, physic-chemical characteristics, microbiological quality

ABSTRACT - Milk quality control and its improvement are crucial for the its important contribution in human diet. This study aims to determine the physico-chemical values of milk (density, protein, lactose and fat content in %, and the freezing point) as well as the determination of microbiological characteristics (total number of mezofile microbes (NPM) and somatic cells number (SCC).) This study took place in the period March-December 2011, where 120 raw milk samples received at milk collection centers in Korca region were analyzed. The obtained results and their confrontation with Dir. Directive 92/46/EEC and the Directive No. 5 dated 25.3.2011 of Ministry of Agriculture “The norms of raw milk collection” helped us to create all-encompassing image of milk quality in this region. Results are considered valuable in terms of consumer health problem, especially regarding the risk of contamination and the necessity of application of HACCP systems for healthy dairy products.

INTRODUCTION - Milk physico-chemical and microbiological analysis are important tools to monitor the its quality². Milk is known as a food with completed nature¹. Beside milk nutritional value, the active biological ingredients such as casein and whey proteins are considered important for physiological and biochemical functions owing to the significant impact on human metabolism and his health^{2,4}. Milk is considered as the main source of dairy products in European developed countries¹. This product in Albania represents an important role for all age-groups diet. Milk hygienic characteristics are fundamental and affect the quality and quantity of processed products. If the somatic cells increases would have an impact on milk ingredient changes³. Products characteristics, method of collection, distribution and consumption dictate the need for a high level of hygienic monitor.

MATERIAL AND METHODS - The milk samples analyzed were collected from collection center in the region of Korça and Pogradec (30 villages). Local farmers of this region supply these centers. The production of dairy products in these centers reaches (7239 Hl milk, 621 liter yogurt, 146 kg butter, 2544kg cheese, etc.) which are collected round 45,217 tons milk (Korça) and 12,112 tons milk (Pogradec).

The study was performed in the period May-December 2011. Sampling was carried out according to standardized criteres. The samples were carried out as duplicate samples and they were collected in milk bulk tank in the factory or dairy centers. Later they are placed in 250 ml sterile containers and were stored in cooling boxes (at 4-6° C). All the collected samples were transported immediately to the laboratory. **Physico-chemical analysis.** The values of temperature, protein, lactose, fat and minerals content as well as freezing point were determined using a milk analyzer (LACTOSCAN S_L). **Microbiological tests.** The

total number of mezofile in milk samples was detected in accordance with ISO4833:2003. Samples were incubated at 30° C for 72 hours. The growing colonies were monitored and analyzed using an electronic counter colonies. PCA culture was used for microorganisms identification, while for the somatic cells number determination DeLaval database kit DCC, was used.

RESULTS - Tests results for the somatic cells number identified 52 positive samples (52/120 samples). It is very important to emphasize that this number is not in accordance with the values specified in Directive no. 92/46/EEC and 94/71/EC as well as on the Directive No. 5 dated 03/25/2011 of the Ministry of Agriculture ($\leq 400,000$ cells/ml). These values indicate that the milk is unhealthy for human consumption and milk industry. We consider very important the influence of seasonal temperature changes. These temperatures affect both in the somatic cells content number and microbial load of milk. The milk microbial load resulted with higher values in the period May-September (55/120 samples analyzed). Milk physical-chemical tests detected that 37 samples had a decrease content of lactose and 55 samples a decrease content of protein.

DISCUSISON - This study proved to be useful in terms of the imperative HACCP implementation systems in order to provide healthy dairy products. Results are considered valuable in terms of consumer health problems, associated with the contamination risk. However, we still have not foodborne outbreaks reports related to human consumption^{4,5}. The production of milk with a high number of somatic cells and microbial cells in tank is associated with the lack of care in managing and a low level of hygiene. This milk is considered a source of potential presence of pathogens or the potential presence of mastitis in animals, which can be problematic for human consumption.

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INDIVIDUAL HAIR CORTISOL AS A TOOL FOR MONITORING HPA ACTIVITY IN HEALTHY DAIRY COWS

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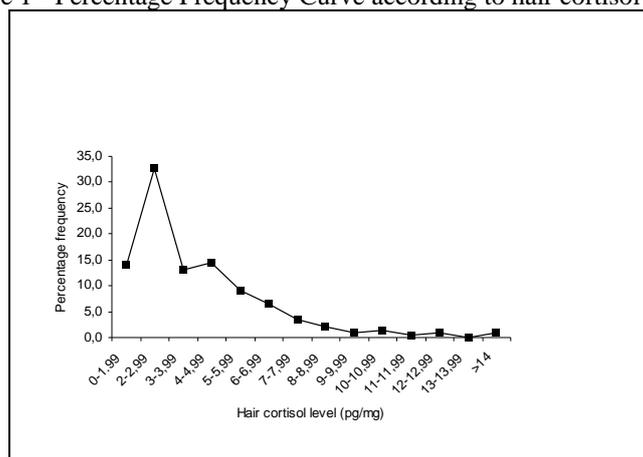
ABSTRACT - Hair cortisol levels were determined in 85 multiparous lactating Italian Friesian dairy cows. The animals were clinically healthy and had not undergone social group changes within the past 3 months. Hair cortisol data were stratified into 14 frequency classes. Data showed a strongly positive asymmetric distribution. In 50% of the animals, cortisol levels were below 3.29 pg/mg while in 33% hair cortisol levels were concentrated in the hair cortisol class 2-2.99 pg/mg. This range of values was suggested as the representative class of hair cortisol levels in healthy animals. Hair cortisol analysis could be a valid, interesting and useful tool for monitoring HPA activity in healthy dairy cows.

INTRODUCTION - Cortisol is a key hormone in the physiological response of the hypothalamic-pituitary-adrenal axis (HPA) and can be determined in blood or, non-invasively, in faeces, urine, milk and saliva. Measuring cortisol levels in hair samples can also be used to assess HPA activity. Cortisol plasma spread from capillaries to cells of the hair follicles and may finally be deposited in the hair shaft¹. Hair cortisol levels are unaffected by circadian hormone variations or by factors inducing short-term variations. The collection of this biological material is simple, non-invasive and the sample obtained does not decompose like other body fluids or tissues². Measurement of hair cortisol has been validated in humans, rhesus macaques, dogs and cats^{3,4,5}. A prior study has addressed the use of hair cortisol determinations in bovine⁶. The aim of the current study was to evaluate hair cortisol levels in healthy Friesian dairy cows.

MATERIALS AND METHODS - Cortisol levels in hair samples were examined on 85 multiparous lactating Italian Friesian dairy cows from 90 to 305 days of lactation. The animals were selected from a single herd, were clinical healthy and no change in social group or any disease occurred in the 3 months before the start of sampling. A single hair sample from each cow was obtained by clippers from the frontal region and stored in dry tubes at room temperature until analysis. Hair strands were washed in 5 ml isopropanol, as suggested by Davenport et al.⁴, and hair cortisol extraction was performed according to the method described by Koren et al.³ modified by Accorsi et al.⁵. Hair cortisol was determined using the RIA method described by Tamanini et al.⁷. The variable hair cortisol concentration was submitted to univariate statistical analysis to establish its distribution, central tendency and dispersion. On samples, mean, standard error, median, sample variance, kurtosis and skewness were calculated. The Kolmogorov-Smirnov test was used to confirm or refute the normal distribution of data. Fourteen classes of data were defined and percentage frequencies for each class calculated.

RESULTS - The hair cortisol concentrations recorded for the 85 dairy cows ranged from 0.76 to 20.41 pg/mg, mean and standard error were, respectively, 3.96 and 0.16 pg/mg. The median, standard deviation and variance were 3.29, 2.48 and 6.17 pg/mg, respectively. In 50% of the animals, concentrations were below 3.29 pg/mg (median). This asymmetric distribution along with high values of kurtosis and skewness obtained indicate that data redistribute according to a non-normal distribution. The Kolmogorov-Smirnov test confirmed that it is unlikely these data are normally distributed. Cortisol levels were classified into 14 classes and percentage frequencies for each class were calculated (Figure 1). The data concentrated in the hair cortisol class 2-2.99 pg/mg.

Figure 1 - Percentage Frequency Curve according to hair cortisol classes



DISCUSSION - The data obtained in this study describe cortisol distribution in healthy Friesian dairy cows from a single herd. Hair analysis can monitor hormone changes over a long period of time (long-term exposure) and provides a window to the past. Assuming a 0.6 - 1 cm per month hair growth rate in the dairy cow, hair analysis may document a historical timeline of hormonal status from the initial development of a strand of hair to the time of its collection. In this study, hair cortisol levels were found to vary widely indicating different individual activation of the HPA axis. Our most interesting finding was that 50% of the animals had hair cortisol levels below 3.29 pg/mg and that the class 2-2.99 pg/mg included 33% of the animals. Considering that hair cortisol concentrations provide long term information, these values are likely to be representative of the physiological range of variation in hair cortisol produced in healthy lactating dairy cows. The results point out also that, in some animals, HPA axis is strongly activated and that there are also animals with a low level of activity of the same axis, findings that require further investigations. This preliminary study suggest that hair cortisol analysis as a valid, interesting and useful tool to monitor HPA axis activity in lactating dairy cows.

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PREVALENCE OF SUBCLINICAL MASTITIS TO A DAIRY COWS FARM IN ALBANIA

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KEY WORDS: Cow, mastitis, microorganisms

ABSTRACT - This study has been carried out in a farm of milk cow husbandry in Ndroq-Tirana. A number of 96 milk samples has been undergone to this study, belonging to 24 cows, half of them in the first 100 days of the lactation period and half at the end of this period, before drying. Analytical results showed an increasing of the somatic cells in the milk, beyond the permitted levels: 430.000-630.000 cells/ml Bacteriological tests demonstrated the presence of mastitis cause: *Staphylococcus aureus* and *Streptococcus agalactie*. The prevalence of subclinical mastitis resulted to 37.5% and more in detail: 20.8% for *Staphylococcus aureus*, 5.2% for *Streptococcus agalactie* and 7.3% for *Eschericia coli*.

INTRODUCTION - The undertaking of this the study was conducted on the basis of a survey on the total number of somatic cells in milk, which showed a significant increase in their numbers over the allowed limit - upon the request of the farm specialists. This study aims also to monitor and highlight the importance of the necessity of drafting and implementing a concrete plan to control mastitis in the farm^{1,2}. Despite the antibacterial barrier of breast sphincter, it cannot be avoided completely the entering of bacteria in the breast, even in small quantities, especially during milking. It is not excluded that the bacteria can reach the breast through the blood. We can say that for every stall clinical mastitis there can be found 10-20 sub-clinical mastitis^{4,5}

MATERIALS AND METHOD - The study is carried out analyzing 96 samples of milk⁶ belonging to 24 cows: 12 cows were taken during the first 100 days of lactation period; 12 cows in the last period of lactation, before drying. All 24 tested cows were not treated with antibiotics in the last two months and all their quarters of breasts were healthy^{7,8}. For every sample has been performed the inoculation in agar ground plates added with blood sheep. The results were read after 18-24 hours. The culture has been classified in accordance with the form of colonies and other characteristics such as intensity and type of hemolysis^{4,2}. There has been performed chemical and physical analysis to the raw milk, such as total acidity, lactose content, fat content and ash.

RESULTS AND DISCUSSIONS - This study aims to put in evidence the impact of bacterial microorganisms in the infection of cows with mastitis, as well. For this purpose, we have undertaken microbiological analyses of the samples, looking for bacterial presence in the milk^{5,3}. Table 1 shows the percentage of milk sample polluted with *Staphylococcus aureus*, *Streptococcus agalactie* and *Eschericia coli*. The tested samples have a number of

somatic cells above the normal value. This content ranges from 430.000 to 630.000 cells/ml. In total, the content in subclinical mastitis was 37.68%.

Staphylococcus aureus was responsible for 21 cases of subclinical mastitis, *Streptococcus agalactie* for 5 and *Eschericia coli* for 7 other cases. So, the mastitis prevalence was caused in the 20.8% of cases from *Staphylococcus aureus* 5.2% of cases from *Streptococcus agalactie* and in 7.3 % of cases from *Eschericia coli*.

Table 1 - Microbiological results of milk samples

Type of microorganism	Positive samples		Negative samples	
	N	%	N	%
<i>Staphylococcus aureus</i>	21	21.8	75	78.2
<i>Streptococcus agalactie</i>	5	5.2	91	94.8
<i>Eschericia coli</i>	7	7.3	89	92.7

Table 2 - Monthly average analytical results of the milk

Parameters	Average results	Parameters	Average results
Acidity	20° Thorner	Lactose	4.7%
Fat	4.15%	Ash	0.8%
Total protein	3.8%		

Analytical results showed an increasing value of total acidity of milk (20° Thorner) towards the normal value of 18° Thorner. This fact is strictly connected to the presence of subclinical mastitis in cows.

CONCLUSIONS - High prevalence of subclinical mastitis caused fro *Staphylococcus aureus*, *Streptococcus agalactie* and *Eschericia coli* favoured from a number of factors in the farm. For mastitis control we recommend the implementation of a rigorous plan. Mastitis can never be eliminated completely, being impossible to eliminate completely the infection sources., but can be controlled: Reducing the sources of infection; Controlling the infection transmitters; Monitoring the favorising factors of infections; Improving the defensive mechanisms of the breast.

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SOMATIC CELL STUDY IN FRESH MILK AND THEIR IMPACT ON THE FINAL PRODUCT

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KEY WORDS: somatic cells, milk products

ABSTRACT - During the period of August 2011-December 2011, 200 samples were collected from collection points which were intended for intensive processing from four of the milk processors in Kosovo, namely 50 samples for each dairy, divided into 10 samples a month, so both summer and winter seasons were covered. The samples were analyzed at the Milk Laboratory of the Food and Veterinary Agency (FVA). The work method is based on regulation no. 12.2011 “for the determination of specific rules of hygiene of food of animal origin” dated November 30th, 2011. Samples were selected so as to cover the entire month. Samples which showed an increase in the number of somatic cells were isolated and analyzed in detail. From samples analyzed we have seen that 24.5% or 49 samples have been categorized in class III (with cell number >500.000/ml, 35 samples were categorized as *out of range*, since the number of S.C. isolated was >700.000/ml). In the About 24% or 48 samples were categorized as II-nd class (number of S.C. isolated >400.000/ml and <500.000/ml). In 1st class 22.5% or 45 samples were categorized, (with S.C. count >300.000/ml and <400.000/ml). On the other hand 29% or 58 samples have been categorized as Extra Class, with cell number <300.000/ml.

INTRODUCTION- Somatic cells present in milk are indicators of milk quality¹. Normal milk contains a limited number of cellular elements: an average is calculated to be about 400.000/ml cell content according to EC directives, no. 92/46/EEC and 94/71/EC. This amount is calculated on the basis of the geometric average over a period of three months with at least one sample per month. Content of somatic cells in milk of healthy animals as a rule is lower than 200,000 cells/ml, but a higher number up to 400,000 cells/ml may be acceptable. The presence of somatic cells in milk can be calculated in the milk of individuals, as well as on the basis of the stable dairy herd¹.

MATERIALS AND METHODS - Study subjects were chosen to be four dairies in Kosovo, which have earned the trust of local consumers. Initially samples were taken for analysis of milk collected in intensive dairy processing plants “B” from FK, “A” from Mitrovica, “Ab” from Prizren and “K” from Gjilane. During the period from August 2011 to December 2011 a total of 200 samples were monitored, respectively, 50 samples from each dairy, separated by samples per month, to watch the seasonal aspect of the falls that are covered during the seasons of summer and winter. The study was based on the research and liaison between the number of somatic cells and the impact that the latter may have on other components of milk such as fat and protein.

RESULTS - From the analyzed samples it is observed that different results are obtained, respectively, samples are categorized in all classes. A summary of the analyzed samples is shown in the table:

Table 1. Summary of samples analyzed: classification by categories, according to dairies and classification based on percentage

Categories	“B”	“A”	“Ab”	“K”	Sample total	Percentage
Extra Class	13	17	13	15	58	29%
Class I	11	9	11	14	45	22.5%
Class II	14	9	15	10	48	24%
Class III	12	15	11	11	49	24.5%
Total	50	50	50	50	200	100%

As seen from Table 1 of the analyzed samples we have seen that about 24.5% or 49 samples are categorized into Class III, which means that the number of somatic cells in these samples was higher than 500.000/ml, out of those about 35 samples were categorized as outside the limit, with a number of somatic cells over 750,000/ml. In Class II are categorized about 24% or 48 samples, with the number of somatic cells of more than 400,000/ml and less than 500,000/ml. In Class I are also categorized about 22.5% or 45 samples, the number of somatic cells greater than 300,000/ml but less than 400,000/ml. While 29% or 58 samples are categorized into Class extra, with a number of cells less than 300,000/ml.

DISCUSSION - The impact of the increased number of somatic cells is versatile. Besides the impact on losses in quantity and quality of milk there are three aspects that should be given special importance to. First of all the impact of the high number of somatic cells in the final product; secondly the impact of somatic cells in processing or in the further processing of milk; and third the impact of the high number of somatic cells on public health².

Among the main negative effects are also, the lowering of the level of lactose and the lowering level of casein, which as we know is the main protein component of mil . Among other important impacts that causes the increased number of somatic cells in milk are: reduction of calcium content, while having the added value or content of plasma enzymes and lipases. These cause the degradation of milk fat by turn in the milk fat component to fatty acids which give the milk a taste and odor of decay³.

We have already said that the casein content is lower and this results in reduced amount of cheese produced, compared with the amount of cheese that would be produced by the same amount of milk with a standard count of somatic cells. Another impact of a high number of somatic cells in milk is the increased levels of lipase content which passes in the yeast and cultures used for fermentation, and so give undesirable odor and taste⁴. We have already seen that several enzymes produced by the destruction of bacteria by somatic cells survive the pasteurization processes. Also according to Osteras⁵, cheese produced from milk with increased number of somatic cells has lower stability of the structure and taste.

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THE PRESENCE OF β -LACTAMS IN FRESH MILK

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KEY WORDS: β -Lactams, SNAP Test, antibiotic resistance.

ABSTRACT - This paper emphasizes the study of the detection β -Lactams in fresh milk in collection points in Kosovo. During the period from November 2011 until February 2012 a total of 1050 samples were collected from four municipalities randomly selected. The milk samples from the four municipalities were collected once a week and were sent to the Food and Veterinary Agency Laboratory (FVAK), where they were analyzed at the drug residues laboratory for the presence of β -Lactams using the SNAP Test. The final results show that nine, which means 0.9% out of 1050 samples, gave positive results for the presence of β -Lactams. In the municipality of Podujevë 279 samples were collected and analyzed, of those one resulted positive translating into a percentage of 0.05%, as far as the municipality of Skënderaj goes, 238 samples were analyzed and three of the samples or 1.3% were positive. Rahovec municipality had four positive samples or 1.1% out of 356 tested, and Fushë Kosovë municipality had one or 0.5% sample from 187 collected, which showed presence of β -Lactams. The presence of β -Lactams has been shown to have a great impact in the buildup of antibiotic resistance in animals and humans alike.

INTRODUCTION - Antibiotics are strong medicines, which fight bacterial infections. When antibiotics are used properly they help save lives and maintain good health, but if these antibacterial agents are misused they can cause serious health problems. Antibiotic resistance is a serious problem that is growing with time. As antibiotic use increases for both animals and humans, bacteria resistant to the drugs used emerge. Moreover, there is evidence that resistance may develop for chemically similar bacteria¹. Mastitis is the most prevalent disease in cattle, which requires antimicrobial treatment^{2,3}. In the Republic of Kosovo, the use of antibiotics in the veterinary profession is not regulated by any laws or administrative instruction, the only measure taken is the obligatory number of samples that the KFVA needs to send to an accredited laboratory for antibiotic testing. The aim of this study was to collect fresh milk samples from collection points and have them screened for the presence of β -Lactams.

MATERIALS AND METHODS - One thousand and fifty samples were collected from four collection points in four different municipalities of the Republic of Kosovo. The municipalities which participated in the study were Podujevë, Skënderaj, Rahovec, and Fushë Kosovë. The method used for the detection of the β -Lactams was the SNAP[®] β -Lactam Test produced by IDEXX Laboratories. The SNAP[®] β -Lactam Test detects the most common antibiotics such as penicillin G, amoxicillin, ampicillin, ceftiofur, and cephapirin. The method used was the one described in the test protocol which is included when you purchase the kit. The sample is prepared and 450 μ l \pm 5 μ l and is added to the sample tube which contains a conjugate pellet at the bottom. The mixture of the conjugate

and milk is shaken gently from side to side 3-4 times, and then the mixture is placed on a heater block and incubated for 5 minutes. After the incubation period, the entire contents of the sample tube are poured into the sample well of the SNAP[®] device to run the test. As the edge of the activation circle on the device begins to disappear, you press down swiftly until you hear a distinct “snap” sound. It is important to note that if activation takes more than 60 seconds the results may be inaccurate; this may be caused by a thick sample or by using less than 450 µl of sample. Then the test is put on an incubation device for 4 minutes. The results are read 30 seconds after incubation either visually, or using the SNAPshot* DSR Reader. A negative sample will result in a darker than or equal spot to the control spot. A positive sample will give a lighter spot than the control spot.

RESULTS AND DISCUSSION - Nine samples or 0.9% of a total of 1050 samples tested resulted positive for the presence of antibiotic residues. Figure 8 shows the number of positive and negative samples for each municipality, which participated in the study. In the Republic of Kosovo, so far, the systematic control of raw milk is not performed therefore there is insufficient data concerning the issue. The figures by municipality are Podujevë one resulted positive translating into a percentage of 0.05%, as far as the municipality of Skënderaj goes, three of the samples or 1.3% were positive. Rahovec municipality had four positive samples or 1.1%, and Fushë Kosovë municipality had one or 0.5% positive sample, which showed presence of β -Lactams. In many EU countries, the percentage of positive raw milk samples for the presence of antibiotics is less than 0.5%⁴. In a study conducted in Montenegro by Nikolić et al., (2011)⁵, out of 6161 samples tested 483 or 7.84% resulted positive for the presence of antibiotics. Since no other study has been previously conducted in Kosovo on the presence of antibiotics in milk we cannot compare our results. This study however, is a good indication that a nationwide monitoring program needs to be put in place to control and reduce the level of antibiotics in milk. Furthermore, it is important that the KFVA which contracts private veterinary practices in each municipality in the Republic of Kosovo raises the awareness of those veterinarians on the use of antibiotics as well as educating farmers on the dangers of treating their animals with antibiotics themselves as well as the appropriate cessation times which are recommended after the use of antibiotics before the milk can be used for consumption.

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NONANTIBIOTIC THERAPY OF *STAPHYLOCOCCUS AUREUS* MASTITIS IN COW

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KEY WORDS: *Staphylococcus aureus* mastitis, dairy cows, nonantibiotic therapy

ABSTRACT - Mastitis caused by *Staphylococcus aureus* is difficult to control due to deficient penetration of antibiotics and *S. aureus*' ability to survive outside of PMNs and inactivate the antibiotics. The perspective lies in nonantibiotic therapy. Lysostaphine is a mixture of proteolytic enzymes with antistaphylococcal action from staphylytical staphylococci. The part of *Staphylococcus simulans* gene was cloned into *E. coli*, and with procedures of genetic engineering, into the bovine embryo. In this way, transgenic cow that secretes lysostaphine directly into milk was produced. *Staphylococcus aureus* produces adhesive matrix molecules (MSCRAMMs). Factor of adhesion, fibronectin, applied artificially, binds to MSCRAMMs and blocks *S. aureus* adhesion to mammary epithelium. Immunization of cows with antimatrix antigen results in inactivation of the bacteria. Ranalexin, a peptide isolated from the skin of american bullfrog, has an inhibitory and antitoxical effect, which makes him important in anticipation of ethiological predomination of MRSA mastitis. Staphyloxanthin, antioxidative pigment that protects *S. aureus* from the reactive oxygen of immune system. Blockers of staphyloxanthin production inhibit *S. aureus* resistance?

INTRODUCTION - It is impossible to control *S. aureus* by antibiotic treatment only, because of several reasons: the medicine can not penetrate to all infected tissues due to abscesses and strictures formation; the bacteria survives outside of PMNs destroying phagocytes and, *S. aureus* produces enzymes that inactivate penicillin. Therefore, antibiotic therapy has no future in combat with mastitis. The main problem lies in *S. aureus* being the imperishable source of infection (in cow, on cow and around the cow), as contagious and environmental mastitis pathogen, which is incurable after the break out of infection. Thus, it is necessary to think about new way of treatment without antibiotics.

MATERIALS AND METHODS - Lysostaphin: a mixture of antimicrobial enzymes that destroy bacterial wall. It originates from staphylytical staphylococci (*S. simulans*), and is an extremely potent antistaphylococcal agent that kills *S. aureus*. *S. aureus* produces adhesive matrix molecules (MSCRAMMs family), **fibronectin** being one of adhesive factors. Artificially applied fibronectin connects strongly to MSCRAMMs and, in this way, blocks the adhesion of *S. aureus* to the epithelium. **Ranalexin**, a peptide isolated from the skin of american bullfrog, has an inhibitory and antitoxical effect, which makes him important in anticipation of ethiological predomination of MRSA mastitis. **Staphyloxanthin**, a carotenoid pigment that causes antioxidative effect which enables the bacteria to avoid destroying effect of reactive oxygen from the host's immune system. It is responsible for the *S. aureus*' golden colour. Perhaps staphyloxanthin allows *S. aureus* to survive attacks of host's immune system?

RESULTS - With the methods of genetic engineering, the cow that secretes lysostaphin directly into milk was created. Lysostaphin destroys *S. aureus* within minute and, in this way, prevents injury and colonization of epithelium, last two being the triggers of inflammation. The part of *S. simulans* gene was cloned into *E. coli*, and with procedures of genetic engineering, into the bovine embryo. Immunization of cows with antimatrix antigen could render the bacteria harmless. Ranalexin has inhibitory effect on MRSA, in combination with antibiotics or lysostaphin; for example in the form of dressings impregnated with these compounds.

Blocators of staphyloxanthin production inhibit *S. aureus* resistance?

DISCUSSION - Above mentioned nonantibiotic methods could result in production of cows resistant to *S. aureus* and of milk free of *S. aureus*, which would prevent the transmission of this pathogen to other cows.

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COMPARED RESULTS OF PROGESTERONE PATTERNS AND FERTILITY IN OESTRUS INDUCED AZAWAK COWS IN NIGER

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KEY WORDS: zebu cattle, oestrus synchronization, Artificial Insemination

ABSTRACT - The aim of the study is to evaluate the efficiency of a protocol for oestrus synchronization using a progesterone intravaginal device in Azawak cows reared in two different ranches in Niger. All subjects were submitted to artificial insemination using frozen semen from selected Azawak bulls. The concentration of progesterone was determined to assess the performance to pregnancy diagnosis, further confirmed by rectal examination. Any statistically significant difference according to oestrus behaviour and fertility was observed between the two husbandry systems.

INTRODUCTION - In the Sahelian area, breeding plays an important key role in terms of socio-economics but this sector is still characterized by low yields. With the aim of enhancing the potential of indigenous cattle a research was developed for the *in situ* conservation of the *Bos indicus* Azawak breed through the diffusion of Artificial Insemination (AI)¹. The aim of this research is to evaluate the efficacy of a protocol for oestrus synchronization using a progesterone intravaginal device (PRID[®]) in relation to oestrus detection and pregnancy rate in two different ranches in Niger: the Experimental Sahelian Station in Toukounous¹, with a semi-extensive breeding system in a semiarid sahelian region, and the Dairy Cooperative in Kirkissoye, with an intensive breeding structure in the periurban area of Niamey, near to the Niger river.

MATERIALS AND METHODS - At the Toukounous Station 14 Azawak cows, aged between 6 and 17 years, were submitted to oestrus synchronization and AI and 18 Azawak cows, aged between 3 and 12 years, were recruited in a control group; at the Dairy Cooperative of Kirkissoye 14 Azawak cows were submitted to oestrus synchronization and AI and 12 Azawak cows belonged to the control group. The synchronization protocol was performed according the following schema: *G0*: insertion of the PRID[®] (1.55 g of progesterone); *G7*: 0.5 mg/subject of sodic cloprostenol (Estrumate[®], Shering Plough Animal Health); *G9*: device removal and administration of 400 IU of PMSG (Folligon[®], Intervet, Italy); *G11*: two fixed time AI at a distance of 12 hours, using frozen semen from Azawak bulls selected at the Experimental Station of Toukounous. Cows recruited in control groups exhibited natural oestrus behaviour and were submitted to twice AI at 12 and 24 hours after oestrus beginning using raw Azawak bull semen. In both the farms, the diagnosis of pregnancy was performed both by transrectal palpation and by progesterone (P₄) dosage using RIA method after blood collection at day 24 and 31 post AI¹. The effects of the protocols on oestrous response and fertility rates, according to the different breeding and feeding managements were studied. Chi-square and Fischer's exact test were performed to compare the obtained data using GraphPad InStat3. Data were considered statistically significant with p<0.05.

RESULTS - Animals in Toukounous had a mean Body Condition Score (BCS) of 3, while in Kirkissoye the mean BCS was 2. None of the animals lost PRID[®] device before Day 9 (Retention Rate – RR: 100%). At the Toukounous Station the oestrus response rate (ORR), that is the percentage of animals that showed oestrous behaviour, was 57.14%. Oestrus behaviour started meanly 50 hours after device removal and lasted 10 to 25 hours (mean 17h). The observed pregnancy rate (PR) after rectal palpation performed 3 months post AI was 28.57% and 75% of pregnant cows showed oestrous behaviour. In the control group the PR was 50%. According P₄ dosage, pregnancy was confirmed in 40% of animals that resulted pregnant after rectal palpation. The comparison with the control groups showed any statistically difference (P>0.05). At the Dairy Cooperative in Kirkissoye, the ORR was 42.85%; oestrus behaviour started mainly 33 hours after PRID[®] withdrawal and lasted meanly 17 hours (11-27 h). The PR after rectal palpation was 57,14% in the experimental group and 33.33% in the control group. The P₄ dosage confirmed the same pregnancy rate as rectal palpation diagnosis. The comparison between the experimental and control groups showed any statistical significant difference (P>0.05), even if PR in the control group was noticeably lower than in the experimental one. The statistical analysis between ORR and PR in the experimental studies in Toukounous and in Kirkissoye showed no differences (P>0.05). Likewise any statistical difference was found between the PR in the control groups.

DISCUSSION – BCS variations were slighter in Toukounous than in Kirkissoye. These variations, in a tropical environment, are related to factors that affect physical condition of animals, such as stress associated to unstable food availability or undiagnosed pathologies. Nutritional status affects fertility: the energy deficiency suppresses the secretion of hypothalamic GnRH and pituitary gonadotropin release by inhibiting the growth of ovarian follicles and interrupting the oestrus cycle². Oestrus synchronization contributes to overcome this problem and improve reproductive performance. The observed PRID[®] RR was higher than in literature³. At the Toukounous station the ORR was greater than in Kirkissoye, according to the correlation between oestrus behaviour and nutritional conditions²; for the same reason the PR in control groups was superior in Toukounous. On the contrary, the PR in the experimental groups was higher in Kirkissoye, though with any statistical validation. This remark could confirm the usefulness of synchronization protocols in difficult conditions. Anyway, observed PR are similar to literature³. Pregnant cows according to rectal palpation showed reliable P₄ concentrations in Kirkissoye but not in Toukounous. This situation can be explained with the presence of a corpus luteum not completely secreting or a mating after AI, possible in a less controlled breeding management. Occurrence of silent, short and nocturnal oestrus is a limit of this study while oestrus was observed only daytime. The use of ultrasound examination may be a support for monitoring ovarian activity and improve reproductive management of cows submitted to AI. According to the results in Toukounous, AI performed on animals in natural oestrus is most effective, as reported in literature⁴; instead in Kirkissoye, where breeding conditions are unfavourable, the protocols for oestrus synchronization were particularly useful.

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BOS INDICUS GENETIC MARKERS IN LOCAL BOS TAURUS CATTLE BREEDS FROM SOUTH AND EAST EUROPE

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KEYWORDS: *Bos taurus*, *Bos indicus*, growth hormone receptor polymorphism

ABSTRACT - Several evidences of the presence of zebuine markers in Podolian cattle breeds have been collected so far. Here we present the results of a polymorphism analysis at the GHR gene in southern and eastern European cattle breeds confirming the presence of zebuine alleles (Sau96I- and LINE-) in Podolian cattle also at the somatotropin receptor gene. These findings are most likely due to the introgressive hybridization that frequently occurred by the time Indian zebu cattle migrated towards Eastern Europe first as wild populations (25-30 thousand years ago) and then following Asian invaders (in late Roman times). The different GHR alleles at taurine and zebuine cattle likely predate domestication, their distribution primarily reflecting separate domestication events, although influence of natural and man-guided selection on shaping present-day distributions should not be disregarded, due to the strong adaptive significance of GHR-mediated processes. Therefore, GHR polymorphism should be taken into great account in evaluating functional variability in cattle breeds for conservation purposes.

INTRODUCTION - The GHR mediates biological actions of growth hormone on target cells by transducing the GH signal across the cell membrane and inducing transcription of many genes, thus playing a major role in metabolism regulation. Several polymorphic sequences have been identified in the bovine GHR gene and variants characteristic of zebuine cattle (*Bos indicus*) have been highlighted^{1,2,3} that may only reflect the separate domestication events of taurine and zebu cattle or also likely have had a differential adaptive significance. Italian Podolian, Ukrainian Grey and Hungarian Grey belong to the Podolian cattle group (*Bos primigenius*) which is named after the Podolian plateau in Ukraine, from where the ancestors of these taurine local breeds seem to have spread out over Europe. Several evidences of the presence of zebuine markers in Podolian cattle breeds have been collected so far at β -globin, α -lactalbumin and casein loci^{4,5} as well as at neutral AFLP and STR markers^{6,7}. Here we add a new evidence resulting by the polymorphism analysis at the GHR gene in southern and eastern European cattle breeds confirming the presence of zebuine markers in Podolian cattle also at the somatotropin receptor gene.

MATERIALS AND METHODS - A total of 254 animals from seven southern and eastern European breeds (Italian Podolian, Ukrainian Grey, Hungarian Grey, Polish Red, Polish White Back, Lithuanian White Backed and Lithuanian Light Grey) were genotyped for the presence/absence of the Sau96I restriction site at position -262 (allele codes: Sau96I+ and Sau96I-, respectively) and the LINE-1 element in the P1 promoter region of the GHR gene (allele codes: LINE+ and LINE-, respectively). LINE-1 PCR primer sequences were

derived by Aggrey et al.⁸ while Sau96I primer pairs were designed on the basis of the bovine GHR gene sequence (GenBank U15731). After Sau96I endonuclease restriction of the 318-bp amplified fragment, products were separated by electrophoresis in 2% agarose and visualized under UV light. The PCR-amplified DNA fragments of the GHR promoter were purified from agarose gels using spin columns and sequenced.

RESULTS AND DISCUSSION - A complete linkage disequilibrium was observed between the LINE-1 and the Sau96I sites (all animals with Sau96I+/+ genotype had LINE+/+ genotype, those with Sau96I-/- genotype had LINE-/- genotype, and those heterozygous at the Sau96I site were also heterozygous with respect to the 1206-bp insertion/deletion of LINE-1 element). Hale et al.¹ found that the Sau96I-/- and the LINE-/- genotype are specific for *Bos indicus*. Later on, the Nguni cattle – a *Bos taurus* x *Bos indicus* crossbreed - was found to be in heterozygous state at the Sau96I and LINE-1 loci⁹. In our study, a comparable and high frequency of the “-” alleles was observed in the Italian Podolica and the Ukrainian Grey breeds (0.30 and 0.25, respectively), followed by the third Podolian cattle breed, the Hungarian Grey (0.10), with homozygous “-/-“ variants found in Podolica and Ukrainian Grey cattle only. On the contrary, the “-” alleles were not detected in the Lithuanian native cattle (Lithuanian White Backed and Lithuanian Light Grey) that are thought to be of North European origin. The detection, at very low frequency, of the “-“ alleles in the two Polish native breeds may be the result of crossbreeding among Polish and Ukrainian cattle, as the Podolian plateau has been, during centuries, part of Poland. These results confirm the influence of zebuine cattle on Podolian breeds, including Italian Podolic, likely mediated by Middle-Eastern and Balkan breeds⁶.

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THE AMOUNT OF METABOLIC ENGERGY IN THE FOOD RATIO AND LAMINITI OF THE COW

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KEY WORDS: Metabolic energy, total energy, alcalic rezerve.

ABSTRACT - The impact of the structure of the food ratio in cows for the appearance of laminiti, has been studied in two groups of experiment cows with a stallor breeding regime (1,5,8). There were taken into consideration factors such as: indicators of the composition of the food ratio, reports of the slight fermented fibers (NDF) and the complex fermented fibers (CF) against the dry matter (DM) and reports of fermented energy (FME) against the total of energy in the ration (ME). The evaluation of the metabolic state was done with the selection for the group animals and individuals with laminit, based on the level of glucose and alcalic reserve in serum. In the group of cows that were fed with portions where the ratio of metabolic energy against the total energy was 87-89% and the content of cellulose less than 23 – 26 % against dry matter, the presence of laminit was detected in 32.1 % of the cows. In the cows in the group where the ratio of metabolic energy against the total energy was 73 – 77 % and cellulose in the food portion was 29-33 %, the laminit was detected in 19.2% of the cows. In the cows that suffered clinically from the laminit syndrome, glucose was avarage 1.85 ± 0.04 mMol/liter. The level of alcalic reserve in mg% NaOH consumed in the titration was within the average values (244.6 ± 8.84 mg%). Glicemia showed strong correlative relationship ($r = -0,686$) and statistically proven ($P > 0.999$) when appearance of laminit. The variations of the level of alcalic reserve did not show any impact in the presence of laminit in the cows.

INTRODUCTION - Laminit in cattles is an aseptic inflammation of the lamelar corium in the walls of the hooves, which is spread in the surface of the subsoil⁶. Laminit accompanied with hitching presents the most common and costly syndrome for cows in farms that breed animals with high volume of milk productivity and a high number of heads (3,4,5). The root causes of laminit are different factors connected even to the way of the animals being fed. Excess carbohydrades in foor portions, or slightly fermented, are commonly a cause for finding the acidose which in time is the cause of birth of lamint in cows (5,1,6). The studys aim is to clarify the linkof cause between the structure of the food ratio and lamint in cows.

MATERIALS AND METHODS - The study was done between June 2010 and June 2011, in a cow farm for the production of milk, where a year through type of food is applied. From the study it was put, the impact of energetic composition and the report against the level of complex fermented fibers against the total of dry patter that cause the appearance of lamint in cows. The structure of the food portion (ratio) was processed with tabelar values from McDonald P. e bp. (1995) and the amount of Dry Matter (DM), amount of slight fermented fibers (NDF), amount of complex fermented fibers (CF), total metabolic energy (ME) and metabolic energy (FME) was set.

For experimets, two groups of cows that were treated with different portions of food that contained different amount of complex fermented fibers and metabolic energy, were used.

In continuity, the levels of metabolic indicators for the level of glucose and alcalic reserve were monitored, to show the level of acidose not only that of rumen ⁽²⁾. Blood samples were analyzed every month during the whole period of the experiment. The level of glucose in blood was determined using the fast method, while the determining of alcalic reserve was done with the amount of NaOH spent for titration. For the groups of cows in the experiment, the presence of laminit was evidenced clinically. The results were processed statistically.

RESULTS - The food portion (ratio) used in the groups of cows, for the first group showed 76.76 MJ (the ratio between slight fermented energy (FME) against total energy (ME) 84.59%) and for the second group 48.96 MJ (68.60%). Contents of cellulose (CF) in ratio with the dry matter was 24.41% in the first group and 35.99% in the second group, while the content of slight fermented fibers (NDF) was 45.12% in the first group and 37.73% in the second group. From the metabolic indicators it was observed that the cows in the first group a lower level of glicemia was detected during the whole time of the experiment. In the level of alcalic reserve there werent much differences between the groups and also in comparesment with the average there were no big differences ^(1,2). The outcome data is statistically proven. In the end of the period of the experiment we concluded clinically the hitching syndrome in 17 heads or 20.98%. From the cows with the hitching syndrome, 9 heads (32.1%) were from the first group and 5 heads (19.2%) were from the second. The biggest number of heads with laminit was observed in the 4th and 5th month, after the begining of the experiment. The statistical processing of the data showed that there is a strong correlation of a negative caracter ($r = - 0.868$) between the level of glicemia in cows and the number of cows with laminit. Strong correlative connection of that of the negative caracter ($r = - 0.898$) was also between the contents of cellulose in food portion and number of cows with laminit, also between the level of metabolic energy in portion (ratio) and number of cows with laminit. The connection was also strong ($r = 0.753$) but of positive carater.

DISCUSSION - The appearance of laminit in cows is a slow process that is closely connected to the ingredients and structure of the food portion (ratio)^{1,5,7,8}. The low level of cellulose, under 25% and hight level of metabolic energy, over 75%, in the foor portion (ratio) are cause for the birth of the laminit syndrome in cows, since these create the conditions for the presence of rumen acidose. The appearance of rumen acidose affects the microbic review of rumens flora, bringing a more active development of the microflora (*S. bovis* and *Lactobacilus*) and the intensification of lactic fermentation¹. In conditions of consuming the food portion with high energetic level for a longer time, the occurrence of lamint in cows goes up to 32.1 %.

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PENILE LESIONS OF YOUNG BEEF BULLS IN SICILIAN SLAUGHTERHOUSES

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KEY WORDS: bull, penis, fibropapilloma

ABSTRACT - Lesions of the penis are underestimated in bulls but they may have important consequences on fertility. Physical defects of the penis, identified during breeding soundness examination, cause the exclusion of the bull as potential breeder. In this study, 825 young beef bulls were evaluated for penile lesions in Sicilian slaughterhouses. These were found in 31 cases (3.8%) including a congenital short penis (0.1%), persistence of the frenulum (0.8%), fibropapilloma (2.3%) and balanoposthitis (0.6%). Specimens were fixed and processed for histological examination. Gross and histological findings were described and compared with literature. Fibropapilloma was the most common penile lesion in young beef bulls. Although the tumour spontaneously regresses or may be surgically treated, the affected bulls can develop pain or penile protrusion failure and should be excluded as breeders to avoid further virus spreading.

INTRODUCTION - Lesions of the penis are underestimated in bulls, but they may have important consequences on fertility. Numerous abnormalities can induce failure of penile protrusion and consequently poor libido¹. Physical defects of the penis and prepuce are identified in yearling bulls during the breeding soundness examination causing the exclusion of the animal as potential breeder. Among congenital diseases, persistence of frenulum is the most common lesion, with a prevalence of 4.4% with differences among breeds². It can reflect a genetic defect, heritable to progeny³. Among acquired lesions, primary or secondary, consequent by trauma, penile infections have been reported but the typical responsible agents (bovine Herpesvirus, *Campylobacter foetus* ssp *venerealis*, *Arcanobacterium pyogenes*, *Corynebacterium renale*)⁴ have been declining with the diffusion of the artificial insemination and prophylactic programs. Fibropapilloma is in absolute the most common penile lesion; it has been observed in young bulls with a 2.8% prevalence, although influenced by age and breed². Fibropapilloma is a multinodular mesenchymal tumour caused by the bovine Papillomavirus type 1⁵, usually localized on the glans penis⁶. Homosexual behaviour in young bulls, which are reared together, is at the basis of the transmission of the disease⁷. Penile fibropapilloma has a benign behaviour and may spontaneously regress⁸. The aim of this study was to evaluate penile lesions in young beef bulls slaughtered in Sicilian slaughterhouses.

MATERIALS AND METHODS - Gross examination of penis of young beef bulls from Sicilian slaughterhouses was performed. Age and breed were obtained from the animal passport. Lesions were identified and tissue samples were fixed in 10% buffered formalin, dehydrated throughout the graded series of alcohol-xylene solutions, embedded in paraffin wax, sectioned at 5 micron and routinely stained with haematoxylin and eosin for microscopic evaluation.

RESULTS - In this study, penis of 821 young beef bulls, were examined. The age ranged from 5 to 36 months, pure and mixed beef breeds (Limousine, Charolais), were mainly represented. All animals had both scrotal testes. Lesions of the penis were seen in 31 cases (3.8%). Marked reduction in length and diameter of the penis (congenital short penis) with an undistinguishable glans penis was detected in 1 case (0.1%). The persistence of frenulum, as a connective tissue band with a width ranging from 0.5 to 2 cm, was found in 7 cases (0.8%). Balanoposthitis was observed in 5 cases (0.6%), probably secondary to trauma. The flogosis involved mainly the glans penis and was characterized by reddish areas, in a case with extensive ulcerations and necrosis. Microbiologic examinations were not available. Histologically, moderate submucosal and perivascular lymphoplasmacellular infiltrates were seen. Fibropapilloma was discovered in 19 (7-18 months) bulls (2.3%), as single (63%) or multiple masses located at the glans penis (10 cases), at the preputial fornix (5 cases) or at the penile body (4 cases). The size ranged from 0.3 to 10 cm in diameter, but in most cases (89%) was under 2 cm. The masses were sessile or pedunculated, round or elongated in shape, with a roughened to cauliflower greyish-pink surface. Ulceration, haemorrhage and secondary infection could be present. Histologically, marked proliferation of fibroblasts covered by a thickened hyperplastic stratified squamous epithelium with epidermal interdigitations was the typical appearance of the tumour. In some cases the mass consisted of fibrous tissue rich of fibrocytes. Inflammatory infiltrates (lymphocytes, plasma cells, macrophages) were often detected under the epithelium. In the epidermis, focal areas of keratosis, hyperkeratosis and acanthosis could be seen, with granular layer cells containing keratoinaline granules. Some intranuclear inclusions could be recognizable.

DISCUSSION - Only 8 congenital penile lesions on 821 cases (1%) of which 7 cases of persistent frenulum were found in this survey. The major prevalence (4.4%) reported by Bruner et al.² may be dependent by the different range of age (5-36 vs 11-15 months), but it possible that the genetic improvement and the diffusion of artificial insemination caused a significant decline of this hereditary pathology. Five (0.6%) cases of balanoposthitis probably secondary to trauma were observed; these lesions generally occur during the pubertal period of young bulls, where homosexual mount are generally experimented. This behaviour has been considered at the base of the venereal transmission of germs, including the Papillomavirus. Fibropapilloma is the most common lesion observed in this survey and in agreement with other studies on young bulls. The gross and microscopic features are in agreement with other reports. The presence of inflammatory infiltrates demonstrates the active immune response against the neoplasm which may regress spontaneously within a year⁸. Penile fibropapillomas are generally asymptomatic but some bulls can be reluctant to protrude the penis and to mounting, showing paraphimosis or phimosis. Although the tumour can be surgically treated, the breeder with a history of fibropapilloma should be excluded to avoid further spreading of the virus.

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HEMATOBIOCHEMICAL INDICATORS INFLUENCED BY SUBACUTE RUMINAL ACIDOSIS (SARA) IN DAIRY COWS

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KEY WORDS: Ruminal fluid, pH, hematobiochemical indicators

ABSTRACT - The study aims to assess the impact of subacute ruminal acidosis (SARA) in dairy cattle in some hematobiochemical indicators. The study was done in period March 2010, March 2011, in 60 cows in the first phase of lactation (35 ± 25 days lactation). Experimentally was followed the performance of ruminal pH content for the first three months after calving and hematobiochemical indicators. From hematobiochemical indicators in blood serum was studied the level of glucose, total protein, total calcium, inorganic phosphorus, magnesium, total bilirubin and creatinine. Significant differences and statistically proved ($p < 0.05$) was observed at the level of glucose, total calcium, total bilirubin and creatinin. The data obtained show that in the course of developing SARA, arise significant changes in some hematobiochemical indicators that can be used for guiding clinical examinations.

INTRODUCTION - SARA condition is displayed with high density on farms of dairy cattle especially in the early period of lactation. This pathology, caused considerable economic damage directly and indirectly as SARA condition, is often underestimated by the absence of clinical signs. Establishing an accurate diagnosis of SARA is complex and relies on food control and analysis of ruminal fluid pH. Appropriate techniques that ensure ruminal fluid collect is rumenocentesis because makes possible the recognition of rumen condition at the time of inspection. Ruminocentesis remains a moderate diagnostic technique that is accepted with difficulty by animal owners. For this purpose the present studies are directed at finding other methods for early and indirect diagnosis of SARA in dairy cows. The study directed at finding new methods to control for SARA condition indirectly, by determining the values of hematobiochemical indicators affected by SARA.

MATERIALS AND METHODS - The study was done in five cows farms with shed breeding mode. In each farm were randomly selected 12 cows in first phase of lactation (on days 35 ± 25), without clinical signs of presence disease and in good body condition. From cows were taken samples as ruminal fluid and blood from jugular vein. Ruminocentesis was chosen as the best possible techniques to obtain ruminal fluid^{2,3}. Ruminal fluid sampling was performed 4-6 hours after the distribution of food rations and pH was measured immediately with pH- meter. Blood samples were collected from the same cow from jugular vein with vacutainer which then centrifuge at 2500 turns per minute, for 20 minutes to win the serum. From hematobiochemical indicators were examined: glucose level, total protein, total calcium, inorganic phosphorus, magnesium, total bilirubin and creatinine with standart methods. According to results obtained in ruminal pH, farm animals in the study divided into groups: Group A: animals with average of ruminal pH 5.8 to 6.2 (group of healthy control animals), and Group B: animals with average of ruminal pH in interval between 5.6 to 5.8 (group of experimental animals with SARA condition). Hematobiochemical indicators were

processed statistically and differences were identified between the two groups of animals, additions and correlative relations. The data were statistically processed with analysis of variance to verify the effects in group and was determined the impact of SARA condition in hematobiochemical indicators. Statistical differences were assessed ($p < 0.05$) for different hematobiochemical indicators.

RESULTS - From the data it seems that there was no significant difference for total protein concentration, total calcium and glucose. Magnesium, creatinine and total bilirubin significantly reduced in animals with condition of subacute ruminal acidosis (SARA). Phosphorus concentrations increased statistically in both groups of cows in the study. Between the values of ruminal pH and total protein level in serum has a strong correlative relations in positive character ($r = 0,628$). Such positive character relations values of ruminal pH, exist also between total calcium and glucose. Significant correlative relations but negative character ($r = - 0,650$) is between the values of ruminal pH and inorganic phosphorus values.

DISCUSSION - Variations between groups in values of hematobiochemical indicators are related with various values of ruminal pH. Study results suggest that variations in ruminal pH can be useful for diagnosis of subacute ruminal acidosis (SARA) in dairy cows, including some changes in hematobiochemical indicators. The presence of SARA condition affects in the decrease of total protein and glucose. The total calcium is more interested and related with conditions or pathologies after calving than with SARA condition⁵. The concentrations of magnesium and inorganic phosphorus seems significantly influenced in cows that suffering from SARA, compared with total calcium level. Linear increase of the concentration of inorganic phosphorus as an indicator for SARA, is much more important than decrease of total calcium level and total magnesium level. In function of indirect indicators for the evaluation of rumen function in cattle, can be utilized values of total protein in serum and glucose values in blood, in the early lactation period. Values of total calcium level and serum inorganic phosphorus are less affected by changes in ruminal pH values.

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HEMATOLOGICAL INDICATORS INFLUENCED BY RUMINAL SUBACUTE ACIDOSIS (SARA) IN DAIRY COWS

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KEY WORDS: Ruminal fluid, hematological profile, leukocyte formula

ABSTRACT - For one year period, was studied the influence of the state of subacute ruminal acidosis (SARA) on hematological indicators of dairy cows with high yields for the period three months after calving. Considered indicators were ruminal pH, total white cell (WBC), red cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume, (MCV), corpuscular hemoglobin (MCH), ratio of red and white blood cells (RDV), total platelets (PLT) and indicators of leukocytes formula (neutrophils, lymphocytes, monocytes, eosinophils and basophils). The data obtained showed that the presence of SARA condition in cows affects the deviation from the norm of some hematological indicators. The biggest impact appears to index of the total white cells (WBC), in hematocrit (HCT), in number of leukocytes, lymphocytes and basophils ($p < 0.05$). Changes in hematological indicators, can be used to control rumen function in cattle.

INTRODUCTION - Subacute ruminal acidosis (below SARA), presents a major problem in dairy cattle farms. SARA condition is often underestimated because not evidence by specific clinical signs and signs that usually can be evaluated are the secondary ones which start to become apparent weeks or months later. Establishing an accurate diagnosis of SARA is complex and relies on fond control and pH of rumen content. Current studies are directed at finding indirect methods for diagnosis of SARA in dairy cattle. This study aims to precisely determine the changes in the hematological indicators during SARA, in order to use them as diagnostic test.

MATERIALS AND METHODS - In the period March 2010 - March 2011, the influence of SARA condition on hematological indicators on 60 high yielding dairy cows, was studied. The study was conducted on five farms and in each of them were selected at random 12 cows in the first period of lactation (35 ± 30 days), without clinical signs of disease and in good body condition. All cows were sampled to obtain ruminal content and blood from jugular vein. Ruminocentesis was chosen as the best techniques possible to obtain ruminal fluid^{1,2,5,6}. Samples of ruminal fluid were taken from 4 to 6 hours after consumption of food ration and pH was measured immediately with portable pH meter. Based on the results of the ruminal pH, animals in the study were divided into groups: Group A, normal with average ruminal pH 5.8 to 6.2 (group of healthy animals or group of control animals) and group B, animals with average ruminal pH in the interval between 5.6 and 5.8 (or group of experiments with animals at risk for SARA). Blood samples were collected from the same cow in tubes with heparin. At the moment three scopes, were prepared. From blood samples were determined: total white cells (WBC), red cells (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), corpuscular hemoglobin (MCH), ratio of red cells with white (RDW) and total platelets (PLT) with standard methods. Hematological profile was performed within two hours of sampling. From blood scopes was

determined (neutrophils, lymphocytes, monocytes, eosinophils and basophils). The data were statistically developed with a variance analysis to verify the effects on the group and to evaluate statistically significant differences between groups in the study.

RESULTS - From the data obtained it appeared that the amount of red blood cells (RBC), hemoglobin (HGB), the ratio of red cells with white blood cells (RDW), total platelets (PLT), mean corpuscular volume (MCV), corpuscular hemoglobin (MCH), monocytes and eosinophils were not significant differences between animals in the experimental groups compared with the values of the rate ratios and the ratios of the values in the control group. The total of white blood cells seem to increase more, especially in the second group (experimental group), (8.09 ± 1.56 versus 5.58 ± 1.57 in control group). Hematocrit values low in the group of the experiment cows (29.07 ± 2.71 versus 29.57 ± 2.8 in control group). The number of erythrocytes almost does not change. The study noted changes dictated by the level of ruminal pH content on leukocyte formula indicators. Changes in some indices of leukocytes formula seems to be influenced by the total white cells. The number of neutrophils increased (4.2 ± 0.8 versus 3.35 ± 0.88) in the blood of experiment cattle, and increases as the number of lymphocytes (2.98 ± 1.01 versus 2.66 ± 0.95). Less visible growth undergoes number of basophils (0.2 ± 0.01 versus 0.04 ± 0.03). The number of monocytes and eosinophils is not affected by pH of ruminal content.

DISCUSSION - Changes in values, of hematological indicators and leukocyte formula between individuals of groups, related to various values of ruminal pH^{4,5}. Study results suggest that variations of the contents of ruminal pH affect the values of hematological indicators and the latter may be useful for the diagnosis of SARA in dairy cows⁷. In the first period of lactation, during SARA condition in dairy cows, the biggest changes seem in increase of number of white blood cells, mainly on account of increased neutrophils and basophils. According Meglia³, the phenomenon of increasing white blood cells detected by increased levels of cortisol that is realized in many different events of the period around the calving.

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PREGNANCY-ASSOCIATED GLYCOPROTEINS (PAGs) AS A SERIC PARAMETER TO DIAGNOSE PREGNANCY AND EMBRYONIC MORTALITY IN BUFFALO COW. PRELIMINARY RESULTS

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KEYWORDS: buffalo cow, pregnancy-associated glycoproteins, pregnancy diagnosis

ABSTRACT - Pregnancy diagnosis is an important part in reproduction management of all ruminants and embryonic mortality has a substantial impact on the fertility of domestic animals. Domestic buffalo are generally regarded as having low reproductive efficiency, furthermore, there are a high rate of embryonic losses above all during the critical phases of embryonic attachment. So, to have a specific, reliable, not expensive method for pregnancy, is particularly important for this species. The concentrations of PAG were determined using a radioimmunoassay system (RIA-706) in fifty buffalo cow from 0 to 80 days of pregnancy and from parturition until day 80 postpartum. In the pregnant buffalo cow PAGs are detectable in the maternal circulation from Day 25 after breeding. In the case of embryo loss, PAG concentration increased between day 23 and day 28 and then declined when embryo occurred. Concentration decrease steadily in the postpartum period reaching undetectable levels only by day 40 postpartum.

INTRODUCTION - Reproductive efficiency is the primary factor affecting productivity. Accurate diagnosis of pregnancy or non-pregnancy and re-enlistment of non pregnant buffalo into an appropriate breeding protocol, are essential component of successful reproductive programs. Various methods aimed at improving detection of pregnancy in buffalo. Non return to oestrus and rectal palpation of reproductive organs have been the common methods adopted for pregnancy diagnosis in cow. Oestrus behaviour in buffalo has a lower intensity than in cows and is therefore much more difficult to detect by observation, thus non return to oestrus is misleading as buffaloes remain silent without being pregnant¹. The rectal palpation is an accurate method only from day 30 to 45 post mating². Transrectal ultrasonography diagnosis in buffalo can be adopted successfully from Day 28-30 after service³. Anyhow, it is necessary restrain the animals and have a proficient operator. Concentration of progesterone in blood at 20 and 24 days post breeding has been used as a tool for early pregnancy diagnosis⁴. However, the accuracy of predicting pregnancy on the basis of high blood progesterone levels at 21 days was only 66.7%⁵. So, in this specie is particularly useful a reliable and accurate method for early detection of pregnancy. Pregnancy-associated glycoprotein constitutes a large family of glycoproteins expressed in the outer epithelial cell layer of the placenta of eutherian species⁶. They are synthesized by mono and binucleate trophoblastic cells, some of them being secreted in maternal blood from the moment when the conceptus becomes more closely attached to the uterine wall and formation of placentomes begins⁷. Recently different chromatography allowed identification of new PAG from buffalo placenta⁸. The PAG concentrations in buffalo species were determined by using heterologous PAG RIA systems^{2,9}.

MATERIALS AND METHODS - The study was carried out at the CRA in Monterotondo (Italy). A total of 117 water buffalo cows were used for PAG determination in the maternal blood. Samples were collected by jugular venipuncture into EDTA-coated tubes from the day of artificial insemination to day eighty and from parturition until 80 day postpartum. Plasma was separated by centrifugation at 2,500xg for 10 minutes and stored at -20°C until analysis. The concentrations of PAG can be determined in buffalo cows (*Bubalus bubalis*) using a heterologous radioimmunoassay systems (RIA-706) according to a method of Perenyi et al.¹⁰. The minimum detection limit, calculated as the mean concentration minus twice the standard deviation of 20 duplicated of the zero standard are 0.1 ng/mL. Animals were confirmed to be pregnant by ultrasound at day 30 after artificial insemination.

RESULTS - In the pregnant buffalo cow (n=52) PAGs are detectable in the maternal circulation from Day 25 after breeding. In pregnant animals, plasma PAG concentration increased during the whole experimental period and was always greater than 0.8ng/mL. In non-pregnant animals (n= 49), PAG levels always remained near zero. In the case of embryo loss (=7), PAG concentration increased between day 23 and day 28 and then declined when embryo occurred. In this study, most embryonic losses occurred between days 25 and 28. Concentration decrease steadily in the postpartum period reaching undetectable levels only by day 40 postpartum.

DISCUSSION - In practice, the measurement of PAG concentrations in peripheral maternal circulation has been used for both pregnancy confirmation and the follow-up of the trophoblastic function. The first aspect can help veterinarians and breeders in the management of reproduction, while the second represents a powerful tool for investigators involved in studying factors affecting embryo and fetal mortality and embryo biotechnology.

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THE INFLUENCE OF STRUCTURE AND BALANCE OF FEED RATION ON THE REPRODUCTIVE INDEX-LIVE WEIGHT OF CALVES AT BIRTH OF BREED BROWN ALPINE.

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KEY WORDS: feed ration, dairy cows, calves, reproductive index

ABSTRACT - Reproductive activity occupies an important place and role in the cattle productivity because it relates to the increasing number of animals and with the growth of animal production. It is quite evident that the unsatisfactory economic indicators that are taken today by cattle farms, compared with animals genetic potential skills, and other unsatisfactory production indicators as low fertility, the high cost of their production, etc, are related to a large extent with the insufficient level of nutrition. In this context, was undertaken this study with the intention to survey the impact of feeding on the calves productivity performance. The analysis of productive indicators as weight of calves at birth, calves vitality, gain of body weight during the first 90 days of the calves, concludes that the cattle feeding systems for the Brown Alpin breed are still very extensive. The farms are suffering from calving and production seasonality, which stipulates the low level of utilization of animal genetic potential. The influence of specific indicators of nutrition on the weight of calves at birth by the simple regression demonstrated very weak relation between them.

INTRODUCTION - Increased economic efficiency in growing cattle farms is related with the effectiveness of the feed used in ration. It is evident that the unsatisfactory economic indicators that are taken today by growing cattle farms, compared with animals genetic potential skills, and other unsatisfactory zotechnical indicators as low fertility, the high cost of production, are related to a large extent with the insufficient level of nutrition. In this context, was undertaken this study, which had the intent to: Physiological assessment of nutrition and its impact on reproduction indicator of brown alpine breed and live-weight of calves at birth.

MATERIAL AND METHODS - The study was conducted in a private farm in the district of Fier. To study the influence of type of feeding on reproductive indicators of cow was followed: daily average consumption of feed ration, ration balancing rate, the influence of concentrate ration level on the weight of calves at birth, vitality, etc.. Impact of feed factors on birth weight of calves in the method is studied through a simple linear regression.

RESULTS AND DISCUSSION - The cattle farm was observed for one year during which there were 67 calving and their distribution was almost uniform but with a minimum for our population in June, 6.2% of cows, while the maximum was in October, in the amount 9.1%. Statistical analysis of our data does not show significant differences, which means that% of normal calving has a performance standard and comply with the breed standard. But we cannot say the same about the live-weight of calves at birth, which was 45% lower

than the breed standard. All this is explained by nutrition in the 8th and 9th month of pregnancy. The analysis of the herd performance indicators, for the period 2009-2010, shows us that we are dealing with a herd of production about two times higher than average milk production in the country.

Table 1 - Production indicators in the herd of BA cows.

Heads	Mean (X) ±Standard Deviation (SD)	Annual milk production Kg	Age at first calving Mo	Cows body weight kg	Mothers milk production Kg
122	X ±SD	4652 ±294	28.8 ±2	502 ±37	5117 ±1174

Average milk production and cows' live weight approaches the breed standards and as such can be called herd with average milk production. Within the herd there's a wide variation of parameters that match common distributions of such herds in systems based on feed ration with limited quantity of concentrates. The feed ration structure of the studied herd was 60% fresh forage, 5% hay and 35% concentrate. This is an inappropriate structure of the feed ration, with very low percentage of hay/roughage feed and high percentage of concentrate and we can say that the ration has a low effectiveness and is unacceptable and uneconomic for Brown Alpine cows in the dry period. The simple linear regression shows very weak relation between feed ration, energy, protein used for the cows and live weight of calves at birth.

From the study of the influence of cows' feed ration on the process of birth and vitality of calves was highlighted very high morbidity percentage (68%), from the 2 to 45th day after calving, which is caused from nutritional imbalances during the dry period of the cows. In the treatment of calves has been observed that those who do not consume more than 1.9 kg of colostrum for the first time can not gain more than 540 gr body weight per day. Calves consuming 2.5-2.9 liters of colostrum gained body weight 750 g per day and those taking over 3 liters of colostrum gained not more than 670 g body weight (all data are in the growth period and not in the fattening phase).

CONCLUSIONS - Systems of feeding cattle and respectively Brown Alpine breed are still very extensive ones, with a low efficiency of their use as a result of wrong feed structures, where are used a lot of concentrate is used. The farms are suffering from calving and production seasonality, which stipulates the low level of utilization of animal genetic potential. Using the same feed ration for milking and drying cows have brought negative effects on the calving process and in the disease incidence of calves from 2-45 days after calving. The influence of specific indicators of nutrition on the weight of calves at birth by the simple regression demonstrated very weak relation between them.

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EFFICACY OF KETOPROFENE (Fenleve®*) FOR THE REDUCTION OF SOMATIC CELLS IN THE MILK OF HIGH PERFORMANCE DAIRY COWS IN ITALY PRELIMINARY STUDY

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KEY WORDS: cow, mastitis, somatic cells, ketoprofene

ABSTRACT - A high count of somatic cells (SCC) in the milk is a great problem for dairy farm breeders over all the world. The aims of this study have been to evaluate the capacity of ketoprofene to select the cows with high SCC due to microbial mastitis from those due to traumatic inflammatory conditions, the efficacy of the drug in decreasing the number of the SCC used alone or in association with the antibiotic of election. In all cases the results have been very satisfactory.

INTRODUCTION - In dairy farm with high milk production the SCC is one of the most heavy problems that farmers of all the world have to face¹. This condition is often due to a sub-clinical infectious mastitis², but in other cases is induced by lameness, skeletal inflammatory condition or myositis³. The aims of this study have been the following: evaluate the efficacy of ketoprofene⁴ in selecting the cows with SCC induced by trauma from those with infectious mastitis, verify the efficiency of ketoprofene in decreasing the number of SCC, control the possibility of using an association of ketoprofene associated with the elective antibiotic in reducing the SCC and recovery the cows from mastitis.

MATERIALS AND METHODS - In this study the Authors considered a sample of 67 dairy cows belonging to a separate group of 130 cows all with history of high SCC, 50 were the treatment group, 17 the control group. The cows belonged to a farm of 3,000 cows and of course the high SCC group was the last to milk. Tab. I shows that 50 cows with SCC of 520,000 to 20.141 million (mean 3.354 million) were treated with ketoprofene (*Fenleve*®)* 2,000 mg (20 ml) i.m. day for three days. The control received 20 ml of Ringer's solution i.m. for three days. After 10 days the SCC were controlled and the cows that kept high SCC or increased were treated with ketoprofene at same dose plus 15mg/Kg ampicillin and 7.5 mg/Kg dicloxacillini i.m. (*Cloxalene plus*°) for three days. If the germ isolated was not sensible to the chosen antibiotic, the treatment was repeated with the elective one. The cows that did non have any reaction to the second treatment and the control that at the second control were still at very high level of SCC were culled. The results were statistically evaluated.

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RESULTS - Table I shows that 34 cows (68%) gave a positive response and the range dropped to 60,000 to 432,000 with an average 247,500. The 16 cows that did not have a positive reaction at the bacteriological examination showed several bacteria like *Staphylococcus spp* excluding *S. aureus*, *Serratia*, *Enterococcus faecium/durans* all

sensitive to ampicillin. Six of these cows reacted positively to the treatment with ketoprofene plus ampicillin plus dicloxacillin. The 10 remaining cows of this group kept high SCC and were culled. The SCC of the control did not change in a significant way so since the udder was not in good condition and the cows were not high producing anymore we decided to cull all of them so the general mean of SCC in the group decrease considerably and the milk could be sold for his proper use. The comparison between the means resulted to be highly significant in both cases (Tab. I).

TAB. I - EFFICACY OF KETOPROFENE (FENLEVE) ON MILK SOMATIC CELL COUNT (SCC) IN HIGH PRODUCING DAIRY COWS.

COWS	SCC x 1000 BEFORE TREATMENT			SCC x 1000 AFTER TREATMENT WITH POSITIVE REACTION			SCC x 1000 BEFORE TREATMENT WITH KETOPROFENE + ANTIBIOTIC			SCC x 1000 AFTER TREATMENT WITH KETOPROFENE + ANTIBIOTIC		
	COWS SAMPLE N°	RANGE	MEAN(μ)	N°	RANGE	MEAN(μ) S.D.	N°	RANGE	MEAN(μ) S.D.	N°	RANGE	MEAN(μ) S.D.
TREATED	50	520-20141	3354 [^]	34	60-432	247.5 ± 84 [^]	16	664-7967	880 ± 75*	6	49-399	226.33 ± 64*
CONTROL	SCC x 1000 BEFORE TREATMENT			NO TREATMENT								
	COWS SAMPLE N°	RANGE	MEAN (μ)	N°	RANGE	MEAN (μ) S.D.						
	17	538-18432	3222	17	437-16832	2889						

[^] The comparison between the to means is highly significant P<0,001

* The comparison between the to means is highly significant P<0,01

DISCUSSION - Ketoprofene has shown to be a very valuable tool to select cows with high SCC in the milk due to trauma or non infectious causes from those due to bacterial sub-clinical mastitis. It has good efficacy in decreasing the number of somatic cell and has the great advantage that does not pass into the milk, so the production of treated cows can be use with a great advantage for the farmer and for the consumers. In association with and elective antibiotic it can be use as an adjuvant for the therapy of bacterial chronic mastitis, but is efficacy is not very high. Anyway after the results obtained in his preliminary study the Authors are very favorable to the use of the protocol described in the present paper.

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COMPANION ANIMALS

CORTISOL HAIR AND NAILS CONCENTRATIONS IN NEWBORN PUPPIES AND KITTENS: PRELIMINARY RESULTS

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KEY WORDS: canine and feline newborns, cortisol, hair and nails

ABSTRACT - Since cortisol (C) plays an important role in foetal multi-organs final maturation, the study evaluated the reliability for hair and nails C measurement in newborn puppies and kittens as a retrospective picture of foetal C accumulation during the last stage of gestation. The results obtained from 32 born dead normal puppies and 8 born dead normal kittens showed that C is higher in the hair of kittens compared to puppies, while no differences were found between nails C levels in dogs compared to cats, between hair and nails C concentrations within each species, between sex of the newborns and, in the dog between the fair and dark color of the coat. This experiment provides a new interesting tool for C non invasive measurement in newborn puppies and kittens, useful for the study of both last intrauterine development foetal stage and for newborn adaptational monitoring.

INTRODUCTION - During the last days of pregnancy, cortisol (C) plays an important role in foetal multi-organs final maturation and for triggering of parturition¹. C levels, usually assessed in blood, saliva, urine and faeces, could be alternatively analysed in the hair of several species, providing a new, non invasive technique for a retrospective picture of previous long-term C accumulation². At authors knowledge, hair C concentration has been previously studied in dogs and cats, but not in newborn puppies and kittens. In canine and feline foetus body hair begins to grow at 45 days of gestation, so that hair shaved at the time of birth reflects C accumulated during the last stage of pregnancy³. Cortisol has also been measured in humans nails, but the authors are not aware of similar studies in puppies and kittens, in which nails are formed by 40 days of gestation. For all these reasons the aim of the present study was to evaluate the reliability for hair and nails C assay in newborn puppies and kittens and to evaluate at what extent factors related to pregnancy or to the newborn could have influenced C incorporation in hair and nails.

MATERIALS AND METHODS - The study was performed on 35 born dead puppies belonging to several breeds and on 8 born dead kittens. A complete clinical record was collected for each subject, focusing on mother gestation and parturition as well as litter characteristics (litter size, body weight, uniformity, sex ratio, etc.). At the time of sampling, a total evaluation of each newborn has been performed, with special attention to the body weight and development according to species, breed and presence of gross malformations. Hair strands were washed in 5 ml isopropanol, as suggested by Davenport et al.⁴, to minimize the risk of extracting cortisol from outside the hair and at the same time ensure the removal of any steroids on the surface of the hair. Hair was shaved at the level of the skin, from the back and the dorsal portion of the neck when a single color coat was detected, or from each colour area when coat was composed by multiple color areas. Hair samples have been collected in dry tubes and stored at room temperature until analysis. Furthermore also the nails have been sampled from each newborn and stored together with the hair of the same subject. Hair and nails C

concentrations were analysed by RIA⁵. A t-test corrected Welch was used to evaluate differences within each species between hair and nails and males and females, and between dogs and cats in hair and nails.

RESULTS - Cortisol concentration was detected in both hair and nails samples belonging to 35 born dead puppies and 8 born dead kittens. Gestation course of bitches and queens, maturity, birth weight and pathologies macroscopically visible of newborns were considered as potentially interfering factors on C hair and nails levels. However, all pregnancies and parturitions were normal, all newborns, except one, were mature and with normal body weight at birth. Only one gross malformation (foetal anasarca) was found, and one foetus was aborted. The data concerning cortisol hair and nail concentrations in the 32 normal born dead puppies and in the 8 normal born dead kittens are reported in Table 1.

Table 1 - Cortisol concentrations (pg/mg) (mean \pm SD) in born dead puppies and kittens hair and nails samples.

Sample	Dog (n=32)	Cat (n=8)	Dog		Cat	
			Male (n=17)	Female (n=15)	Male (n=4)	Female (n=4)
Hair	64 \pm 27.16*	87 \pm 13.95*	71 \pm 27.90	57 \pm 25.17	92 \pm 10.68	82 \pm 16.26
Nails	66 \pm 55.41	76 \pm 62.20	59 \pm 58.05	73 \pm 53.19	49 \pm 28.37	103 \pm 79

*p<0.01

When the coat color was considered, puppies hair samples were divided in fair and dark, but statistics did not show any significant difference (68 \pm 27.04 vs 61 \pm 27.56 pg/mg, respectively). C hair and nails concentrations were very high in two puppies: the one affected by foetal anasarca (hair C=175.6 pg/mg, nails C=195.6 pg/mg) and another puppy born prematurely, according to the ultrasonographic prediction date of parturition (hair C=325.1 pg/mg, nails C=404.6 pg/mg). In one dog foetus, aborted at about 40-45 days of pregnancy, C was measured only in nails, because hair had not begin to grow (nails C=50.2 pg/mg) and resulted within the range observed in born dead puppies.

DISCUSSION - The preliminary results of the present study showed that C concentrations are detectable in hair and nails of newborn puppies and kittens. The statistical analysis evidenced significant higher hair C levels in kittens compared to puppies. No significant differences were found between hair and nails C evaluations within each species and also between canine and feline nails C concentrations. As concerns at the comparisons between sexes neither hair nor nails C levels differed in the two sexes in each species. On the basis of coat color, statistics failed to show significant differences between C concentration in fair and dark hair, in contrast to the results obtained in a previous study (2). This experiment provides a new interesting tool for C non invasive measurement in newborn puppies and kittens, useful for the study of both last intrauterine development foetal stage and for newborn adaptational monitoring.

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IGF-I, NEFA AND CORTISOL CONCENTRATIONS IN FETAL FLUIDS OF DOGS AND CATS: PRELIMINARY DATA

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KEY WORDS: dogs and cats, fetal fluids, hormones and metabolic factors

ABSTRACT - Knowledge about fetal fluids composition could be useful for fetal well being assessment also in small animals, but few studies focused on this topic. In the present study IGF-I, NEFA and Cortisol (C) levels were evaluated in amniotic and allantoic fluids collected from dogs and cats at mid pregnancy (MP) and from dogs at term pregnancy (TP). A total of 11 canine amniotic and 8 allantoic MP samples, 24 canine amniotic and 26 allantoic TP samples, and 18 feline amniotic and 4 allantoic MP samples were collected and analyzed for IGF-I and C by RIA and for NEFA by enzymatic-colorimetric methods. The results showed significant differences between the two fluids within the canine specie only in C levels at TP, with higher levels in the amniotic fluid. When the different stages of pregnancy were considered in the dog, IGF-I levels declined, while C levels increased significantly at TP in the amniotic fluid. The comparison between the two species evidenced that, at MP, IGF-I levels were lower in the cat in both amniotic and allantoic fluids. NEFA seems to show no significant differences between the two fluids, between the two stages of pregnancy and also between dogs and cats.

INTRODUCTION - Fetal and neonatal well being are assessed in humans by ultrasonography, but also by fetal fluids evaluation. This implies a full knowledge of both normal and abnormal fetal fluids composition, which is still not completely studied in dogs and cats. To date the ultrasonographic assessment of fetal viability is the major tool for fetal assessment in small animals clinics. The full knowledge of fluids composition, in relation to fetal maturation and well being, could be helpful for pregnancy management, especially when planned Caesarean sections are required. Among several parameters, IGF-I and NEFA are important factors involved in the fetal and perinatal growth and maturational processes. Cortisol (C) is known to play an active role in the process of birth and parturition, but little is known about its possible role in the central stage of intrauterine fetal development in dogs and cats. For these reasons, the aim of the present study was to evaluate the presence of IGF-I, NEFA and C in fetal fluids of dogs and cats at different stages of pregnancy.

MATERIALS AND METHODS - The study was performed on 7 bitches submitted to Caesarean section at term of pregnancy (TP), 3 bitches and 6 queens submitted to ovariohysterectomy at mid pregnancy (25-35 days) (MP) for mismating and control of stray-pet population. During surgery, the amniotic and allantoic fluids were aseptically collected by puncture of each fetal sac, placed in a glass sterile tube and, after a gross description of each fluid (volume, color, turbidity), immediately centrifuged at 1000 x g for 10 minutes. The supernatant was immediately stored at -20° C until analysis. At TP a full evaluation was also performed for each newborn, while at MP just the presence and size of the fetus was evaluated. The neonates were also evaluated for viability by APGAR score¹, for maturity by hair distribution and completeness, for sex, for absence of gross physical defects and weighted before nursing. IGF-I levels were evaluated

using a modified RIA technique², while enzymatic-colorimetric methods were used to determine plasma concentrations of NEFA. C concentration was evaluated by RIA. The t-test corrected Welch was used for the statistical analysis for: a) differences in IGF-I, NEFA and C concentrations at each stage of pregnancy between each fetal fluid within each species, b) differences in IGF-I, NEFA and C concentrations in each fetal fluid between different stages of pregnancy for each species, c) differences in IGF-I, NEFA and C concentrations in each fetal fluid and at different stages of pregnancy between dogs and cats.

RESULTS - At TP a total of 24 canine amniotic and 26 allantoic samples, while at MP 11 canine amniotic and 8 allantoic samples were collected from bitches belonging to several breeds with litters ranging between 1 and 7. MP feline samples were collected from 6 cats for a total of 18 amniotic samples and 4 allantoic samples. As easily expected, at MP the major fluid was the amniotic, while at TP the allantoic was also abundant. However, in the course of Caesarean section, the main attention was paid to the newborns, so that the collection of especially allantoic fluid was often impossible or difficult, at least for the complete volume collection. All the neonates (15 males and 11 females) were alive and viable, healthy, mature, without physical defects and their body weight was in the range for the specific breed. As respect for MP, all the vesicles contained the fetus with no evidence of size discrepancies within litters. At a gross description, MP provided a volume of about 1 ml amniotic fluid, always clear, while at TP, amniotic fluids volume ranged between 0.5-6 ml and was frequently characterized by little whitish flocculations. Allantoic fluid volume ranged between 0.5 and 10 ml and was frequently citrine. As concerns at the IGF-I, NEFA and C concentrations, these hormones were detected in both canine and feline samples, in both types of fluid, in both stages of pregnancy in the dog. Mean (\pm SD) IGF-I, NEFA and C in amniotic and allantoic fluids in the collected samples are reported in table 1.

Table 1- Mean \pm SD IGF-I, NEFA and C in amniotic and allantoic fluids in the collected samples

	IGF-I (ng/ml)		NEFA(μ Eq/L)		CORTISOL (ng/ml)	
	amniotic	allantoic	amniotic	Allantoic	amniotic	allantoic
Canine MP	5.6 \pm 1.83 \clubsuit \S	4.3 \pm 2.3 \diamond	262.7 \pm 82.51	244.4 \pm 22.89	6 \pm 3 \clubsuit	6.6 \pm 1.52
Canine TP	4.2 \pm 1.5 \S	5.6 \pm 3.2	342 \pm 98.63	275.4 \pm 25.9	10 \pm 3 \clubsuit	5.9 \pm 1.4*
Feline MP	2.6 \pm 1.79 \clubsuit	1.8 \pm 0.63 \diamond	268 \pm 102.87	258.7 \pm 56.1	4.4 \pm 1.62	8.8 \pm 2

* \S \clubsuit \diamond \blacklozenge $p < 0.05$; \spadesuit $p < 0.001$

DISCUSSION - The preliminary results of the present study showed that IGF-I, NEFA and C are detectable in fetal fluids of dogs and cats at MP and of dogs at TP. The statistical analysis showed significant differences between the two fluids within the canine specie only in C levels at TP, with higher levels in the amniotic fluid. The lack of cats samples collected at TP did not allow to verify whether or not the same finding could have been possible also for the cat. When the different stages of pregnancy were considered in the dog, IGF-I levels declined, while C levels increased significantly at TP in the amniotic fluid. The comparison between the two species evidenced that, at MP, IGF-I levels were lower in the cat in both amniotic and allantoic fluids. NEFA seems to show no significant differences between the two fluids, between the two stages of pregnancy and also between dogs and cats. The results suggest that C increases in the amniotic fluid toward end of gestation and this finding is in agreement with what reported in humans, in which the increased level of C is responsible for fetal lung maturation. IGF-I increase toward the end of pregnancy confirm their role in fetal and neonatal growth³, while could be interesting to better investigate the possible differences in IGF-I levels between dogs and cats at MP.

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THE ESTRUS DETECTION THROUGH VAGINAL CYTOLOGY IN BITCHES

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KEY WORDS: bitches, vaginal cytology, estrous, seasonality

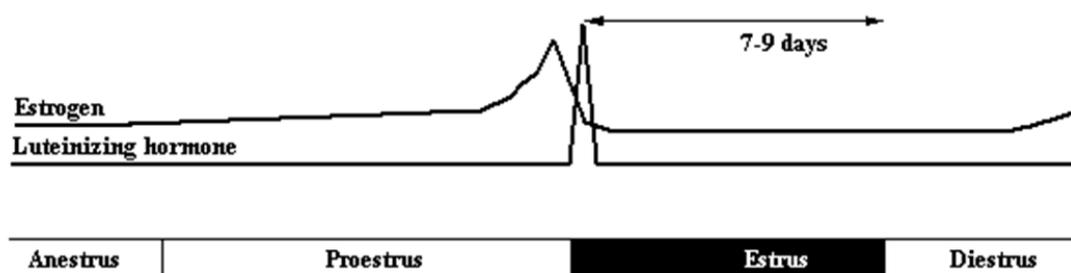
ABSTRACT - The bitches are considered monoestrous pets. In our country, the majority of them show estrus in late winter-early spring (February-March) and autumn (October-November). Usually, the appearance of estrus is not attended from the owners. For this purpose, during the months January-March, we assessed the different stage of estrus cycle through vaginal cytology. The samples were collected from 24 bitches of different breeds in one Private Veterinary Clinic for Small Animals, in Tirana. The evaluation of the samples was conducted in the Diagnostic Laboratory of the Faculty of Veterinary Medicine, Tirana. Vaginal cytology in bitches is a good test, which reflects the morph-functional changes in the genital tract epithet, under the influence of the hormonal profile changes. As it results from the study, about 86.4% of small breeds (two or three breeds) and medium breeds, were in the last period of anestrus (4 heads), in the starting of proestrus (6 heads) and in the estrus period (7 heads). Just one head or 13.6% was in the stage of deep anestrus. While large breeds (7 heads), during this period, were found in diestrus (6 of them) and only one head was in the estral period. This study was intended to the changes of vaginal cytology for evaluation of the physiological status of the bitches in favor of their successful reproduction.

INTRODUCTION - Vaginal cytology is a simple technique that can be used by practitioners to help characterize stages of the reproductive cycle of the bitch or to evaluate certain diseases of the genital tract. Vaginal cytology usually is used in conjunction with the physical examination, clinical history, vaginoscopy, and hormonal assays to determine the stage of the reproductive cycle^{1,3,4,6,7}. This is especially important if artificial insemination is to be performed^{2,3,6}. Other uses of vaginal cytology and ancillary testing include determination of the whelping date, diagnosis of inflammation of the vagina, and identification of some types of neoplasia involving the vaginal vault and urethral orifice. Cytology changes through the canine estrous cycle reflect changes in blood concentrations of estrogen. As depicted below, and described in more detail in the section on, estrogen levels rise prior to and during proestrus and fall in conjunction with the preovulatory surge of luteinizing hormone^{2,5,7}. Rising levels of estrogen induce the “cornification” that is characteristic of smears examined during estrus^{1,3,6}. Ovulation occurs two days after the LH surge.

MATERIAL AND METHODS - In this study were included 24 bitches which were presented randomly in a private veterinary clinic for small animals in Tirana, during the months of January-March, 2012. All the bitches came to clinic by the owner and were in a good health. Seventeen heads of them were small and medium breeds and the others (7 heads) were large breeds. The bitches were of different ages (2-8 years) and with normal

reproductive status. Materials used for sampling are as follows: cotton-tipped swabs (six inch variety); microscope slides: methanol or commercial spray fixative for cytology (can also use ethanol); staining solution: Giemsa. The evaluation of the vaginal slides was performed in the Diagnostic Laboratory of the Faculty of Veterinary Medicine, in Tirana.

RESULTS - The data obtained by microscopic evaluation of each sample is based on the type of cells that dominate on the slides. In the different stages of the cycle, under the effect of different hormonal level, appear different epithelial cells in the vagina. During the study, we detected that the bitches of small and medium breeds (5-25 kg) were found in the last phase of anestrus; 4 heads were predominated by intermediate cells in the microscopic field. The P₄ level was low and ovarian activity was virtually absent. Secretions were minimal and very tenacious or absent. The cervix was tightly constricted and the vaginal mucosa was pale. At this stage, 6 bitches that were evaluate in proestrus, were dominated by the large intermediate and superficial cells and a few blood cells. These changes in cell profiling are the result of hormonal profile that appears at this stage. Estradiol concentration increases as ovarian follicles mature and the uterus enlarges. The vaginal epithelium proliferates accompanied by diapedesis of erythrocytes from uterine capillaries. 7 bitches were found in the estrous phase with dominance (90%) of the superficial cells on the microscopic field. During estrus, glandular secretions increase, the vaginal epithelium becomes hyperemic, and ovulation occurs. This stage of the reproductive cycle is influenced mainly by estrogens. A bitch of the group was seen in the stage of deep anestrus and it was dominated by parabasal cells. In terms of the bitches of large breeds (25-40 kg), the vaginal slides reveal that 6 of them were in the anestrus stage and only one was in estrous.



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CYTOLOGICAL EVALUATION OF SMALL MAMMARY NODULES IN THE BITCH

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KEY WORDS: bitch, mammary tumour, cytology

ABSTRACT - Mammary tumours are extremely common in the bitch and the diagnosis is generally clinical and confirmed by histopathology of the removed tissues. Small mammary nodules are considered less aggressive or benign, and, in the practice, they are not always removed. This study was aimed to cytologically evaluate 48 small (under 2.5 cm) mammary nodules in 43 bitches. All the cases were also T1N0M0, according to the TNM grading scale. Each nodule was aspirated 1 up to 3 times and the slide was stained with Diff-Quick. Cytological evaluation registered: cellularity, cellular types, inflammatory cells, presence of clusters or aggregates, cellular and nuclear pleomorphism, nucleolar abnormalities, mitoses. Sixty-five per cent of nodules were malignant, the remnant cases were diagnosed as benign, hyperplastic or inflammatory lesions. This result demonstrates that it does not exist a cut off for mammary nodules in the bitch, to distinguish between benign and malignant lesions. Small nodules have always to be aspirated and if the malignancy is confirmed they should be removed. Only benign small nodules may be monitored, taking in account that there is evidence that benign mammary lesions are precancerous in the bitch.

INTRODUCTION - Mammary tumours are extremely common in adult bitches. The diagnosis of mammary tumours in the bitch is based on signalment, reproductive history and clinical signs; however, a definitive diagnosis requires the histological examination of the surgically removed tissues. Cytological evaluation of aspirates has been evaluated as a sensitive diagnostic tool^{1,2} to have a preoperative diagnosis. In these papers, cytology was realized when the clinical diagnosis had been assessed and the surgical treatment had been planned. Mammary masses over 5 cm have always to be considered malignant and removed³. In these cases or when malignant features of the mass are easily recognizable (ulceration, infiltration, lymph node involvement, metastasis) cytology has a limited significance, while, in smaller nodules, the technique may differentiate benign and malignant growths. In TNM classification, regarding the size, the first stadium (T1) of mammary tumour is under 3 cm and the second stadium (T2) is between 3 and 5 cm⁴. In a recent study it has been reported that mammary nodules less than 2.1 cm are generally benign and over 4.7 cm are generally malignant⁵. Small mammary nodules, in the practice, are not always removed. Considering the above reported data, this study was aimed to cytologically evaluate mammary nodules under 2.5 cm, in order to identify the benign/malignant lesion ratio.

MATERIALS AND METHODS - During routine clinical examinations, performed in surgeries of Sicily, mammary nodules under 2.5 cm were selected for this study. The nodules had to be also without lymph node involvement and evidence of far metastasis (T1N0M0 according to TNM grading scale). Each nodule was aspirated 1 up to 3 times (depending by the size) using a 22-gauge needle attached to a 5-ml syringe. Full suction was applied to the syringe, and the needle was moved back and forth as well as in different directions. The aspirated material was deposited onto glass slides. These were

air-dried and stained with Diff-Quick. Cytological evaluation registered: cellularity, cellular types, inflammatory cells, presence of clusters or aggregates, cellular and nuclear pleomorphism, nucleolar abnormalities, mitoses. Statistical analysis was done using the Fisher's exact test. Values less than 0.05 were considered statistically significant.

RESULTS - Forty-eight mammary nodules belonging to 43 bitches were evaluated. Mixed breed (13 cases), German shepherds (4 cases) and miniature breeds (19 cases) were the most prevalent breed classes of the sampling. The mean age was 9 ± 3 years. The mean size of the nodule was 17 ± 6 mm, and it affected the right or left mammary line without significant difference. The inguinal mammary gland was the most affected ($p<0.01$) while the cranial thoracic one the least ($p<0.01$). Cytological evaluation confirmed that 28/43 (65%) of nodules showed signs of malignancy (7 carcinomas, 10 carcinosarcomas, 7 sarcomas, 3 hemangiosarcomas, 1 liposarcoma), the remnant cases were diagnosed as benign, hyperplastic or inflammatory lesions. In this group the inadequate samples ($n=7$) were also included.

DISCUSSION - The cytological procedure had no complication and was well-accepted by owners. For the purpose of this study, each nodule also belonging to the same animal was considered as a different nodule, considering that when multiple tumours are present, different tumoural types are often diagnosed^{2,5}. The nodules were found in 64% of cases in breeds considered to have a high risk to develop mammary tumours⁶ and this may have interfered with results. In this study, the high prevalence of malignant tumours (64%) demonstrates that it does not exist a cut off for mammary nodules in the bitch, to distinguish between benign and malignant lesions. Small nodules must always be aspirated and if the malignancy is confirmed they should be removed. In the remnant group (36%) all the benign lesions were included for which a continue monitoring should be applied, informing the owner that benign lesions are considered to be precancerous in this species⁵. The inadequate samples ($n=7$) were classified as not malignant, this assumption is justified only by the fact that high cellularity was considered a criterion of malignancy, but, in the practice, the aspirate should be repeated. This study did not include a follow-up and only few cases underwent surgery followed by histological confirmation of the tumour type. In 2012, there is still scepticism among owners on the efficacy of surgical treatment of mammary tumour for different reasons (costs, long postoperative care, median survival after surgery). Treating small tumours may represent the success key but new therapeutic approaches must be improved. The frequent occurrence of mammary tumours in our country is explained by the low diffusion of early neutering in pets, which is, at the moment, the unique effective prophylactic treatment⁷.

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MONITORING OF WEIGHT LOSS AND GAIN IN NEWBORN PUPPIES OF BOXER BREED

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KEY WORDS: puppies, loss and gain weight

ABSTRACT - In the first days after birth is common to see weight loss in puppies that should not exceed 10% of weight at birth. The main causes of weight loss are urine and meconium issued followed by non-recovery of fluids expelled. In our study within 3-5 days the puppies retrieved birth weight and got back a weight gain that led to double in two weeks. We observed a mean weight loss of 2.27% in the first two days of life and they start recovery the birth weight on the fourth day (mean values). The puppies that regained birth weight earlier were those that showed a greater weight gain. The puppies with higher birth weight had lower weight losses.

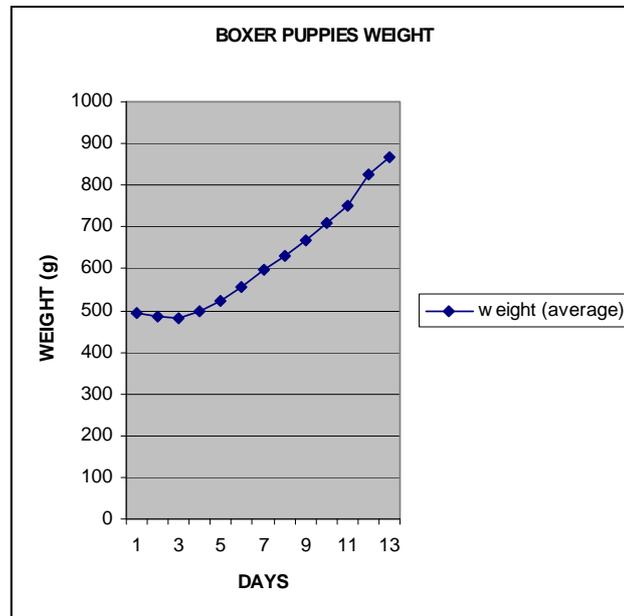
INTRODUCTION – In healthy subjects the weight loss at birth is a physiological event related to meconium and urine issue. The recovery of the weight loss must be fast (in the first 2-3 days of life) and must be less than 4-5%. In some cases it may reach 10% and represents a negative prognostic significance for survival of the puppies. The aim of the study has been to monitor changes of the newborns weights in the first two weeks of life.

MATERIALS AND METHODS - In our study we selected a sample of 142 puppies relative to 37 pregnancies in Boxer breed bitches all from the same farm. The pregnant bitches were bred under standard conditions with dry maintenance feed at a dose equal to an increase of 50% of the normal dairy requirement. The feed was gradually replaced from day 40 of pregnancy with a puppies feed until weaning. No pharmacological treatments were used to promote milk production. All animals received an integration with folic acid (*Folina*[®]) 5 mg/die. After each delivery a oxytocin dose (0.5 IU / kg) was administered to increase uterine involution. Puppies that died during the first two weeks were not included in the study. The puppies were weighted immediately after birth and four times a day for 14 days.

RESULTS - The bitches in our sample have completed 37 pregnancies with an average of 3.86 weaned pups. Females were 75 (52.8%) and males 67 (47.2%). The average weight of puppies at birth was 495.3 g with a doubling in 13 days. At 3th day mean weight loss was 2.27%, on the fourth day the growth curve was reversed with the recovery of birth weight. The growing was linear during the monitoring. The study shows a positive correlation ($r=0.92$; $p<0.05$) between birth weight and the weight on the second day, indicating that the larger puppies suffer from a minor weight loss. Even the growth curve of percentiles is kept parallel to the curve of average weight.

DISCUSSION - In the first days after birth is common to see weight loss in puppies that do not exceed 10%. The main causes of weight loss are represented by the issue of urine and meconium and non-recovery of fluids expelled. Normally after the first 3-5 days the puppy retrieves birth weight and get a weight gain that will lead to double weight in two weeks with some differences depending on the breed¹. Breed specific

variations in puppies growth patterns are related to differences in secretion of circulating growth hormone. Poor nutrition during the first four months can have direct consequences for future growth and development. During this period alimentary excesses could be dangerous as deficiencies¹. It seems to be well established that low birth weight directly correlates with the extent of neonatal disease or death². In healthy puppies weight loss should not exceed 5%, and in any case more than 10% weight loss has negative prognosis for survival of pups before weaning.



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THERAPEUTIC ULTRASOUND AS A POTENTIAL MALE DOG CONTRACEPTIVE: STATE OF THE ART

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KEY WORDS - dog, contraception, ultrasound

ABSTRACT - The purpose of this review article is to present a 50-year perspective of research on mechanical method for limiting male dog reproduction by the use of therapeutic ultrasound. Ultrasound's potential as a male contraceptive was first reported by Fahim et al. in 1977 where it was shown that a single application could result in a reversible dramatic loss of germ cells. If the method can be made permanent, a non-invasive method for controlling domestic pet populations could be developed, although standard treatment is not yet identified. More recent studies in 2000s by our research group demonstrated that tree treatment in a week of 5 minutes at 1MHz leads to an irreversible testis damage.

An ideal male dog contraceptive would be reliable, not reversible, with no side effects and inexpensive. For many years, traditional neutering has been accomplished through surgical methods of sterilization namely orchidectomy. However not all owners have their pets surgically sterilized. In addition, when considering feral dog populations where permanent sterilization is desired, surgical methods can be too time consuming and expensive to be performed on a large-scale. Currently, several alternatives to surgical sterilization are being investigated. Considered on technical and practical merits alone, ultrasound is one of the most promising forms of male dog contraception. In this paper, researches investigating mechanical approach to sterilization are reviewed. Ultrasound (US) consists of inaudible high frequency (greater than 20 kHz) mechanical vibrations (pressure waves). The waves produced, are transmitted by propagation through molecular collision and vibration, with a progressive loss of the intensity of the energy during passage through the tissue inducing the tissue heating. The scrotal testis of mammals is normally at a temperature several degrees (3-4°C) below that of the body core. The concept that an elevation of testicular temperature results in impairment of spermatogenesis is widely accepted. Basing on this principle, it is possible to affect the sperm production by raising testicular temperature. US, as a suppressant of spermatogenesis, was found to be more effective at lower temperature because of its combined effects thermal and mechanic¹. For mechanical contraception ultrasound waves are of the same frequencies, powers and a duty cycle associated with the therapeutic use.

Ultrasound's potential as a male contraceptive was first reported by Fahim et al. in 1977¹. In a series of publications, it was shown that a single application of ultrasound could result in a dramatic loss of germ cells from testes and that this loss of germ cells was reversible. In 1980 Fahim stopped research on US. The information on ultrasound languished for decades.

More recently from 2005 our research group has began investigating on US as we believe that if the method can be made permanent, a non-invasive method for controlling domestic pet populations could be developed. Although, standard treatment is not yet identified: the least number of applications needed, the shortest interval among applications, the best testis area where to treat by US, the ultrasonic frequency or power, and so on, are treatment parameters to be investigated. A first study in 2009²

demonstrated that three treatment in a week of 5 minutes at 1MHz and 1.5 watt/sq cm leads to an irreversible testis damage, while 3MHz in the dorso-cranial portion of the testis is ineffective³. Reducing the number of applications at two times in alternate day (1.5 watt/sq cm, 2.5 cm² transducer, 1 MHz) but increasing the treatment length at 15 minutes⁴ is ineffective on dog fertility as well as three treatments of 5 minutes in a day (one every 5 minutes, 2.5 cm² transducer, 1 MHz, 1.5 watt/sq cm)⁵.

No notable side effects other than infertility and no influence on testosterone production were reported during these studies. Fahim⁶ reported that his treatment method did not affect genetic safety in future generations of animals.

In conclusion, our results demonstrate that a short exposure to therapeutic ultrasound is an effective method for depleting testes of spermatogenic cells; US application was supposed to create an environment not suitable for spermatogenesis¹.

Optimizing the treatment conditions, studying the safety of repeated use, the duration of its contraceptive effect and its reversibility and are the next required steps to establish whether therapeutic ultrasound can serve as the basis for a new, irreversible male contraceptive.

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REPRODUCTIVE TRAITS, VIABILITY AND GROWTH OF KANGAL SHEPHERD DOGS

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KEY WORDS: dog, kangal, reproductive traits

ABSTRACT - The purpose of this research was held to determine the reproductive traits, viability and growth characteristics of Kangal Dogs, housed in a farm located in Ankara. The study was conducted with 39 bitches and 163 puppies. Average oestrus time was 408, 5 days whereas the number of the oestrus 18, 14, 11 and 7 for summer, winter, autumn and spring respectively. The effect of the season is significant. The intervals were 236.6 days in pregnant and 182.6 days in non-pregnant. Average proestrus and oestrus periods were 7.5 and 4.9 days respectively. Average live weight change between prior to their gestation and the weaning period was 2.6 kg. The average gestation period was 60,8 days where as the rates of oestrus 100%, with a 8.6% pseudo pregnancy and 91.4% birth rate and the average number of the litter is 5.9. Average rate of still births was 6.7%, whereas 8.1% in birth types with 7-9 puppies and 4.7% with 1-3 puppies. Stillbirth rates in autumn-winter and spring-summer were determined 8.0% and 3.9 % respectively and 19.2% for the puppies of 5-7 years of bitches. Maternal age and seasons effects are considerable. Viability rates at 120 days were determined as 86.8. The effect of the season is significant up to day 30. The average birth weights in male and female puppies are 0.54 and 0.52 kg, respectively.

INTRODUCTION - Kangal is a well known native dog breed of Turkey. It is classifying among large dog groups as shepherd dog¹. It has been reported that first oestrus time between 6-18 months, frequencies of oestrus signs and ratios vary according to the seasons. Average oestrus ratio 94.7% on Kangal, 64.3% - 83.7% on other breeds. Oestrus frequencies were from 3.5 to 13 months, Average mating age for larger dog breeds is 18 months time and average copulation time is 2 to 4 days following the end of proestrus². The highest gestation ratio has been obtained at day 11.9 as of proestrus start, and the copulation period has no effect for the length of gestation. Breed and number of the young have an effect on gestation periods whereas the maternal age has none. Gestation length is 58-63 days on Kangal, 58-69 on different dog breeds³. Birth rates are 75% - 95.9% on Kangal, 61.5% - 100% on different breeds. Pseudo-gestation ratio is 50-75 % on dog breeds although some breeds have more such as Afghan Greyhound, Beagle, Labrador and Retriever. Average litter number on large, medium and small breeds are respectively 7-8, 67 and 3-4 whereas 6.2-8.9 on Kangals⁴. Stillbirth rate varies 1.8% to 16.1% on different breeds. Viability rate is between 72%-91% and the effect of seasons, maternal age is significant². The live weight is between 32.2-59 kg and should be at least the same as in pre-pregnancy post lactation on female Kangal dogs. There seems a high correlation between the live weights in pre-pregnancy and weaning periods². The average birth weights on male and female Kangal young are 550 and 525 g respectively. Decrease on the number of young increases the birth live weight and maternal age has a significant effect on birth live weight up to the age of 6 months^{1,3}.

MATERIAL AND METHODS - Research was held with 30 bitches at the age of 2 to 7 and 163 puppies. Adult male and females were fed 1,5 to 2 kg, 2 or 3 times/day with tank age and mixtures of stale bread, barley meal and dry food. Young were given breast milk for the first 30 days followed by water, milk and dry food with broth for the next 15 days and chum with bone meal after the day 45 in 350 – 400 g meals 4 times a day. Ambient temperature was 26°C to 34°C, average snow depth was 15 cm. Standard formulations to calculate the reproductive trait criteria, live birth changes between the pre-pregnancy and weaning periods and viability rate were used in this study. Birth weights have been measured by scales, precise to 10 and 50 g. Variance analysis, T Test and chi square test have been used in accordance with the criteria of the data used for the comparison of multiple groups. For the significant differences among groups, Duncan test is used.

RESULTS - Average of the first oestrus exhibit is $408,5 \pm 5,3$; for those born in autumn-winter 386, 50 ± 6 , 99 and for spring-summer seasons $423,11 \pm 5$, 27 days ($p < 0,001$). Average period between the estruses is $228,50 \pm 17,32$; $236,62 \pm 19,79$ for the pregnant, $182,65 \pm 18,51$ for non-pregnant. Proestrus and oestrus periods are respectively 7.52 ± 0.71 and $4,90 \pm 0,50$ days. Number of oestrus in winter, spring, summer and autumn are 14 (28%), 7 (14%), 18 (36%), 11 (22%) respectively ($p < 0,001$). Average gestation length is 60.78 ± 0.33 days. This period has been observed on 2-3 ages as 60.63 ± 0.26 ; 5 - 7 ages $61,10 \pm 0.31$ days. Live weight change, from average gestation and weaning periods, is 2.63 ± 0.19 kg; this becomes 3.60 ± 0.34 for 1-3; 5-6 and 7-9 young dogs and 2.56 ± 0.23 and 2.70 ± 0.29 for autumn-winter and spring-summer seasons respectively, 2.70 ± 0.20 and 2.56 ± 0.32 kg for mature dogs of 2-3 and 5-7 respectively. Birth type showed a significant effect ($p < 0,001$). Oestrus rate was 100% in this study. Birth rate was 91.4 % and pseudo-gestation was 8.57%. The number and ratio of stillbirth are 11 and 6.7%. These values are 1, 4, 6 and 4.7%, 5.9 % and 8.1% on 1-3; 5-6 and 7-9 young dogs respectively. It is much higher in autumn-winter period (8%) in respect to spring-summer time (3.9 %) and for the maternal of 5-7 ages (9.2%) in respect to the ones between 2-3 ages. Viability rates at 15, 30, 45, 60, 90 and 120 days are 96.1%, 90.8%, 88,8%, 86,8% and 86.8%, respectively. Females have higher viability in all periods.

DISCUSSION AND CONCLUSION - First oestrus exhibit age was found to be shorter in autumn-winter. Proestrus time is lower than the values reported for some other dog breeds. It can be advantage for the breeder as this period is short. It can be said that the oestrus time obtained in this study are efficient for optimum reproductive traits. Oestrus rate (100%) is higher than those reported for Kangal and other dog breeds. Pseudo-gestation rate (8.57%) is usually lower than the values reported by various sources (50-75%), especially very low when compared to some dog breeds like Afghan Greyhounds, Beagles and Labrador Retrievers. Stillbirth rate (6.7%) is observed similar to, higher or lower than the values reported for Kangal and larger breeds. Viability rate at day 120 (86.8%) is similar to, higher than or very high than the values reported for Kangal and some other dog breeds. This study confirms that, the reproductive traits, growth and viability rates on Kangal Shepherd Breed are similar or better to other dog breeds, type of birth, maternal age and seasons have different effects on these criteria on different bases, the breed has a very low rate of pseudo-gestation and it is extremely essential to do more researches on this subject.

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FELINE MAMMARY HYPERTROPHY/FIBROADENOMA COMPLEX: A RETROSPECTIVE STUDY OF TWENTY-FIVE CASES

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KEY WORDS: mammary hypertrophy/fibroadenoma complex, cat, retrospective study

ABSTRACT - Feline mammary hyperplasia is a benign and progesterone responsive condition. Twenty-five cases of mammary fibroepithelial hyperplasia spontaneous (19) or after treatment (6) with progestin are described: 1 with pyometra, 2 were sisters and other 2 were mother and daughter. In 14 cats we observed bilateral enlargement of all mammary glands whereas in the other 11 cases the enlargement involved only few glands. It is possible the pathology has genetic predisposition. The pathology does not prevent the galactopoiesis and suckle function. The condition can give recurrences and can also spontaneously regress. The difference between numbers of mammary glands involved suggests the involvement of local factors in the individual gland.

INTRODUCTION - Feline mammary hypertrophy/fibroadenoma complex (MH/FC) is a growth disturbance in cats is a non-neoplastic, progesterone-responsive condition characterized by rapid proliferation of mammary stroma and duct epithelium in one, several or all the mammary glands¹. The lesion is associated with endogenous and exogenous progesterone; it appears usually in young queens after the first oestrus, during gestation and pseudopregnancy or also after the administration of progestogens². It can also develop in male cats treated with synthetic progestin during the course of treatment for sex-related behavioural problems or dermatological conditions³. Progesterone or its synthetic analogues induce local synthesis of growth hormone (GH) and insulin-like growth factors (IGFs) in mammary epithelial cells stimulating the local proliferation of epithelial cells³. Typical clinical signs include a diffuse, firm swelling which rapidly develop in mammary glands without sign of inflammation⁴. Hyperplastic mammary tissue may undergo spontaneous regression, after ovariohysterectomy, or may regress once progesterone levels decline. The purpose of the present study is to report a retrospective analysis of twenty-five cases of MH/FC.

MATERIALS AND METHODS - From 1990 to 2011 twenty-four female and one male cat with mammary fibroepithelial hyperplasia were admitted to the Obstetrics and Gynecology Clinics, Department of Veterinary Medicine, University of Sassari. Breed, age, spontaneous development of pathology (pregnancy or pseudopregnancy) or development after treatment with progestin, number and site of mammary glands affected, family relationships and recurrence, evolution of lesions and treatment were examined.

RESULTS - Breeds included 21 European shorthair, two Siamese and two Persian cats. The age of the animals ranged from 3.5 months to 12 years. In nineteen cases fibroadenomatosis had developed spontaneously, the other six cases had been treated with synthetic progestin: three queens with acetate medroxyprogesterone (MPA), 2 with megestrol acetate (MA) and one male with MPA. One cat was treated with MA for 8 years (last treatment 30 day before the MH/FC), one animal was pregnant when MPA was given and the male had been treated after neutering. Of the animals in which fibroadenomatosis had developed spontaneously, eight were pregnant (from 20 to 45 days); ten were in pseudopregnancy and 1 with pyometra. In 14 cats we observed

bilateral enlargement of all mammary glands whereas in the other 11 cases (Table 1) the enlargement involved only few glands. The hypertrophic mammary glands ranged from 1.1 cm x 1 cm to 8.7 cm x 7.8 cm. Two cats were sisters and other two were mother and daughter. The mother presented MH/FC in the first pregnancy and after partum had suckled kittens. Therapeutic surgical procedures for fibroadenomatosis included neutered (ovariectomy n=8, ovariectomy n=5) and mastectomy (n=9) or only one mastectomy (n=1). One queen was treated with antiprogesterins (Alizine; Virbac, Italy) and in one animal has occurred spontaneous resolution. The definitive diagnosis was confirmed by tissue biopsy, histopathological evaluation after mastectomy or mammary hyperplasia regression after treatment.

Table 1 - Cats with partial involvement of mammary glands

cat n°	mammary glands								cat n°	mammary glands							
	right				left					right				left			
	M 1	M 2	M 3	M 4	M 1	M 2	M 3	M 4		M 1	M 2	M 3	M 4	M 1	M 2	M 3	M 4
1,2		X	X	X		X	X	X	7			X		X	X		
3				X				X	8		X			X			X
4				X				X	9			X					X
5	X	X	X	X		X	X	X	10					X	X		
6				X		X	X	X	11		X	X					

DISCUSSION - According to previous studies our results confirm that the fibroadenomatosis is a consequence of stimulation by endogenous progesterone or may be induced by exogenous progestogens^{2,3,5}. The difference between numbers of mammary glands involved, only few glands rather than the entire mammary system suggests an individual mammary sensibility to circulating progesterone or difference in production of GH and IGFs, variation in local hormonal receptors or other factors not known yet. The family relationships finding in two cases, one mother and daughter and the other in two sisters with MH/FC at the same time suggests the possibility that the pathology has a genetic predisposition. The condition can give recurrences⁶ and can also spontaneously regress⁷. The pathology does not prevent the galactopietics and suckle function.

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PROGESTERONE MONITORING IN HYPOFERTILE BITCHES

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KEY WORDS: progesterone assays, infertility, German shepherd dogs.

ABSTRACT. This work was designed to evaluate the use of two methods for progesteronemia measurement in a breeding kennel with problems of infertility. Thirteen estrous cycles were monitored either through a commercial colorimetric kit (Ovulation[®] test, BVT groupe, Virbac, France) (Group 1: 8 cycles) or through chemiluminescence (Immulite[®], Diagnostic Product Co., Los Angeles, USA) (Group 2: 5 cycles). Upon ultrasonographic examination, 37.5% of Group 1 bitches were determined not pregnant whereas 100% of the bitches in Group 2 were pregnant. One of the pregnant bitches of Group 1 that delivered a single pup was mated at least 4 days before ovulation. Accurate progesterone measurement during estrous may help to correct inappropriate breeding management.

INTRODUCTION. The main cause of infertility in bitches is inappropriate breeding timing (1). Timing of ovulation by using serum progesterone (P4) measurement may increase the chance of conception. Serum P4 concentration can be assessed either with colorimetric methods, for direct 'field' use, or through quantitative assays. The aim of this work was to investigate conception results in a breeding facility where serum P4 concentration has been measured by either of the two methods.

MATERIALS AND METHODS. The study involved German shepherd bitches housed in a breeding kennel where infertility was the main complaint. The bitches were usually brought to males abroad, for a single mating. All the bitches were negative for both *Brucella canis* and CHV-1. Thirteen estrous cycles were monitored by serum P4 measurement. In 8 cases (Group 1), P4 concentration was assessed by using a commercial colorimetric kit (Ovulation[®] test, BVT groupe, Virbac, France) and the day of breeding was 2-3 days after the colorimetric reaction indicating a P4 concentration between 2.5 and 8 ng/ml.

In 5 cases (Group 2), serum P4 concentration was measured by chemiluminescence (CLIA) (Immulite[®], Diagnostic Product Co., Los Angeles, USA), an assay validated for the canine species (2). The day of LH peak was considered corresponding to a P4 value between 1 and 2.5 ng/ml. In both cases, blood samples were collected once every 2 days, beginning 4-7 days after the onset of proestrus.

Pregnancy was diagnosed echographically (General Electrics Medical System Logic P5/A5, Soligen, Germany) using a convex probe (6-10 MHz) 20 days after mating.

In both groups, the day of the LH peak was retrospectively determined by calculating 65±1 days from parturition date (3), to evaluate whether the bitches were mated on the days of maximum fertility.

RESULTS AND DISCUSSION. In Group 1 (commercial kit), 3 of 8 bitches (37.5%) did not become pregnant, whereas all 5 bitches in Group 2 (CLIA) were pregnant. In 10 cases it was then possible to retrospectively estimate the day of LH peak. Table 1 shows mean±SD of the interval between mating and LH peak for the pregnant bitches belonging to the two groups.

The distance between the mating day and the LH peak day was -2/+6 days (8 d) for group 1 and +2/+6 days (4 d) for Group 2. The highest fertility is known to be in the interval 3-5 days after the

LH peak (4); although our data were not adequate for a statistical analysis, we observed that Group 1 values were more variable (SD=3.3) than those of Group 2 (SD=1.8); furthermore, one of the pregnant bitches of Group 1, mated two days before the LH peak, delivered only one puppy. An error in performing or reading the test by the breeder may be presumed (See Fig. 1). The range of puppies per bitch born in Group 1 and 2 was 1-12 and 4-9, respectively.

CONCLUSION. Serum P4 monitoring, by detecting the maximum fertility period may help optimizing breeding results, particularly in kennels where only a single mating is allowed. The interpretation of the colorimetric results may be misleading. Even if few cycles were monitored in this study, conception results with CLIA were far better than with the colorimetric kit.

Fig. 1. Progesterone concentration in a pregnant bitch of Group 1. Day 0 indicates the day of LH peak; mating (→) took place prior to LH peak and ovulation (···). The bitch whelped a single puppy.

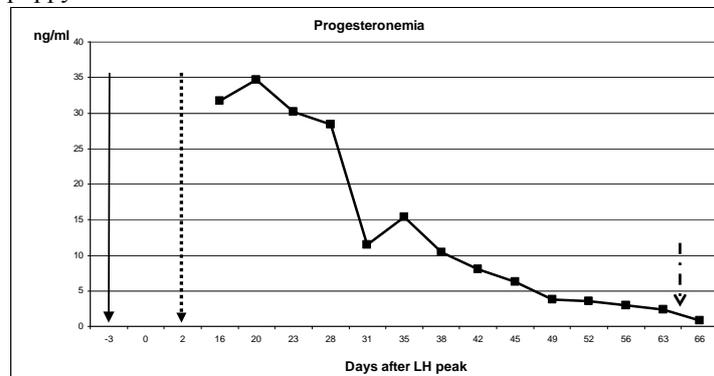


Table 1. Mean interval between day of LH peak and day of mating (mean±SD) and range of litter consistency in the two groups (Group 1: P4 measured by commercial colorimetric kit) (Group 2: CLIA).

	Group 1	Group 2
Mating-LH peak interval (Days; Mean±S.D.)	3.2±3. 3	4.8±1. 8
Range of litter consistency	1-12	4-9

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PARTURITION DATE PREDICTION IN BITCHES OF DIFFERENT BREEDS THROUGH FOETAL HEAD MEASUREMENT AND BLOOD PROGESTERONE DOSAGE

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KEY WORDS: canine parturition date prediction, foetal head diameter, maternal progesterone

ABSTRACT - Aim of this study was to assess the accuracy of parturition date prediction through the use of all the different formulas available in literature, applied in the last days of pregnancy. Progesterone concentration was measured daily till parturition. Parturition occurred on the expected date in a minority of bitches, ranging from 40% to 0% depending on bitch size and equation; also progesterone concentration resulted only indicative.

INTRODUCTION - Accurate prediction of parturition date could be very useful for the optimal management of the whelping bitch and the organization of veterinary assistance. Ultrasonography can be of help: during late pregnancy, the easiest foetal parameter that can be measured is the maximum head diameter in transverse plane, i.e. the biparietal diameter (BPD)¹. Also serum progesterone concentration can be useful to predict how parturition is imminent^{2,3}.

Aim of this work was to assess the accuracy of parturition prediction by means of all the different equations available in literature, applied to bitches of various breeds in the last days of pregnancy. Also progesterone concentration was evaluated.

MATERIALS AND METHODS - The work was done on 22 pregnant bitches. Table 1 shows the number of bitches for each breed and the relative size. In the last days of pregnancy, at least two foetal heads were measured for each bitch using Prosound 2[®] (Aloka Co., LTD) with 2.5-6.0 MHz transducer. A blood sample was collected from 11 bitches daily until parturition to measure progesterone concentration through a chemiluminescent assay. The mean BPD was used in all the available formulas, according to bitch size category. The equations are reported in Table 2. The accuracy of parturition date prediction was determined by calculating the difference between the actual and the predicted parturition dates, with each formula for each breed and/or bitch size.

Table 1 - Number and size of the bitches of different breeds

N	Breed	Size
5	West Highland White Terrier	Small
11	Staffordshire Terrier	Medium
2	Lagotto	Medium
1	Border Collie	Medium
1	Italian Segugio	Large
2	American Staffordshire Terrier	Large

RESULTS - Small size dogs were all West Highland White terriers: 40% of them whelped on the expected date, using the formulas A and C; 20% with D; 0% with B. A total of 80% bitches whelped with a precision of ± 2 days, with formulas A, C and D.

The formula B gave worse results, with 40% of bitches whelping more than 3 days later than expected. About 18% of the Staffordshire terrier bitches whelped on the expected day (formula G), 9% with formulas D and E and 0% with formulas F and C. The difference between actual and predicted parturition dates was ± 2 for 72.8% of the bitches with the formula G; for 45.5% bitches with the formula D. The other formulas showed a lower precision, with more than 70% (C) and 80% (F) whelping ± 3 from the expected date. The formula D was the most accurate for the other medium size bitches, that is two Lagotto bitches (± 2 and ± 4 , respectively) and a Border Collie one (± 1). The equation used for large breed bitches gave a prevision that was 4 days late in all our cases, meaning an underestimation of gestational age. Table 3 shows blood progesterone concentration on the day before parturition: 3 bitches had a concentration ≥ 5 ng/ml and in other 6 cases, the value was between 2 and 3 ng/ml

Table 2 - Formulas to calculate whelping date from foetal head diameter (BPD) in the last part of gestation of bitches of different size

Small	$63.2 - (24.36 + 1.54 \times \text{BPDmm})$	A [4 (Maltese)]
	$63.4 - (23.89 + 1.63 \times \text{BPDmm})$	B [4 (Yorkshire terrier)]
	$(\text{BPDmm} - 25.11) / 0.61$	C [5]
	$(\text{BPDmm} - 2.49) / 0.006$	D [6]
Medium	$45 - (15 \times \text{BPD})$	E [7]
	$43.92 - (14.88 \times \text{BPDcm}) + (0.11 \times \text{BPD}^2 \text{cm})$	F [8]
	$\text{BPD}_{\text{mm}} = 23.138 + 1.098x + 0.00016 x^2$	G [9]
	$(\text{BPDmm} - 29.18) / 0.7$	C [5]
	$(\text{BPDmm} - 2.49) / 0.006$	D [6]
Large	$(\text{BPDmm} - 29.18) / 0.7$	C [5]

Table 3 - P₄ concentration (ng/ml) 24 h before parturition in 11 bitches

Progesteron (ng/ml)											Mean \pm SD
6.5	2.2	2.4	5.0	2.5	5.6	1.6	2.6	2.4	2.1	0.43	3.03 \pm 1.85

DISCUSSION - On the whole, prediction accuracy was lower than in the original studies; this means that formulas calculated on a breed can not be used on different ones, especially in late gestation, when there may be a high variability in foetal growth rates across breeds. To increase precision, more foetal parameters could be measured, like body diameter or the deep portion of telencephalic vesicle¹⁰, but this could very unpractical under clinical conditions. Our data show that the prediction of parturition date is not accurate enough with the available formulas, especially in medium and large size bitches. Progesterone concentration was higher than expected (≥ 5 ng/ml) in almost 30% of cases. The measure of BPD near term can give an indication of gestational age, that can be very useful when the reproductive history is unknown, but it cannot accurately predict the end of pregnancy.

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UNUSUAL CASE OF SYNDACTYLY IN A DOG

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KEY WORDS: syndactyly, radiography, dog

ABSTRACT - A case of syndactyly in all four limbs is described in a 3-month-old male inbred dog. Clinical and radiographic findings are described. Syndactyly is a rare condition in most animal species. In dogs, this condition has been infrequently reported. To the authors' knowledge, the malformation described here is the first case documented in a dog.

INTRODUCTION - Congenital deformities are structural or functional congenital defects arising from errors during development, they can affect an isolated portion of a body system, the complete system or parts of several systems.

The condition results from a failure of digital separation from apoptosis during embryogenesis¹. Complete syndactyly extend to the digital tips, whereas incomplete syndactyly ends proximal to the finger tips Simple syndactyly refers to digits connected only by skin and soft tissue. Complex syndactyly denotes bony fusion between adjacent phalanges. Complicated syndactyly refers to the interposition of accessory phalanges or abnormal bones between digits².

This classification has also been used for syndactyly human and canine patients

The syndactyly has been infrequently reported in canine species^{3 to 9}.

The aim of this report is to describe the macroscopic and radiographic findings of complete simple syndactyly observed in both forelimbs and hindlimbs of the puppy dog.

MATERIALS AND METHODS - The study was performed in a 3-month-old male inbred, referred for clinical evaluation to the Department of Veterinary Public Health at the Faculty of Veterinary Medicine, Messina, Italy. The anamnestic data revealed that the animal was born with a deformed both forelimbs and hindlimbs. The sites of deformation was assessed by physical examination and radiographed in lateral and dorso-palmar/plantar views; X-ray settings of 50 kV, 8 mAs at a film focus distance of 1 m, without grid, were used.

RESULTS - Physical examination revealed a lack of separation between the digits in both feet; lameness not detected. The nails on all the fingers, were normal. On the palmar/plantar aspect of the paw, the pads were normal. In radiographs, the bones of the all paws showed to be of normal shape and size.

DISCUSSION - The present report of complete simple syndactyly in a dog provided novel information about congenital limb malformations, being the first report of such malformation in both forelimbs and hindlimbs of the puppy dog.

Embryologically, the primary step in limb formation is the aggregation of the somatic mesoderm cells of the hypomere beneath the surface ectoderm, forming the limb bud. During development of limb buds, the superficial ectoderm becomes thicker along the

distal ridge, the so called apical ectodermal ridge. This ridge persists until the condensation of the digital mesenchyme. The critical period for the development of the entire limb in the canine embryo is between the 3rd and 4th weeks of pregnancy, when tissues are more susceptible to external influences¹⁰.

Although congenital limb deformities in dog have been documented previously, the present case is, to the authors' knowledge, the first report of total canine complete simple syndactyly, representing an addition to the scant literature on this subject.

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LECTURES
And
WORKSHOP

CONTROL OF REPRODUCTION IN SHEEP AND GOATS

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It's been several years since the sheep and goat dairy sector is experiencing a deep crisis. Crucial to this has been the increase in prices of raw materials used in farming, both for food and farm management, and the simultaneous value decline of sheep and goat's milk and meat. These economic processes have motivated the breeders to streamline production processes and the vets to develop new strategies that may have a significant economic impact on breeding. A scrupulous reproductive management allows a significant increase in profits for the farm, and a higher quality of professional services, and thus a higher productive efficiency. With the correct application of these methodologies the veterinarian is no longer a cost, but becomes an active part of the budget. The management actions conducted with the help of specialists (Veterinarian and Nutritionist) will require a different involvement for breeders but it will improve the farm's production and economic efficiency. Sardinia has 3,314.66 sheep and 294,430 goats (Laore, 2011). In 2011 more than 3,400.000 quintals of sheep's milk were produced. The average production per ewe is about 130 liters rather than the expected 250 liters; thanks to surveys carried out on farms, it has been shown that this difference is due to the presence of many animals that remains unproductive, not pregnant or animals that begins the production too late.

From investigations made by the ARAS technicians, it was confirmed that such a low average production is determined by a large number of unproductive sheep. So a new technical-veterinarian expertise is necessary, to take care of reproductive management by using diagnostic ultrasound in daily business planning. The technician-veterinarian definitively binds itself to sheep or goat's farm. He will control in a dynamic way every animal in the farm and monitor their physiological status with the purpose to create groups: males good for breeding and females for each reproductive status and so on. Two months before mating, males are clinically examined and checked by ultrasound; later, not pregnant females at the ultrasound control are treated to return in estrus thanks to the right health management and a proper food. This will reduce the number of unproductive females and will bring down prices and increase farm's productivity.

This management flexibility allows adapting the intervention to different situations and different needs of sheep and goats breeding. Manage playback properly and rationally give the vet the opportunity to maximize farm income. Comparing the results obtained from farms similar in herd size and management, it was seen that a difference in fertility of 20% in eight hundred animals, determines a lost of fifty thousand Euros. Another example, in two flocks of three hundred animals with a 25% disparity in their fertility, there is a variation of PLV of nearly twenty thousand Euros. Most of the time an increase in PLV, variable and directly proportional to the size of reproductive problems, not leads to any additional investment. Considering that an animal reproduce itself when all the basic needs are satisfied, it's possible to say that good reproductive results confirm that all the physiological needs are met. Therefore veterinary cost will be on charge of the positive budget and will not be on charge of the breeder. The exact vet performance value has been estimated. It should varies from 5% to 20% of the

increase that the technical intervention produces which is about €5.00 per head. The wide variability between sheep and goat farms, about reproductive efficiency, indicate that these animals have different sensitivity to different breeding condition and therefore their productive performance can be improved by applying appropriate management techniques. With this purpose, the first interventions to be realized, is to regulate the reproductive phases and concentrate the parturitions in autumn; it is also essential to ensure an adequate remount management, particularly in terms of food, to ensure a good body condition that is essential to an early reproductive activity start and that anticipates the age at the first birth.

CONTROL OF REPRODUCTION IN AQUACULTURE

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Control of reproduction in captivity is essential for the sustainability of commercial aquaculture production, but most cultured fishes exhibit some form of reproductive dysfunctions. The fish reproductive cycle is separated in the growth (gametogenesis) and maturation phase (oocyte maturation and spermiation), both controlled by reproductive hormones of the brain, pituitary and gonad. Although the growth phase of reproductive development is concluded in captivity in most fishes oocyte maturation (OM) and ovulation in females, and spermiation in males may require exogenous hormonal therapies. In some fishes, exogenous hormonal manipulations are used only as a management tool to enhance the efficiency of egg production and facilitate hatchery operations, but in others exogenous hormones are the only way to produce fertilized eggs reliably. Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad, or synthetic agonists of gonadotropin releasing hormone (GnRH α) that act at the level of the pituitary to induce release of the endogenous LH stores, which, in turn act at the level of the gonad to induce steroidogenesis and the process of OM and spermiation. After hormonal induction of maturation, broodstock should spawn spontaneously in their rearing enclosures, however, the natural breeding behaviour followed by spontaneous spawning may be lost in aquaculture conditions, due to absence of the necessary environment (e.g., substrate, water depth, large water volumes). Therefore, for many species it is also necessary to employ artificial gamete collection and in vitro fertilization. In the last three decades, hormonal therapies have been used successfully in many cultured fishes to control or enhance reproductive function, and enable the production of good quality gametes to support hatchery operations for the production of fingerlings for grow-out.

ANGIOGENESIS AND OVARIAN FUNCTION IN RUMINANTS

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The ovarian cycle is characterized by repeating patterns of cellular proliferation, differentiation and transformation that accompany follicular and corpus luteum (CL) development, function and regression of the corpus luteum. The purpose of this work is to highlight the important steps of ovarian regulation and especially of angiogenesis during follicle development and maturation as well as during corpus luteum (CL) development, function and regression in ruminants. Angiogenesis is defined as the generation of new blood vessels through sprouting from already existing blood vessels. Of the numerous promoters of angiogenesis that have been identified, the most important factors appear to be members of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and angiopoietin family members. Experiment 1: antral follicle classification occurred by follicle size and oestradiol-17 β concentration in the follicular fluid (FF) into 5 groups (<0.5, 0.5-5, 5-40, 40-180 and >180 ng/ml). Granulosa cells (GC) and theca interna (TI) were investigated separately. Experiment 2: CL were assigned to the following stages; days 1-2, 3-4, 5-7, 8-12, 13-16, >18 (after regression) of estrous cycle and of pregnancy (month 1-2, 3-4, 6-7, >8). The mRNA expression, protein concentration and protein localization of examined factors in different follicle and CL groups was analysed by RT-PCR, RIA and immunohistochemistry. Analysis of the VEGF transcripts by RT-PCR shows that follicle and CL tissue express predominantly the smallest isoforms (VEGF120 and VEGF164). VEGF mRNA expression in both follicle compartments, theca interna (TI) and granulosa cells (GC), VEGF protein concentration in total follicle tissue and VEGF protein concentration in follicular fluid (FF) increased significantly with developmental stage of follicle growth. As shown by immunohistochemistry, VEGF protein was clearly localized in TI and GC of preovulatory follicles. Follicular FGF1 mRNA expression was relatively high in TI and lower in GC, and without any regulatory change during final follicular growth. The FGF1 protein could be localized predominantly in the cytoplasm of GC, in smooth muscle cells of blood vessels, and to a lesser degree in TI. FGF2 mRNA in TI increased significantly in large follicles and was low and without any regulatory change in GC. Our results suggest that VEGF and FGF family members are involved in process of folliculogenesis and especially during final growth of the preovulatory follicle by stimulation of angiogenesis and granulosa cell survival and proliferation. The highest mRNA expression for VEGF and VEGFR2 mRNA in corpus luteum (CL) was detected during the early luteal phase followed by a significant decrease afterwards. VEGF protein concentration in CL tissue was significantly higher during the early luteal phase followed by a decrease at the late luteal phase and after CL regression. The mRNA of FGF1 in CL increased significantly during mid-luteal stage. In contrast, FGF2 and FGFR expression is highest during early luteal phase and decrease thereafter to a lower level. During the early stages FGF2 was strongly expressed in cytoplasm of capillary endothelial cells and in smooth muscle

cells of arteries. In contrast, FGF1 is very weak in the cytoplasm of luteal cells during early luteal phase, followed by a stronger staining during mid-luteal stage and regression. The levels of ANPT1 mRNA in early and regressing CL were lower than those in mid and late CL, whereas ANPT2 mRNA expression did not change during the estrous cycle. Consequently, the ANPT2/ANPT1 ratio (an index of instability of blood vessels) was high in the early and regressing CL. In conclusion, our data indicate that the VEGF, FGF and ANPT system members are involved in the local mechanisms regulating angiogenesis and angiolysis of the newly forming corpus luteum after ovulation and the maintenance of capillary function during CL development, function and regression (luteolysis).

CONTROL OF REPRODUCTION IN STRAY DOGS

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In many countries more attention is being given to problems linked with straying animals; health and agricultural organizations together with organizations for environmental protection meanwhile the same mass media is pays great attention to this theme through organization of information campaigns aimed at sensitizing public opinion. The world health organization handles the problem by providing guidelines as a solution to problems created by straying animals in urban areas. By “straying” we intend wandering around of domestic animals that have been abandoned or lost by their owners and left. The possibility of transmission of various diseases from animals to man has been proven to be possible, particularly from stray dogs due to their living conditions, major possibility of contact with domestic pets, wild animals and man himself. Possibilities which grant them the role of an “ecologic interface”. Additionally, their eating habits, the lack adequate sleeping places, night activities coupled with inadequate prophylactic treatments render these animals vulnerable to various pathogenic and parasitic agents which are a frequent source of zoonoses such as; rabies, echinococcal/hydatid diseases, leishmania, heartworm disease, spotted fever etc. Besides zoonoses other aspects of public health linked with the presence of stray dogs are represented by aggressions from man, road accidents caused by crossing dogs and environmental contamination. The Humane Society of the United States estimates that each year between 8 and 10 million dogs and cats enter shelters and 4–5 million of these animals are euthanized. Euthanasia, as a means of controlling the overpopulation of dog and cats is costly in many respects; financial to the authorities that must oversee these activities, emotional to those who must actually perform the euthanasia and dispose of the carcasses, and societal to all who care about this disturbing problem. There also are ethical implications of euthanizing millions of animals each year. Other methods include poisoning, infection with fatal diseases, and hunting. Many veterinarians recommend surgical sterilization for population control in dogs and cats. However, when considering feral cat and dog populations where permanent sterilization is desired, surgical methods can be too time consuming and expensive to be performed on a large-scale. Currently, the pharmacological contraception is one alternative and reversible method for blocking fertility. Hormonal treatments can be divided in: estrus suppressors (progestins, androgens and analogs of GnRH) and pregnancy termination (estrogen, antiprogestins, prostaglandin F₂alpha, corticosteroids and prolactin antagonists). Progestins: megestrol acetate (2.2 mg/kg body weight orally for 8 days beginning in early proestrus), Medroxyprogesterone acetate (during the anestrus 2 mg/kg IM every three months) and proligestone (during the anestrus 10-30 mg/kg SC., with repeated administration 3 and 7 months later). Androgens: Mibolerone (the dose is weight and breed dependent (0.5-12 kg, 30 µg/d; 12-23 kg, 60 µg/d; 23-45 kg, 120 µg/d; >45 kg, 180 µg/d; and German shepherd type-bitch, 180 µg/d PO; treatment can continue for a maximum of 2 years. Analogs of GnRH: nafarelin (32 µg/days via an implanted osmotic pump or daily injections), deslorelin (subcutaneous administration of deslorelin via a slow-releasing implant). Estrogen: oestradiol benzoate (0.01 mg/kg SC on day 3, 5 and 7 after mating), oestradiol cypionate (0,44 mg/kg IM of 1 mg once during estrus).

Antiprogestins: aglepristone (10 mg/kg SC twice, 24 h apart). Prostaglandins: natural (25 µg/kg day 1, 50 µg/kg day 2 and 3, 100 µg/kg day 4) and synthetic (2.5 µg/kg SC day 1, 3 and 5) PGF₂α. Corticosteroids: dexamethasone (beginning on day 30 post LH-surge 0.2 mg/kg PO BID for 10 days). Prolactin antagonists: bromocriptine (0.1 mg/kg PO SID or BID for 6 days) and cabergoline (5 µg/kg PO for 8 days). Over the past two decades, efforts have been made to develop a vaccine that could suppress fertility in both female and male canids and felids. Several targets of immunocontraception have been identified, including GnRH, LH and its receptor, sperm antigens, and oocyte zona pellucida proteins (ZP). Many of these treatments can cause different side effects. In particular the prolonged use of progestins determine polyphagia, leading to weight gain; lethargy or restlessness, marked mammary stimulation with hyperplastic and/or neoplastic changes and increase: incidence of uterine pathology (CEH- pyometra complex), secretion of growth hormone and thus the risk of acromegalic changes, the risk of local skin alterations finally clinical and pathologic changes typical of diabetes mellitus and adrenocortical suppression. As regards the androgens the most common side effects reported in dogs are clitoral hypertrophy and vaginitis. Other side effects include increased body odor, urinary incontinence and urine spraying, mounting behavior, cervical dermis thickening and epiphora. Mibolerone is also contraindicated for use in Bedlington terriers due to an increased risk of hepatic dysfunction. The estrogens used for pregnancy termination may produce the CEH- pyometra complex, medullar hypoplasia followed by aplastic anemia, further sterility and abnormal behavior. The prostaglandin F₂ alpha determine gastro-intestinal effects (emesis and diarrhea), hyper-salivation; however tachycardia and broncho-constriction were reported. Finally corticosteroids (mainly dexamethasone) are not recommended for pregnancy termination in Europe due to many side effects (brownish vulvar discharge, polydipsia and polyuria due to transient adrenal suppression). The administration of vaccine derived from native GnRH results in no antibody production or a short-lived, weak response because the animal is tolerant to its own hormones. However, if GnRH is altered, for example coupling it with another molecule, in a way that induces recognition as a foreign material, with many antigenic determinants, an IgG response will occur, but it is difficult to realize. Sperm antigen immunization has not resulted in a satisfactory control of fertility because the entire spermatozoa cannot be used for vaccine development because it shares several antigens with other somatic cells. Bitches immunized with partially purified ZP proteins had ovarian cysts that were lined by a basement membrane with a clump of luteinized cells. These histologic changes provided an explanation for estrous cycle aberrations (prolonged proestrus bleeding and estrous behavior). Among immunosterilization methods, the immunocontraception targeting LH and its receptor have been successful in domestic carnivores because it has no side effects.

Unwanted dogs and cats or wild animals may be reservoirs or vectors of transmissible diseases to man and to economically valuable domestic species and its can give problem in agriculture. Furthermore, the stray cat and dog can: kill several species of animals in their territory, assault human people and cause car accident. The pharmacological contraception and the use of an immune vaccine represent a possible alternative to lethal measures (euthanasia, poisoning and hunting) or to surgical sterilization. An ideal immunocontraceptive would have a high margin of safety for treated animals, be effective in a high percentage of treated animals, have a rapid onset and long duration of activity following a single treatment, inhibit sex hormone production, be efficacious in all animals regardless of sex or age, be simple to deliver in the field and have a low cost. In conclusion the ovariohysterectomy remains the

procedure of choice in stray dogs because determine permanent sterilization and in the young bitches has been proven to reduce incidences of mammary tumors. Commercial development of drugs for hormonal down-regulation with exogenous steroid hormone, GnRH agonists, antiprogestins, prolactin antagonists and immunocontraception provide non-surgical alternatives for contraception and pregnancy termination. The relatively slow use of contraceptive vaccines for dogs and cats reflects the complexity in vaccine development, but is important to realize more study for improving the efficiency of immunocontraception.

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MECHANISMS INVOLVED IN RESUMPTION OF OVARIAN ACTIVITY IN POST-PARTUM DAIRY COWS.

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Ovarian function in mammals depends on the activity of both hypothalamus and pituitary, which secrete GnRH and gonadotrophins (LH and FSH), respectively; in addition, a local production of a variety of factors (among them IGF-I and IGF-BPs) ensures a fine modulation of both follicular development and luteal function. In particular, GnRH is released in a pulsatile manner (low pulsatility during the luteal phase, when P4 levels are high and high pulsatility during the follicular phase, when production of E2 is prevailing) and promotes a pulsatile LH release. Follicular growth (about 3-4 months) can be divided into two phases: gonadotrophin-independent and gonadotrophin-dependent (Webb et al., 2004). The second one, in cattle, occurs in waves (Savio et al., 1988; Sirois and Fortune, 1988); each wave of growth includes emergence (or recruitment), selection and dominance that is followed by either ovulation or atresia. Emergence consists in a growth of a cohort of follicles (± 5 mm in diameter) and is coincident with an increase of FSH release (Sunderland et al., 1994). Selection reduces the cohort of follicles to the ovulatory quota (generally one in cows); during the selection process, FSH concentration is low, granulosa cells develop receptors for LH (Bao et al., 1997) and intrafollicular IGF-I increases (as a consequence of IGF-BPs reduction; Canty et al., 2006). During dominance, the single selected follicle (dominant follicle, DF) suppresses FSH secretion and inhibits all other follicle growth; the fate of DF depends on LH pulse frequency. If P4 concentrations are high (luteal phase; see above), LH pulse frequency is low and DF undergoes atresia; the increase of LH pulsatility (follicular phase) stimulates the final maturation of DF and its E2 production which exerts a positive feedback on GnRH (and thus LH) secretion, leading to ovulation (Sunderland et al., 1994). A follicle wave lasts 7-10 days; therefore, during a normal oestrous cycle there are usually 2-3 waves.

During the first two trimesters of pregnancy, follicular growth continues (Ginther et al., 1996) at 7-10 day intervals but in late pregnancy high P4 and E2 levels (from both CL and placental origin) strongly suppress FSH release (Crowe et al., 1998), thus inhibiting follicular growth. During the last 20-25 days of pregnancy the ovaries are quiescent. At parturition, both P4 and E2 concentrations dramatically decrease, thus allowing an immediate (within 3-5 days) resumption of the recurrent increases of FSH levels. The first FSH increase induces the first post-partum wave of follicle growth which produces a DF by 7-10 days post-partum (Crowe et al., 1993); E2 secretion depends in turn on LH pulsatility during the dominant phase of the follicular wave as well as on both size and IGF-I availability of DF (Canty et al., 2006). Therefore, the fate of this DF (ovulation or atresia) during the post-partum period is mainly dependent on LH pulse frequency (one LH pulse per hour is required to stimulate a DF to ovulate). In dairy cows, ovulation of the first post-partum DF generally occurs in 30-80% of animals; DF undergoes atresia in 15-60% of animals, while becoming cystic in 1-5% (Beam and Butler, 1997; Sakaguchi et al., 2004; Sartori et al., 2004). In most of the cases first ovulation, in both dairy and beef cows, is silent (Kyle et al., 1992) and is followed by a short cycle in that premature PGF2 α release (Peter et al., 1989) shortens luteal phase. It is likely that E2 produced by the post-ovulatory DF (days 5-8 of the

cycle) stimulates E2 (and oxytocin) receptors (Zollers et al., 1993), thus inducing a premature CL regression; the second ovulation (usually associated with oestrus expression and a normal luteal phase) occurs approximately 9-11 days after the first one.

Dairy cows may present two, three or four follicle waves during the oestrous cycles occurring in the post-partum period (Sartori et al., 2004); but usually they tend to have two. As mentioned above, LH pulse frequency is mainly affected by P4 whose concentrations tend to be lower in lactating cows than in cyclic heifers (Sartori et al., 2004) allowing an increase of LH pulse frequency and a prolonged growth of each DF (Savio et al., 1990). Cows with prolonged luteal phases often present a fourth follicle wave. Both number of follicle waves and rate of turnover of DF are related to the duration of dominance of the DF; nutrition, by altering the metabolic clearance of P4, affects both duration of dominance and number of follicle waves per cycle.

The main factors affecting resumption of ovarian activity in dairy cows include BCS (Body Condition Score), energy balance (which, in turn, depends on energy intake, BCS and milk production), diseases and difficult calving (Beam and Butler, 1997; Opsomer et al., 2000; Wathes et al., 2007). Most of these factors contribute to reduce LH pulsatility. The most determinant risk is a dramatic reduction of BCS between calving and first AI, that prolongs the post-partum anoestrus. It is therefore important to prevent a decline in energy balance and reduce the post-partum negative energy balance period. Cows which are actively mobilizing tissue present high non-esterified fatty acids plasma levels but low concentrations of insulin, glucose and IGF-I (Grummer et al., 2004) as well as a lower E2 production. Monitoring of BCS from before calving to first AI is very important for an optimal reproductive management; this monitoring may be performed via determination of IGF-I plasma levels, which are an useful index of nutritional status (Zulu et al., 2002). As for diseases (among these, severe lameness, persistent subclinical mastitis, retained placenta, endometritis and metritis, which delay uterine involution), they may also condition follicle growth and E2 production; all these acute stressors inhibit GnRH release and hence LH pulse frequency resulting in a longer interval from calving to onset of the first luteal phase.

Post-partum dairy cows are often affected by ovarian cysts (DFs which fail to ovulate), that continue to grow to diameters >20-25 mm in the absence of a CL (Hatler et al., 2003); this growth seems to be due to a lack of positive E2 feedback on LH/FSH surge. As a consequence of the reduced CL function, P4 concentrations are low while E2 levels are high, resulting in exhibition of oestrous behavior by cows; oestrous behavior usually lacks during the last phase of cyst lifespan. High E2 and inhibin levels suppress FSH release; as a consequence, no new follicle waves emerge during the early period of ovarian cyst. When the cyst becomes E2-inactive, a new follicle wave can emerge; the DF of this new wave may ovulate, undergo atresia or become cystic. Ovarian cysts are often associated with low insulin (Vanholder et al., 2005) and IGF-I levels and high non-esterified fatty acids concentrations.

As above mentioned, ovulation in post-partum dairy cows mainly depends on GnRH (and hence LH) pulse frequency. GnRH (or its analogs) is effective in inducing the ovulation only when administered during the growing phase of DF (Ryan et al., 1998); on the contrary, GnRH administered at emergence of a follicular wave does not induce any effect and the cohort of follicles continues to develop unaffected. Therefore, the effectiveness of the treatment is fully dependent on the stage of follicle development.

Treatment with progesterone is also effective in shortening the post-partum anoestrus and in inducing oestrous expression and a normal luteal phase (Rhodes et al., 2003); P4 administration may be accompanied by eCG to ensure ovulation.

In conclusion, in dairy cows follicular growth generally resumes within 7-10 days post-partum as a consequence of a FSH rise occurring within 3-5 days of calving; first ovulation is generally silent and followed by a short cycle. Delayed resumption of ovulation depends on a lack of LH pulse frequency and resumption of ovulation is optimized by an appropriate pre-calving nutrition and management.

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ENERGY BALANCE AND REPRODUCTION IN HIGH YIELDING DAIRY COW

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Dairy cow genetic selection by herdsman has the primary purpose to improve milk's production and therefore the production of its main constituents such as fat and proteins. Up till recently some new parameters such as resistance to specific diseases and fertility have been introduced in the main Friesian selection's functional index. It's well know that genetic improvement of fertility is a long way and less significant if compared to what could be achieved by improving the environment, management, nutrition and health or rather the other components that contribute, with genetic, at phenotypic expression of this performance. For long time the idea that sub-fertility was the "price" to pay to improve the Friesian's production prevailed. The numerous published epidemiologic analyses and the empirical farm's experience not identify a "strong" negative correlation between production and fertility moreover there isn't hormonal and physiological condition supporting this hypothesis. Fertility, however, do has a negative effect on production because it's the main factor that affects the average days of lactation in a farm. The lengthening of the calving-to-conception interval and inter pregnancy interval reduces the number of new born and "fresh" cows that go on producing milk during a timeframe. The average lactation days lengthening, or the greater average distance from the previous birth, can result in a lower farm's productivity. Genetic selection has rewarded those reproducers able to give a progeny that produces more milk, more fat and more proteins giving these subjects the advantage of a greater diffusion of their genetic heritage. For a long time we wondered what were the hormonal and metabolic differences among high genetic potential animals (HMG) and the rest of population. The most productive cows present a higher concentration of growth hormone and a lower concentration of insulin. This "hormonal structure" allows the udder to have a greater availability of nutrients such as glucose, amino acids and fatty acids for the milk and its main constituent's synthesis. GH is a well know hormone both for specific studies conducted on it and for the knowledge arising from the possibility of using it in a synthetic form (bST) in many countries, United States particularly. GH increases the udder blood's flow while the lower insulin production make less available glucose to the tissues that use and have its receptors. Genetic selection could easily operate because the mammary tissue has a limited presence of insulin's receptors or, better, the glucose uptake by the mammary alveolar cells is not strictly dependent on this hormone. A lower insulin production compared to the blood glucose stimulates lipomobilization useful to the udder to have long-chain fatty acids required for fat's milk synthesis. M. Lucy (2006) compared diabetes type I, type II and the cow in post-partum. Cow makes a series of homeostatic regulation during the transition between dry period and lactation as to maximize milk production after parturition called lactation peak usually occurs between 30-45 days after birth. For evolutionary biology this condition is explained by the mother "will" to ensure the greatest amount of nutrients to calf before it can take those presents in surrounding environment. The farmers exacerbate this condition to their advantage by selecting cow with a higher maternal attitude. In metabolic terms this translates in a hormonal

structure that makes milk's production a priority and making the reproduction "suspendable" only when nutrients will be available to allow the cattle to make the "decision to breed", which it does only after a careful short, medium and long term metabolic reconnaissance. After parturition, in fact, there will be a sudden increase of pituitary GH concentration and also in part of prolactin, while both insulin and IGF-1 will increase very gradually their blood concentration. Compounding this physiological situation can contribute insulin-resistance various degrees that periparturient acute and chronic inflammatory processes may trigger in cow. The ingestion ability of dairy cows in the firsts week is not commensurate with its nutrients requirements, so it needs to draw on all available nutrients reserves to be allocated to hepatic gluconeogenesis. It will draw upon the fatty acids and glycerol stored in adipose tissue, upon labile proteins, or glucogenesis amino acids that may have accumulated during the dry in tissue and upon hepatic glycogen. Admitted that the dry nutritional management has the opportunity to cow to build these reserves (with exception of those lipidic), however they are of short duration. Within a few days the HMG cow is able to empty all the available amino acids and glucose reserves. Lipidic reserves are exhausted in a much longer time. Physiological and pathological ingestion limitations and the big boost productivity establish a metabolic status called "negative energy balance" (NEBAL) although more properly it should be supplemented with protein deficiency characteristic of these first week of lactation. Cow ensures the priority metabolic functions such as production, thermoregulation and locomotor activity while suspending the non-priority functions such as reproduction and growth during the period of negative energy and protein balance. This condition leads to a lengthening of ovarian activity resumption after parturition, affects negatively on the follicles quality or result in a parturition-conception interval lengthening and so on cow fertility.

The negative energy and protein balance so is able to affect the GnRH hypothalamic production and so the pituitary gonadotropins production. Moreover, the minor deficiency of essential amino acids are able to influence the hepatics genes expression that govern the IGF-1's synthesis now identified as the most powerful follicular growth factor. The limited nutrients availability in the first weeks of lactation, caused by the large upper uptake, reflects directly on follicles and influences both the oocytes quality and granulose ability to produce estrogen and progesterone. Certain metabolites or molecules bound to NEBAL as NEFA and BHBA also have directly negative effects on follicle. It was found that a higher NEFA and BHBA blood concentration may adversely affect oocyte quality and therefore the ovarian follicles quality. On this structure have negative effects the lower insulin concentration also. NEBAL can be considered a physiologic status and so cannot be eliminated in the HMG cow. Instead it can possible to manage its amplitude and its duration from the last weeks of gestation until to the resumption of the uterus receptivity to a new pregnancy or 50-60 days after parturition. There are several techniques in order to assume at cow the greatest possible quantity of nutrient during the transition phase and during the lactation peak in order to overcome all the chemical, hormonal and mechanicals limitation to ingestion ability. Several factors are able to influence the cow ingestion ability but are often difficult to assess. Today goal is to increase the ability to diagnose the NEBAL amplitude mainly in the initial phases. Determination of nutrition status (BCS) is one of the most popular and most used system. Detection of BCS or rather the score difference from parturition until first weeks of lactation, although economic and simple to perform, has limitations related to the survey subjectivity and the low sensibility of the method. Today we are looking for low cost biomarkers able to quantify the extent of NEBAL and related metabolites. The fat and protein's milk concentration and their relationship in the first

75 days of lactation are interesting because they have adequate sensitivity and specificity. A fat percentage more than 4.8% is an expression of high weight loss and a fat/milk protein ratio >1.4 indicates a ketosis risk. The latter biomarker has a more than 50% sensitivity and specificity. A company specializing in milk analyzers has developed a fat milk dissection infrared technique (FTIR). It is well known that fat's milk is made by different length and saturation fatty acids. If in the milk of first weeks of lactation prevail the long-chain saturated fatty acids ($>C:16$) we could think that they stem mainly from fat stores rather than from the rumen where are produced short-chain saturated fatty acids such as acetic and butyric acid. The same technique has been used for the quantitative determination of individual milk's BHBA. The direct measurement of NEFA blood cows in the first weeks of lactation is a reliable system but it's more expensive. The paradox of sub-fertility in dairy-cows is in its hormonal structure. In theory a higher GH concentration makes the cows more fertile due to the increased IGF-1 hepatic production that it entails. Cows with these hormonal and metabolic structures are more likely to increase in length and amplitude the post-parturition negative energy and protein balance. The deep and full critical review of the breeding techniques and HMG dairy cow's nutrition with selective extra care could make compatible the dairy cow's production capacity and fertility.

PERIPARTAL STRESS: INTERFERENCE WITH REPRODUCTION

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It is well known that dairy cow fertility has decreased while milk yield increased over the last 50 years. Periparturient and production diseases are among the main stress factors affecting fertility. The number of days to onset of pregnancy in healthy cows is ~86 days, for those treated for hypocalcaemia, 95 days; mastitis, 100 days; dystocia or retained fetal membranes, 108 days; endometritis, 115 days; lameness, 123 days (Borsberry & Dobson 1989). In parallel with the decrease in fertility, the percentage of cows expressing full oestrus behaviour (standing to be mounted; STBM) has halved from 80% to 40%, as well as a decrease in the average duration of STBM (from 15 hours to ~2 - 5 hours).

We asked if there was an association between production diseases and behaviour. To do this, we used a precise scoring system to quantify oestrus intensity and found that severely lame cows had a much lower total oestrus score than healthy cows; lame cows had reduced mounting activity and displayed less sniffing behaviour (Walker et al 2008a). Lame cows also spent more of the day lying down but less time standing, walking and displaying oestrus behaviour (Walker et al 2008b). Hormonal profiles revealed that progesterone and oestradiol concentrations were lower in lame cows; non-ovulating lame cows had fewer LH pulses in the late follicular phase and the LH surge was absent (Morris et al 2011).

Further detailed studies on oestrus behaviour used several different methods simultaneously to quantify the intensity of oestrus behaviour in the same cows on the University commercial farm: researcher observation of cow behaviour every 15 min throughout the follicular phase, usual observations by farm staff, heat mount detectors, pedometers, neck collars and milk progesterone measurements. Scratchcards were only 36% efficient at identifying potential oestrus events (periods of low progesterone in cyclic cows); KaMaRs, farm staff and neck collars each similarly identified 56-63%; the most efficient was the combination of farm staff observations and pedometers or neck collars, 75% (Holman et al 2011). On another commercial farm, pedometers detected more potential events than the farm staff (71% versus 55%) and a combination of farm staff and pedometers identified 78% (Russell et al, in preparation). The most important finding on both farms was that 20-25% events were not identified by any method – in other words, no signs of oestrus were expressed. And for how many of these events do we (vets and advisors) blame the farm staff for not being good at oestrus detection? There were more ‘silent’ potential oestrus events in lame cows, in those with low body condition scores (<2) and those with higher milk yields. Progesterone concentrations were lower for 10 days prior to the potential event, and lower for one day longer in the follicular phase. Irrespective of the type of production disease, another investigation revealed that two moderate clinical conditions extended the post partum onset of luteal activity by 10 days, a similar delay to one severe affliction (Peake et al 2011).

Some cows do, however, display oestrus and are inseminated but do not establish a pregnancy – they become repeat breeders. Ten percent more repeat breeders had previously had an untreated vulval discharge – indeed, most treatments are focused on the ovary (Canu et al 2011). One approach to establishing a pregnancy in repeat breeders was to inseminate for a 4th time then add an embryo 6 or 7 days later – this

increased the pregnancy rate from 30% to 51% (Canu et al 2011). However, simply one injection of GnRH on Day 11 after the 4th insemination increased the pregnancy rate in repeat breeders from 24% to 52% (Sheldon and Dobson 1993).

On reflection, rather than either of the above treatments, it would be better to reduce the incidence of production diseases. A review of the literature reveals that the incidence of retained fetal membranes, hypocalcaemia and dystocia has reduced for each from ~10% to 2 - 4% over the last 30 years (Hayes et al 2012). This is probably due to a combination of genetic progress and improved animal husbandry. However, the incidence of lameness and mastitis has stubbornly remained at 30% and 15%, respectively (Hayes et al 2012).

In conclusion, the dairy industry relies on efficient reproduction for its future. A better understanding of the exact problems reducing this efficiency will help to overcome the situation. Reproduction does not take place in isolation. There is considerable interaction with the environment. If an animal is under too much stress, she will subdue her usual responses until conditions improve. Reductions in fertility are usually temporary and the underlying mechanisms need to maintain a reversible aspect.

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DRUGS AND HORMONES IN BOVINE REPRODUCTION

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ABSTRACT - Bovine constitutes the most important species on global level for the provision of the food, protein and energy sources for human nutrition. More than ¼ of the world production and consumption of meat and milk is provided by bovines. Hormonal pharmacotherapy, in comparison with the other groups of medicaments, is used to modify the physiological processes such as: superovulation, estrus synchronization, as well as the prevention and treatment of several main uterine pathologies after calving. The values of a hormonal therapy are related to the renewal of the normal activity of an organ, the interruption of actions that are not related to a normal physiological activity, (e.g the use of a progestin in higher doses than the physiological ones, leads to anestrus and lack of estrus which is exploited e.g in female dogs, mares, etc) or anti-inflammatory effect of the corticosteroids. Hormonal drugs are also characterized by dose dependent effects, e.g Progesterone in low doses favours the release of hypophysial gonadotropins, whereas in high doses it prevents their secretion, or low levels of estrogens. In milking cows, during the period between two estruses, it stimulates the mammary tissue by favouring milk production, whereas high levels of estrogens in cows during estrus or after their use reduce milk production.

INTRODUCTION - Pharmacotherapeutic substances influencing the sphere of reproduction, depending on their effects might be grouped as drugs or hormones of substitute therapy, of pathogenetic therapy as well as of pathogenic cause. The values of a hormonal therapy are related to the renewal of the normal activity of an organ, the interruption of the actions not related to a normal physiological activity, (e.g the use of a progestin which leads, higher than physiological doses, to anestrus and lack of estrus which is exploited e.g in female dogs, mares, etc) or anti-inflammatory effect of the corticosteroids. The majority of the hormones are characterized by a really short $t_{1/2}$ (Progesterone 4', ACTH 6') because they are metabolized rapidly. Thus there have been attempts to extend the period that they stay in an organism, such as acetylation of steroid hormones.

The drugs and hormones that are used and recommended nowadays in the field of reproduction are numerous and by far the most widely studied, however research to find single use substances as well as combinations with a lot of simultaneous effects is still going on. It is not a coincidence that nowadays combinations of antibiotic with anti-inflammatory or combinations of sulphamidic with anti-inflammatory etc, are still used even in reproductive pathologies of bovines.

While classifying or grouping different pathologies of reproduction in milking cows I think that it would be better if we considered the grouping of pathologies according to the anatomical parts that make up the reproductive organs in cows (Turmalaj et al., 2010).

Hormonal therapy in this pathology still remains considerably attentive and diverse (follicular lutein cysts) ovary, thus not escaping the context of their possible causes and the mechanism of action for the substance chosen for intervention. Several substances might be used in this pathology, such as: Progesterone, LH or LH analogues, GnRH, GnRH and agonist (cystorelin, buserelin, lecirelin), PGF 2α and its analogues (dinoprost, cloprostenol, D-cloprostenol, alfaprostol, luprostitol, fenprostalen), the complex calcium-naloxone, etc. Each of these hormonal substances is used for a certain effect or purpose, e.g GnRH and its synthetic analogues induce the release of a LH similar-ovulation peak which is able to cause luteinisation of the cyst or ovulation of the present follicle followed by the emergence of a luteal phase; or the complex calcium-naloxone is able to compete directly with the G proteins, the receptors for opioides, i.e. they remove the internal opioides connected to the ovaries; or GnRH + PGF 2α to produce firstly luteolysis and later luteinization (Selami et al., 2010).

The pharmacotherapeutic efficiency of these pathologies is also influenced by the reduction or inhibition of the inflammatory processes of the tubular part of genital organs. Considering numerous studies carried out on ethiopathogenesis of the repeated estruses it results that progesteronemia plays a key role, especially during the first week after insemination. The low level of progesterone in this period retards cellular development and differentiation.

Delayed embryonic development might inhibit recognition of pregnancy by the mother for lack of protein synthesis, especially of the interferon τ , which prevents luteolin. The most efficient intervention even in these disorders is done by means of hormonal therapy such as progesterone, GnRH, hCG, somatotropine, etc. Somatotropine obviously promotes embryonic development and concentration of the circulatory IGF $_1$ even in the uterus, which promotes embryonic growth and differentiation by modulating simultaneously protein synthesis, especially τ interferone.

Numerous studies have been carried out to evidence the effect of progesterone related to the evolutionary characteristics of the dominant follicle by treating animals with low doses of intravaginal progesterone (PRID). These have evidenced such indices as growth, speed, static, domination, size and ultrasound view of the above mentioned follicles. The results have shown that the influence of progesterone is inalterable in these processes.

Uterus infections otherwise known as metritis, include inflammation of the uterus as a result of the damaging effects of different bacterial causes. Uterus infections constitute an important problem for the industry of milk production by influencing the reduction of reproductive capacities of cows as well as the reduction of milk production (BonDurant, 1999). Cows suffering from uterus infection immediately after post calving, exhibit a lower level of fertilization and an increase of the copulation index. Metritis in cows directly influences other reproduction pathologies, such as: cervical and ovary infection, the creation of cysts, infection of mammary glands (mastitis) and in some advanced stages even suppuration of the uterus (pyometra), mummification as well as maceration and putrefication of the fetus. Bacterial infections of the uterus influence the ovaries through the effect on the main endocrine gland of hypothalamic-hypophyseal (Sheldon, 1998).

Accurate diagnosis of uterine pathologies through direct examination with vaginoscope, rectal palpation and later laboratory diagnosing makes up the essence of management of all pathologies of reproduction (Loder, 1993). Diagnosis of uterine infections within 10 days after calving is relatively slight and might be done through direct infection of the uterus by means of a vaginoscope (Williams et al., 2005). A cow suffering from uterus

infection has clinical signs, such as temperature, suppurative discharge from the uterus or vagina as well as delay of the uterus involution (LeBlanc et al., 2002).

Traditionally uterus infection has been classified into acute endometritis, with inflammation of the uterus mucus only, with turbulent discharge and terrible odour, appearing up to 14 days after calving. Subacute and chronic endometritis with limited discharge, lack of clinical signs appears after 14 days following calving. Inflammatory metritis of the three uterus strata is accompanied with macropurulent discharge up to suppuration of the uterus (pyometra) (Selami et al., 2009). The definition of the stage of uterus infection is based on the system of evaluation with points of the endometritis according to Sheldon I.M (2005). The basic criteria of this evaluation are based on the character of the mucus; clear, with drops of pus, white pus, creamy pus or pus containing blood (0-1-2-3 pike), as well as based on the presence of smell in the mucus, dividing it into odourless and with strong odour (0-3 pike, Sheldon et al., 2002).

Final evaluation in several cases must be accompanied with additional laboratory evidence, such as cytological by the vaginal smears, antibiogram to identify bacterial sensitivity of cultures to antibiotics. Advanced diagnosing is carried out through biopsy, which provides complete information on the degree of inflammation, the functional status of endometrium and formulates the respective prognosis (Bonnett et al., 1993).

The incidence of uterus infection observed in studies carried out recently is variable, conditioned by a series of factors.

The factors that influence the increase of incidence of uterus infection include:

- Lack of correct implementation of the program of monitoring cows during the period of drying up, especially two months after calving up to uterus involution.
- Lack of identification of subclinical metritis,
- Lack of treatment in time other reproductive pathologies, such as: vaginitis, ovaritis, cystitis and mastitis.
- Shortcoming in the implementation of breeding conditions, food balance, milking hygiene, technical rules during insemination, vaginal and rectal controls, and insufficient care for heifers reproducing for the first time.

Uterus infection in cows has an incidence up to 14% of the total of diseases treated in milking cows. Uterus infection includes an incidence up to more than 35% of reproductive disease. The incidence of uterus infection influences the increase of incidence of other pathologies of the reproductive organs. Cows with acute endometritis increase the incidence of their copulation index up to 8-12%. The highest incidence of uterus infection appears starting from two weeks up to two months after calving with uterus involution. In this period the incidence increases up to 10% compared to the period post partum. In cows of advanced age with more than six lactations the incidence is higher, up to 15-18% compared to the new cows. The economic damages caused in milking cows by the presence of uterus infections are considerable in comparison with other pathologies of reproductive organs, or all the treated pathologies. The main damages include decrease of milk production and meat.

They increase copulation index, decrease fertilization, increase the cost of milk and meat production, decrease the stage of exploiting food portion, increase the expenses for veterinary treatment, increase the incidence infertility up to damage of the cow (LeBlanc et al., 2002).

Studies based on the information by NAHRS (National Animal Health Reporting System) have managed to draw the real cost of reproductive diseases. Cost evaluations have been compared to cattle used for meat production and milking cows. Assessment of total annual costs is based on fertility, abortions/failures, distocya, placenta retention,

uterus infection to the amount of 441-502 million \$ for meat production and 473-484 \$ for milk production.

The part about aggregate expenses is added to this cost, thus going up to 1 billion \$, expenses per year. The annual cost of uterus infection results up to six times higher in relation to the cost of respiratory pathologies. Specifically $\frac{3}{4}$ of the reproductive diseases costs go to infertility, uterus infection and more specifically to the damage of calves within the 24 first hours of life. The national aggregate cost in USA has been calculated between the expenses for meat production with the expenses for milk production. The final result highlights that the expenses about milk production have been almost three times higher than in milking cows (52.60 \$/head/year), than in cattle for meat production (14.00 \$/head/year) (Gilbert and Schwark, 1992). The final result is not contradictory, because the number of head for meat production is three times higher in relation to the head for milk production.

The main factors that influence the increase of costs for milking cows are summarized in the loss of milk production as a result of uterus infection, dystocia and placenta retention. Loss of milk production by the uterus infection is calculated with $\frac{1}{3}$ of all milk production costs. To improve the costs of milk production the implementation of the programme of uterus infection treatment is of great interest, in order to have the highest possible degree of fertility.

The causes that predispose uterus infection are numerous. Depending on their origin they might be: environmental, nutritive, or by uterus damages during assistance. The basic causes are bacterial, viral, thrush and protozoal infections (Sheldon et al., 2005). The above mentioned causes or their combination influence on the appearance of uterus infection. The nutritive factors include protein imbalance in dried cows, calcium deficiencies, vitamins A and D, selenium, food of conjugated fatty acids (CAL), which influence the recovery of the uterus and normal involution. The main factors that cause viral uterus infection are yet pathogenic, which have found conditions for growth and colonization in the vagina and uterus disrupt the balance of the usual microflora by damaging the useful bacteria, such as lactobaciles etc.

The main pathogenic bacteria that cause viral infection include *E. coli*, *A. pyogenes*, *Peptostreptococci spp.*, for acute endometritis, as well as *A. pyogenes*, *P. vulgaris*, *F. necrophorum* for subclinical endometritis. The disruption of the vaginal and uterus microflora enables the appearance of uterus infection, and the reduction of the degree of immunity of the epithelial cells of the endometritis and the overall immunity. The immunological imbalance is the result of disruption of the function of neutrophils, disruption of the useful bacterial microflora in vagina and uterus.

A predisposing factor that stimulates the growth of pathogenic bacteria are irregular treatments of cows two months after calving. In the cases of treatment with progesteron and glucocorticoid the formation of a new yellow body is encouraged with an increase of the progesterone concentration in blood, with the stimulation of the pathogenic bacteria to the defensive mechanisms of the uterus (Selami et al., 2009). Treatment of uterus infection has been improved in the last decade in relation to the traditional treatments used a long time ago. Treatment according to the traditional method included massive infusions in the uterus inflamed with disinfectant permanganate solution, rivanol accompanied by antibiotic and direct sulphamid to the uterus. The time of treatment of uterus infection is based on the destruction of pathogenic bacteria by using antibiotics of a wide spectrum, of relatively not long period to eliminate bacterial resistance. Studies show that the use of oxytetracycline, ceftiofur, cefquinone are of great interest for acute endometritis and cefapirine (Metricure), oxytetracycline, for the subacute and chronic cases (Chenault et al., 2001).

The use of chemotherapy improves the contracting force of the uterus to provide the extraction of pathologic content by the uterus.

Over the recent years treatment of uterus infection has been carried out in combination with the prostoglandines with the estrogenic. Removal from use of the estrogenic esters is recommended for this purpose, because the estrogenic esters are accompanied with residues in the product taken by the treated animal, and for this reason it is forbidden by the respective references of FDA.

The most effective scheme in using chemotherapy always includes the use of prostaglandin, which helps in eliminating the immunosuppressive influence that progesterone has on the epithelial cells of the endometrium. Prostaglandin influences the elimination of the corpus luteum, it simultaneously improves uterus contraction and eliminates the immunosuppressive effects of progesteron (Lewis, 2003). Studies prove that the use of prostaglandin in cows with uterus infection directly stimulates the immune function of the endometrium cells. Contraction effects of prostaglandin on the uterus by promoting the extraction of pathologic content out of the uterus, they simultaneously eliminate pathogenic bacteria established within the endometrium crypts. If these bacteria are not eliminated in the presence of progesterone they are colonized and bring back uterus infection by increasing the incidence of recedes.

Studies show that prostoglandin F2 alfa serves primarily as the pro-inflammatory molecule to stimulate production of proinflammatory cytokins, with reinforcement of leucotrien production B4. Proinflammatory cytokins and L-B4 reinforce phagocytosis and the function of lymphocytes. Interdependence is thought to be present between prostaglandin F2 alfa and the level of L-B4, proinflammatory cytokins, phagocytosis, function of lymphocytes and the elimination of the negative effects of progesteron, as an immunosuppressor of the endometrium cells. The used F2 alfa prostaglandin encourages luteolysis, stimulates contraction of the uterus by giving efficiency in healing uterus infection (Dhailwall et al., 2001). Among the most successful chemotherapeutic treatments for uterus infection yet is the combined use of F2 alfa prostaglandin, with antibiotics of a wide spectrum with a priority of the new generation of the ceporins, quinolons, macrolids and oxiteraciclins.

CONCLUSIONS

1. In milking cows contamination of the uterine cavity after calving is inevitable and the presence of pathogenic bacteria usually causes clinical diseases of vaginitis, endometritis, metritis, as well as pathologies of the ovaries.
2. These pathologies are accompanied by reduction of fertility, delay in uterine involution and the time of the first ovulation after calving. These pathologies influence the appearance of the yellow body and reduction of the pregnancy rate with the first mating.
3. All these factors require efficient diagnosing and treatment in time and with efficient schemes by implementing the program of control and management of reproductive diseases.
4. Stimulation of the endocrine environment also stimulates immune responses by showing the interaction between the immune and endocrine system as well as the basic mechanisms to preserve a healthy and functional uterus. The application of prostaglandin plus oxytocin enables stimulation of the immune and endocrine system.

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