A030 Folliculogenesis, Oogenesis and Superovulation

Selection of porcine viable oocytes by brilliant cresyl blue test (BCB) at different exposure times

A.A. Borges, M.L.T Santos, L.B. Queiroz Neta, M.V.O Santos, A.K.N. Feitosa, A.F. Pereira

UFERSA.

Keywords: G6PDH, oocyte quality and morphology, staining test.

In vitro embryo production (IVEP) is an excellent tool for genetic improvement and basic research in several species. Several parameters affect the efficiency of the technique, as the immature oocyte quality before in vitro development. In general, the brilliant cresyl blue (BCB) test has been used as a method of selection of these viable oocytes, which is based on the capability of glucose-6-phosphate dehydrogenase (G6PDH) to convert the BCB stain from blue to colorless. This enzyme is active in the growing oocyte, but has decreased activity in grown oocytes. Studies show that structures can be incubated for 90 min before maturation. However, the reduction of exposure time would be important to optimize IVEP. Therefore, the aim of this work was to select porcine viable oocytes using different exposure times with the BCB. Ovaries were recovered from a slaughterhouse and transported in saline solution at 30-35°C. All follicles (2-8 mm) were aspirated using 5 mL syringe with 22 G needle. The oocytes recovered were classified under stereomicroscope by morphological criteria as viables (≥ 1 layer of compact cumulus cells and homogeneous cytoplasm) and non-viables (< 1 layer of compact cumulus cells and heterogeneous cytoplasm). Subsequently, the structures were distributed in four exposure times to the staining agent (15, 40, 60 and 90 min) and incubated in PBS containing BCB (26 µM, Sigma, USA) at 38.5°C. After each period, the oocytes were washed in PBS and classified as positive BCB (BCB+) or negative BCB (BCB-), according to blue or colorless cytoplasmic staining, respectively. All data were expressed as percentage and analyzed by the Fisher exact test using GraphPad Instat 3.06 software ($P<0.05$). After four repetitions, a total of 79 ovaries resulted in 614 structures, obtaining an average of 7.8 oocytes/ovary. Of these, 340 and 274 oocytes were classified as viable and non-viable, respectively, according to morphological criteria. The percentages of viable oocytes by BCB test were 29.5% (39/132), 73.8% (121/164), 83.8% (140/167) and 76.8% (116/151) for 15, 40, 60 and 90 min, respectively. Exposure times to BCB of 40 and 60 min were as effective as 90 min for the selection of porcine viable oocyes ($P>0.05$). However, the incubation of the structures for 15 min was not effective for oocyte selection ($P<0.05$). Additionally, except for the exposure time of 15 min, the percentage of viable BCB+ structures was higher when compared to BCB- oocytes (15 min: 33.8% (23/68) vs. 66.2% (45/68); 40 min: 67.8% (61/90) vs. 32.2% (29/90); 60 min: 90.1% (82/91) vs. 9.9% (9/91); 90 min: 97.7% (86/88) vs. 2.3% (2/88), $P<0.05$). In conclusion, oocyte staining with BCB may be useful for selection of porcine viable structures only if exposure time is greater than 15 min.

A031 Folliculogenesis, Oogenesis and Superovulation

Effect of alpha lipoic acid on developmental competence of equine preantral follicles

A.G. Lindquist[1], R.G. Gomes[1], L.A. Lisboa[1], C.B. Silva[2], M.C. Max[1], R.L. Oliveira[1], S.M. Gonzalez[1], P.C. Marino[1], T.R.R. Barreiros[3], M.M. Seneda[1]

[1]Universidade Estadual de Londrina; [2]UNOPAR/UEL; [3]Universidade Estadual do Norte do Paraná.

Keywords: alpha lipoic, equine, in vitro culture.

The aim of this study was to evaluate the effect of adding different concentrations of alpha-lipoic acid on in vitro culture of equine preantral follicles. Ovaries (n = 5) from mares in the anovulatory season, were collected at a local slaughterhouse and washed in 70% ethanol and PBS. The internal region (parenchymal) was divided into 9 pieces of approximately 3x3x1 mm. One fragment was immediately fixed in Bouin (control) and the other 8 pieces were transported for one hour to the laboratory in PBS plus penicillin (200 IU/mL) and streptomycin (200 mg/mL) at 4°C. At the laboratory, 8 fragments were individually cultured in culture dishes at 39°C in an atmosphere with 5% CO2 and air with saturated humidity for two (D2) or six (D6) days in 1 ml of minimum essential medium (MEM) - (Gibco BRL, Rockville, MD, USA) (osmolarity 300 mOsm/L pH 7.2) supplemented with ITS (6.25 ng/mL insulin, 6.25 ng/mL transferrin, 6 25 ng/mL selenium), 0.23 mM pyruvate, 2 mM glutamine, 2 mM hypoxanthine, 1.25 mg/mL bovine serum albumin, 200 IU/mL penicillin and 200 mg/mL streptomycin (MEM+). Treatments consisted of MEM+ supplemented with different concentrations of alpha-lipoic acid (50, 100, or 250 ng/mL). Medium was replaced every two days. After culture, ovarian fragments were fixed in Bouin and processed for histology. Follicles were classified according to their stage of development in primordial, primary, secondary and antral follicles, and according to their morphology in normal or degenerated. Data were compared by nonparametric analysis of variance (Kruskal Wallis test), considering 5% significance level. Overall, 795 slides were evaluated containing 5,205 sections. Follicles were present in 37.6% (1957/5205) of the histological sections, and 56.3% of them were morphologically normal. Regarding follicular development, fragments treated with MEM (D2) 50 ng/mL of alpha lipoic acid (ALA 50 D6), ALA 100 (D6) and ALA 250 (D6) had a higher percentage of developing follicles in comparison with primordial follicles. The control group showed a higher proportion of primary (84.4%) than developing follicles (15.6%). We conclude that alpha lipoic acid at 50 ng/mL was effective to promote the development of equine primordial follicles in culture for six days.

A032 Folliculogenesis, Oogenesis and Superovulation

Kit ligand promotes the transition from primordial to primary follicles after *in vitro* culture of ovine ovarian tissue

A.Y. Cavalcante, B.B. Gouveia, R.S. Barberino, T.L. Lins, L.P. Santos, R.J.S. Gonçalves, M.H.T. Matos

UNIVASF.

Keywords: activation, ovary, sheep.

In vitro studies have showed that Kit Ligand (KL) maintains survival, promotes growth (Jin et al, Mol. Reprod. Dev. 70, 82–90 2005; Reynaud et al, Mol. Reprod. Dev. 56, 483–494, 2000) and activation (Celestino et al, Mol. Reprod. Dev. 77, 231 - 240, 2010; Lima et al, Cells Tissues Org. 195, 260–271, 2012; Parrott e Skinner, Endocrinology 140, 4267-4271, 1999) of ovarian preantral follicles in different species. However, the effect of different concentrations of KL on in vitro development of preantral follicles enclosed in ovine ovarian tissue has not been evaluated. The aim of this study was to evaluate the effect of KL on the morphology and development of ovine preantral follicles in vitro. After collection of ovaries (n=8) in a slaughterhouse, one fragment of ovarian tissue was fixed for histological analysis, corresponding to fresh control. This fragment was dehydrated, diafanized and stained with Hematoxylin-Eosin. The remaining fragments were cultured for 7 days in α-Minimal Essential Medium (α-MEM – GIBCO, Invitrogen, St Louis, EUA) supplemented with hypoxantine, glutamine, ascorbic acid, bovine serum albumin (BSA) and ITS (insulin, transferrin and sodium selenite) (Sigma Chemical Co., St. Louis, MO, USA) in the absence (control medium) or presence of KL (1, 10, 50, 100 or 200 ng/mL; Sigma Chemical Co., St. Louis, MO, USA). After culturing, the morphological analysis of preantral follicles was performed by histology, and follicles were classified as normal or atretic according to the absence or presence of cytoplasmic retraction, nuclear pycnosis and/or disorganization of granulosa cells, as well as primordial, primary and secondary follicles. The percentages of normal follicles and follicles at each stage of development were compared by ANOVA and Tukey's test ($P<0.05$). After 7 days of culture, all the treatments reduced ($P<0.05$) the percentage of morphologically normal follicles compared to fresh control. In addition, a decrease ($P<0.05$) in the percentage of primordial follicles and an increase in the percentage of primary follicles was observed at the concentration of 100 ng/mL KL, compared with the fresh control, control medium (α-MEM) and the other concentrations of KL. In conclusion, 100 ng/mL KL promotes the transition from primordial to primary follicles (follicular activation) after in vitro culture of ovine ovarian tissue.

A033 Folliculogenesis, Oogenesis and Superovulation

Proliferative activity of multi-oocytes follicles in ovine ovaries

C.B. Costa[1], R.L. Oliveira[2], C.B. Silva[3], L.A. Lisboa[2], D.F Higashi[2], F.D. Sarapião[2], S.M. Gonzalez[2], M.M. Santos[2], I. Búfalo[2], M.M. Seneda[2]

[1]UEL; [2]Universidade Estadual de Londrina UEL; [3]UNOPAR/UEL.

Keywords: cpna Ki-67, folliculogenesis, immunohistochemistry.

Multi-oocyte follicles (MOFs) have been often described in studies of follicular population. However, their development and proliferative activity are poorly studied. The purpose of this study was to determine the frequency and activity of multi-oocytes follicles in ovine ovaries. Pairs of ovaries (n = 32) obtained from a slaughterhouse were evaluated for cell proliferative activity through the expression of Proliferation Cell Nuclear Antigen (PCNA) and the protein detected by the antibody Ki-67. The analysis for CPNA was performed by histological score and due to the absence of normality and homoscedasticity, data were analyzed using the Wilcoxon test for dependent samples. There was immunostaining for PCNA in all stages of follicular development and there was no difference ($p > 0.05$) between the immunoblots of oocytes from follicles containing one oocyte and multi-oocytes follicles. Multi-oocytes follicles were found in greater amounts in the left ovary ($p = 0.03$). There was no positive immunostaining for Ki-67 in primordial follicles. However, in all other development stages, Ki-67 immunostaining was observed in follicles with one oocyte and MOFs. Thus, MOFs in sheep ovaries showed proliferative activity and are possibly able to develop to antral stages.

A034 Folliculogenesis, Oogenesis and Superovulation

Comparison of morphological and morphometric characteristics between follicles with one or more oocytes in sheep ovaries

B.P. Novelli[1], R.L. Oliveira[2], K.C.S. Santos[2], F.D. Sarapião[2], J.V.R. Bueno[2], I. Búfalo[2], S.M. Gonzalez[2], W. Blaschi[1], M.M. Seneda[2]

[1]UENP; [2]UEL.

Keywords: follicular population, folliculogenesis, preantral follicles.

The investigation of morphological characteristics of the population of preantral follicles is important for the understanding of physiological processes such as folliculogenesis and for new reproductive strategies. The aim of this study was to morphologically characterize the population of sheep ovarian follicular reserve. Ovine ovaries (n = 32) were histologically processed and analyzed by light microscopy. The follicles and oocytes showed the following average diameters: (primordial with one oocyte) 30.96 ± 4.6 m and 25.09 ± 1.61 mm; (multioocyte primordial) 48.62 ± 5.66 m and 24.31 ± 3.35 mm; (primary) 50.96 ± 8.52 m and 34.42 ± 4.50 mm; (secondary) 86.48 ± 21.85 m and 49.01 ± 11.49 mm; (antral) 226.26 ± 69.93 m and 66.26 ± 11.01 micrometers. Data were compared were using the Wilcoxon test for dependent samples. There was no difference between oocyte diameter in primordial follicles with one or more oocytes. Multioocyte follicles at the primordial stage were observed in greater amounts than secondary follicles, and their prevalence was similar in the right and left ovaries ($p = 0.792$). Regarding the macroscopic analysis, data were subjected to Spearman correlation test. There was a positive correlation between follicular population and ovarian diameter ($p < 0.01$), ovarian weight ($p < 0.05$) and ovarian volume ($p < 0.05$). The was not correlated with the other variables. However, correlation of total number of multioocyte follicles with follicular average population approached significance ($p = 0.056$). These results suggest that multioocyte primordial follicles have morphology and developmental capacity similar to primordial follicles with one oocyte.

A035 Folliculogenesis, Oogenesis and Superovulation

Immunohistochemical assessment of cellular proliferation in multioocyte follicles from *Bos indicus* ovaries

C.A. Gutjahr[1], R.L. Oliveira[2], K.C.S. Santos[2], F.D. Sarapião[2], M.M. Santos[2], C.B. Silva[2], S.M. Gonzalez[2], W. Blaschi[1], M.M. Seneda[2]

[1]UENP; [2]UEL.

Keywords: folliculogenesis, nelore, PCNA.

The objective of this study was to evaluate by immunohistochemistry cellular proliferation in multioocyte follicles from bovine adult ovaries. Ovaries (n=12) from Nelore heifers obtained at a slaughterhouse were fixed in Bouin and submitted to histological processing. Slides were prepared in duplicate (PAS staining-Schiff and Immunohistochemistry) with sections of 5 µm. Screening for multioocyte follicles was performed on PAS stained slides. All multioocyte follicles were submitted to immunohistochemistry with anti-PCNA (Clone PC1O, ZYMED® Laboratories). The level of PCNA-positive cell proliferation was assessed in granulosa cells (score ranging from one to four) and oocytes (qualitative assessment). Data were evaluated only for qualitative aspects and therefore will not be expressed numerically. Data were subjected to ANOVA ($p \leq 0.05$). From 12 ovaries examined, we found 12 multioocyte follicles, containing from two to three oocytes. These structures were found predominantly in primordial follicles (n = 10) and primary follicles. There was no difference ($p > 0.05$) in cell proliferation of granulosa cells from primordial and primary follicles. However, there was a trend of increase in mitotic activity of granulosa cells from follicles multioocyte compared to other follicles. For the mitotic activity in oocytes, PCNA immunostaining was positive in follicles at different stages of development. Immunostaining was stronger in oocytes from multioocyte follicles ($p < 0.05$), reinforcing the proposition that multioocyte follicles are in constant proliferation. Based on these results, we concluded that adult Bos taurus indicus cattle present multioocyte follicles in early stages of development. The oocytes enclosed in multioocyte follicles do not display the same pattern of PCNA immunostaining, suggesting they are in different stages of development and/or there is some kind of competition or dominance between them.

A036 Folliculogenesis, Oogenesis and Superovulation

Effect of different concentrations of EGF on in vitro culture of equine ovarian preantral follicles: preliminary results

C.B. Silva[1], M.C. Max[2], R.G. Gomes[2], L.A. Lisboa[2], P.C. Marino[2], R.L. Oliveira[2], S.M. Gonzalez[2], M.M. Santos[2], T.R.R. Barreiros[3], M.M. Seneda[2]

[1]UNOPAR/UEL; [2]Universidade Estadual de Londrina; [3]Universidade Estadual do Norte do Paraná.

Keywords: EGF, equine, in vitro culture.

The aim of this study was to evaluate the effect of different concentrations of EGF on in vitro culture of preantral follicles enclosed in equine ovarian fragments. Ovaries (n=5) from mares in seasonal anoestrus were collected from a local slaughterhouse, washed in PBS and ethanol 70% and transported for one hour in PBS plus penicillin (200 IU/mL) and streptomycin (200 mg/mL) at 4°C. At the laboratory, the internal part (parenchyma) of the ovary was divided into 11 fragments of approximately 3x3x1 mm (125-135 mg). One fragment of each ovary was immediately fixed in Bouin (control, D0). The other 10 fragments were individually cultured in culture dishes containing 1mL aliquots of MEM (Gibco BRL, Rockville, MD, USA) (osmolarity 300 mOsm/L, pH 7.2) supplemented with penicillin (100 IU/mL) streptomycin (100 mg/mL), bovine serum albumin (1.25 mg/mL- Gibco BRL, Rockville, MD, USA), ITS (insulin - 6.25 µg/mL transferrin - 6.25 µg/mL selenium - 6.25 ng/mL), pyruvate (0.23 mM), glutamine (2 mM) and hypoxanthine (2 mM), which is referred as MEM+. Culture was performed at 39°C in an atmosphere with 5% CO2 in air and saturated humidity for 2 days. Medium was supplemented with different concentrations of EGF (10, 50, 100 and 200 ng/mL). After culture, fragments were fixed in Bouin and processed for histology. Follicles were classified according to their stage of development as primordial, primary, secondary and, and according to their morphology as normal or degenerated. Data were compared by nonparametric analysis of variance (ANOVA, $P<0.05$). A total of 450 slides containing 1800 sections were analyzed including the control group and treatment groups. Overall, 198 follicles were identified, of which 93 (47%) were primordial, 105 (53%) were developing and 58% were classified as morphologically normal. Follicular development was observed in all tested concentrations of EGF (MEM - 75% (3/4), 10ng/mL - 100% (4/4), 50ng/mL - 85,7% (12/14), 100ng/mL - 100% (6/6) e 200ng/mL - 96% (24/25)). We concluded that MEM+ medium supplemented with these concentrations of EGF allowed development of follicles in equine ovarian fragments cultured for two days.

A037 Folliculogenesis, Oogenesis and Superovulation

Preservation of preantral follicles of domestic cats (*Felis catus*)

D. Missio[1], N.C. Carvalho[1], F.Q. Rosa[2], A.C.G. Guimarães[2], F.G. Leivas[2], D.S. Brum[2]

[1]Programa de Educação Tutorial- Pet Veterinária- Universidade Federal do Pampa; [2]Laboratório de Biotecnologia da Reprodução- Biotech-Universidade Federal do Pampa.

Keywords: cats, ovaries, preantral follicles.

The Felidae family consists of 37 species, of which only the domestic cat is not at risk of extinction. Preantral follicles (PAF) are a source of gametes with the possibility to produce thousands of oocytes for assisted reproduction biotechnologies. However, for this purpose, to maintain the quality of the follicle after removal and transport of the ovaries to the laboratories is a challenge. In an attempt to improve the conditions ofor conservation of PAF, several media have been tested at different temperatures and times. However, most of this research has been done with ovaries from livestock animals; there are no reports in the literature on how to deal with feline ovaries. Thus, the aim of this study was to investigate the effectiveness of TCM 199 and PBS in the conservation of feline PAF at 4ºC for 24 hours. Ten ovaries of five cats (Felis catus) aging between 8 and 24 months were obtaneid following ovariohysterectomy. Immediately after surgery, ovaries were washed with saline 0.9%, and the ovarian cortical layer was dissected and divided in six fragments of 3mm3: two fragments for the control group; two fragments preserved in TCM 199 (Sigma Aldrich, St. Louis MO, USA; T1) and two fragments preserved in PBS (Dulbecco's Modified DMPBS Flush - Nutricell, Campinas, SP, Brazil; T2). The control fragments were fixed in Carnoy, dehydrated in ethanol and embedded in paraffin. Ovarian fragments from T1 and T2 were kept at 4°C for 24 hours and then fixed in paraffin as in the control group. Histological sections from the three groups (control, T1 and T2) were stained with PAS-hematoxylin. 400 PAF were evaluated per treatment, using a optic microscope with 400x magnification. PAFs were classified according to the stage of development in primordial, primary and secondary. Additionally, they were classified as normal or degenerated follicles. Data were analyzed by chi-square test ($P < 0.05$). The percentage of viable follicles after transport for 24 hours was 63%, 59% and 55% for the control, T1 and T2 groups, respectively. The viability of PAF in T1 was similar to the control group, but greater than in T2 ($P > 0.05$). T1 and T2 did not differ. Primordial follicles maintained higher viability rates when compared to developing follicles, regardless of treatment. We conclude that it is possible to maintain the viability of PAF from cat ovaries transported at 4°C for 24 hours using TCM 199; and primordial follicles have higher viability when compared to developing follicles.

A038 Folliculogenesis, Oogenesis and Superovulation

Effect of different energy sources on growth and maturation of oocytes from caprine preantral follicles cultured *in vitro*

D.M. Padilha[1], R.T. Padilha[1], A.B. Duarte[2], G.M. Silva[3], J.L. Revilla[3], C.A. Leal[1], H.H. Correia[3], D. Rodina[3], J.R. Figueiredo[3]

[1]Universidade Potiguar; [2]Universidade Federal do Ceará; [3]Universidade Estadual do Ceará.

Keywords: energy supplies, goats, preantral follicles.

The aim of this study was to evaluate the effect of different energy sources on survival, growth and maturation of oocytes from goat preantral follicles cultured in vitro. For the experimental design, before being slaughtered, goats used in the current experiment were fed during 30 days with: 1) carnaúba, 2) cassava e 3) cashew. The ovaries from six animals/group were obtained in a local abattoir. Preantral ovarian follicles were isolated from the ovarian cortex and individually cultured for 36 days in basic medium (α-MEM supplemented with bovine serum albumin (BSA), insulin, transferrin and selenium, glutamine, hypoxanthine, ascorbic acid and FSH at increasing concentrations (D0-D6: 100 ng/mL; D6-D12: 500 ng/mL; D12-D36: 1000 ng/mL). Every 6 days, follicular development was evaluated to determine follicular survival, antral cavity formation, follicular diameter and oocyte growth. At the end of culture, oocytes were submitted to in vitro maturation (IVM). COCs were incubated during 32 in 100 µL drops of TCM 199 supplemented with 1 µg/mL 17-β-estradiol, 5 µg/mL LH, 0.5 µg/mL rFSH, 10 ng/mL EGF, 1 mg/mL BSA, 22 µg/mL piruvate, 50 ng/mL IGF-I, and 100 µmol/L cisteamine. Follicular survival, retrieval of grown oocytes for IVM, antrum formation and meiotic resumption after in vitro culture were compared using Chi-square. Results are expressed as mean ± standard deviation (SD) and differences were considered to be significant when $p<0.05$. In all groups tested, the percentage of antrum formation and follicular diameter increased from Day 0 (0% - 152 µm) to Day 6 (40% - 312 µm) ($p<0.05$). Moreover, a reduction in follicular survival was also observed in all groups from Day 0 (100%) to Day 12 (85%) of culture ($p<0.05$). However, follicular survival (70%), follicular diameter (532 µm), antrum cavity formation (88%) and number of oocytes reaching 110 µm after culture (30%) did not differ between diets ($p>0.05$). In conclusion, with these culture conditions, goat preantral follicular development is not influenced by energy sources.

A039 Folliculogenesis, Oogenesis and Superovulation

Effects of treatments to induce puberty and increase pregnancy rate in heifers in native pastures of Pantanal.

E. Nogueira[1], U.P.G. Abreu[1], L.O.F. Oliveira[1], F.B. Santos[2], J.C. Borges[1], L.F.M. Pfeifer[3]

[1]EMBRAPA CPAP; [2]USP; [3]EMBRAPA CPAFRO.

Keywords: ovulation induction, P4 intravaginal device, prostaglandin.

Early puberty and age at the onset of reproductive life are factors that increase the profitability of cattle production. In extensive pasture systems with low nutritional pastures, such as the Pantanal, the mating age in Nelore heifers is about three years. Ovulation in Bos taurus prepubertal heifers using prostaglandin F2α (PGF2α) has been previously reported. This work aimed to evaluate two hormonal protocols to promote first ovulation in prepubertal heifers and to elevate pregnancy rates in Nelore heifers. 222 Nelore noncyclic heifers (absence of CL after two gynecological exams 11 days apart) with ages averaging 715 days and weighting 275.57 ± 10.43 were submitted to three treatments: Control (n = 73)- heifers received two applications of 2 ml physiological solution on the same days of treatments in T1 group; T1 - PGF (n=74) , heifers received two doses of PGF2α (0.530 mg sodium cloprostenol; Sincrocio; Ouro Fino) 5 days apart, the first coinciding with D0 in T2 group; T2 - CIDR (n = 75)- heifers received an intravaginal device (P4) previously used 3 times (24 days; CIDR, Zoetis, São Paulo, SP) on D0, which remained for 8 days and an injection of PGF2α (0,530 mg; Sicrocio; Ouro Fino) on the day of implant removal. After the treatments the diameter of the preovulatory follicle and ovulation rate were evaluated in 4 transrectal ultrasonography examinations 3 days apart. After the evaluation period, heifers were grouped and mated with bulls previously evaluated at a 1:20 bull:cow ratio. Pregnancy diagnosis was performed 40 days after the breeding period by transrectal ultrasonography and pregnancy rates were assessed by logistic regression using PROC LOGISTIC of SAS software. The ovulation rate was not increased in T1 and T2 groups compared to the control ($P > 0.05$), and pregnancy rates did not differ ($P > 0.05$) between the control group (36.99%), PGF (35.14%), and CIDR (46.67 %), despite the 10% difference found in the T1 compared to T2. In conclusion, alternatives like the reutilization of P4 devices or PGF2α to induce ovulation and increase pregnancy rates were not effective in pre pubertal Nelore heifers kept in native pastures in Pantanal.

A040 Folliculogenesis, Oogenesis and Superovulation

Follicular development in ovarian autografts in the perimetrium and subcutaneous tissue of Balb-c mice

F.A. Santos, M.M.L. Pimentel, P.D. Brito, G.H.F. Moura, M.F. Macedo, M.B. Bezerra

UFERSA.

Keywords: autograft, Balb-c female mice, follicle proportion.

The aim of this study was to evaluate quantitatively and qualitatively follicles derived from autografts allocated in the subcutaneous tissue and perimetrium of mice. Twelve Balb-c female mice were randomly divided into three experimental groups with 4 animals in each group. Group I - control (non-transplanted animals), group II - animals that received the ovarian fragment in perimetrium, group III - animals that had the abdominal subcutaneous tissue as the receptor site. In transplanted animals, the left ovary was removed and used for other studies, while the cortex of the right ovary was fragmented and reimplanted according to the group description. Thirty days after surgery, mice were euthanized, transplants removed and histologically processed. The percentages of ovarian follicle categories (normal and atretic) were compared using the chi-square test ($p < 0.05$). Follicles were classified qualitatively (normal and atretic) and morphologically (primordial, transitional, primary, secondary, early antral, antral and pre-ovulatory). No statistical difference was observed in the percentage of atretic follicles. The percentages of follicles in differente morphological categories did not vary either, except for pre-ovulatory follicles (group I = 1.4%, group II = 2.6%, group III = 11.3%). This difference was due to one of the subcutaneous transplants bearing only 1 follicle, which was preovulatory. In all groups, the largest population of normal follicles was constituted of preantral follicles (group I = 84.8%, group II = 83.5%, group III = 69.4%), with the highest percentage of primordial follicles (group I = 28.2%, group II = 31.3%, group III = 25.8%), primary (group I = 27.5%, group II = 26.1%, group III = 21.0%), followed by transitional (group I = 14.9%, group II = 17.4%, group III = 11.3 %) and secondary (group I = 14.2%, group II = 8.7%, group III = 11.3 %). This was likely due to their small size and simple structure; they also have lower metabolism favoring survival in ischemic environments. In addition, they are predominantly located to the periphery of the ovarian cortex favoring the benefit from revascularization after transplantation. Thus, we can conclude that ovarian autotransplantation maintains the viability of the organ, allowing survival and development of follicles to more advanced stages including preovulatory.

A041 Folliculogenesis, Oogenesis and Superovulation

Follicular and luteal dynamics of buffalo (*Bubalus bubalis*) during breeding and non-breeding seasons

J.R.S Torres Júnior[1], A.S. Albuquerque[1], J.A.T Souza[2], I.G. França[1], H.G. Pereira[1], A.A. Coelho[1], C.S. Santos[1]

[1]Universidade Federal do Maranhão; [2]Universidade Federal do Piauí.

Keywords: anestrus, photoperiod, seasonality.

This study aimed to assess ovarian follicular and luteal dynamics of Buffalo (Bubalus Bubalis) during breeding and non-breeding seasons. The study was conducted in the Equatorial Amazon (4º Latitude South, State of Maranhão, Brazil). Reproductive seasonality has not been assessed in the region of the study. Based on records of conceptions/births from the previous three years, a reproductive favorable season (FAV; July 2013) and an unfavorable season (UNFAV; January 2013) were determined. Twenty two multiparous nonlactating Murrah female buffaloes (Bubalus bubalis), with average body score 3.25±0.6 (Ranging 1 to 5) and bearing at least one follicle ≥ 8.5mm denoting ovulatory capacity were used. The same buffaloes were evaluated in both periods (january and july) and treated with two doses of PGF2alpha with a 14 day interval (0.50 mg of Cloprostenol, Sincrocio®, IM; Ouro Fino, Brazil). Ovarian dynamics was monitored daily starting 48 hours after the second dose of PGF. Jugular blood samples from all animals were collected daily to measure serum progesterone concentrations. Parametric variables were analyzed by ANOVA using SAS®. Breeding seasons and follicular waves were compared using Student's t test (PROC GLM). Hormonal concentrations were analyzed as repeated measures (PROC MIXED). Non- parametric variables were analyzed using the Wilcoxon test (PROC NPAR1WAY WILCOXON) and the binomial distribution by PROC GLIMMIX. The significance level was 5%. The general rate of cyclicity (presence of corpus luteum at the first PGF) differed significantly between seasons (13.6 % for UNFAV vs 72.7% for FAV, P<0.05). The presynchronization protocol was not effective in UNFAV, and was followed by anovulatory waves and turnover of dominant follicles up to 9.1 ± 0.2 mm in all animals. In FAV, only 18 females (81.8%) ovulated at regular intervals indicating occurrence of physiological estrous cycles. Of these, 15 females (83.3%) exhibited a two follicle wave pattern, two (11.1%) exhibited one follicular wave and only one (5.6%) had three follicular waves. Serum progesterone concentrations differed between UNFAV (0.9 ± 0.1 ng/mL) and FAV (7.2 ± 0.7 ng/mL) (P<0.05). Mean values for females with One vs Two vs Three follicular waves were, respectively: interovulatory interval (22.5 ± 2.5 vs 21.9 ± 0.6 vs 22.0 days), maximum diameter of the ovulatory follicle (12.5 ± 1.5 vs 12.1 ± 0.3 vs 9.0 mm), and length of luteal phase (10.5 ± 2.5 vs 13.0 ± 0.8 vs 14.0 days). In summary, the FAV period was characterized by cyclic ovarian activity with predominance of two follicular waves and the UNFAV period by turnover of anovulatory waves with persistent follicles. We concluded that nonlactating buffalo females display distinct reproductive patterns in favorable and unfavorable reprudcutive periods.

A042 Folliculogenesis, Oogenesis and Superovulation

Effect of GDF-9 on *in vitro* culture of equine preantral follicles: preliminary results

L.Z. Bergamo[1], P.C Marino[1], C.B. Silva[2], L.A. Lisboa[1], M.C. Max[1], F.D. Sarapião[1], S.M. Gonzalez[1], R.L. Oliveira[1], R.G. Gomes[1], M.M. Seneda[1]

[1]Universidade Estadual de Londrina; [2]UNOPAR/UEL.

Keywords: equine, GDF-9, *in vitro* cultivation.

The aim of this study was to evaluate the effects of different concentrations of GDF-9 on in vitro culture of equine ovarian fragments. Ovaries (n=5) from mares in the anovulatory season were collected at local slaughterhouse and washed in 70º ethanol and PBS, and transported for one hour in PBS plus penicillin (200 IU/mL) and streptomycin (200 mg/mL) at 4ºC to the laboratory, where the internal portion of the ovary was divided into 9 pieces of approximately 5 x 5 x 1mm. One fragment of each ovary was immediately fixed in Bouin (control group, D0). The other 8 fragments were cultured individually in culture dishes containing 1mL of MEM (MEM control) or base medium supplemented with GDF-9 (0, 50, 100 or 200 µg/mL). MEM+ was MEM (Gibco BRL, Rockville, MD, USA – 300 mOsm/L osmolarity, pH 7.2) supplemented with pyruvate (500 µL), glutamine (500 µL), hypoxanthine (500 µL), ITS, BSA (1.25 mg/mL-Gibco BRL, Rockville, MD, USA), penicillin (500 µL) and streptomycin (500 µL). Culture was performed at 39°C in an atmosphere with 5% CO2 in air and saturated humidity for 2 or 6 days. Medium was replaced every 2 days. After culture, fragments were fixed in Bouin and processed for histology. Follicles were classified according to their stage of development as primordial, primary, secondary and antral follicles, and according to their morphology as normal or degenerated. Data were compared by nonparametric analysis of variance (Kruskal Wallis test), considering significant differences with P<5%. Overall, 675 slides were evaluated with an average of 4 ovarian fragments in each. We found 552 follicles, of which 68 (12.3%) were developing follicles in the control group (MEM+), 355 (64.3%) were primordial, 197 (35.7%) were developing and 379 (68.6%) were morphologically normal. Fragments cultured with 50 µg/mL of GDF-9 for two days of had higher proportion of developing follicles (9%; 50/552). We concluded that MEM supplemented with 50 µg/mL of GDF-9 provided better follicle development during in vitro culture of equine ovarian fragments for two days.

A043 Folliculogenesis, Oogenesis and Superovulation

Different specifications of neddle and syringe used for OPU affect the number of recovered oocytes from slaughterhouse bovine ovaries

L.B. Queiroz Neta, M.L.T Santos, A.A. Borges, M.V.O Santos, A.K.N Feitosa, A.F. Pereira

UFERSA.

Keywords: developmental competence, *in vitro* embryo production, oocyte retrieval.

The quantitative and qualitative efficiency of OPU with slaughterhouse bovine ovaries depends on several factors, as the specification of the needle and syringe. This procedure can cause morphological changes in the oocyte due to the physical impact generated by the procedure. These alterations are related to oocyte quality and competence and number of viable structures, which are important for the success of in vitro embryo production. Therefore, the aim of this work was to evaluate the effect of needle diameter and syringe volume on quantity and quality of bovine oocytes derived from slaughterhouse bovine ovaries. Follicles (2-8 mm) were aspirated using needles 18 G or 21 G and syringes 5 mL or 20 mL containing phosphate buffered saline solution, providing four experimental groups: 18G5, 18G20, 21G5 and 21G20. After OPU, the follicular content was assessed under a stereomicroscope and oocytes were classified according to the number of cumulus cells layers and homogeneity of cytoplasm as: Grade I (≥ 3 layers of compact cumulus cells and homogeneous cytoplasm), Grade II (1-2 layers of compact cumulus cells and homogeneous cytoplasm), Grade III (< 1 layer of cumulus cells and heterogeneous cytoplasm) and Grade IV (degenerated oocyte). All data were analyzed by the Fisher exact test ($P < 0.05$). After 16 repetitions (four repetitions/ system), OPU from 114 ovaries resulted in 382 recovered structures, providing 3.3 oocytes/ovary and an overall recovery rate of 44.2% (382/864). The percentages of retrieved oocytes per aspirated follicle were 25.3% (43/170), 35.9% (103/287), 65.2% (103/158) and 53.4% (133/249) for groups 18G5, 18G20, 21G5 and 21G20, respectively. Differences were observed in all aspiration systems, and the system 21 G needle with the 5 mL syringe promoted the recovery of the largest number of structures ($P < 0.05$). For oocyte quality, the use of the 20 mL syringe (26.2%, 27/103) resulted in a lower percentage of grade I structures, when compared to the 5 mL syringe (41.9%, 18/43) with the 18 G needle ($P < 0.05$). However, no difference was observed between syringe volumes using a 21 G needle for percentage of grade I oocytes (21G5: 36.9%, 38/103 vs. 21G20: 48.1%, 64/133, $P > 0.05$). Additionally, differences in grade IV structures were only observed between syringe volumes with the 21 G needle (21G5: 33.0%, 34/103 vs. 21G20: 18.8%, 25/133, $P < 0.05$). Finally, no difference was observed between the systems for the percentage of grade II and IIII oocytes ($P > 0.05$). In conclusion, the system 21 G needle and 5 mL syringe was quantitatively the most efficient, although all systems performed similarly qualitatively.

A044 Folliculogenesis, Oogenesis and Superovulation

Thermographic evaluation of ovarian tissue in autotransplanted Balb-c mice

M.B. Bezerra, F.A. Santos, M.M.L. Pimentel, A.C.G. Texeira, N.O. Santos, M.F. Macedo, R.G. Izzo, A.R.F. Ricarte

UFERSA.

Keywords: ovary, thermography, transplantation.

Infrared thermography has been tested in veterinary medicine as it is a fast and non-invasive method that allows a more precise diagnosis of diseases and physiological changes in tissues. This method consists in capturing the infrared radiation emitted by tissues and permits to infer about blood perfusion, which may indicate inflammatory or necrotic processes and if local perfusion is physiological. Thus, the present study aimed to assess tissue perfusion in autologous ovarian fragments transplanted under the kidney capsule, through the surface temperature obtained by infrared thermography during different phases of the autograft procedure in Balb -c mice. Sixteen sexually mature Balb-c females mice were anesthetized and submitted to laparotomy. An infrared thermographic camera (FLIR b60, InfraCAM Thermal Imager®) was used, the images were obtained 20cm from the tissue to be transplanted at three different moments: before tissue collection, when the ovary was exposed after incision (T1); immediately after transplantation, when fragments of approximately 1mm3 were transplanted under the kidney capsule (T2); and 30 days after the procedure (T3), when the animals were submitted to laparotomy. Images were transferred to the Flir Quick Report 1.2 Software (Copyright© 2009) for the evaluation of surface temperatures in ovarian fragments at different times. The statistical analysis was performed using BioEstat 5.3, the results were expressed as means and standard deviations, and submitted to ANOVA and Tukey test for comparisons between T1, T2 and T3. Significant differences were considered when $P < 0.05$. The mean values of surface temperatures at different times were: 29.7 ± 1.4 for T1, 27.3 ± 1.4 for T2 and to 28.8 ± 1.4 for T3. A significant reduction in the temperature was observed after the transplant (T2), which is compatible with the period of tissue ischemia. Thirty days after transplant (T3), temperature was similar to that observed in T1 and there were no signs of tissue death. In this study, infrared thermography was able to detect ischemia and reperfusion in the transplanted autologous fragment, indicating that this method can contribute to monitor tissue perfusion and reestablishment after transplantation.

A045 Folliculogenesis, Oogenesis and Superovulation

Morphological evaluation of ovarian autotransplantation to the perimetrium and subcutaneous tissue in Balb-c mice

M.M.L. Pimentel, F.A. Santos, P.D. Brito, R.G. Izzo, M.F. Macedo, M.B. Bezerra

UFERSA.

Keywords: autograft, Balb-c female mice, ovarian morphology.

The aim of this study was to morphologically evaluate ovarian fragments derived from autografts allocated in the subcutaneous tissue and perimetrium of mice. Twelve Balb-c female mice were randomly divided in three experimental groups with 4 animals in each group. Group I was the control group (non-transplanted animals), group II consisted of animals that received the ovarian fragment in perimetrium, group III included animals that had the abdominal subcutaneous tissue as the receptor site. The left ovary was removed and used in other studies, while the cortex of the right ovary was fragmented and reimplanted according to groups description. Thirty days after surgery, mice were euthanized, transplants removed and taken to histology. Ovarian morphology data was expressed descriptively. Follicles were classified qualitatively (normal and atresic) and morphologically (primordial, transitional, primary, secondary, early antral, antral and pre-ovulatory). Presence of corpora lutea and neovascularization in transplants was reported as percentages. All follicles, corpora lutea and blood vessels reveal characteristics described by the literature (Pedersen, T. Journal of Reproduction and Fertility, vol. 17th, p. 555-557, 1968) and no morphological differences were observed between these structures from fragments transplanted into the subcutaneous abdominal, into the perimetrium or from control nontransplanted tissue, suggesting that both sites allow ovarian development. Presence of corpora lutea was observed in 25% of the transplants located in the subcutaneous tissue and in 50% of those located in the perimetrium, indicating return to ovarian activity in these fragments. The presence of blood vessels was observed in 75% of the transplants to the perimetrium and 50% of those to the subcutaneous tissue, indicating neovascularization. A higher percentage of neovascularization and presence of corpora lutea was observed in animals having the perimetrium as the receptor site. Thus, it is concluded that ovarian autologous transplants allocated to the subcutaneous tissue and perimetrium are feasible and do not change the morphology of the transplanted tissue. Moreover, perimetrium was a better receptor site compared to subcutaneous tissue.

A046 Folliculogenesis, Oogenesis and Superovulation

Effect of body condition score on final follicular dinamics in Mangalarga Marchador mares

M.P. Bottino, J.N.S. Sales

Universidade Federal de Lavras.

Keywords: BCS, fertility, folicular growth.

The objective of this study was to evaluate the effect of body condition score on final follicular dynamic in adult Mangalarga Marchador mares. The study enrolled 52 mares (35 lactating and 17 non lactating) with body condition score (BCS) ranging from 4.5 to 8 (1-9 scale). Mares were divided into two groups according to BCS (High BCS Group - BCS≥7 and Low BCS Group - BCS<7). Animals with a corpus luteum received 6.71mg of dinoprost tromethamine (Lutalyse®, Pfizer, Brazil) intramuscularly. After the dominant follicle has reached a diameter between 33 and 35 mm, animals were examinated daily by ultrasound evaluation to determine the diameter of the dominant follicle and uterine edema degree (0 to 3 scale). Mares with a dominant follicle with a diameter equal or greater than 37 mm were given 1000 IU of hCG (Chorulon®, Merck, Netherlands) intravenously to induce ovulation. The determination of ovulation was made by ultrasound identification of a luteinized structure in the same place where the dominant follicle was located in previous examinations. Pregnancy diagnosis was performed 12 to 14 days after ovulation by ultrasound examination. All data were analyzed by GLIMMIX procedure of SAS. There were no differences between groups relative to initial diameter of the dominant follicle [High BCS Group (33.9±0.17mm) and Low BCS Group (33.56±0.24mm); P=0.18], maximum diameter of the dominant follicle [High BCS Group (45.73±0.84mm) and Low BCS Group (45.36±0.88mm); P=0.84], maximum diameter of the ovulatory follicle [High BCS Group (44.59±0.83mm) and Low BCS Group (44.92±0.90mm); P=0.75], follicular growth [High BCS Group (4.18±0.27mm) and Low BCS Group (3.83±0.29mm); P=0.17] reduction in pre ovulatory dominant follicle diameter [High BCS Group (0.69±0.21mm) and Low BCS Group (0.13±0.08mm); P=0.07], follicular diameter at hCG administration [High BCS Group (44.08±0.70mm) and Low BCS Group (43.88±0.96mm); P=0.69], follicular diameter at prostaglandin administration [High BCS Group (25.17±1.78mm) and Low BCS Group (24.06±1.98mm); P=0.76], uterine edema at induction of ovulation [High BCS Group (2.3±0.14mm) and Low BCS Group (2.21±0.18mm); P=0.69], uterine edema after ovulation [High BCS Group (0.59±0.13mm) and Low BCS Group (0.33±0.12mm); P=0.09] and pregnancy rate [High BCS Group 61.3% (19/31) and Low BCS Group 63.6% (7/11); P=0.89]. Therefor, BCS did not interfere in final follicular dynamics of Mangalarga Marchador mares.

A047 Folliculogenesis, Oogenesis and Superovulation

Efect of inhibition of L-ARG/INOS/NO pathway by aminoguanidine on in vitro maturation of bovine oocytes-cumulus complexes (COCS) in the presence of follicular wall hemisections

N.F. Torres, M.C. Caldas-Bussiere, K.S. Nogueira, D.F. Dubeibe, C.S.P. Carvalho, G.B. Aguiar, V.L. Maciel, C.L.M. Souza

UENF.

Keywords: *in vitro* maturation, nitric oxide, nucleotides.

The knowledge of the mechanisms involved in oocyte-cumulus complex (COC) maturation is important for the progress of reproductive biotechnologies and assisted reproduction techniques. Nitric oxide (NO) is involved in the process of in vitro maturation of bovine COCs (Viana, K. S. et. al., Anim. Reprod. Sci. 102, 217-22, 2007). The synthesis of NO occurs from L- arginine, which is catalysed by three isoforms of nitric oxide synthase (NOS) (Ignarro, L.J., 2000, Nitric Oxide, Biology and pathobiology, 845p). Studies support that NO derived from inducible nitric oxide synthase (iNOS) affects oocyte maturation (Matta, G. S. C. et.al, 2009 Anim. Reprod. Sci. 111, 189-201). This study aimed to evaluate the role of iNOS in the maturation of COCs by adding the selective inhibitor aminoguanidine (AG) to the culture medium. Groups of 20 COCs (120 COCs/treatment) were cultured with eight follicular wall hemi-sections (HS) at 38.5°C and 5 % CO_2 in 200 ml of maturation medium (TCM 199/BSA) supplemented with different AG concentrations (1, 10, 50 , 100 and 150 mM). Controls consisted of COCs cultured in the presence (control -) or absence of HS (control +). Nuclear maturation was assessed by staining with 2% acetic orcein and plasma membrane integrity of cumulus cells by propidium iodide staining after 22h of culture. A randomized design with 6 replicates (120 COCs/treatment) and 7 treatments (1, 10, 50, 100 and 150 mM AG and two controls) was used. The percentage of cellular integrity was subjected to ANOVA and multiple linear regression, nuclear maturation was assessed by ANOVA and means were compared by the Tukey test ($P < 0.05$). The integrity of cumulus cells of the group of oocytes cultured without HS (control +) (85.9 ± 2.3 %) differed in relation to the control group - (71.2 ± 3.7 %), and to groups treated with 1, 10, 50, 100 and 150 mM AG, (57.8 ± 12.1, 66.3 ± 4.2, 58.2 ± 4.6, 55.3 ± 4.3, 48.3 ± 3 3, respectively) ($P < 0.05$). The addition of 150 mM AG promoted the lowest number of viable cells (48.3 ± 3.3%; $P <0.05$). The presence of HS (Control -) decreased the percentage of oocytes that reached metaphase II (MII) (41.0 ± 4.0%) compared to the control group + (78.5 ± 3.9%). However, the addition of 100 and 150 mM AG blocked the progression of meiosis to MII compared to other treatments and controls ($P <0.05$). These results suggest that activity of iNOS is directly linked to cumulus cells integrity and resumption and progression of meiosis to MII.

A048 Folliculogenesis, Oogenesis and Superovulation

Caracterization of follicular dynamics in Morada Nova sheep

R.N. Assis, J.F. Silva, E.J.F.S Araújo, E.M.S.T. Melo, L.M. Almeida, M. Fernandes, W.H.C. Pequeno, A.G.F. Santos, L.R. Souza, J.N.S. Sales

UFPB.

Keywords: follicular growth, follicular wave, ovulation.

The aim of this study was to characterize follicular dynamics of Morada Nova ewes. The experiment was conducted at the experimental station of São João do Cariri – Praíba, where 13 Morada Nova ewes with body scores ranging 2.0-3.5 and maintained in semi-intensive system were used. On a random day of the estrous cycle, ewes were synchronized with an intravaginal progesterone (P4) device. Ten days later, the P4 device was removed and 250µg cloprostenol was administered intramuscularly. From this day, ultrasound examinations were performed every 24 hours for 24 days or until ovulation. Statistical analysis was performed using the statistical analysis system (SAS, 2000). The length of the estrous cycle was 16.0 ± 0.5 days and 3.7 ± 0.3 waves per estrous cycle were observed. The maximum diameter of the ovulatory follicle at the end of the estrous cycle and synchronized wave were similar (5.6 ± 0.3mm and 6.1 ± 0.3 mm; P=0.39). The ovulatory follicle present at the end of the estrous cycle and synchronized wave showed similar growth rates (0.4mm/day and 0.3mm/day; P=0.37). Moreover, the interval between P4 device removal and ovulation was 72.0 ± 11.8 hours. The number of ovulations at the end of the estrous cycles was lower than that observed in synchronized waves (1.0 ± 0.0 and 2.0 ± 0.2; P=0.002). We conclude that Morada Nova ewes display follicular dynamics patterns similar to those observed in other breeds.

A049 Folliculogenesis, Oogenesis and Superovulation

Improvement of the *in vitro* development of equine preantral follicles by ascorbic acid supplementation

S.M. Gonzalez[1], R.G. Gomes[1], R.L. Oliveira[1], M.M. Santos[1], F.D. Sarapião[1], I. Búfalo[1], A.G. Lindquist[1], D.F. Higashi[1], T.R.R. Barreiros[2], M.M. Seneda[1]

[1]Universidade Estadual de Londrina; [2]Universidade Estadual do Norte do Paraná.

Keywords: equine, *in vitro* culture, sacorbic acid.

The aim of this study was to evaluate the effect of adding different concentrations of ascorbic acid to in vitro culture of equine preantral follicles. Ovaries (n = 5) from mares in the anovulatory season were collected at a local slaughterhouse and washed in 70% ethanol and PBS. The internal region (parenchymal) was divided into 9 pieces of approximately 5x5x1 mm. One fragment was immediately fixed in Bouin (control) and the other 8 pieces were transported for one hour to the laboratory in PBS plus penicillin (200 IU/mL) and streptomycin (200 mg/mL) at 4°C. At the laboratory, 8 fragments were individually cultured in culture dishes at 39°C in an atmosphere with 5% CO2 in air with saturated humidity for two (D2) or six (D6) days in 1 ml of minimum essential medium (MEM) - (Gibco BRL, Rockville, MD, USA) (osmolarity 300 mOsm/L pH 7.2) supplemented with ITS (6.25 ng/mL insulin, 6.25 ng/mL transferrin, 6 25 ng/mL selenium), 0.23 mM pyruvate, 2 mM glutamine, 2 mM hypoxanthine, 1.25 mg/mL bovine serum albumin, 200 IU/mL penicillin and 200 mg/mL streptomycin (MEM+). The treatments consisted of MEM+ supplemented with different concentrations of ascorbic acid (25, 50, or 100 µg/ml). Medium was replaced every two days. After culture, ovarian fragments were fixed in Bouin and processed for histology. Follicles were classified according to their stage of development in primordial, primary, secondary and antral follicles, and according to their morphology in normal or degenerated. Data were compared by nonparametric analysis of variance (Kruskal Wallis test), considering 5% significance level. Overall, 951 slides were evaluated containing 4,450 sections. Follicles were present in 4,85% (216/4450) of the evaluated histological sections. We classified 43.5% of the follicles as morphologically normal. The control group showed a higher proportion of primary (89 %) than developing follicles (11 %). Regarding follicular development, fragments treated with MEM (D6) and 25 µg/ml of ascorbic acid (AA 25D6) presented a higher percentage of developing follicles in comparison with primordial follicles. This group showed greater follicular development (26.3% of primordial follicles and 73.7% of developing follicles) compared to the other groups analyzed ($P \leq 0.05$). We conclude that ascorbic acid at 25 µg/ml was effective to promote the development of equine primordial follicles for six days of culture.

A050 Folliculogenesis, Oogenesis and Superovulation

Immunohistochemical localization of active caspase-3 in the bovine fetal ovary

S.S.D. Santos[1], K.N.L. Brito[1], M.A.P. Ferreira[1], L.F.S. Oliveira[1], N.N. Costa[1], T.V.G. Silva[1], P.P.B. Santana[1], M.S. Cordeiro[2], M.S. Miranda[1], O.M. Ohashi[1]

[1]Universidade Federal do Pará; [2]Instituto Federal do Pará.

Keywords: apoptosis, caspase-3, folliculogenesis.

Caspases (cysteine - dependent aspartate - specific proteinases) belong to the family of cysteine proteases that have the ability to recognize and cleave substrates having aspartate residues. There are 14 known caspases, and six (caspases -3, -6, -7, -8, -9, -10) are involved in apoptosis. During the mitotic period, the number of oogonia in bovine ovaries reaches two million per fetus and after this period the oogonia enter meiosis until prophase I. However, approximately 90 % of the oogonia are lost and at birth the follicular population is approximately 200,000 (Beckers et al., Reprod. Dom Anim., 31, 543-548, 1996). Waves of degeneration were observed during this period in humans (Baker, T.G Proccedings of the Royal Society, Series B, 158, n 972, 417-433, 1963) and cattle (Erickson, B.H, J. Reprod Fertil, 10, 97-105, 1966). The objective of this study was to evaluate the occurrence of apoptosis during folliculogenesis in bovine fetuses by immunohistochemistry for caspase -3. Ovaries from 15 bovine fetuses aged between 5 and 9 months (Abdel-Raouf M , El-Naggar MA . 1968. Biometry of the Egyptian buffalo fetus. UARJ Vet Sci, 5:37-43) were obtained in a slaughterhouse and were fixed in 10% formalin solution for 24 hours, embedded in paraffin and sliced in 5 m thick histological sections. After deparaffinization, sections were submitted to immunohistochemistry for caspase -3. Bovine fetal ovaries showed immunostaining for active caspase -3 at 5, 6, 8 and 9 months of age, indicating the occurrence of apoptosis. At 5 months, there was immunostaining for active caspase -3 only in the oocytes. At 6 and 8 months, immunostaining for active caspase -3 was observed in follicular cells and oocytes, and at 9 months immunostaining was observed in theca cells of antral follicles. This is the first study reporting immunostaining for active caspase -3 in bovine fetal ovaries and this technique was effective to detect ovarian apoptosis, especially in oocytes as evidenced at 5 and 8 months. This contrasts with previous data in which Tunel only detected apoptosis in follicular cells. We conclude that immunohistochemistry for active caspase -3 can be used to detect apoptosis in bovine fetal ovaries, with the benefit of evaluating both follicular cells and the oocyte. More studies are needed to better characterize the occurrence of apoptosis in bovine fetal ovaries.

A051 Folliculogenesis, Oogenesis and Superovulation

Differences in the lipid profile of the oviduct of Aberdeen Angus and Nelore heifers with high and low follicular population

V. Pinheiro[1], K.R.A. Belaz[2], M.N. Eberlin[2], E.M. Razza[1], C.M. Barros[1], M.F.G. Nogueira[1]

[1]UNESP; [2]UNICAMP.

Keywords: follicle, oviduct, reproduction.

Follicular growth in cattle occurs in a wave pattern varying from 2 to 3 waves during each estrous cycle, and is characterized by the initial development of a synchronous group of antral follicles, from which only one will become dominant. The number of recruited follicles is variable among individuals and breeds; however, it is highly repeatable in individuals. Literature reports indicate that the number of follicles recruited in each wave is higher in zebu breeds compared with taurine. A relationship between the follicular population and the oocyte competence has been suggested, and ultimately the follicular population could influence the fertility of the animal (Ireland et al., 2007, Human Reproduction 22:1687-1695). The objectives of this study were: i) to identify females in Nelore (*Bos indicus*) and Aberdeen Angus (*Bos taurus*) breeds that have high and low population of recruited antral follicles per wave and ii) to characterize, in both breeds, the differences in lipid profile of oviductal sections (infundibulum, ampulla and isthmus) 24 h after ovulation (D1). We used nulliparous Nelore heifers [n=4, 24 to 30 months old for both groups (high and low)] and Aberdeen Angus [n=4, 16 to 20 months of age for both groups (high and low)] with body condition score above 3.5 (range 1-5 points) and their antral follicular population was assessed by ultrasonography (3 exam.). All animals had synchronized ovulation and were slaughtered at the same time (D1) to collect oviducts. Oviductal sections were subjected to matrix-assisted laser desorption/ionization mass spectrometry to assess their lipid profile. Lipids were ranked and analyzed by ANOVA (mixed model, Proc PRINCOMP, SAS).There was no difference in the oviductal lipid profile between animals with high versus low follicle count. However, there were differences in lipid profile between breeds (P=0.0462) and segments of the oviducts (P = 0.001). Between breeds, some specific lipids such as phosphatidylcholine [PC (34:1), PC (38:7) and PC (38:4)] were more abundant in Nelore but sphingomyelin [(SM) (16:00)] was more abundant in Aberdeen Angus. Among oviductal sections, there were higher abundances of PCs (34:1, 38:7 and 38:4) in the isthmus and of SM (16:00) in the infundibulum. Hence, the mass-spectrometry evaluation detected differences in phospholipids (e.g., PC and SM) associated with the number of carbons and the levels of unsaturation of the carbon chain related to oviductal section and breed.We conclude that there are differences in lipid profiles of oviductal sections in B. taurus and B. indicus. Ongoing studies will compare the differences in the presence of oxidative stress, and relate them to fertility in normal conditions or heat stress between the two breeds.

FAPESP: #2013/02201-3, #2012/50533-2 and #2012/23409-9.
Opinions, hypotheses and conclusions or recommendations expressed in this material are authors' responsibility and do not necessarily reflect the views of FAPESP.