A021 Folliculogenesis, Oogenesis and Superovulation

Effect of replacing ECG for FSH on follicular dynamics in crossbred wooless sheep during seasonal anestrous: preliminary results

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The equine chorionic gonadotropin (eCG) is a hormone commonly used to synchronize estrus and ovulation induction in sheep. An alternative is the use of Follicle Stimulating Hormone (FSH), because it has lower immune response, greater commercial availability and lower cost. The objective of this study was to evaluate the effect of FSH to replace eCG on ovarian follicular development crossbred wooless sheep. The experiment was conducted in the State University of Mato Grosso do Sul (UEMS), Aquidauana University Unit - MS (20º 28'S, 55º 48' W). It were used adult females (n = 13), multiparous, randomly distributed into two experimental groups that received intravaginal device impregnated with 60 mg of medroxyprogesterone acetate (MAP; Progespon ®, Tecnopec) for 10 days. Upon device withdrawal animals received different intramuscular hormonal treatments: G1 (n = 6) - 500UI eCG (Novormon ®, Syntex, Argentina) and G2 (n = 7) - 10mg of FSH (Folltropin®, Bioniche, Canada). To characterize follicular development, ovaries were examined 12 h after hormones insertion and then at intervals of 12 h until 96 h, using an ultrasound (Pie Medical Aquila, Benelux B.V) provided with a 5.0 MHz transvaginal transducer. Diameters of the largest follicle and their respective growth rate were recorded. The largest follicle average diameter from animals treated with eCG was 4.18 ± 0.52 mm, whereas the animals treated with FSH was 3.76 ± 0.33 mm. The growth rate was 1.44 ± 0.26 and 0.86 ± 0.15 mm/day, respectively, for females treated with eCG or FSH. These preliminary results show that eCG can possibly be replaced by FSH in induction protocols of estrus in female sheep wooless in a period of seasonal anestrus.

A022 Folliculogenesis, Oogenesis and Superovulation

Ultrastructural analysis of goat preantral follicles after long-term *in vitro* culture in dynamic medium containing growth differentiation factor 9 (GDF-9) and follicle-stimulating hormone (FSH)

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Keywords: caprine, folliculogenesis, ovary.

The aim of this study was to evaluate the growth differentiation factor 9 (GDF-9) and follicle-stimulating hormone (FSH) association effects, in a dynamic medium, on the caprine preantral follicles ultrastructure when cultured in ovarian tissue for 16 days. Ovarian fragments were cultured in αMEM+ alone or supplemented with GDF-9 (200 ng/ml) added during the first half of culture period (days 0–8) followed by addition of FSH (50 ng/ml) (days 8–16) (GDF-9/FSH) at 39° C and 5% CO_2. Non-cultured (control) and cultured fragments were processed for ultrastructural analyses. The follicles from non-cultured control and from treatment with GDF-9/FSH showed preserved oocytes and intact both plasma and nuclear membranes as well as large oocyte nuclei. In addition, the organelles, especially mitochondria and endoplasmic reticulum, were uniformly distributed in the ooplasm. However, after 16 days of culture, the GDF-9/FSH treatment promoted discreet increase of vacuoles in comparison to follicles from the control group. Granulosa cells were normal and well organized around the oocytes, presenting elongated nuclei and a high nucleus-to-cytoplasm ratio. Moreover, the mitochondria and endoplasmic reticulum were most of the organelles in oocyte and granulosa cells in follicles from both groups. Severe ultrastructure alterations suggesting degeneration were noticed in follicles cultured in αMEM+ alone for 16 days. Follicles contained high numbers of vacuoles spread throughout the cytoplasm of all cells, which often fused to create large vacated areas. In conclusion, the supplementation with GDF-9 and FSH maintains follicular ultrastructure of goat preantral follicles during the long-term in vitro culture.

A023 Folliculogenesis, Oogenesis and Superovulation

Expression of androgen producing enzymes in low and high follicle count Nellore cows

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Keywords: androgens, follicle, nelore.

During follicle development androgens are synthesized by tecal cells under LH influence through the enzymatic conversion of the androgens precursors. In bovines, androgen concentration in the ovary is positively correlated with the number of antral follicles (Mossa et al., Reproduction 140:713-20, 2010). This experiment was design to evaluate steady-state levels of androgen producing enzymes (*CYP11A1, CYP17A1, HSD3B, HSD17B)* and FSH receptor (*FSHR*) mRNA in high follicle count (HFC) and low follicle count (LFC) cows. Sixteen Nelore heifers (24 months old approximately) were kept in *Brachiaria brizantha* grass and were supplemented with a mix of grains. Estrous cycle was synchronized with two doses of PGF2α 11 days apart. To determine the number of follicles for each animal an ultrasound device (US; Mindray Vet DPS 2200, Brazil) equipped with a 7.5-MHz probe was used to perform three exams one day after ovulation of subsequent cycles. Animals were slaughter approximately 24 h after ovulation of the last cycle. Ovaries were collected and transported in saline solution (0.9%) on ice. Three follicles from 2-4 mm in diameter were dissected from the CL contra lateral ovary of each cow. Total RNA was extracted using RNeasy Microarray Tissue Mini Kit (Qiagen, USA). Gene expression was evaluated using oligo-dT reverse transcription, followed by Sybr Green Master Mix reaction chemistry and a Step One Plus (Applied Biosystems, USA) Real-Time PCR Detection System. Each sample was analyzed in duplicate reactions. All CT values for genes of interest were normalized to the housekeeping gene *PPIA* using the ΔC_T method. The mean ΔC_T from the three follicles of each cow was analyzed using median and Ansari-Bradley tests from the Proc NPAR1WAY procedure of SAS (SAS 9.2). There were considered the group effects (LFC x HFC) and follicle diameter. Mean follicle number for Nelore cows were 30. Ten animals were considered to be in the LFC group (Mean = 16.3; 10<20) and six animals were in the HFC group (Mean = 53.3; 43<67). Expression of *CYP11A1, CYP17A1, HSD3B* was greater (P<0.05; Fold change 1.62, 1.81, 1.23, respectively) for follicles derived from HFC group when compared with follicles derived from LFC group. There were no difference in expression of *HSD17B* and *FSHR*. There was no effect of follicle diameter in the expression of the genes of interest. In conclusion, high follicle count might be the result of higher expression of androgen producing enzymes, as no change in *FSHR* was observed.

A024 Folliculogenesis, Oogenesis and Superovulation

Cluster analysis and artificial neural network on the superovulatory response prediction in mice

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Keywords: artificial neural network, cluster analysis, superovulation.

In mice, research involving embryo production needs to induce several animals to death. These not always provide a significant amount of embryo after the superovulation (SOV) process. Thereby, the objective, with this work, was to develop math methods to predict the amount of embryo that females (M. musculus) produce after SOV and copulation. The ultimate goal is to avoid unnecessary deaths and adhere to the 3Rs ethic principles. It was utilized the artificial neural network (ANN) and the cluster analysis (CA) as prediction models. The ANN elaboration was performed by the software MATLAB while to the CA were utilized the softwares Microsoft Excel 2010, XLstat and PAST. It was superovulated 118 mice (Swiss Webster strain) by hormones administration (eCG and hCG; 10IU/0.1mL, via i.p. with an interval of 48 h apart each other), with the following entrance variables evaluated to each SOV: female (F) and male (M) weight (g) at the embryonic recovery (ER) day; copulatory plug presence; reflux presence after eCG and/or hCG injection; F and M age (days) at the day of ER; animal facility location (A or B); F and M origin (in-house or out of the Campus); viable embryo amount (VEA) obtained per F; season in which the embryo was recovered; light intensity (lux) and temperature (ºC) in the F cage after SOV and previously to copulation. The best simulation obtained from ANN was 87% accurate to predict VEA (97 cases in 118). In the CA the aim is from values of the entrance variables to group females that demonstrate the same pattern to obtain a determined VEA. To the CA it was used a similarity matrix, utilizing the Gower's similarity index and algorithms from the Aglomerative Hierarchical Method to cluster the animals. The entrance variables utilized were not robust enough to discriminate the animals in distinct groups. The SOV involve undetectable variables (odor into the cages, animal manipulation, noise, stress, etc) and unknown that might be very important to the superovulatory response and to cluster the animals. The ANN was effective in the prediction of the response after SOV mainly ranging from 0 to 3 viable embryos per ER. The ANN use to predict mice VEA on embryo recovery could, at least partially decrease unnecessary death of superovulated females which produce none or few viable embryos. Results partialy presented at the 1st Workshop on Veterinary Biosignals and Biodevices (Portugal, 2012).

A025 Folliculogenesis, Oogenesis and Superovulation

Identification of SIRT 1 and BCL11A in maturation of bovine oocytes submitted to heat stress: preliminary results

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Keywords: heat stress, maturation, oocyte.

The sirtuins are a group of enzymes related to binding or silencing of genes involved in a feedback mechanism that preserves the cells viable longer when subjected to stressful situations. Among mammalian sirtuins, SIRT1 controls cell differentiation and survival and are implicate in transcriptional repression mediated by both BCL11A (Senawong, Arch Biochem Biophys, 434: 316-31). The aim of this work was to identify SIRT1 and BCL11A in the bovine oocytes maturation process when submitted to heat stress in vitro. Bovine ovaries were collected at a local slaughterhouse and transported to the laboratory. Only excellent quality cumulus-oocyte complexes (COCs) were selected and stained by Brilliant Cresylblue (BCB - Catalá, Reproduction, 142:517-527) for 90 minutes and then classified in BCB+ (stained blue cytoplasm showing cytoplasmic maturation) and BCB - (cytoplasm unstained). After that COCs BCB+ and BCB- were placed in an incubator at high humidity and 5% CO_2 in air at temperatures of 37°C, 38.5°C and 40°C for 24 h. For the control group it were used COCs not matured. The identification of BCL11A and SIRT1 and immunohistochemistry were performed by confocal microscopy. The samples were prepared with the pipetting of COCs so that only the first cell layers stayed attached to the oocytes; oocytes and cumulus cells were evaluated in parallel. COCs were exposed to primary antibodies, monoclonal antibody to SIRT1 or polyclonal antibody to BCL11A (1:100; antibodies-online, Aachen, Germany), staining the nucleus (SYBR Green, Molecular Probes, Goettingen, Germany; 1:300) and then exposed to secondary antibody (Alexa Fluor 546; Molecular Probes, Goettingen, Germany; 1:1000). It was observed signal for both SIRT 1 and BCL11A in the oocyte and cumulus cells at all temperatures. However, the staining pattern was altered between groups. For SIRT1 was observed gradual increase of cell labeling with the increase of maturation temperature. However, when we used BCL11A antibody, an increase in cumulus cells and a decrease in oocyte labeling, were observed as the maturation temperature increased. Apparently there was no difference between BCB+ and BCB-, showing that these proteins probably are not influenced by oocyte quality. These results supported the hypothesis that there is a relationship between sirtuins, BCL11A and COCs in response to heat stress adaptation, which could affect the bovine oocytes development and competence potential.

A026 Folliculogenesis, Oogenesis and Superovulation

Evidence for the participation of SIRT 1 and 2 in the processes involved in the maturation of bovine oocytes under conditions of *in vitro* heat stress - preliminary results

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Keywords: heat stress, maturation, oocyte.

One cause of poor performance of farm animals in heat stress situations is the adaptation that they suffer after exposition to high ambient temperatures. The precise regulation mechanisms at cellular level, especially in farm animals are poorly known. Sirtuins act as a switch between energy metabolism and cellular signaling pathways associated with other physiological processes, among them aging and apoptosis. The aim of this study was to find evidence of the SIRT 1 and SIRT 2 participation in the bovine oocytes maturation under in vitro heat stress. Bovine ovaries were collected at a local slaughterhouse and transported to the laboratory. Only excellent quality cumulus-oocyte complexes (COCs) were selected and stained by Brilliant Cresylblue (BCB; Bhojwani et al., Theriogenology 67:341, 2007) for 90 minutes and then classified in BCB+ (stained blue cytoplasm showing cytoplasmic maturation) and BCB- (colorless cytoplasm). After that COCs BCB+ and BCB- were placed in an incubator at high humidity and 5% CO_2 at temperatures of 37°C, 38.5°C and 40°C for 24 h. For the control group non maturated COCs were used. The SIRT1 and SIRT2 identification of was performed by immunohistochemistry for confocal microscopy. The samples were prepared by pipetting of COCs so that only the first cell layers stayed attached to the oocytes; oocytes and cumulus cells were evaluated in parallel. This material was fixed in 3% paraformaldehyde and stored in refrigerator until sample processing. After permeabilization and blocking the COCs were exposed to primary antibodies, monoclonal antibody to SIRT1 or polyclonal antibody to SIRT 2 (1:100; antibodies-online, Aachen, Germany), staining of the nucleus (SYBR Green, Molecular Probes, Goettingen, Germany; 1:300) and were then exposed to secondary antibody (Alexa Fluor 546; Molecular Probes, Goettingen, Germany; 1:1000). The slides were individually mounted for COCs. It was observed signal for both SIRT 1 and 2 in the oocyte and cumulus cells at all temperatures. However, the labeling pattern between the groups modified by increasing gradually as the maturation temperature increased. For SIRT 2 intensity of labeling was lower when compared to a SIRT 1, especially in cumulus cells. This shows that there is a possibility of greater demand for SIRT 1 and 2 in heat stress situations. Apparently there was no difference between BCB+ and BCB-, showing that these proteins probably do not suffer influence of oocyte quality. These results support the hypothesis that there is a relationship between sirtuins and COCs in response to adaptation to heat.

A027 Folliculogenesis, Oogenesis and Superovulation

Use of deslorelin plus hCG under different administration routes for inducing ovulation in mares during the spring transition period

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Keywords: deslorelin, hCG, ovulation.

The use of ovulation inducing agents in mares is an essential tool during the breeding season for maximizing the reproductive efficiency. Both hCG and deslorelin are good examples of ovulation inducing agents that may present unsatisfactory results on the reproduction transition period. Deslorelin may present flaws in the transitional period due to limited stock of LH at the time. And hCG, when administered in several doses during breeding season, may present decrease in ovulation rates due to antibody production against the molecule (Squires, E.L. J Eq Vet Sc. v.28, n.11, p.627-34, 2008). This study purpose is to verify the efficiency of deslorelin association plus hCG for ovulation inductiong in submitted mares, comparing i.m. or i.v routes for hCG injection. 50 cyclic mares were used between July and September (spring transition) under artificial lights in open field, aging from 3 to 18 years old (10.5-year-old average) of Paint Horse and Quarter Horse breeds. The mares were sorted in two groups, with homogenous distribution of age and breed. In Group I (n = 50) it was injected i.v. 1,667 UI of hCG plus deslorelin (1.5mg) i.m.. In Group II (n = 50) hCG (1,667 UI) plus deslorelin (1.5mg) were injected i.m.. The drug administration occurred after identification of the first follicle diameter $\geq$ 35mm and uterine edema level 3 (scale1-3) of season. One hundred and four procedures were conducted in Group I and 96 in Group II. After 36 hours after hormone injection, ovulation was detected ultrasonography. In Group I, 87.5% (91/104) of the mares presented ovulation after 36 hours, while in Group II, values were of 80.2% (77/96) for the same evaluation. The hCG injection routes, when associated with deslorelin did not produce any difference (P>0,05). The 80 and 87.5% ovulation rate detected is in accordance to the literature (75 to 84%) with ovulations accouring up to 48 h after the separate injection of either one or the other ovulation inducing agent, independent of the injection routes (Samper, J.C. Therio v.70, p.445-7,2008), but not during the transition period. This current study observed satisfactory ovulation rates in a critical breeding season period. Despite of cost increase in, it was possible to get satisfactory ovulation rates during the transition period, regardless the hCG injection routes.

A028 Folliculogenesis, Oogenesis and Superovulation

Ovarian follicular dinamycs in bovine females with high, medium and low antral follicular counts: preliminary results

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Keywords: antral follicular population, cattle, follicular dynamic.

This research evaluated the antral follicle population in bovine females comparing the follicular growth among high, medium and low antral follicular counting, Braford females (5/8N elore x 3/8 Hereford, n = 137, 9±1-mo-old) were examined by ultrasonography (Áquila PRO, Pie medical, Maastricht, The Netherlands) using a 7.5-convex array transducer, interval of 60d (D0, 60, 120, 180, 240, 300), and antral follicle$\geq$3mm were counted. After evalua tion, 66 females were not used and the remainder were assigned to three groups: G-High antral follicular counting (AFC, n = 24, $\geq$40 follicles), G-Medium (n = 24, 20-25 follicles) and G-Low (n = 23, $\leq$10 follicles). When females (n = 71) reached 24 mo of age, they were submitted to an ovulation synchronization protocol. Randomly during their estrus cycle (D0), they received an auricular device (Crestar®, Intervet-Schering Plough, Brazil) and 2mg EB (Estrogin®, Farmavet, Brazil), IM. At device removal (D8), they were injected with 2mg PGF2α (Ciosin®, Intervet-Schering Plough, Brazil), 300IU eCG (Novormon®, Syntex SA, Argentina) and 1mg EC (ECP®, Pfizer, Brazil), IM. Follicles count and largest follicle diameter were monitored on D0, D5 and daily (every 12h) after the device withdraw until ovulation, and also six days later to the CL diameter evaluation. Data were analized by ANOVA and Tukey test. From the 137 animals, 25 had more than 40 follicles in all ultrasound scans (G-High), 87 females, 11 to 39 follicles (G-Medium), and 25 animals, less than 10 follicles (G-Low). The mean uterine horn diameter (mean±SD) on D0 and 300 was 0.97±0.1 and 1.29±0.2 (G-High), 1.09±0.2 and 1.38±0.3 (G-Medium), and 1.07±0.1 and 1.41±0.3cm (G-Low; p>0.05). The mean body weight on D0 and 300 was 186±22 and 301±28 (G-High), 194±33 and 303±27 (G-Medium), and 188±27 and 300±35kg (G-Low; p>0.05). After synchronization, the mean number of antral follicles on D5 was 47±9.9 (G-High), 24±9.9 (G-Medium) and 9±3.9 (G-Low; p<0.05). The mean diameter of the largest follicle on D5 was 0.66±0.3 (G-High), 0.70±0.2 (G-Medium), and 0.80±0.2cm (G-Low; p>0.05). The mean diameter of the preovulatory follicle differed (p<0.05) between G-High and G-Low (1.15±0.2 vs. 1.32±0.2cm). The interval from the device removal and ovulation was 69.33±5.1 (G-High), 71.25±3.0 (G-Medium) and 70.50±4.1h (G-Low; p>0.05) and the mean ovulation rate was 75 (18/24, G-High), 67 (16/24, G-Medium) and 70% (16/23, G-Low; p>0.05). The mean CL diameter was 1.93±0.3 (G-High), 1.97±0.3 (G-Medium) and 2.04±0.3cm (G-Low; p>0.05). We conclude that there is repeatability in numbers of antral follicles and there are differences in the follicular dynamics between females with high and low AFC submitted to estrous synchronization.

A029 Folliculogenesis, Oogenesis and Superovulation

The intake of white tea does not interfere in the number of corpora lutea in superovulated rats

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Keywords: camellia sinensis, reproduction, white tea.

The white tea originated from *Camellia sinensis* plant is considered a healthy drink, because it is associated with prevention of cancer, cardiovascular disease and osteoporosis and its catechins possess antioxidative, antibacterial and antitumoral activity. White tea is minimally processed; due to this, it has high catechins concentration, approximately 99 mg/g. Despite many potential benefits of white tea consumption, the catechins present in white tea can significantly inhibit proliferation, steroidogenesis and VEGF production by swine granulosa cells, so it is also important to get an insight on the possible reproductive-related consequences. Thus, the aim of this work was to verify the white tea influence in the ovulation rate in superovulated rats. For that, the Wistar rats were sorted in two groups, the control group (n = 30) drinking water ad libitum and the treated group (n = 30) drinking only commercial white tea (Chá & Cia, Brazil) at 2.5% *ad libitum* in an experimental situation as realized by Yang *et al*. (Eur J Cancer Prev, 12:391–395, 2003) e Niwattisaiwong *et al*. (Drug Metabol Drug Interact., 20:43-56, 2004). The medium consumption of white tea was 32.37±2.70 mL/day. This study was approved by ethics committee to be realized. The experiment had lost for three months, and in the end of each month, 10 animals of each group were superovulated with 150UI/Kg of eCG (Folligon®, Intervet Schering-Plough, Brazil) and 150UI/Kg of hCG (Vetecor®, Hertape Calier, Brazil) and sacrificed. After that, the corpora lutea were counted. The chosen statistical analysis was the unpaired t test with Welch's correction and the moments in each group were analyzed by variance analysis, the differences were considered when $p<0.05$. No differences were observed between the groups in the moments observed. Medium value for corpora lutea number in both groups were: first month (control = 61.10±10.94 and treated = 64.10±25.02); second month (control = 64.90±27.77 and treated = 67.60±20.58); and third month (control = 62.30±20.17 and treated = 43.80±20.08). The chronic consumption of white tea for three months does not interfere with the ovulation in superovulated rats.

A030 Folliculogenesis, Oogenesis and Superovulation

Taurine, zebu and bubaline heifers show differences on follicular and luteal dynamics during the estrous cycle when compared at the same environment and nutritional management

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Keywords: follicular deviation, follicular dynamics, heifers.

Considering the impact that nutrition and environment have on reproduction, it is essential to compare the reproductive cycle between bovine (taurine and zebu) and bubaline heifers at the same time and under the same nutritional management. Therefore, the objective of this project was to study the follicular dynamic of Holstein (n = 15), Gir (n = 11) and Buffalo (n = 15) heifers kept under the same conditions. All heifers were housed at the Department of Animal Reproduction, (CBRA/FMVZ/USP), Pirassununga Campus. They were pre-synchronized with two doses of prostaglandin F2α (PGF; D-cloprostenol, 150µg, IM, Prolise®), fourteen days apart (D0 = day of first ovulation). From D0 daily ultrasound was performed (Aloka SSD500, 7.5MHz) to scan the ovaries and track its follicular dynamics (follicles≥ 3mm) until the the second ovulation occurrence. After the second ovulation, the follicular dynamic was performed twice daily to detect the time of deviation of the first follicular wave. The variables were analyzed by PROC GLIMMIX, SAS. No difference was found among the inter-ovulatory interval (22.2±0.7; 22.1±1; 24±0.7 days) between Holstein, Gir and Buffalo, respectively. Differences were present (P<0.05) in at least one of the genetic groups, Holstein, Gir and Buffalo, respectively, for: number of follicular waves (2.8±0.2a, 3.4±0.24b, 2.8±0.13a waves), number of follicles recruited after first (27.7±2.2a, 64.2±17.1b, 22.7±1.2a follicles), and second ovulation (38.1±7.2a, 60.3±11.8b, 27.1±2.8a follicles), diameter of first wave DF (16.4±0.3a, 12.3±0.5b, 12.2±0.3b mm), diameter of second wave DF (11.8±0.5a, 9.4±0.7b, 10.8±0.5ab mm), ovulatory follicle diameter (15±0.4a, 13.7±0.7ab, 13.4±0.4b mm), maximum CL diameter (26.9±0.5a, 22.4±0.8b, 19.2±0.5c mm) and luteal phase length (13.8±0.5a, 16.0±0.8b, 15±0.5ab days). No differences were found among the breeds for the moment of deviation. However, Holstein heifers presented larger DF and SF diameter (FD: 7.9±0.3; FS: 6.9±0.2mm; P<0.05) at deviation when compared with Gir (FD: 6.7±0.3; FS: 6.1±0.3mm) and Buffalo (FD: 6.4±0.4; FS: 5.8±0.3mm). The results herein presented show that heifers from different genetic groups (Bos taurus, Bos indicus and Bubalus bubalis), kept under the same nutritional and environmental status, have different folliculogenesis and luteal growth that are specific to their breed.

A031 Folliculogenesis, Oogenesis and Superovulation

Effect of parity, days in milk and milk yield on the superovulatory response and conception rate of in *vivo* produced embryos of Holstein cows

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Keywords: Holstein, milk production, superovulation.

The aim of this study was to evaluate the effects of parity (1st, 2nd, or 3rd lactation), days in milk (DIM1: 65 to 100 days and DIM2: 101 to 150 days in milk) and daily milk average production (low: up to 23.9, medium: 30.5 and high production: 45.5 L of daily milk production) on the response to a superovulatory (SOV) protocol and conception rate at 30 days of pregnancy (TC30) for in vivo embryos produced in Holstein donors. The study was conducted in a commercial farm (Fazenda Santa Rita - Agrindus S/A) located in Descalvado - SP and the data set had 554 SOV performed from 2007 to 2010. Donors were superstimulated and inseminated at fixed time (P36) with an ovulation inducer (GnRH) 24h after P4 ear implant removal. Immediately before the flush process (7 days after GnRH), the number of CL was recorded. Statistical analysis was performed using SAS with the PROC GLIMMIX. Parity did not affect superovulation (≥ 2 CL P = 0.64), total CL (P = 0.16) and number of recovered structures (P = 0.60) nor the TC30 (P = 0.58). However, 1st, 2nd, and $\geq$3rd lactation affected the recovery [72.2b (1,617/2,239) vs. 77,6a (2,086/2,688) vs. 81.5%a (796/977), P = 0.03], viable embryos [47.6a (769/1,617) vs. 40.2 b (838/2,086) vs. 50.3% a (400/796), P <0.0001] and unfertilized structures rates [42.9b (695/1,617) vs. 50.3a (1,050/2,086) vs. 40.7%b (324/796), P <0.0001], respectively. The days in milk did not affect the superovulation (P = 0.47) and recovery rates (P = 0.27) nor the TC30 (P = 0.93). However, cows before 100 days in milk had a lower percentage of viable embryo [41.9 (127/303) vs. 51.4% (284/552), P = 0.008] and a higher proportion of unfertilized structures [49.5 (150/303) vs. 39.1% (216/552), P <0.0001]. Milk production did not affect superovulation rate (P = 0.94) and TC30 (P = 0.50). However, total CL numbers were reduced in high-producing cows (10.1 ± 0.7) when compared to lower producers (13.5 ± 0.9, P = 0.01). Further, the daily milk average changed the total structures recovered (10.9 ± 0.9a vs. 9.9 ± 0.7a vs. 7.2 ± 0.6b, P = 0.005), recovery [79.4a (1,244/1,567) vs. 78.0a (1,262/1,618) vs.71, 2%b (830/1,165), P <0.0001] viable embryos [47.4a (590/1.244) vs. 43.8b (553/1.262) vs.44.9%b (373/830), P <0.0001] and unfertilized structures rates (43.2b (538/1,244) vs. 48.5a (613/1,262) vs.44.3%b (368/830), P <0.0001); low, medium and high production, respectively. In conclusion, 1st lactation cows have lower recovery rates of oocytes and embryos. In addition, cows in early lactation (<100 days in milk) have lower viable embryo rate. Finally, increased milk production reduces the in vivo embryo production performance of Holstein donor cows.

A032 Folliculogenesis, Oogenesis and Superovulation

Comparisson of the antral and preantral ovarian follicle population between nelore (*Bos indicus*) and ½ nelore x angus (½ *Bos indicus* x *Bos taurus*) females

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Keywords: bovine, follicle antral, follicle preantral.

The aim of the present study was to compare the population of antral and preantral follicles in Nelore (Bos indicus) and ½ NeloreXAngus (Bos indicusXBos taurus) of high and low follicular count. Bos indicus (Nelore, n = 20) and Bos indicusX Bos taurus (½ NeloreXAngus, n = 20) females (72-90m) were subjected to follicular aspiration randomly during their estrous cycle (D0) to withdraw all follicles ≥ 3 mm and to induce the growth of a new follicular wave. Ovaries were examined by ultrasonography (Áquila PRO, Pie medical, Maastricht, The Netherlands) using a 7.5-convex array transducer, during days 4, 19, 34, 49 and 64, and antral follicles ≥ 3 mm were counted. After these evaluations cows were assigned to two groups: high antral follicular counting (AFC, mean ≥ 30 follicles; Bos indicus, n = 7 and Bos indicusXBos taurus, n = 6) and low AFC ≤ 15 antral follicles; Bos indicus, n = 6 and Bos indicusXBos taurus, n = 6). After D64, ovaries were collected at abattoir, processed for histology and serially sectioned at 7μm. One at every 120 sections was mounted and stained with periodic acid Schiff (PAS) and hematoxylin. The number of preantral follicles (primordial, primary and secondary) was estimated using a correction factor (Gougeon e Chainy, 1987. J Reprod Fertil, 81:433-442). Only one ovary per female was analized. There was an attempted to correlate the number of antral follicles with the population of preantral ones. Data was compared using the Mann–Whitney test ($P \leq 0.05$) and correlation analized using cubic polynomial regression. There was repeatability in the numbers of antral follicles during follicular waves per individual of high and low AFC. The mean number of antral follicles (mean±SD) was 35±9 (Bos indicus) and 38±6 follicles (Bos indicusXBos taurus for the high AFC group, and 10±3 (Bos indicus) and 12±2 follicles (Bos indicusXBos taurus) for the low AFC. A large variation in numbers of preantral follicles was observed among individuals within the same group and between breeds. The mean number of preantral follicles in Bos indicusXBos taurus of high AFC (116.226±83.156) was greater (P<0.05) compared to Bos indicus (63.032±58.705). However, there was no difference (P>0.05) between the average number of preantral follicles of Bos indicus (28.324±24.525) and Bos indicusXBos taurus (85.748±129.628) of low AFC. The average number of preantral follicles from Bos indicus of high AFC did not differ (P>0.05) from that of Bos indicusXBos taurus of low AFC. There was no correlation between antral and preantral follicles. We conclude that both the influence of Bos indicus cattle and the taurus breed may contributted for differences between Nelore and 1/2 NeloreXAngus in numbers of antral and preantral follicles.

A033 Folliculogenesis, Oogenesis and Superovulation

Recovery of buffalo cumulus-oocyte complexes in different phases of reproductive activity

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Keywords: buffaloes, ovary, pregnancy.

This experiment aimed to contribute to the national research about folliculogenesis in buffaloes. This study evaluated ovaries of 86 crossbred Murrah/Mediterranean buffaloes obtained at slaughterhouses located in Lençóis Paulista (Frigol®), Rancharia (Better Beef®) and Cajati (Frivale®), in Sao Paulo state. The origin of the animals was not considered; however, the reproductive status (pregnant or non-pregnant) and the stage of pregnancy (early, middle and final) were estimated. From the 86 buffaloes, 33 were pregnant and 53 were non-pregnant. From pregnant females, 13 were in the early, 10 in the middle and 10 in the final stage of pregnancy. Ovaries were transported to Advanced Reproduction and Cell Therapy laboratory of FMVZ - UNESP - Botucatu in plastic bags with saline solution heated at 36°C added of penicillin and streptomycin. The cumulus-oocyte complexes (COC) were recovered by follicular aspiration using a 30 x 8 mm needle and 10 mL syringe. The Mann-Whitney test was used to compare the variables with the reproductive status and the Kruskal-Wallis test was used to compare the variables with the period of pregnancy, considering statistical difference when $p < 0.05$. In total, 714 COC were recovered - 185 (25.9%) grade I (GI), 219 (30.7%) grade II (GII), 73 (10.2%) grade III (GIII), 133 (18.6%) denuded and 104 (14.6%) expanded. The mean/ animal of COC classified as GI, GII, GIII, denuded, expanded and the total number recovered were respectively 2.2, 2.5, 0.8, 1.5, 1.2 and 8.3. When compared pregnant and non-pregnant buffaloes, the latter category presented the greatest number of GI (37 vs. 148, respectively, $p= 0.007$) and of total recovered (194 vs. 520, respectively, $p= 0.001$). The proportion of GI was higher in non-pregnant animals than in all stages of gestation ($p= 0.048$). The proportions of denuded ($p= 0.025$) and of total recovered ($p= 0.013$) were distinct between pregnant and in the early pregnancy stage females. The difference in the number of retrieved COC can be explained by the presence of corpus luteum (CL) in one of the ovaries of all pregnant buffaloes. The CL presence reduced the recovery rate of denuded oocytes ($p= 0.027$) and the total number of COC aspirated ($p= 0.028$/ Mann-Whitney test). This event occurred because the non-pregnant females, which had fewer CL and less frequently, had more follicles in ovarian surface (25.8 ± 16.2 - non-pregnant and 14.7 ± 5.0 - pregnant, $p < 0.001$/ Mann-Whitney test). It was found that when the CL was present, occupied a large area of the ovary, leaving less tissue to follicular development.

A034 Folliculogenesis, Oogenesis and Superovulation

Effect of profertil® on LH profile, ovulation and conception rates in nelore cattle

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Keywords: GnRH, LH peak, ovulation

The aim of the present study was to evaluate the effect of the im injection of Profertil® on LH profile and ovulation rate in Nelore (Bos indicus) cattle previously subjected to follicular wave synchronization. At random stages of the estrous cycle (D-10), Nelore (Bos indicus) cows (n = 95; BCS:3,13±0,03; BW:438,7±5,5kg) and pubertal heifers (n = 125; BCS:3,02±0,01; BW:329,4±2,8kg; age:26,8±0,4 months) were treated with a norgestomet ear implant (Crestar®, Intervet, Brazil) and 2 mg estradiol benzoate (Sincrodiol®, OuroFino, Brazil). On D-2, the implant was removed and 1 mg estradiol cypionate (E.C.P. ®, Pfizer, Brazil), 150 μg D(+)cloprostenol (Prostaglandina Tortuga®, Tortuga, Brazil) and 300 IU eCG (Folligon®, MSD, Brazil) were injected. Cattle were inseminated 48h after implant removal (D0). On D6, only cows and heifers with follicles ≥ 8,5mm (ultrasonography) were allocated into one of three treatment groups: 100μg Profertil® (gonadoreline acetate, Tortuga, Brazil), 100μg Fertagyl® (gonadorelina acetate, MSD, Brazil) or placebo (Profertil® diluent). Cows and heifers were homogenously allocated among groups. The occurrence of ovulation (absence of the dominant follicle) was evaluated on D8 and conception rate on D40. Serial blood collection (-30, 0, 60, 120, 180, 240 and 300 min in relation to treatments with GnRH or placebo) were done in a subset of animals to evaluate LH profile. Ovulation and conception rates were analyzed using PROC GLIMMIX, and LH concentration using PROC MIXED from SAS. No interaction was found among category (cow and heifer) and treatment. The dominant follicle diameter the on D6 was similar (P= 0.81) among animals treated with placebo (11.1±0.2), Profertil® (10.8±0.2) and Fertagyl® (11.3±0.3), demonstrating homogeneity before treatment. Cattle from control group did not have nor LH peak neither ovulation. However, all animals treated with GnRH had a peak of LH. The LH profile was similar between animals treated with Profertil® and Fertagyl®, with average concentration during the peak of 2.4±0.5 and 2.8±0.4ng/mL, respectively, occurring 120 min after treatment. Control animals had any increase on LH concentration, averaging 0.40±0.01 ng/mL during the whole period. Ovulation rate was 48.3% (58/120a) and 28.4% (8/28a) when Profertil® and Fertagyl® were given, respectively, differing from the control group [0% (0/72b); P = 0.0001]. Pregnancy rates were similar (P=0.43) among groups (control: 62.5%, Profertil®: 52.9% and Fertagyl®: 53.6%), regardless formation or not of accessory CL (50.3 vs 50.0%; P = 0.7). In conclusion, 100μg Profertil® is able to induce ovulation in Nelore cows and heifers with previously synchronized follicular wave emergence and presence of follicle ≥ 8.5 mm at treatment.

A035 Folliculogenesis, Oogenesis and Superovulation

Ultrasound biomicroscopic image attributes of ovarian follicles in heifers

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Keywords: follicles, images, ultrasound biomicroscopic.

High-resolution ultrasound biomicroscopy (UBM) with high-frequency transducers (20-50 MHz) has enabled imaging of small antral follicles (<1 mm) and acoustic characterization of the follicular antrum and wall. Quantitative echotextural analysis of the images generated by UBM provides detailed functional information about the granulosa and theca layers of follicle walls. The objectives of our study were to compare biomicroscope image echotextures between dominant and largest subordinate follicles during the first follicular wave and to characterize UBM attributes of the ovulatory follicle. Hereford crossbred heifers (14-16 months old, n = 12) were used. Ovarian examinations were performed transvaginally from Day -4 or -3 (Day 0 = ovulation) to Day 10 using a UBM imaging instrument (Vevo 660; Visual Sonics Inc., Toronto, Canada) equipped with a 25 MHz end-fire transducer. Echotextural assessment was performed by spot metrics of the follicle antrum and follicle wall by using a series of custom-developed computer algorithms optimized for ultrasonography (Synergyne©, Version 2.8, WHIRL, Saskatoon, SK, Canada). Although, subordinate and dominant follicles development were tracked from D-2 (around 1 mm in diameter) to D10, the ovulatory follicle was tracked only from D-4 to ovulation. All data were analyzed by analysis of variance for repeated measures using the mixed procedure (Littell et al., 1998) in the Statistical Analysis System software package (SAS version 8.2 for MS Windows; SAS Institute Inc., Cary, NC). No differences were detected between dominant and largest subordinate follicle in the mean pixel value (MPV) or pixel heterogeneity (PH) of the antrum. However, the MPV of the wall of dominant follicles was greater and PH tended to be greater than that of largest subordinate follicles (P = 0.05 and P = 0.1, respectively). PH of the wall the ovulatory follicle decreased (P = 0.03) from D-4 to D-1; however, no changes in MPV were observed. Similarly, no changes were detected in MPV or PH of the follicular antrum. In conclusion, although no differences in antral characteristics of dominant vs subordinate follicles were detected, mean pixel value and pixel heterogeneity of the follicular wall were higher in dominant follicles, and pixel heterogeneity of follicular wall of ovulatory follicles decreased over days. Quantitative pixel analysis from follicular wall images using UBM could be applied to evaluate follicles in live animals. However, more studies using this technique are necessary.

A036 Folliculogenesis, Oogenesis and Superovulation

Maintenance of isolated bovine preantral follicle viability after *in vitro* preservation in an oocyte transportation machine

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Keywords: culture, moifopa, ovary.

Follicular viability maintenance after the collection and transportation of the ovaries to the laboratories specialized in reproductive techniques is a limitant factor for the success of the in vitro culture of preantral follicles in different species, including bovine. In this way, the objective of the present study was to evaluate the viability of isolated bovine preantral follicles cultured in vitro after preservation for 12 or 24 hours in the oocyte transportation machine® 12 – Compact, (TO; Wta – São Paulo, Brazil). After collecting the bovine ovaries (n = 7), 91 secondary follicles with diameter 200 mm were mechanically isolated and destined to the preservation in TO at 39°C, in TCM 199 supplemented with 20 mM HEPES, 1% bovine serum albumin, 3 mM glutamine, 10 μg/mL insulin, 2,5 μg/mL transferrin, 4 ng/mL selenium, 50 μg/mL ascorbic acid and 100 ng/mL recombinant FSH. Subsequently, the follicles were destined to the in vitro culture in the same preservation medium. A total of five treatments were used: preantral follicles cultured after isolation (T1 – cultured control); isolated follicles preserved for 12 (T2) or 24 h (T3) and follicles cultured after preservation for 12 (T4) or 24 h (T5). The follicles were individually cultured in drops with 100 μl of medium, at 39°C and 5% CO_2 for 7 days. Every other day, 60 of the medium were replaced by a refreshed one. At the end of the preservation and/or culture, all follicles were destined to analyses of follicular viability using the fluorescent markers calcein-AM (4 μM) and ethidium-homodimer-1 (2 μM) for viable and non viable preantral follicles, respectively. Data of viability were analysed by qui-square test and values were considered significant when $P<0.05$. The results demonstrated that follicles from the control, T1, T2 and T4 showed 100% of follicular viability and only the T3 treatment showed 96% of viability after preservation (12 h) and culture for 7 days. However, there were no differences between the treatments in regard to the follicular viability. In conclusion, isolated bovine preantral follicles can be successfully preserved for up to 24 h in the TO, keeping its viability after 7 days of in vitro culture.

A037 Folliculogenesis, Oogenesis and Superovulation

Influence of the ovarian fragment size on the apoptosis rate of ovine preantral follicles preserved *in vitro*

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Keywords: ovary, sheep, tunel.

The aim of the present study was to evaluate the effect of the size of ovine ovarian fragment on the apoptosis of preantral follicles preserved in vitro. 10 ovine ovaries were collected in the slaughterhouse and, for each animal, one of the ovaries was divided into two halves. Subsequently, on half was divided into two fragments (1/4 of the ovary). One of the fragments was subdivided into two fragments of 1/8 of the ovary, being one of them immediately fixed for histology (control). The remaining fragments and the whole ovary were preserved in MEM-HEPES supplemented with antibiotics at 4°C for 24 h. The morphology of ovine preantral follicles preserved in situ was evaluated using histology and the follicles were classified as primordial, intermediate, primary and secondary, as well as in normal or atretic. TUNEL technique was performed in the control and in treatments that showed the best results of follicular survival after preservation, using an in situ cell death detection kit, following the manufacturer´s instruction (Roche Diagnostics Ltd.), with some modifications. Cell apoptosis in the different follicular compartments (oocyte, granulosa and theca cells) was evaluated and cells showing a brown staining were considered TUNEL positive or apoptotic. The rates of follicular survival after in situ preservation were analysed through ANOVA and Scheffé´s test. Apoptosis rate were compared using qui-square test and values were considered significant when $P < 0.05$. The results showed that after preservation, there was a significant decrease in the percentage of normal preantral follicles in all treatments when compared to the control. The percentage of normal follicles after conservation in 1/8 of the ovary was similar to that observed in 1/4 of the ovary, and significantly higher than 1/2 and the whole ovary. For follicular apoptosis detection, 1347, 1676 and 1262 cells were analysed in the control and in 1/8 and 1/4 of the ovary, respectively. There was no significant difference in the percentage of the total of TUNEL positive cells among the different treatments. After analysing each follicular compartment, no apoptotic oocytes were observed and there was no significant difference in the percentage of TUNEL positive granulosa cells. However, a significant higher percentage of apoptotic theca cells was observed after preservation of 1/8 (22.5%) and 1/4 (24.8%) of the ovary compared to the control (5.2%). In conclusion, preservation of smaller ovarian fragments (1/8 or 1/4) at 4°C for up to 24 h did not influence apoptosis rate in oocyte and granulosa cells of ovine preantral follicles. In addition, theca cells seem to be more sensitive to apoptosis after preservation of ovine ovarian tissue. Therefore, due to the greatest presence of primordial and intermediate follicles in ovarian tissue, i.e., follicles without theca cells, and due to the usefulness, it is suggested to preserve ovine preantral follicles in fragments of 1/4 of the ovary, in MEM-HEPES, at 4°C for up to 24 h.

A038 Folliculogenesis, Oogenesis and Superovulation

Follicular survival after *in vitro* preservation of ovine ovarian tissue in the oocyte transportation machine (12-compact®)

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Keywords: ovarian, preantral, sheep.

Maintenance of quality of preantral follicles after ovaries collection and transportation a limiting factor for the success of subsequent in vitro culture, since, in most cases, the female donors, are far from laboratories specialized in reproductive biotechnologies. In this way, the aim of the present study was to evaluate the effect of different periods and media for the preservation of ovarian tissue in the oocyte transportation 12 – Compact® (TO) on the survival of ovine preantral follicles. For this, after the ovaries collection from females without defined breed (n = 5), the ovarian cortex was divided into 13 fragments, being one of the fragments directly fixed in 4% buffered formaldehyde for histological analyses (control). The remaining fragments (one fragment for treatment) preserved for 6 or 12 h in the TO, at 35ºC, in cryotubes containing 2 mL of two different media: The Minimal Essential Medium supplemented with HEPES, 100 mg/mL of penicillin and 100 mg/mL of streptomycin (MEM-HEPES) or MEM-HEPES supplemented with BSA (3 mg/mL), glutamine (2 mM), hypoxantine (2 mM), ITS (6,25 µg/mL of insulin, 6,25 µg/mL of transferrin and 6,25 ng/mL of selenium), ascorbic acid (50 µg/ml) and recombinant FSH (50 ng/ml), corresponding to the MEM-HEPES+ medium. Thus, the experimental design was performed according to the following treatments: fragments of ovarian cortex without preservation (control), preserved for 6 h in MEM-HEPES (T1) or MEM-HEPES+ (T2) and preserved for 12 h in MEM-HEPES (T3) or MEM-HEPES+ (T4). After the preservation periods, the fragments were fixed in 4% buffered formaldehyde and destined to histology. The media pH was measured before and after the preservation periods. Follicles were classified as primordial or developing (intermediate, primary and secondary), as well as in normal or atretic, according to the morphology of the oocyte and granulosa cells. Data of pH of the media were submitted to qui-square test and data of survival to ANOVA and Tukey´s test. Values were considered significant when $P<0.05$. The results showed that there is no significant difference in the initial and final (after the preservation periods) media pH (7.37 and 7.21, respectively). Regarding to follicular survival, a total of 750 preantral follicles were analysed, being 76.6% normal in the control, 62.8% in T1, 70.8% in T2, 54.0% in T3 and 58.0% in T4. All treatments were able to maintain follicular survival after the preservation periods when compared to the control. It can be concluded that two medium (MEM-HEPES and MEM-HEPES+) was efficient to maintain ovine preantral follicle survival after preservation at 35 ºC for up to 12 h in the TO.

A039 Folliculogenesis, Oogenesis and Superovulation

Expression of melatonin receptor (MEL-1A-R) in ovine ovaries

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Keywords: immunohistochemistry, protein, sheep.

The hormone melatonin seems to be involved in follicular development due to the presence of its receptors in ovaries of different species. However, the presence of melatonin type 1 receptor (MEL-1A-R) in ovine ovaries is not known. In this way, the present study aims to verify the MEL-1A-R presence in different stages (primordial, primary, secondary, early and later antral) and follicular compartments (oocyte, granulosa and theca cells) in ovine ovaries. The ovaries (n = 8) were collected at a slaughterhouse and fixed in 4% paraformaldehyde for 18 h. Sections of 5 μm were mounted in glass slides for the immunohistochemistry technique. The slides were incubated in citric acid at 98-100oC for 7 min and endogenous peroxidase was blocked using 1% mouse normal serum, diluted in Phosphate Buffered Saline (PBS). Subsequently, the sections were incubated for 24 h at 4oC with the polyclonal anti-MEL-1A-R antibody(Santa Cruz Biotechnology, USA) (1:40). Then, the sections were incubated for 45 min with biotinylated mouse anti-IgG secondary antibody (Santa Cruz Biotechnology, USA) , diluted 200 times in PBS, containing 1% mouse normal serum. The sections were incubated for further 45 minutes with avidin-biotin complex (1:600). The protein localization was demonstrated with diaminobenzidine and the sections were counterstained with hematoxylin. The negative control was performed through the replacement of the primary antibody by an IgG of the same species in which the primary antibody was produced. The follicles were classified in primordial, primary, secondary, early and late antral. In the different follicular compartments, the immunostaining was classified as absent, weak, moderate and strong. The results showed that there is no expression of the protein for the MEL-1A receptor in primordial, primary and smaller secondary follicles (less than 6 layers of granulosa cells). In addition, it was observed a weak immunolocalization in the granulosa cells of largest secondary follicles (more than 6 layers of granulosa cells) and early antral follicles, and strong in later antral follicles. There was no expression in oocytes and theca cells of any follicular stage. In conclusion, granulosa cells of largest secondary and antral follicles showed the protein presence for the MEL-1A receptor in ovine ovaries.

A040 Folliculogenesis, Oogenesis and Superovulation

Localization of fibroblast growth factor (FGF-2) protein in ovine ovaries

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Keywords: folliculogenesis, immunolocalization, preantral.

The fibroblast growth factor-2 (FGF-2) is an intraovarian peptide involved in folliculogenesis in different species. However, the expression of FGF-2 in ovine ovaries is not known, especially in preantral follicles. In this way, the aim of the present study was to evaluate the expression of FGF-2 in the different follicular stages (preantral: primordial, primary and secondary; antral: early and late) and compartments (oocyte, granulosa and theca cells) in ovine ovaries through immunohistochemical technique. Ovine ovaries (n = 6) were collected in a slaughterhouse and fixed in 4% paraformaldehyde for 18 h, dehydrated and embedded in paraffin wax. Then, sections of 5 µm were mounted in glass slides. The epitopes were activated by the incubation of the slides in citric acid at $98\text{-}100^{\circ}\text{C}$ for 7 min and non-specific binding was blocked using 1% mouse normal serum, diluted in Phosphate Buffered Saline (PBS). Subsequently, the sections were incubated for 24 h at 4°C with polyclonal rabbit anti-human FGF-2 antibody (1:40; Santa Cruz Biotechnology). Then, the sections were incubated for 45 minutes with biotinylated mouse anti-rabbit IgG secondary antibody (Santa Cruz Biotechnology), diluted 200 times in PBS, containing 1% mouse normal serum. The sections were incubated for further 45 minutes with avidin-biotin complex (1:600). Finally, the protein localization was demonstrated with diaminobenzidine and the sections were counterstained with hematoxylin. The negative control was performed through the replacement of the primary antibody by an IgG of the same species in which the primary antibody was produced. Immunostaining was classified as absent, weak, moderate or strong in the follicular stage and compartment described above. According to the results, a moderate immunolocalization of the protein for FGF-2 was observed in the oocytes of primordial and primary follicles, however, the protein was absent in the granulosa cells of these follicles. It was verified a weak expression of the protein for FGF-2 in the oocyte and granulosa cells of secondary follicles. In addition, a weak immunostaining was observed in granulosa cells of early and late antral follicles. There is no expression in oocytes of antral follicles neither in theca cells of any follicular stage. In conclusion, oocytes from preantral follicles and granulosa cells from secondary and antral ovine follicles showed expression for the protein for FGF-2.

A041 Folliculogenesis, Oogenesis and Superovulation

Estimation of the population of ovarian preantral follicles in prepubertal and adult female dogs: preliminary results

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Keywords: bitch, preantral follicles, prepubertal.

The number of preantral follicles in domestic mammals is established during fetal life. Information on distribution, quantification and size of preantral follicles in bitches is scarce. The aim of this study was to estimate the population of preantral follicles in the ovaries of prepubertal and adult female dogs. Eight pairs of ovaries were obtained from eight small and medium size bitches (prepubertal, n = 4; adult, n = 4; 3 ± 1 years-old) undergoing ovariohysterectomy using conventional surgical procedures and pain relief therapy. Immediately after collection, ovaries were cut longitudinally into two halves and immersed in Bouin's fixative for histological processing. Ovaries halves were dehydrated in alcohol, cleared with xylene, embedded in paraffin, and serially sectioned at 5 ųm. Every 70th histological section was mounted and stained with periodic acid-Schiff (PAS) and hematoxylin. The number of preantral follicles was estimated by counting the follicles in each section using the nucleus of the oocyte as a marker and a correction factor (A Gougeon and GBN Chainy 1987 J. Reprod. Fertil. 81, 433-442). Preantral follicles were classified according to the developmental stage as primordial (one layer of flattened granulosa cells surrounding the oocyte), primary (one layer of cuboidal granulosa cells) or secondary (two or more layers of cuboidal granulosa cells). The average number of preantral follicles (mean ± SD) was 3,070 ± 1,560 in prepubertal females and 1,903 ± 481 follicles in adult ones. Preliminary results suggest that there was difference between the number of preantral follicles in the ovaries of prepubertal and adult female dogs.

A042 Folliculogenesis, Oogenesis and Superovulation

Nuclear maturation kinetics of sheep oocytes cultured *in vitro*

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Keywords: *in vitro* maturation, oocytes, sheep.

It has been used various methods to increase in vitro maturation rate, such as, addition of growth factors, fetal bovine serum, serum of female in heat, hormones and follicular fluid to the medium and also co-culture with granulosa and cumulus cells, although these conditions are not similar to the ones presented in vivo. The present study's aim was to analyze the nuclear maturation kinetics of sheep oocytes cultured in vitro. It were cultured 968 oocytes with compact cumulus oophorus and divided into four different treatments: T1 – TCM 199 with 10% of fetal bovine serum (BFS) and 50 μg/mL of gentamicin (basic medium); T2 – basic medium and 10 μg/mL of FSH and 10 μg/mL of LH; T3 – basic medium, 10 μg/mL of FSH and 10 μg/mL of LH and 10 UI of human chorionic gonatropin (hCG) (Pregnyl® – Organon); T4 – basic medium, 10 μg/mL of FSH and 10 μg/mL of LH and 10 UI of hCG and 1 μg de 17b-estradiol/mL, in times 20 to 22h, 23 to 24h, 26h and 28 hours of cultivation in CO_2 incubator at 5% and temperature of 39ºC. The table of chi-squared was used to analyze data with 5% significance level. In the times of 20 to 22h, 23 to 24h, 26h and 28 hours of cultivation, respectively, it was observed in T1 that 45.43%, 44.32%, 53.73% and 54.04% of the oocytes restarted meiosis, showing that the medium without additions makes the oocytes restart meiosis later. In T2, meiosis' restart rate were 53.42%, 58.94%, 53.11% and 68.78%, we could observe that gonadotropin association with the highest cultive time restarts the meiosis quicker. In T3, the percentage was 69.50%, 72.60%, 74.94% and 78.85% giving evidence to lower results of buffalos by Santos et al. (2002, Nuclear maturation kinetics in vitro of buffalo oocytes, 39:271-275). Other than that, it was observed that the addition of gonadotropins to cultive medium allows higher percentage of nuclear maturation with treatments 1 and 2 with significant difference ($p<0.05$). In T4 the rates were 83.42%, 79.46%, 87.47% and 87.76% thus the maturation rates obtained in different times were higher than the ones found in bovines by Bevers et al. (Therio, 47:13-22,1997). It can be concluded that the supplemented media provides higher rates of meiosis' restart in less time.

A043 Folliculogenesis, Oogenesis and Superovulation

The role of insulin-like growth factor-I on heat-induced cytoskeletal changes in bovine oocytes

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Keywords: cytoskeleton, heat shock, IGF-1.

Adverse environmental conditions such as elevated temperature and humidity compromises productivity and reproductive efficiency in cattle. The low fertility associated to hyperthermia is a multifactorial problem since it affects physiological and cellular functions in several tissues. However, heat stress promotes, among several physiological and cellular alterations, changes in the reproductive tract microenvironment, compromising oocyte nuclear maturation, fertilization and embryo development to the blastocyst stage. It has been demonstrated that the insulin-like growth factor (IGF-1) plays a termoprotector role in the bovine oocyte. Therefore, the objective of this study was to: 1) determine the effect of heat shock (in vitro elevated temperature) on bovine oocyte cytoskeletal changes and 2) evaluate the termoprotector role of IGF-I in this context. Cumulus-oocyte complexes (COCs) collected from slaughterhouse-crossbred ovaries were subjected to the heat shock model (control: 38.5°C for 22 hours and heat shock: 41°C for 14 hours followed by 38.5°C for 8 hours) in the presence of 0 or 100 ng/ml of IGF-I during in vitro maturation. COCs were mechanically denuded by repeated pipetting to remove cumulus cells, fixed in 3.7% formaldehyde and permeabilized in 0.1% Triton-X 100. Then, oocytes were incubated in 50 µL Phalloidin toxin conjugated with alexa fluor 594 (5 IU/mL) for one hour at room temperature to localize actin filaments. Oocytes were evaluated by fluorescence microscope (Olympus IX81) equipped with Texas Red filter. Fluorescence intensity (FI) was quantified in the cortical (FI1) and central (FI2) region of each oocyte using the software Image J version 1.45s . Data were subjected to least-squares analysis of variance using the PROC GLM procedure of SAS. The dependent variable was FI1/FI2 ratio. The statistical model considered all the main effects and all possible interactions. Pdiff procedure was used to establish meaningful comparisons between the means. In the absence of IGF-I exposure of bovine oocytes to heat shock decreased (p <0.001) the FI1/FI2 ratio from 1.02+ 0.91 to 0.91+ 0.02 fluorescence arbitrary units (AU) for the control and heat shock groups, respectively. However, in the presence of IGF-I FI1/FI2 ration was 0.98+ 0.02 and 0.93+ 0.02 AU (IGF x temperature interaction, p = 0.06) for control and heat shock , respectively. These results indicated that addition of IGF-I to maturation medium minimized the negative effect of elevated temperature on actin filaments organization in the cortical region of the oocyte.

A044 Folliculogenesis, Oogenesis and Superovulation

Influence of a protected fat diet on the postpartum cyclicity in primiparous nellore cows (B*os taurus indicus*)

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Keywords: heifers, post partum anestrous, supplement.

This experiment was conducted to test the hypothesis that fat supply on primiparous heifers during the final third of gestation will increase the preovulatory follicle diameter and the number of follicles reducing postpartum calving interval and increasing pregnancy rate. Contemporary heifers (n = 19; 24-27 months of age) with average live weight of 366±3.6 kg were used. After TAI heifers were inseminated with sexed semen from two different bulls. Body weight was measured every 10 days pre-partum and every 7 days postpartum. After nutritional adaptation the treatments began 60 days before expected parturition: Control Group (CG, n = 9) without fat only concentrate (2 kg/heifer/d, plus 250g grinded corn) and Fat group (FG, n= 10) with fat (100 g/heifer/d) and concentrate (2 kg/heifer/d), the diets were isocaloric. After parturition all animals were fed the common pre-partum diet. The ovaries examination and blood samples were performed every 24 h from the second week up to ninety (90) days during the postpartum period and every 4 days for 30 days thereafter. During the diet treatment, forage intake of was evaluated individually. There was no difference (p = 0.60) in live weight between the CG and FG either during (468±42 kg and 462±30 kg) or after treatment (429±38 kg and 417±27kg). At all six cows ovulated, three from FG (at 109, 120 and 149 days) and three from CG (at 57, 98, 114 days). Cows from Control group (90± 29 days) ovulated earlier (p = 0.05) than Cows from FG (126 ± 21 days). The weight at first ovulation was greater (p = 0.03) in CG 438±32 kg than in FG 388±30 kg. During the nutritional treatment period there was no difference (p = 0.52) in food consumption between CG and FG (9.86±2 and 10.15±2 kg/day/animal). The mean number of follicles (≥ 3 mm) did not change (p = 0.16) between groups, during 15 until 90 days postpartum (CG 11±3 and FG 13±3). Largest follicle diameter did not differ (p = 0.89) between CG (9.80±2 mm) and FG (9.9±1.8 mm) on the other hand the second largest follicle average diameter during 100 days after parturition was higher in the CG (7.14±1.5 mm) than in FG (6.36±1.6 mm). It was not possible to observe a positive effect of fat supplementation during pre-partum period reducing cyclicity return in primiparous cows after parturition.