A090 OPU - IVF and ET

Fertilization rate and developmental kinetics of bovine embryos produced using semen from high and low *in vitro* fertility bulls

Mayra Elena Ortiz D´Ávila Assumpção, Tamie Guibu de Almeida, Leticia Signori Castro, Adriano Felipe Siqueira, Vivian da Silva Cardoso Pinto, Thaís Rose dos Santos Hamilton, Luana de Cassia Bicudo, Camilla Mota Mendes, Mayra Fernanda Alves, Andrea Cristina Basso

FMVZ/USP, Sao Paulo, SP.

In vitro embryo production is a well-established and commercial technique, being nowadays the best choice for embryo production in some breeds. Therefore, better results can be achieved with the selection of high in vitro fertility bulls. Understanding why a high fertility bull produce more blastocysts than a low fertility one is the key for bull selection. Since embryo kinetics can be linked with embryo viability (Edwards, 2003), it could be a powerful tool to identify differences between these two groups of bulls. The aim of this study was to evaluate early embryo kinetics and fertilization rate from bulls with high (HF) and low (LF) in vitro fertility. For bull selection, a commercial laboratory database was assessed. Bulls were ranked based on blastocyst/cleavage rate (embryo development rate). Ten bulls with high (n=5) and low (n=5) in vitro fertility were used for 5 manipulation of in vitro embryo production, as described by Pontes et al. (2010). Ten oocytes from each manipulation (n=50) were stained with 1 mg/ml Hoechst 33342 (Sigma), washed, mounted on microscope slides and examined with epifluorescence microscopy (Olympus IX80, Olympus Corporation, Tokyo, Japan). The presumptive zygotes were classified in three categories: negative fertilization (1 PN); normal fertilization (2 PN), and polyspermy (>2 PN). Embryos were classified by their specific stage of development (2; 3-4; 6 or 8 cell stage) at 24, 36, 48, 60, 72 hpi (hours post-insemination). Cleavage rate was assessed at day 3 (D3) of embryo culture, viable blastocysts and embryo development rates were assessed at day 7 (D7). Data were analyzed using PROC GLIMMIX of SAS (SAS ® 9.3 Institute Inc., Cary, NC, USA, 2003). Blastocyst rate was higher in the HF group (29.4%) than in the LF (16.0% - $P<0.0001$), similarly to embryo development rate (HF = 34.0%; HL = 189%; $P<0.0001$). There was no significant difference in cleavage rate (HF=86.7%; LF= 84.9%; P= 0.2581), neither in embryo kinetics, in all of the evaluated periods ($P>0.05$). No difference was found in negative fertilization (HF=10.69%; LF= 8.80%; P=0.9925), nor in polyspermy between groups (HF=16.18%; LF=29.20%; P=0.6066). However, normal fertilization was higher in the HF group (72.0%) than in LF group (62.0%) (P=0.0332). In conclusion, early embryo kinetics could not explain the difference in blastocyst rate between high fertility (HF) and low fertility (LF) bulls. Nevertheless, HF bulls had more normal fertilization than LF bulls.

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A091 OPU - IVF and ET

Effect of insulin: glucose ratio on oocyte and embryo production and pregnancy rate in lactating dairy cows

Tiago Leiva[1], Reinaldo Fernandes Cooke[2], Umberto Pardelli[1], Rodrigo de Oliveira Rodrigues[1], Jose Luiz Moraes Vasconcelos[1]

[1]FMVZ/UNESP, Botucatu, SP; [2]Oregon State University, Burns.

Insulin:glucose ratio has been used to determine insulin resistance in dairy cows, which may affect reproductive outcomes in dairy cows. So, the aim of this study was evaluate the insulin:glucose ratio on oocyte and embryo production and pregnancy rate in lactating dairy cows. Follicles of two-hundred-six non-pregnant lactating dairy cows between 80 to 280 days in milk were aspirated in random days of the estrus cycle for embryo production. At the time of follicle aspiration, cows were scored for body condition, and blood samples were collected for determination of plasma concentrations of glucose and insulin. Data was analyzed using a MIXED and GLM procedure of SAS. Insulin:glucose ratio concentrations were classified using its median distribution (0.05 and 0.32 ± 0.01 for I:G below [Be] or above [Ab] or median, respectively). There was no effect of insulin:glucose ratio on oocyte production (13.02 vs. 13.47 ± 1.4 oocyte for Be and Ab, respectively; P = 0.80), embryo production (1.48 vs. 0.98 ± 0.28 embryo for Be and Ab, respectively; P = 0.15) and pregnancy rate (US1 = 53 vs. 47 ± 5% [open vs. pregnant] for Be and Ab, respectively; P = 0.40; and US2 = 57 vs. 43 [open vs. pregnant] for Be and Ab, respectively; P = 0.48). However, there was tended (P = 0.06) for embryo produced per oocyte collected, where cows with insulin:glucose ratio below of median had higher number of embryo produced per oocyte collected compared with cows Ab (0.11 vs. 0.07 ± 0.17, respectively). There was no effect of regression model for insulin:glucose ratio per oocyte production P = 0.20; I:G per embryo production P = 0.96 or I:G per embryo produced per oocyte collected P = 0.55. In conclusion, lower insulin: glucose ratio increase the number of embryo produced per oocyte collected, but they was not efficient to change any others reproductive variables.

A092 OPU - IVF and ET

Hostein and Gyr gametes contribute differently to the embryonic development morphokinetics

Clara Ana Santos Monteiro[1], Gabriela Ramos Leal[1], Renata Pasolini[2], Agostinho Jorge dos Reis Camargo[2], Raquel Varella Serapião[2], André Luís Rios Rodrigues[1], Luiz Altamiro Garcia Nogueira[1], Ana Maria Reis Ferreira[1], Clara Slade Oliveira[3]

[1]Universidade Federal Fluminense, Niterói, RJ; 2.Pesagro-Rio, Niterói, RJ; [3]Embrapa Gado de Leite, LRA/CESM, Valença, RJ.

In the present study we aimed to assess the possible differential contribution of Holstein (Bos taurus taurus) and Gyr (Bos taurus indicus) gametes on morphokinectical embryo development. To do so, we collected oocytes from 16 purebred donors, Holstein (H n=8) and Gyr (G n=8), using the ovum pick up (OPU) technic, and produced in vitro F1 embryos by Holstein and Gyr gametes cross fertilization (HG and GH, in which the first letter represents donor's breed and second bull's breed) and parthenogenetically activated embryos (H and G). Then, assessed the morphokinects development using a Multi-embryo Chamber (MEC), an in vitro culture (IVC) system based in group culture, but allowing individual assessment, in order to compare embryos (HG vs GH and H vs G) cell numbers at 48, 96 and 144 hours post fertilization. The data were expressed as mean ± SD being the cell number means compared using unpaired T-test and Mann-Whitney for non-parametrical data. Analyses were performed using GraphPad Instat software, at 5% significance level. Additionally, only embryos that reach the blastocyst stage of development were compared between groups (HG n=6, GH n=5, H n=8 and G n=9), in order to compare gametes breed contribution, isolated from development potential. The F1 embryos from Holstein oocytes with Gyr spermatozoid cross-fertilization (HG) develop faster than its reverse at 48 h, GH (48 HPI: 5.00 ± 0.63 vs 3.40 ± 0.89 P = 0.0170), however were similar at 96 HPI (6.67 ± 1.21 vs 5.80 ± 0.84 P = 0.2103) and at 144 h HPI (83.45 ± 21.68 vs 60.44 ± 29.08 p = 0.2632), suggesting gametes differential contribution. While embryos produced by Holstein and Gyr oocytes activation presented similar cell number at all time-point (H vs G; 48 HPI: 4.13 ± 0.64 vs 4.11 ± 0.93; 96 HPI: 7.79 ± 2.81 vs 8.99 ± 4.13; 144 HPI: 72.39 ± 27.48 vs 68.70 ± 27.99), which may indicate that Holstein and Gyr oocytes have similar contribution in embryo development. Based on results, we conclude that Holstein and Gyr gametes differently contribute in the kinetics of embryonic development and suggest bull contribution as the main factor for pointed differences. Financial support: CAPES, FAPERJ and FAPEMIG.

A093 OPU - IVF and ET

Effect of the stage of the estrous cycle of domestic cats on the oocyte maturation competence

Fernando Pedro Perea Ganchou[1], Enmar Monasterio[2], Silvana Mendez[1], Carla Osorio[2], Amaru Pirela[2], Jose Atilio Aranguren[2], Hugo Hernandez[2]

[1]Universidad de Cuenca, Cuenca; [2]Universidad Del Zulia, Maracaibo.

Objective: In vitro fertilization (IVF) can be used in the domestic cat (Felis catus)in order to generate valuable information that can be applied in reproductive physiology of wild feline endangered species (Luvoni, C. Reprod. Nutr. Dev. 40:505-512. 2000). In vitro Maturation (IVM) of Cumulus Oocyte Complexes (COCs) in cats is considered as key in the advancement of IVF in these wild endangered species. The objective of this research study was to evaluate the oocyte competence of COCs obtained at different stages of the estrous cycle of domestic cat females. Materials & Methods: Eighteen clinically healthy 8 months to 2.5 years aged female cats were included in this research. All females were housed under similar conditions (food, water, light) 72 hours prior to surgery (ovaryectomy, OVX). Cats were previously classified according to the stage of the estrous cycle through a vaginal cytology performed hours before the OVX. Three groups resulted according to the previous criteria: a. Follicular Phase (FP), b. Luteal Phase (LP) and Anestrous (A). After OVX, COCs were recovered from follicles using a mixed technique of punction-fragmentation of the ovarian cortex. COCs were thereafter washed in 100 µL droplets (no more tan 25 COCs/droplet) of wash and maturation media supplemented with FSH and LH (Folltropin, Bioniche, Belleville, Ontario, Canada), 17β- Estradiol, BSA, sodium pyruvate and gentamycin. After 30 hours of IVM, COCs were mechanically denuded and fixed in a solution of ethanol:acetic acid (3:1) during 24-72 hours at 4°C. The degree of nuclear maturation was evaluated by staining oocytes with a 1% Aceto-orcein solution (45% of acetic acid) during 30 minutes. Oocyte maturation was characterized as an oocyte in telophase I or metaphase II. Data was analyzed using ANOVA and Chi-square of SAS. Results & Conclusions: The proportion of COCs morphologically fit to initiate IVM did not differ among the different stages of the estrous cycle. In this study 13,7% (41/298) of the oocytes (all groups included) reached nuclear maturation. The maturation rate (%) for oocytes matured in the FP, LP and A were 12,3% (11/89), 17,3% (25/144) y 7,7% (5/65), respectively (P>0,05). Although the difference was not statistically significant, there was a maturation rate 2,5 times greater in the LP than in the A phase (P=0,065). In conclusion, oocytes obtained in different stages of the estrous cycle have similar capacity of reaching maturation under in vitro conditions.

A094 OPU - IVF and ET

Identification of CD46 and CD59 in Brahman Bull cryopreserved sperm cells

Enmar Coromoto Monasterio[1], Venus Lira Vilchez[1], Daniel Argudo[2], Manuel Soria[2], Juan Pablo Hernandez[1], Hugo Hernandez[1]

[1]Universidad Del Zulia, Maracaibo; [2]Universidad de Cuenca, Cuenca.

The main role of the complement system is to protect and defend the host from the attack of pathogenic micro-organisms. Previous studies report the existence of several complement regulatory proteins expressed in different tissues (Harris & Morgan, In: The Complement System: Novel Roles in Health and Disease, pp. 129–166, 2004). These proteins are related with reproduction and are expressed in human and mice sperm cells (Fusi et al. Mol Reprod Dev 29(2):180-188, 1991; Rooney et al. Immunology 75(3):499-506, 1992. The objective of this study was to determine the localization of CD46 and CD59 in frozen-thawed Brahman bull semen, using the Indirect Immunofluorescence Technique (IFI). Materials & Methods: Semen from four ejaculates of five different Brahman bulls was used. Capacitation was induced with heparin while acrosome reaction (AR) was induced with calcium ionophore; and both were evaluated with the chlortetracycline stain (CTC). Afterwards, IFI was used to determine the presence of CD46 and CD59. The statistical analysis was performed using S.A.S for Windows, version 8.2 (S.A.S. Inst. Inc.; Carry, NC, USA). Data were analyzed through a general lineal model ANOVA and results are shown as means ± standard deviation. Results & Conclusions: Data analysis demonstrated that CD46 and CD59 are expressed in 35.93 ± 10.49% and 33.80±12.66%, respectively in bull sperm cells. The expression of both proteins increased significantly after treatment with heparin (CD46: 68.26± 5.29%; CD59: 71.33± 6.24%) and it varied among bulls. It was determined that both proteins are located predominantly in the acrosomal region, which coincides with its location in humans and mice. When bull effect was analyzed, it was observed that the calcium ionophore increased the expression of CD46 and CD59 in all the bulls in comparison to heparin and control groups. However, the effect of heparin was different, and this depended of bull and the antigen. In comparison to control group, heparin only increased the percent of positive sperm to CD46 in two of five bulls, while heparin increased expression of CD59 in all the bulls. In conclusion, the bovine sperm expresses both CD46 and CD59 proteins, mainly in the acrosomal region and this expression was related with the capacitation and AR processes.

A095 OPU - IVF and ET

Effect of spermatozoa concentration on *in vitro* production of bovine embryos

Wasim Al Al Sebli[1], Antônio Santana dos Santos Filho[2], Cláudio Coutinho Bartolomeu[1], Sebastião Inocêncio Guido[2]

[1]UFRPE, Arcoverde, PE; [2]Instituto Agrônomico de Pernambuco, Arcoverde, PE.

The aim of these experiments was to study the effect of different insemination doses on PIV; the semen samples were utilized from two bulls one with a history of low in vitro embryo production (B1) and the other with a good one (B2). The experiment was conducted at the laboratory of animal breeding and reproduction in the experimental station of the Agronomic Institute of Pernambuco (IPA) in the city of Arcoverde. 180 oocytes were aspirated from antral follicles (3-8 mm) of ovaries from recent slaughtered cow and then matured for 24 hours in TCM199 medium. Two Girolando 5/8 bulls (B1,& B2) were the donors of cryopreserved semen; the semen was capacitated by 90% percol gradient. 90 oocytes were divided in 6 groups of 15 oocytes and fertilized as follows, (B1T1), (B1T2), and (B1T3) were fertilized by 2*106, 3*106, and 4*106 respectively, and (B1T1), (B1T2), and (B1T3) the doses and groups were as B1 (Each treatment was repeated 2 times).18 hours after IVF the presumptive zygotes were washed after then cultured in SOF medium. The incubation was in the temperature of 38,5°C, with 5% CO2, and 95% of humidity. The cleavage rate was observed 48 hours after IVF; and 7 days after the IVF the blastocyst rate was observed. The data were analyzed by chi-square test at 5% of signigicance. The cleavage rates in (B1 T1) (B1 T2), and (B1 T3) were 52,17%, 69,23%, and 60% respectively, and in (B2 T1), (B2 T2), and (B2 T3) were 75%, 59,2%, and 82,7% respectively. 7 days after the fertilization the blastocyst rate in B1 groups was 8,7%, 15,3%, and 8 % in (B1 T1), (B1 T2), and (B1 T3) respectively; the blastocyst rate in B2 groups was 18,7%, 25,9%, and 31 % in (B2 T1), (B2T2), and (B2 T3) respectively, as well as 33,3% of the blastocysts of (B2 T1) were hatched. Our results have proved the effect of different concentrations of capacitated bovine spermatozoa and its difference between the two bulls on the blastocyst rate. The increased concentration of spermatozoa of B1, increased the cleavage rate in T2 and T3, however blastocyst rate was increased in T2. In B2T3 the increased spermatic concentration increased the production of blastocysts, as well as the cleavage rate in B2T2 was less than B2T1, and B2T3; however, Heeres et al., 1996 (Theriogenology, v.45, p.266). the best cleavage rate and embryos production were observed when the spermatic concentration of 0.5*106 sperm/1 ml, these observed differences can be related to genetic factors of the bulls.(First and Parrish, J Reprod Fertil, v.34, p.151-165, 1987); The utilization of spermatic concentration which is higher than threshold in IVF can induce increased abnormal fertilization, principally the polyspermy, without changing in the monospermatic fertilization. In this research we have concluded that the individuality of bulls is an important factor to produce bovine embryos in vitro, because we observed that the bull of the history of low IVP increased the production of embryos two times when the spermatozoa concentration was increase from 2*106 to 3 *106, but the spermatic concentration of 4*106 had no effect on IVP, on the other hand the increased spermatic concentration of the bull of history of good IVP, increased the IVP; so more researches are needed to verify the paternal importance in IVP.

A096 OPU - IVF and ET

Ovum pick up and quality of oocytes in stimulated donors with equine corionic gonadotrofin

Felipe Costa Gonçalves[1], Carlos Antônio de Carvalho Fernandes[1], Ana Cristina Silva de Figueiredo[2], Ester Siqueira Caixeta[3], Miller Pereira Palhão[1], Jairo Pereira Neves[1], Deiler Sampaio Costa[4]

[1]Universidade de Alfenas, Alfenas, MG; [2]Biotran - Universidade de Alfenas, Alfenas, MG; [3]Unifal, Alfenas, MG; [4]UMFS, Campos Grande, MS.

Until recently ovum pick up (OPU) in cattle was generally done at random times of the estrous cycle. However, the increasing need to improve efficiency and increase in demand for OPU at less favorable situations leads to an increase in the use of pre-aspiration protocols. In vitro embryo production (IVP) efficiency has been compromised by poor oocyte quality. The aim of the study was to develop a pre-aspiration protocol to synchronize the follicular wave and stimulate follicular development using equine chorionic gonadotrophin (eCG). Twenty Nelore breed cows were randomly assigned to two treatments in a crossover design with two replicates. T1: CTRL (N = 20): D0 - intravaginal implant of progesterone (DIB™, MSD Animal Health, Brazil) and 2mg of Estradiol Benzoate IM (Gonadiol™, MSD Animal Health, Brazil) and OPU in D5. T2: TRAT (N = 20): Same protocol above with the inclusion of 400UI of eCG (Folligon™, MSD Animal Health, Brazil) IM in D3. In D5 follicular measurement and follicular aspiration were done using an ultrasound equipment (Mindray®-M5). The oocytes classified as viable were sent to IVF in maturation medium, in gassed cryotubes transported in carrier device at 37.5°C. IVF procedures was performed in the same Laboratory using standard protocols. Fertilization was performed 22 to 24 hours after placement of the oocytes in the maturation medium. A semen from the same bull and bath was used. The embryos were cultured for seven days in an atmosphere of 5% CO2 and 38.5°C. After seven days of culture the embryos classified as grade 1 were counted. We evaluated following variables: mean number and size of follicles at D5, quantity and quality of oocytes recovered and embryos. The data were submitted to normality tests and means of treatments were compared by ANOVA considering 5% of probability as significance. No differences were showed in the total follicle mean in D5 (27.1 and 24.9, for T1 and T2). However the diameter was higher in T2 (3.6 + 0.7 and 3.2 + 0.6 - $P < 0.05$). Mean of total oocytes (23.2 + 14.3 and 25.6 + 13.4 for T1 and T2) and viable (14.6 + 11.2 and 16.6 + 9.4 for T1 and T2) did not Differed between treatments. Total embryos (2.6 + 1.7 and 3.5 + 2.8) were higher ($P < 0.05$) in females treated with eCG. The results showed that the amount of oocytes nor their morphological quality was altered by the treatment. However, due to the greater production of T2 embryos, probably the intrinsic developmental capacity of oocytes obtained in animals from the group treated with eCG is better. It was concluded that the stimulation of bovine donors with eCG does not improve the quantity or morphological quality of the aspirated oocytes, but increases the potential of embryo production by the PIVE technique.

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A097 OPU - IVF and ET

Evaluation of different incubation systems on bovine *in vitro* embryo production

Camila Muniz Cavalcanti[1], Iana Sales Campelo[1], Mirelly Mirna Alves de Sousa Silva[1], Thiago Victor Damasceno Teixeira[2], João Victor da Silva Albuquerque[1], Luciana Magalhães Melo[1], Vicente José de Figueirêdo Freitas[1]

[1]Universidade Estadual do Ceará, Fortaleza, CE; [2]Universidade Federal do Ceará, Fortaleza, CE.

The use of reproductive biotechniques, such as *in vitro* embryo production (IVP) may contribute to the genetic improvement of the bovine herd. In this context, many efforts have been made to obtain culture systems that are less harmful to embryos. This study aimed to evaluate both the maturation rate and bovine blastocyst production using three different incubation systems. Follicles of 2-8 mm were punctured from bovine ovaries obtained in local slaughterhouses. The obtained *cumulus*-oocyte complexes (COCs) were randomly divided into three groups according to the incubation system: conventional bench incubator - CONV (Thermo, Thermo Fischer, Waltham, USA) with high oxygen tension (5% CO_2), mini-bench incubator - MINIb (Eve, WTA, Cravinhos, Brazil) with low oxygen (5% CO_2, 5% O_2, 90% N_2) and mini portable incubator - MINIp (LabMix, WTA) also with low oxygen tension. *In vitro* maturation (IVM) was performed in TCM-199 (Sigma-Aldrich, St. Louis, USA) supplemented for 22 h at 38.5 °C. After this, a sample of oocyte was stained with Hoechst 33342 (Sigma-Aldrich) and analyzed by fluorescence microscopy (Eclipse E400, Nikon, Tokyo, Japan) in order to determine the maturation rate. For *in vitro* fertilization (IVF), COCs were incubated with frozen/thawed semen in Brackett-Oliphant medium (BO) for 6 h at 38,5 °C. *In vitro* culture (IVC) was performed for 8 days in synthetic oviduct fluid (SOF) at 38.5 °C. Cleavage rate was verified on day two (D2) and blastocyst rate on days seven (D7) and eight (D8) of culture. Data were analyzed by the Student *t* test with 5% of significance level. No significant differences were found for the maturation rate, which was 70.4%, 50.8% and 57.6% for CONV, MINIb and MINIp, respectively. A total of 1067 (CONV = 359; MINIb = 356; MINIp = 352) presumable zygotes were submitted to culture. Concerning cleavage rate, CONV (71.0%) produced the better results ($P < 0.05$) when compared to MINIb (44.1%) and MINIp (35.8%). Similarly, significant differences ($P < 0.05$) were verified for blastocyst production in D7 (CONV = 32.0%, MINIb = 21.1%, MINIp = 11.4%) and D8 (CONV = 32.0%, MINIb = 21.1%, MINIp = 12.8%). In conclusion, the incubation system provided by CONV resulted in higher rates for embryo production. However, it is necessary more repetitions for definitive conclusions.

A098 OPU - IVF and ET

Evaluation of lipid content and gene expression in bovine embryos produced *in vitro* treated with natriuretic peptide C (NPPC)

Amanda Fonseca Zangirolamo[1], Camila Bortoliero Costa[1], Paula Alvares Lunardelli[1], Elisa M. Pioltine[2], Bruna Lopes Cardoso[3], Andrea Cristina Basso[3], Marcelo Fabio Gouveia Nogueira[2], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina, Londrina, PR; [2]Universidade Estadual Paulista, Botucatu, SP; [3]In Vitro Brasil Ltda, Mogi Mirim, SP.

The use of natriuretic peptide C (NPPC) has been described to elevate cAMP levels blocking meiosis in oocytes (Zhang, W. Journal of Cellular Physiology, v 230, pp. 71-81, 2015). However, there are no reports on the use of NPPC in the activation of metabolic processes in embryos. Considering the changes in lipid metabolism by increasing cAMP concentrations, possible benefits would happen by activation of protein kinase A (PKA). The present study aimed to evaluate the effect of NPPC supplementation in the culture medium on the development, lipid content and, transcript levels of genes related to the quality and cellular metabolism of bovine embryos produced in vitro. Ovaries (n = 420) from Nelore cows (n = 210) were obtained from the slaughterhouse. On day 5 (D5) of in vitro culture, the embryos of the experimental group were treated with 100 nM of NPPC and the control group did not receive this substance. The evaluation was performed based on blastocyst and hatching rates in D7, D9 and D10. For the evaluation of the lipid concentration, blastocysts (D7; n = 10 / group) were stained with the Sudan Black B. Also, the gene expression analysis of GPX1 (involved in development), POU5F1 (pluripotency), HAND1 (differentiation and implantation), HP1 (homodimerization and chromatin interaction), IFNT2 (maternal fetal interaction), AKR1B1 (glucose metabolism), SREBF1 and SCD (both involved in lipid metabolism) were made by qRT-PCR. The statistical analysis of in vitro culture was performed by ANOVA test with the SAS system; For the lipid content, the ANOVA test was used by Tukey test and, in the analysis of the gene expressions was used the JMP software. The results were considered significant when $P \leq 0.05$. The lipid concentration was similar for both groups, 883 AU (arbitrary unit) in control group and 881 AU in NPPC group ($P > 0.05$). The gene related to lipid metabolism - SREBF1 - showed lower transcript concentrations for embryos in the NPPC group, P=0.06. Another difference was found on IFNT2 gene, with a higher expression for embryos treated with NPPC, P=0.06. There were no differences in blastocyst rates between embryos cultured with NPPC (44.4%) and control group (39.5 %). Interestingly, the rates of hatching at D10 were higher for NPPC group (63.9%) when compared to the control group (54.6%; P=0.09). In conclusion, our data suggested a possible effect of NPPC on embryo development.

A099 OPU - IVF and ET

Goat incubator: The doe as a life incubator of bovine oocytes - first step

Ribrio Ivan Tavares Pereira Batista, Luiz Sérgio de Almeida Camargo[2], Joanna Maria Gonçalves de Souza Fabjan[3], Jader Forquim Prates[4], Juliane Teramachi Trevizan[4], Felipe Zandonadi Brandao[3], Jeferson Ferreira da Fonseca[4]

[1]Embrapa Caprinos e Ovinos, Núcleo Regional Sudeste, CEJHB–Embrapa Gado de Leite, Coronel Pacheco, MG; [2]Embrapa Gado de Leite, Juiz de Fora, MG; [3]Faculdade de Medicina Veterinária, Universidade Federal Fluminense, Niterói, RJ; [4]Embrapa Caprinos e Ovinos, Núcleo Regional Sudeste, Coronel Pacheco, MG.

Despite significant improvements in the in vitro production of cattle embryos, the suboptimal in vitro culture environment still limits the embryo quality and production. Techniques that associate the advantages of in vivo and in vitro systems, such as intrafollicular transfer of immature oocytes, have been proposed mainly to increase the embryo quality. In this context, we tried to use a goat as live incubator and associated nonsurgical embryo transfer techniques in small ruminants to perform ex situ (in vivo) maturation of bovine oocytes. For this, immature bovine cumulus-oocyte complexes (COCs) of grade 1 and 2 were randomly distributed into two groups for in vitro (IVM; n = 38) and ex situ (ESM; n = 40) maturation. The IVM was performed for a period of 24 h in TCM-199 medium (Gibco Life Technologies, Inc., Grand Island, NY, USA) supplemented with 20 mg/mL of FSH (Pluset, Calier, Barcelona, Spain), 0.36 mM sodium pyruvate (Sigma Chemical, St. Louis, MO, USA), 10 mM sodium bicarbonate (Sigma Chemical, St. Louis, MO, USA) and 50 mg/mL streptomycin/penicillin (Sigma Chemical, St. Louis, MO, USA) at 38.8 ºC in an atmosphere of 5% CO2 in air with maximum humidity. For ESM, a pre-synchronized nulliparous goat (12 months old) received 40 immature COCs in the uterine horn apice by transcervical route (Fonseca et al., 2014 Arq. Bras. Med.vet. Zootec) and 24 h after the procedure the structures were retrieved by the uterine flushing (Fonseca et al., 2013 Small Rumin Res). For analysis of the nuclear maturation rate and lipid quantification, the oocytes were denuded (0.1% hyaluronidase), fixed (4% paraformaldehyde) and stained with 10 µg/mL Hoechst 33342 and 10 µg/mL Nile Red (Molecular Probes, Inc., Eugene, OR, USA) dissolved in physiological saline (0.9% NaCl) with 1mg/mL polyvinylpyrrolidone. Oocytes displaying metaphase II plate were considered matured. The lipid amount was inferred by measuring the fluorescence intensity using the ImageJ program and fluorescence intensity were compared by Student's t-test. Forty-seven percent of the structures were recovered after uterine flushing (19/40). The nuclear maturation rate was 94.5% (18/19) and 81.6% (31/38) for ESM and IVM groups, respectively. In vitro-matured oocytes contained more lipid droplets, expressed as a higher ($p < 0.05$) amount of emitted fluorescence light (858 ± 73 arbitrary fluorescence units) than ex situ-matured oocytes (550 ± 64 arbitrary fluorescence units). This is the first report associating nonsurgical embryo transfer techniques with goat as live incubator for maturation of bovine oocytes. We conclude that transcervical transfer of bovine oocytes to uterine goat may be an alternative to in vitro maturation aiming the reduction of lipids without compromising nuclear maturation. Further studies are required to improve the oocyte recovery rate.
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A100 OPU - IVF and ET

Genetic multiplication center for small farmers

Delcio Bueno da Silva[*1], Carlos Antônio de Carvalho Fernandes[2], Jose Antonio D. Garcia[3], Ana Cristina Silva de Figueiredo[3], Jairo Pereira Neves[3], Eduardo Ramos de Oliveira[4], Juan Henrique Ferreira Nobrega[1]

[1]IF Sul de Minas, Muzambinho, MG; [2]Biotran - Universidade de Alfenas, Alfenas, MG; [3]Universidade de Alfenas, Alfenas, MG; [4]Biotran, Alfenas, MG.

The small farmers are the ones who could most benefit from embryo technology, but they are the ones who are farthest from the difficulty of genetics, financial resources, opportunity, knowledge and technical ability. The objective of this technological extension project was to eliminate these factors and verify the technical and economic viability of an innovative way of applying the biotechnology. A Biotechnology Centerwas created, where the recipients of the small farmers were taken. Location with adequate infrastructure, trained staff, where all activities were on a larger scale, ensuring good results at relatively lower cost. It was offered to subsidized values: the genetics of donors of the Gir breed and sexed semen of Holtein bulls, the Ovum Pick up (OPU) services, embryo production (IVP), preparation of the recipients, inovulation and ultrasonographic examinations. With the support of the Farmers´ Union and Emater, 13 technical meetings were held to present the Project to small farmers. A veterinary team visited those interested farmers and selected the females with the following characteristics: heifers, weight between 290 and 380 kg, negative to brucellosis and tuberculosis tests and without alteration by rectal palpation and ultrasonography evaluation. Twenty-two small dairy farmers from the southern state of Minas Gerais were selected. The selection criteria were: to have a maximum of 150 animals in the herd; Have the dairy farming as the main source of income of the property; Have conditions for calf creation. When the project completed the 211 recipients, their initial goal, the meetings were discontinued. Each small farmer sent four to fifteen heifers. Twenty-three fixed time protocols for the preparation of recipients were made and respective innovulations. A total of 435 embryos transfer were made and 189 pregnancies were obtained, with an average rate of 43.4% and 90.5% (N=171) of female pregnancies. Each producer paid for the pregnant recipient the fixed value of R$ 300.00 per gestation of female, R$200.00 per gestation of male, added to the heifer weight difference between the entrance and exit of the Central. For those 22 non-pregnant recipients only the difference in weight was paid. The total cost of each pregnant recipient for small producers, including transportation costs was R$384.72. Regardless of the value of donor genetics, donated at no cost by a local breeder, the production cost of each gestation, including fees for the different activities involved, materials for recipient preparation, aspiration, embryo production and semen was R$987.54. The average difference of R$602.87 per gestation was funded by the CNPq Project, Process: 468954/2014-7 and the participating Institutions. We conclude that the methodology used is technically viaible and economic viability depends on cost-benefit analyzes for each situation.
Acknowledgments: CNPq, Biotran, Fapemig and Capes.

A101 OPU - IVF and ET

Nuclear maturation kinetics of immature oocytes into preovulatory dominant follicle

Ana Paula Castro[1], Luiz Manoel Souza Simoes[1], Miguel Pizzolante Bottino[1], Thiago Takiya Pontes[1], Sarah de Andrade Dias Rodrigues[2], Felippe Manoel Costa Caixeta[2], Carolina Capobiango Romano Quintão[3], Luiz Sérgio de Almeida Camargo[3], Margot Alves Nunes Dode[4], José Nélio de Sousa Sales[1]

[1]Universidade Federal de Lavras, Lavras, MG; [2]Universidade de Brasília, Brasília, DF; [3]Embrapa Gado de Leite, Juiz de Fora, MG; [4]Embrapa Recursos Genéticos e Biotecnologias, Brasília, DF.

The objective was to evaluate the effect of maturation time on immature oocytes injected into preovulatory dominant follicle by intrafollicular injection of immature oocytes (IIFOI) on nuclear maturation kinetics. Immature bovines cumulus oocyte complexes (COCs; n=438) of grade 1, 2 e 3 from slaughterhouse were randomly assigned to one of three groups: (I) Control (n = 111), the oocytes were matured *in vitro* for 22 hours; (II) Mat20 (n=172) and (III) Mat30 (n=155), 30 oocytes were injected with the aid of a transvaginal guide with convex probe (7.5MHz) into preovulatory dominant follicle of previously synchronized oocyte recipient cows. In the Mat20 group, oocytes were matured in the dominant follicle for 19.8 ± 0.1 hours and in the Mat30 group for 28.3 ± 0.1 hours. In both experimental groups, cows received 12.5µg LH (Lutropin, Bioniche, Canada) at the time of IIFOI (Mat20 Group) or 10 hours after IIFOI (Mat30 Group). Oocytes from Mat20 and Mat30 groups were aspirated 20 hours after LH administration to evaluate the recovery rate. Oocytes from the experimental groups were denuded, fixed and stained by lacmoid to evaluate maturation kinetics as: germinative vesicle, metaphase I, anaphase I, telophase I, metaphase II, parthenogenetically activated and abnormal [chromosomal aberrations and degenerate (presented diffuse or undefined chromatin)]. Statistical analyses were performed by GLIMMIX procedure of SAS. Oocyte recovery rate after OPU was different between the Mat20 [52.9% (91/172)] and Mat30 [72.9% (113/155); P = 0.001]. The rate of oocytes in germinative vesicle state (P = 0.94), metaphase I (P = 0.98), anaphase I (P = 0.99) and telophase I (P = 0.20) were similar between the experimental groups. However, there was difference between groups for oocyte rates in metaphase II [Control - 81.0% (90/111)a, Mat20 - 74.5% (35/47)a and Mat30 - 41.6% (32/77)b; P = 0.001], of abnormal [Control - 5.4% (6/111)c, Mat20 - 21.3% (10/47)b and Mat30 - 48.1% (37/77)a; P = 0.001] and parthenogenetically activated [Control - 0.0% (0/111)b, Mat20 - 0.0% (0/47)b and Mat30 - 9.1% (7/77)a; P = 0.001]. In conclusion, oocytes injected and maintained in preovulatory dominant follicle for 20 hours presented nuclear maturation similar to oocytes matured *in vitro*.

A102 OPU - IVF and ET

Kinetics of embryonic development according to the follicular population in *Bos indicus* donors

Osnir Watanabe[1], Julia Gleyci Soares[2], Fabio Morato Monteiro[3], Guilherme Fazan Rossi[4], Bernardo Marcozzi Bayeux[2], Aline de Sousa Oliveira[5], José Nélio de Sousa Sales[6], Edson Guimarães Lo Turco[7], Marcelo Fabio Gouveia Nogueira[8], Yeda Fumie Watanabe[5], Pietro Sampaio Baruselli[2]

[1]WTA, São Paulo, SP; [2]Departamento de Reprodução Animal, FMVZ/USP, São Paulo, SP; [3]Centro APTA Bovinos de Corte, Instituto de Zootecnia, Sertãozinho, SP; [4]FMVZ/UNESP, Jaboticabal, SP; [5]Vitrogen – YVF Biotech, Cravinhos, SP; [6]Departamento de Medicina Veterinária, UFL, Lavras, MG; [7]Centro de Pesquisa em Urologia, UNIFESP, São Paulo, SP; [8]Faculdade de Ciências e Letras, UNESP, Assis, SP.

The present study aimed to evaluate the effect of the number of follicles aspirated from Nelore (Bos indicus) donors on the development kinetics of embryos produced in vitro. At random day of the estrous cycle, a total of 30 Nelore cows were synchronized with an intravaginal progesterone device associated with treatment with estradiol benzoate (2.0 mg im). Prostaglandin F2α (2.0 mg im) was also administered for CL removal at the time of ovum pick-up (OPU). Five days later, all donors underwent the OPU procedure. Oocytes were classified and submitted to the in vitro embryo production. Blastocysts production and hatching were verified on days 7, 8 and 9. Data were analyzed by the GLIMMIX procedure of SAS 9.3®. The donors were classified into three categories according to the follicular population: Low (18.1 ± 0.8; n = 10); Medium (30.8 ± 1.5, n = 10) and High (54.3 ± 5.1, n = 9). There was no difference in the rate of viable oocytes (64.3 vs. 65.4 vs. 68.5%, P = 0.59) between the groups of Low, Medium and High follicular population, respectively. However, was verified an increase in the cleavage rate (56.4b vs. 63.5ab vs. 64.6%a; P = 0.05) in the animals of High follicular population, when compared to the Low ones. The blastocysts rates, in relation to recovered oocytes, produced in D7 (25.7 vs. 25.7 vs. 33.3%, P = 0.10), in D8 (29.3 vs. 30.8 vs. 35.7%; P = 0.30) and in D9 (15.7 vs. 21.0 vs. 17.6%, P = 0.41) did not differ between the groups of Low, Medium and High follicular population, respectively. Furthermore, no differences were observed in the rates of hatched blastocysts in D7 (0 vs. 0 vs. 0.1%, P = 0.26), in D8 (52.4 vs. 41.8 vs. 53.4, P = 0.30) and in D9 (20.0 vs. 35.1 vs. 23.6%, P = 0.13), in the respective groups. The rate of total blastocysts production, in relation to the recovered oocytes, (32.1 vs. 34.6 vs. 40.6%, P = 0.15) and the rate of hatched blastocysts (71.1 vs. 74.3 vs. 80.2%, P = 0.36) was similar among the animals of Low, Medium and High follicular population, respectively. It was concluded that the kinetics of embryo development was not influenced by the follicular population of Nelore (Bos indicus) donors submitted to OPU-IVEP.

A103 OPU - IVF and ET

Comparison of *in vitro* production, lipid content and gene expression of embryos from *Bos indicus* cows cultivated with or without forskolin supplementation

Nathalia Covre da Silva[1], Camila Bortoliero Costa[1], Paula Alvares Lunardelli[1], Patrícia Kubo Fontes[2], Camila Oliveira Rosa[1], Andrea Cristina Basso[3], Mateus José Sudano[4], Marcelo Fabio Gouveia Nogueira[2], Marcelo Marcondes Seneda[1]

[1]Universidade Estadual de Londrina, Londrina, PR; [2]UNESP, Botucatu, SP; [3]In Vitro Brasil, Mogi Mirim, SP; [4]UNIPAMPA, Uruguaiana, RS.

The aim of this study was to evaluate the effect of supplementation on the culture medium with forskolin on the embryo development, lipid content and transcript levels of genes related to the quality and cellular metabolism of Bos indicus embryos produced in vitro. For IVP, ovaries (n = 420) of 210 Nellore females were collected from local slaughterhouse and transported in saline solution at 35°C to the laboratory. After follicular aspiration, the oocytes were selected (level I and II), MIV, FIV with semen from a single bull previously tested, and CIV. On day 5 of CIV the probable zygotes were randomly divided into the experimental groups: a) control group, cultivated with common medium (n = 447); B) forskolin group (10 µM forskolin supplementation; n = 432). The blastocyst and hatching rates were evaluated in D7, D9 and D10. To evaluate the lipid concentration, blastocysts on D7 (n=10/group) were collected and stained with Black Sudan B stain. The genes expressions of the GPX1, SREBF1, POU5F1, AKR1B1, HAND1, HP1, IFNT2 and SCD were analyzed by RT-PCR. For the statistical analysis, the IVP rates were compared using the ANOVA test from SAS system, for the lipid content the ANOVA test was used followed by Tukey test, and for the analysis of the gene expression data, JMP software was used. The differences were considered significant if $p \leq 0.09$. The blastocyst rate between the groups was 39.5% for the control group and 37.9% for the forskolin group ($p > 0.09$), the hatching rates in D9 and D10 were, respectively, 43.5% and 48.4% for the control group and 55,7% and 62.9% for the forskolin group, showing a significant difference in D10 ($p = 0.09$). The lipid concentration in the treatments was 883 AU (arbitrary unit) in the control group and 772 UA forskolin group, the lipid concentration/ area (AU x 10-10/µm2) was 1.1 ± 0, 8 in the control group and 0.9 ± 0.7 in the forskolin group, and the lipid concentration/ volume (AU x 10-13/µm3) 5,4 ± 4,7 and 4,3 ± 4,0 in the control and forskolin groups ($p > 0.05$). The genes related to pluripotency (POU5F1), development (GPX1), homodimerization and interaction of chromatin (HP1) and differentiation and implantation (HAND1) obtained higher concentrations of transcripts in the embryos from the control group. Genes related to maternal-fetal interaction (IFNT2) and lipid metabolism (AKR1B1 and SCD) presented higher expression in the group supplemented with Forskolin, however for the gene SREBF1, also related to metabolism, we found no difference in expression. In conclusion, the use of Forskolin on the CIV of embryos Bos indicus evidenced differences on genes related to the initial and lipid metabolism, suggesting the beneficial effect of this substance on the IVP of embryos. The lipid concentration in the embryos did not differ with the use of forskolin and the blastocyst rates remained constant, but the D10 hatching rates demonstrated superior results in the forskolin group, thus demonstrating favorable results with supplementation on the in CIV.

A104 OPU - IVF and ET

Antral follicle count (AFC) and its association with reproductive traits in cows

Jesús Alfonso Sánchez[1], Gisvani Lopes de Vasconcelos[2], Marcelo Siqueira El Azzi[1], Nathália Alves[1], Ana Lecia Aparecida Patto Lara Ribeiro[1], Renata Maculan[1], Gabriel Miranda Moreira[1], Cintia Vanin Ribeiro[1], Anita Soares Barbosa Guimaraes[3], José Camisão de Souza[1]

[1]Univresidade Federal de Lavras, Lavras, MG; [2]Universidade Federal de Lavras, Lavras, MG; [3]Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA.

This study was conducted to determine if the antral follicle count (AFC) is positively associated with the total AFC, oocyte maturation stage, viable oocytes, total oocytes, and ovarian volume. Materials and Methods: Ovaries were collected in a slaughterhouse from 105 mixed breed cows (predominantly Bos taurus taurus) that were cycling, multiparous, and with body condition 3 and 4 (1-5) from 3 to 10 years of age. The groups were divided into high (≥25 follicles) (H), intermediate (16-24 follicles) (M), and low (≤15 follicles) (L) AFC. The cumulus ooctye complexes (COCs) were aspirated from follicles from 3 to 8 mm diameter. Only oocytes with quality 1 to 3 were used for the experiment. In vitro maturation (IVM) of oocytes of the AFC classes was conducted in an incubator for 24 h at 38.7ºC with 5% CO2 in a humidified atmosphere. The oocytes were examined every 2 days using Nomarski differential interference contrast (200-400 X) in a Nikon Diaphot DTM microscope (Nikon, Tokyo, Japan). Analyses were made by the SAS® statistical program (SAS Institute, Cary, NC, USA). Analysis of variance to test the reproductive variables (total AFC, oocyte maturation stages M1, M2, and M3, viable oocytes, total oocytes, and ovarian volume). The GLM was preceded by the Tukey test for differences between the individual mean values. The mean and the standard error of the mean were used. P was significant when < 0.05. The total AFC, stage 1 ooctyes, viable ooctyes, total oocytes, and ovarian volume were greater in ovaries H (69.69±2.144, 7.86±0.603, 30.97±5.173, 12.60±0.739) ($p<0.001$) compared to M (36.73±2.383, 4.85±0.670, 13.05±1.235, 38.32±5.750, 10.10±0.750) and L (20.65±2.580, 3.27±0.726, 8,07±1,337, 20.81±6.226, 8.09±0.858). In relation to ovarian volume, there was a significant reduction ($P<0.001$) in ovarian weight and size (length and height) in ovaries L (8.09±0.858) compared to ovaries H (12.60±0.739). In relation to the oocyte category, the number of oocytes with degree of maturation 2 and 3 were higher ($p<0.001$) in H ovaries (4.02±0.388 in G2; 2.09±0.389 in G3) compared to M (2.76±0.432 in G2; 2.48±0.439 in G3). For its part, L (1.24±0.467 in G2; 1.27±0.468 in G3) was similar to M ($p=0.009$ for G2 and $p=0.007$ for G3). The AFC is directly associated with oocyte quantity and quality and with ovarian volume, indicating that these traits can be easily used in selection processes of cows.
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A105 OPU - IVF and ET

Correlation between the concentration of DNA and the call rate of genotyping of bovine embryos biopsied samples

Júlia Martins[1], Carolina Capobiango Romano Quintão[1], Luiz Sérgio de Almeida Camargo[1], Luiz Altamiro Garcia Nogueira[2], Clara Slade Oliveira[1]

[1]Embrapa Gado de Leite, Valença, RJ; [2]Universidade Federal Fluminense, Niteroi, RJ.

The use of embryos in genomic selection has been discussed through the application of high density marker genotyping panels. This possibility arose from the evolution of the techniques of genotyping and pre-amplification of small samples that made the use of the whole-genome amplification (WGA). The objective of the present study was to evaluate the DNA quality of the amplified biopsy sample and to associate it with the call rate obtained after genotyping. The call rate is a quality parameter of the genotyping, which indicates the fraction of SNPs found in relation to the total SNPs tested in each sample. For this, biopsies were removed from PIV blastocysts (d7) by the manual section of the trofectoderma fragment (10-20% of the embryo, Bioniche blade). The samples were frozen in nitrogen, and subsequently the DNA was extracted and amplified using the Single Cell GenomiPhi DNA Amplification kit (Illustra, Buckinghamshire, United Kingdom). The quality of the amplified material was analyzed by the 2100 Bioanalyzer instrument (Agilent Technologies, Santa Clara, CA, USA). Twenty-two biopsy specimens were analyzed. The criteria of total DNA concentration and concentration of DNA fragments greater than 7.000 bp prior to genotyping were adopted. According to the Bioanalyzer, the total DNA concentration reached the minimum value of 3.58ng/ul and the maximum of 726.03ng/ul, average of 25.26 and standard deviation of 155.01. Regarding the concentration of DNA fragments higher than 7,000 bp, the minimum value was 0.98 ng/ul and the maximum was 53.62 ng/ul, the average was 4.98 and the Standard deviation was 12.19. The samples were sent for genotyping using the Bovine SNP50k assay. After the genotyping, the average call rate of each sample was compared with the DNA quantification parameters obtained in the Bioanalyzer. Regarding the call rate, a range of 0.41 to 0.96 (minimum and maximum, respectively) was obtained, an average of 0.79 and a standard deviation of 0.23. The correlation analysis between the total amount of DNA in the Bioanalyzer and the call rate was not significant (R = -0.19 and P = -0.36, Spearman). Similarly, there was no correlation in the parameter concentration of fragments greater than 7,000 bp and the call rate (R = -0.04 and P = 0.85, Pearson). Thus, we can conclude that high total concentration or fragments greater than 7,000 bp of DNA in samples of amplified embryonic biopsies does not suggest that the sample will present a high call rate after genotyping.
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A106 OPU - IVF and ET

Decreased Percoll volume does not influence fertilization rates and early embryonic development of IVP embryos using sexed semen

Daniele Missio[1], Natália Picoli Folchini[1], Hirya Fernandes Pinto[1], Cecília Isabel Inês Urquiza Falcão Machado[2], Fábio Gallas Leivas[1], Daniela dos Santos Brum[1]

[1]Lab. de Biotecnologia da Reprodução (BIOTECH) - UNIPAMPA, Uruguaiana, RS; [2]Renascer Biotecnologia, Barra do Quaraí, RS.

Percoll is the most widely density gradient medium used for sperm selection in bovine embryos *in vitro* production. Though, with increased use of sexed sperm, changes in Percoll protocols have been proposed, aiming to enhance the sperm recovery rate and optimize the use of semen. Among these modifications, previous studies have shown that reduction of Percoll volume increases the sperm recovery rate (Missio *et al*., Anim. Reprod., v. 12, p.718, 2015). However, there are no researches evaluating the influence of this reduction in fertilization and early embryo development. This study aimed to evaluate the effect of different Percoll volume in fertilization rate and embryo development kinetics in bovine embryos IVP. Eight replicates were conducted from a pool of sexed semen from two bulls. The sperm selection was performed by discontinuous gradients Percoll (30, 60, 90%; Folchini et al. Rev. Bras. Reprod. Anim., v. 36, p.239-244, 2012), with volumes adjusted as treatments: Control: 300 µL and Treatment 1 (T1):100 µL for each gradient. At the first and second centrifugation a force of 2.200 x *g* was used during 5'and 1', respectively. After the selection process, a dose of $1x10^6$ spermatozoa/mL of each group was utilized for IVF of previously matured oocytes (Day 0). At 18 h post-insemination (hpi), the probable zygotes were divided into two groups for evaluation of the fertilization rate and kinetics of embryonic development. For evaluation of fertilization rate, the presumptive zygotes (93 and 94 for Control and T1, respectively) were stripped and incubated in a Hoechst 33342 solution (10mg/mL), being considered fertilized zygotes that had two pronucleus formation and extrusion of the second polar body or that had extrusion of the second polar body and sperm in decondensation. To evaluate the kinetics of embryonic development, the presumptive zygotes (54/treatment) were individually cultured in SOFaaci in an embryonic monitoring system (Primo Vision, Cryo Mangement Ltd., Hungary) for up to 48 hpi. The embryos were individually evaluated on day 2 for cleavage, moment of first cleavage and number of cells. Data were analyzed by chi-square (X^2; $P<0.05$). The fertilization rate after Percoll gradients were similar ($P>0.05$) between the Control and T1 (78.5±4.3 and 71.3±4.7, respectively). No difference ($p>0.05$) in the cleavage rate, the average time of the first cleavage and cell number after 48 hpi between the group Control (81.5±5.3; 32.9±1 and 4.16±0.33) and T1 (68.5±6.4; 35.1±0.99 and 3.77±0.30). The results of this experiment suggest that the Percoll volume decreased to 100 µL does not affect the fertilization rates and early embryonic development. Percoll volume reduction can be used as a routine method for the sperm selection of sexed semen for IVF.

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A107 OPU - IVF and ET

FSH dose and strategy of administration during ovarian stimulation alter the gene expression profile in ovine cumulus-oocyte complexes

Gláucia Mota Bragança[1], Ribrio Ivan Tavares Pereira Batista[1], Joanna Maria Gonçalves de Souza Fabjan[1], Vivian Angélico Pereira Alfradique[1], Luana Rangel Côrtes[1], Luiz Sérgio de Almeida Camargo[2], Jeferson Ferreira da Fonseca[3], Felipe Zandonadi Brandao[1]

[1]Universidade Federal Fluminense, Rio de Janeiro, RJ; [2]Embrapa Gado de Leite, Juiz de Fora, MG; [3]Embrapa Caprinos e Ovinos, Sobral, CE.

Ovarian stimulation is an important tool to increase the number of oocytes obtained by laparoscopy for the in vitro production of embryos (IVP). In sheep, different concentrations of FSH administered in single dose (SD) or multiple doses (MD) have been adopted. In parallel, the oocyte quality is fundamental for IVP success, so strategies to produce more competent oocytes have been evaluated. The aim of this study was to evaluate the gene expression profile of BCB+ COC from different hormonal protocols of ovarian stimulation in Santa Inês ewes. To achieve that, a cross-over design was used, where 12 pluriparous ewes had their follicular wave synchronized (Balaro et al., Domest Anim Endocrinol, 54: 10-14, 2016). At 80 h after progestogen implant removal, all ewes received a new vaginal sponge and it started the stimulation by administration of: 80 (Group 1 - 80-SD) or 120 (Group 2 - 120-SD) mg FSH (Folltropin-V®, Bioniche Animal Health, Ontario, Canada) and 300 IU of eCG both in single dose, or 80 (Group 3 - 80-MD) or 120 (Group 4 - 120-MD) in decreasing doses (50/30/20%) every 12 h. The COCs were recovered by laparoscopy and classified morphologically in grade I / II (homogeneous ooplasm and more than 3 cumulus cells layers), III (homogeneous ooplasm and less than 3 cumulus cells layers or partially denuded) and IV (heterogeneous ooplasm or degenerate). For inference of the development competence GI, II and III COCs were stained with bright cresyl blue (BCB) and classified into: BCB+ (competent) and BCB- (non-competent). These variables were compared by ANOVA followed by Tukey test. The abundance of mRNA that encodes proteins associated with steroidogenesis (STAR, FSHr, LHr and ERα), oocyte quality (MATER, BMP15, GDF9 and ZAR1) and apoptosis (BAX and Bcl-2) was assessed by real-time qPCR normalized with GAPDH in BCB+ COCs. The abundance of gene transcripts associated with steroidogenesis was down-regulated ($P < 0.05$) with increasing FSH concentration, when administration was performed in a single dose (80-SD and 120-SD). On the other hand, when the administration was performed in MD, only the LHr was down-regulated (80-MD and 120-MD). In the 80-MD group, FSHr and Erα were down-regulated ($P < 0.05$) in comparison with 80-SD. For genes related with oocyte quality, 80-MD showed up-regulation ($P < 0.05$) to MATER (when compared to 80-SD), ZAR1 and MATER (compared to 120-SD). Nonetheless, apoptosis genes were not affected. These data demonstrate that the FSH dose and strategy of administration affect the gene expression profile in ovine COCs. Subsequent studies are necessary to assess the effect of this change on maturation rate and developmental competence.

A108 OPU - IVF and ET

eCG stimulation prior to ovum pick-up on expression of oocyte quality markers in immature cumulus-oocyte complexes

Carlos Antônio de Carvalho Fernandes[1], Felipe Costa Gonçalves[2], Ester Siqueira Caixeta[3], Ana Cristina Silva de Figueiredo[2], João Henrique Moreira Viana[2], Josiane Rossi Ribeiro[4], Raisse Marina da Silva[4]

[1]BIOTRAN - Universidade de Alfenas, Alfenas, MG; [2]Universidade de Alfenas, Alfenas, MG; [3]Universidade Federal de Alfenas, Alfenas, MG; 4.Biotran Ltda, Alfenas, MG.

The success of in vitro embryo production is related, at least in part, to the quality of cumulus-oocyte complexes (COCs) harvested by ovum pick-up (OPU). The aim of this study was to evaluate the effect of ovarian stimulation with eCG prior to OPU on the mRNA abundance of TGFβ superfamily components and other markers of oocyte quality in COCs. A total of 30 Nellore (Bos indicus) cows were randomly divided into control (n=15) and stimulated (n=15) groups in a cross-over design. On a random day of the estrous cycle, all cows received an intravaginal P4 device (DIB™, MSD Animal Health, Brazil), 2 mg IM of estradiol benzoate (Gonadiol™, MSD Animal Health, Brazil). On Day 3 morning, the stimulated group received 300 IU of eCG IM (Folligon™, MSD Animal Health, Brazil). On the morning of Day 5, the P4 device was removed and OPU was conducted in both groups. Oocytes and cumulus cells (CC) were mechanically separated from pools of 25 immature COCs of control (n=4 pools) and stimulated (n=4 pools) groups. Total RNA was extracted from pools of 25 oocytes and their respective CC using an RNeasy® kit (Qiagen). Target gene expression in oocytes, including bone morphogenetic protein 15 (BMP15), growth differentiation factor 9 (GDF9), SMAD signal transduction factors (SMAD1, 2, 3, and 5), follistatin (FST), oocyte-derived JY-1, and cathepsins (CTSB and CTSZ), and in CC, including FST, CTSB, CTSK, CTSS, CTSZ, amphiregulin (AREG), epiregulin (EREG), and hyaluronan synthase 2 (HAS2), were assessed by real-time RT-PCR using Power SYBR® green master mix and normalized to the levels of cyclophilin (CYC-A). Relative quantification of mRNA abundance was determined using the ΔΔCt method. Effects of ovarian stimulation with eCG on the expression of target genes in oocytes and CC were analyzed by unpaired t test and $P<0.05$ was considered significant. In oocytes, mRNA encoding BMP15, and SMAD1, 2, and 3 was higher ($P<0.05$) in the stimulated group than in the control group. Moreover, the relative mRNA abundance of CTSZ, a member of the cathepsins family functionally related to reduced oocyte competence, was lower ($P<0.05$) in the stimulated group than in the control group. Similarly, cumulus cell CTSS and CTSK mRNA abundance was lower ($P<0.01$) in the stimulated group compared with control group. However, the relative abundance of AREG and EREG mRNA was higher ($P<0.05$) in CC recovered from stimulated group. No differences on mRNA abundance of GDF9, SMAD5, FST, JY-1, and CTSB in oocytes and FST, CTSB, CTSZ, and HAS2 in CC were demonstrated among different groups. In conclusion, eCG stimulation prior to OPU modifies the profile of mRNA abundance of genes related to oocyte quality in COCs, and it may contribute to the improvement of oocyte competence. Acknowledgements: Fapemig, CAPES, CNPq and Biotran.

A109 OPU - IVF and ET

Effect of the oocyte competence on the *in vitro* development of embryos of repeat-breeder dairy cows subjected to drying and subsequent induction of lactation

Rodolfo Daniel Mingoti[1], Flávia Morag Elliff[1], Roberta Machado Ferreira[1], Bernardo Marcozzi Bayeux[1], Romulo Germano Rezende[1], Anibal Eugênio Vercesi Filho[2], Enilson Geraldo Ribeiro[2], Aline de Sousa Oliveira[3], Yeda Fumie Watanabe[3], Pietro Sampaio Baruselli[1]

[1]VRA/FMVZ/USP, São Paulo, SP; [2]Instituto de Zootecnia, Nova Odessa, SP; [3]WTA FIV (Vitrogen), Cravinhos, SP.

The objective of the present study was to evaluate the IVEP of Holstein cows (*Bos taurus*) of different categories. A total of 34 cows were used, homogenously distributed in four groups: cows in the beginning of lactation (BL; n=9), repeat-breeder cows (RB; n=7), dry cows (DC; n=9) and cows with induced lactation (IL; n=9). IL cows went through the following drying process: after the last milking, the antisepsis of the teats was done using cotton and 70% alcohol. Next, Ciprolac® (Ciprofloxacin; Ourofino, Ribeirão Preto, Brasil) was administered, the teat was massaged, and, on sequence, Sellat® (Bismuth Subnitrate; Ourofino) was administered on each teat. Also, the drying process included the Mastitis vaccine (5mL of Mastiplus-BR® SC; Vitafort Animal Defense, Ribeirão Preto, Brasil) on the moment of drying and 30 days after. The 60d drying period was concluded for the posterior induction of lactation of the IL cows. The IL group consisted of RB cows that received the induction of lactation protocol 30 days previous to OPU [500mg of bSTr (Boostin®, MSD, São Paulo, Brasil) on D0, 7 and 20; 30mg/cow/d of BE (Sincrodiol®, Ourofino) and 300mg/cow/d of P4 (Sincrogest®, Ourofino) IM from D0 to 7; 20mg/cow/d of BE from D8 to 14; 0,530mg/cow of PGF (Sincrocio®, Ourofino) on D15; 40mg/cow/d of dexametasone (Cortiflan®, Ourofino) from D18 to 20; 5 min daily massage of the teats and the udder from D16 to 19; milking started on D20; after the onset of lactation, cows received 500mg/cow of bSTr every 14d]. The other groups did not receive any treatment. All cows from the four categories went through three OPUs, with a 30d interval. The antral follicle population count (AFC) was done using US in each OPU. Data were analyzed using the Generalized Linear Mixed Models (PROC GLIMMIX) of SAS (*v9.4*). The AFC was lower (P=0.03) in IL cows (9.3±0.9b) when compared to RB (15.9±2.2^{a}) and DC (17.7±2.3^{a}), not differing from the BL (12.7±1.6^{ab}). However, the quantity of recovered oocytes from BL cows (7.15±0.7^{b}) differed (P=0.008) only from the DC (14.3±2.0^{a}), and was similar to RB (12.9±1.8^{ab}) and BL (10.8±1.2^{b}). There was difference on the number of viable oocytes (P=0.02) between groups (DC=7.8±1.3^{a}; RB=7.3±1.0^{ab}; BL=3.38±0.4^{bc} and VL=4.9±0.7^{c}). No difference was found (P=0.30) on oocyte recovery rate between groups [IL=77.2% (186/241); BL=78.5% (270/344); RB=80.9% (271/335); DC=81.0% (387/478)]. The cleavage rate (cleaved/total of recovered) was greater (P=0.009) on RB cows [48.7%^{a} (132/271)] in relation to the others [IL=29.6%^{b} (55/186); BL=33.0%^{b} (89/270) and DC=39.3%^{b} (152/387)]. The blastocyst rate (blastocyst/total of recovered) was higher (P=0.02) in RB [24.7%^{a} (67/271)] when compared to IL [9.1%^{b} (17/186)], but similar to the other categories [BL=16.3%^{ab} (44/270); DC=17.6%^{ab} (68/387)]. It is possible to conclude that drying followed by induction of lactation was not efficient to improve IVEP of RB cows.

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A110 OPU - IVF and ET

Effect of high density lipoprotein during *in vitro* oocyte maturation on initial embryo development in bovine

Joao Alveiro Alvarado Rincón[1], Jorgea Pradieé[2], Josiane de Oliveira Feijó[1], Mariana Härter Remião[3], Morgana Alves Borges[3], Isadora Andre Rosa Lopes[1], Marcio Nunes Correa[1], Ligia Margareth Cantarelli Pegoraro[2], Augusto Schneider[4]

[1]Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), UFPEL., Pelotas, RS; [2]Embrapa Clima Temperado, Pelotas, RS; [3]Grupo de Pesquisa em Oncologia Celular e Molecular (GPO), CDTEC, UFPEL, Pelotas, RS; [4]Faculdade de Nutrição, UFPEL, Pelotas, RS.

High-density lipoprotein (HDL) is the only lipoprotein present in follicular fluid and is responsible for providing cholesterol for steroidogenesis. In addition, it has anti-inflammatory, antioxidant and cytoprotective properties, mainly derived from its lipid composition, apoliprotein-AI and enzymes such as paraoxonase-1. In this sense, intrafollicular levels of HDL-cholesterol have been positively associated with improved embryonic quality in women. The aim of this study was to evaluate the effect of increasing doses of HDL during oocyte maturation *in vitro* on the initial embryonic development in cattle. The IVP was carried out in an incubator with 5% CO_2 at 39 °C using commercial media (Progest Biotecnologia em reprodução Animal, Botucatu, SP, Brazil). Cumulus oocyte complex (COCs) were obtained from slaughterhouse bovine ovaries, washed and selected for morphology. COCs of grade I, II and III were randomly distributed into three groups (n=50 COCs/group) according to the addition of HDL protein in the IVM medium (G0: 0 mg/mL; G1: 0.5 mg/mL; G2: 1.5 mg/mL HDL, SIGMA-ALDRICH®, St. Louis, MO, USA). IVM occurred for 22 hours. The IVF (day 0) was performed with a concentration of $1x10^6$ spermatozoa/mL for 20 hours. After this period, the presumptive zygotes were washed and cultured in IVC media covered with mineral oil for 7 days. On day 3 the cleavage rate (cleaved/inseminated) was evaluated and 70% of the culture media was renewed, which was repeated on day 5. On day 7 the rate of embryonic development (blastocysts/inseminated) was evaluated. Therefore, 9 replications were performed, totaling 450 inseminated COCs/group. The effect of HDL on the cleavage rate and embryonic development was analyzed through the repeated measures ANOVA followed by the Tukey post-hoc test. The highest dose of HDL had a negative effect on the cleavage rate ($P=0.0003$) and embryonic development ($P=0.02$). The cleavage rate from G0 (68.8%) and G1 (68.1%) was not different, but G2 cleavage rate (56.3%) was lower in comparison to the other groups ($P<0.05$). Likewise, the embryo development rate was not different between G0 (29.4%) and G1 (29.2%), but G2 (19.5%) had a lower development rate compared to the other groups ($P<0.05$). It is concluded that despite the antioxidant and cytoprotective properties of HDL, when in high concentrations it can negatively affect the initial embryonic development in cattle, since the higher concentration of HDL tested in this study decreased the cleavage and embryonic development rates.

A111 OPU - IVF and ET

The effect of donor breed on the *in vitro* production of bovine embryos efficiency

Izabelle Pereira de Lacerda[1], Carla Braga Martins[1], Marcelo Machado de Souza Lima[2], Breno Fonseca Guerra[2], Eduardo Silva Costa[2], José de Oliveira Carvalho[1]

[1]Universidade Federal do Espírito Santo, Alegre, ES; [2]Embryopec Reprodução Bovina Ltda, Juiz de Fora, MG.

The in vitro embryo production (IVEP) is applied in different breeds of bovine herds in Brazil, seeking greater yield efficiency in each of them. Therefore the purpose of this study was to determine whether the donor breed Gir, Holstein or ½ Holstein-Gir, influences the mean oocytes retrieved per OPU session, blastocyst rate, and blastocyst and expanded blastocyst pregnancy rate. For this, 363 Gir donors (n = 2047 OPU), 109 Holstein donors (n = 265 OPU) and 144 ½ ½ Holstein-Gir (n = 465 OPU) were used. The aspirated oocytes were classified according to the IETS criteria and grade 1, 2 and 3 oocytes were considered viable. After OPU, the oocytes were matured, fertilized (D0) with sexed sperm and cultured in vitro for 7 days (D7). For in vitro fertilization, different previously known fertility bulls (n=71) were used in the laboratory routine. In D7, the embryos produced were transferred to previously synchronized Holstein-Gir cross recipients. The pregnancy status was determinated by transrectal ultrasonography at 28 days and confirmation at 60 days of gestation. Kruskal-Wallis test (P≤0.05) was used to compare the groups. Donor breed effect was observed in the mean of viable oocytes aspirated from ½ Holstein-Gir (19.3 ± 0.63a), Gir (14.8 ± 0.23b), and Holstein (9.00 ± 0.45c) donors. In relation to the blastocyst rate in D7, donors Gir (27.1% ± 0.55a) and ½ Holstein-Gir (24.3% ± 1.08a) presented higher rates than Holstein donors (21.3% ± 1, 46c). However, there was no difference in the pregnancy rate of embryos from Gir (37.8% ± 0.85), ½ Holstein-Gir (35.6% ± 1.67) and Holstein (35.1% ± 3.02) donors. In relation to pregnancy rate according to the stage of embryo development, a higher pregnancy rate was observed when transferring expanded blastocyst (40.2%, n = 6581) in relation to the pregnancy of embryos transferred in the blastocyst stage (29, 32%, n = 1954). The pregnancy rates at 30 and 60 days were similar, and no embryo loss was observed between the two gestation diagnoses. It is concluded that donors of the breed ½ Holstein-Gir provide a greater number of oocytes per OPU than donors Gir, which is superior to the Holstein donors. In addition, Gir and ½ Holstein-Gir donors show a higher blastocyst rate when compared to Holstein donors, without any influence of the donor breed on the pregnancy rate at 30 and 60 days. Furthermore, embryos transferred in the expanded blastocyst stage are more successful in the pregnancy rate than embryos transferred in the blastocyst stage.

A112 OPU - IVF and ET

Effect of different times of forskolin exposure to induce lipolysis in *in vitro* matured bovine oocytes

Daniela Martins Paschoal, Katia Lancellotti Schwarz[1], Fernanda Cavallari Castro[1], Hugo Fernandes[1], Letícia Schefer[1], Mateus José Sudano[2], Alício Martins Jr[3], Fernanda Cruz Landim Alvarenga[4], Cláudia Lima Verde Leal[1]

[1]FZEA/USP, Pirassununga, SP; [2]UNIPAMPA, Uruguaiana, RS; [3]FMVA/UNESP, Araçatuba, SP; [4]FMVZ/UNESP, Botucatu, SP.

In the present study, the effect of forskolin exposure time to induction oocytes lipolysis was evaluated in order to improve the rate of *in vitro* production of bovines. Oocytes from zebu cows obtained from commercial slaughterhouse were selected (N=708) and in groups of 20-25, they were transferred to drops of 90 µl of IVM medium (TCM 199) with 10% FBS according to the period of 50 µM forskolin (FSK) exposure during IVM: Control (without FSK, N=167), FSK-6h (6h MIV with FSK and 18h without, N=168), FSK-18h (18h MIV with FSK and 6h without, N=178) and FSK-24h (24-hour MIV with FSK, N=194). The IVM was performed in incubator with 5% CO_2 in air, temperature of 38.5 °C and humidity for 24 hours. The oocytes from the different groups were fertilized *in vitro* with frozen sperm from the single Nellore bull (*Bos taurus indicus*). Semen was selected by Percoll gradient (Nutricell) and the final concentration was adjusted to 2x106 sperm/mL. The probable zygotes were cultured in SOFaa medium plus 5 mg/mL BSA, 2.5% FBS and 0.11 mg/mL sodium pyruvate. Another part of the oocytes (N=210) was stained with 1 µg/ml of Nile red fluorescent dye to assess lipid content. The pictures obtained from the stained oocytes were taken on an epifluorescence microscope with a magnification of 20X and had the fluorescence intensity measured in the Image J. program. The lipid content of the oocytes was presented by the fluorescence intensity mean per µm2 (IF/µm2). For evaluate embryo quality (N=72), the fluorescent dye of TUNEL kit (*Terminal deoxinucleotil transferase Uracil Nick End Labeling*) was used. Green fluorescent nuclei (fluorescein isothiocyanate (FITC) were considered cells with DNA fragmented and blue fluorescent (Hoechst 333342) indicated the presence of the cell nucleus. To know the rate of apoptosis the number of cells stained in green was divided by the total number of blue cells and multiplied by 100. For statistical analysis, the dependent variables were submitted to ANOVA by the least squares method using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC, USA). If the ANOVA was significant, the data were analyzed using the SEM. The level of significance was 5% ($P<0.05$). The control group (23.5 ± 1.8^{a}) had a higher accumulation of lipids than the other: FSK-6h (19.7 ± 1.2^{b}), FSK-18h (17.2 ± 0.7^{b}) and FSK-24h (18.8 ± 0.9^{b}) - ($P<0.05$). There was no difference in the cleavage rate: Control (83.8±4.4 - 140/167), FSK-6h (77.3±5.0 - 131/169), FSK-18h (58.1±8.3 - 99/178) and FSK-24h (61.2±7.41 - 18/194). The blastocyst rate of the Control group (46.4 ± 3.9^{a} - 77/167) and FSK-6h (33.0 ± 6.6^{ab} 54/169) were similar, but FSK-18h (19.8 ± 2.2^{b} - 35/178) and 24h-FSK (16.7 ± 2.3^{b} - 32/194) were lower than Control - ($P<0.05$). Regarding the total number of intact cells, the Control (144.7 ± 8.3^{a}), FSK-6h (145 ± 9.4^{a}) and FSK-8h (130.5 ± 14.9^{ab}) were similar, but the FSK-24h (92.5 ± 6.3^{b}) group had a lower number of cells ($P<0.05$). The rate of cellular apoptosis was similar in all groups: Control (8.0±1.4), FSK-6h (6.1±2.1), FSK-18h (3.5±0.9) and FSK-24h (8.5±3.1). Although all times of drug exposure decreased lipid content, without influencing cell apoptosis, the FSK-18h and FSK-24h groups had a decrease in the total production of blastocysts. In conclusion, 6h of oocytes exposure to FSK is sufficient time to reduce lipids without prejudice the later development and quality of the embryos.
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A113 OPU - IVF and ET

Effect of butafosfan in expression of genes associated oocyte quality

Lucas Teixeira Hax[1], Joao Alveiro Alvarado Rincón[1], Janaina Fadrique da Silva[2], Jorgea Pradieé[3], Ligia Margareth Cantarelli Pegoraro[3], Augusto Schneider[4], Marcio Nunes Correa[1]

[1]Núcleo de Pesquisa, Ensino e Extensão Em Pecuária (NUPEEC), UFPEL, Pelotas, RS; [2]Programa de Pós-Gradução em Veterinária, UFPEL, Pelotas, RS; [3]Embrapa Clima Temperado, Pelotas, RS; [4]Faculdade de Nutrição, UFPEL, Pelotas, RS.

Butafosfan is an organic phosphorus molecule that has been studied as a metabolic modulator. Phosphorus is fundamental for the growth, differentiation and cellular integrity, it acts in the processes of phosphorylation and dephosphorylation of proteins and cellular signals, as well as in the cycle ADP/ATP. Associated with cyanocobalamin, butafosfan had positive effects on cow folliculogenesis. In view of this, butafosfan becomes a viable alternative to improve oocyte metabolism and thus the acquisition of competence. The aim of this study was to evaluate the effect of butaphosphan addition in the maturation medium in expression of genes associated with apoptosis, cumulus cells expansion, resumption of meiosis and energy metabolism. Bovine ovaries were collected from a local slaughterhouse and transported to the laboratory in NaCl 0.9% solution with gentamicin 0.5% at 30 °C. Complex cumulus oocytes (COCs) were aspirated from follicles (3-8 mm in diameter) using a stereo and then, washed three times in washing medium (Animal Biotechnology®, Brasília, DF, Brazil). In total of 809 COCs (n = 809) were randomly assigned to groups of ± 60 COCs/group/routine as supplemented with butafosfan in IVM medium (GC: 0 mg/ml, T1: 0.05 mg/ml; 0.1 mg/ml and T3: 0.2 mg/ml butafosfan, Bayer Animal Health, São Paulo, SP, Brazil). The maturation occurred in 500 µL drops of MIV-TCM medium (Animal Biotechnology®) supplemented with 10% fetal bovine serum at 39 °C in 5% CO_2 atmosphere and at maximum humidity for 24 h. After the IVM, 15 COCs from each group were stripped through successive pipings, the rest of COCs continued in the PIVE routine for further analysis. Cumulus cells and oocytes were stored separately in microtubes containing 100 µL TRIzol (Invitrogen, Carlsbad, California, USA) at -70 °C until analysis of gene expression. In this way 3 routines were conducted. Total RNA was extracted from cumulus and oocyte cells using TRIzol and quantified on NanoVue spectrophotometer (General Electric Healthcare Limited, UK). The cDNA synthesis was performed using iScript Reverse Trascription Supermix (Bio-Rad, Hercules, California, USA) according to the manufacturer's instructions. Real-time PCR reactions were conducted on Applied Biosystems 7500 (Applied Biosystems, Foster City, USA) using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, USA). Expression of the genes: BAX and BCL2 as markers of apoptosis; AREG and EREG as genes related to the expansion of cumulus cells and resumption of meiosis; GDF9 and BMP15 as indicators of oocyte quality and GLUT1 and PFKP related to energy metabolism in oocytes. The results were analyzed using the 2-ΔΔCT method, using the H2A gene as internal control. Statistical analysis was performed in the SAS 9.0 program (SAS, Cary, NC, USA) using the General Linear Model test to determine the linear, quadratic or cubic effect of the supplementation with 0.0, 0.05, 0.1 and 0.2 mg/ml butafosfan in the maturation medium. The relative expression of the genes studied was similar between the groups in both oocytes and cumulus cells (P> 0.05). In conclusion, supplementation of the IVM medium with different doses of butafosfan does not improve oocyte quality.

A114 OPU - IVF and ET

Effect of the number of recovered oocytes by OPU on *in vitro* embryo production of Holstein (*Bos Taurus*) cows

Bernardo Marcozzi Bayeux[1], Rodolfo Daniel Mingoti[1], Flávia Morag Elliff[1], Luciano Mundim de Carvalho[2], Pedro Meirelles do Nascimento Castro[2], Fernando Morais Salles Prestes[2], Ana Claudia Teixeira de Oliveira[2], Pietro Sampaio Baruselli[1]

[1]Universidade de São Paulo, São Paulo, SP; 2.Sexing Technologies, Sertãozinho, SP.

Effect of the number of recovered oocytes by OPU on in vitro embryo production of Holstein (Bos taurus) cows
An analysis of 1256 follicular aspirations (OPU) performed by the same veterinarian in 1021 Holstein oocytes donors for in vitro embryo production during the period of 2011 to 2013 in 10 commercial farms was conducted. A total of 16259 oocytes were fertilized using semen from 8 Jersey bulls. For data analysis, donors were divided according to the amount of oocytes on the moment of the OPU into Group 1 (Q1) – lower quartile (n=314); Group 2 (Q2) – intermediate lower quartile (n=314); Group 3 (Q3) – intermediate higher quartile (n=314) and Group 4 (Q4) – higher quartile (n=314). Data were analyzed using the PROC GLIMMIX procedure of SAS (9.4 version) and "interactive data analyses" of SAS was used to calculate probability. There was difference within groups in relation to the number of recovered oocytes (Q1=5.7±0.1; Q2=9.9±0.1; Q3=14.4±0.1 and Q4=26.8±0.5; P<0.0001) and blastocyst production (Q1=1.4±0.6; Q2=1.7±0.7; Q3=2.7±0.9 and Q4=4.5±1.3; P<0.0001) per OPU. The cleavage rate (number of cleaved oocytes/number of viable oocytes) was greater on Q3 (56.3%; 2331/4041) in relation to Q1 (50.3%; 843/1654) and Q2 (53.0%; 1502/2873), P<0.001. The Q4 (53.7%; 4373/7691) did not differ from the other groups. The blastocysts rate [number of blastocyst/number of viable oocytes; (Q1=25.5; 426/1654; Q2=18.7%. 538/2873; Q3=21.2%, 855/4041; Q4=18.3%, 1408/7691; P<0.23)] and the pregnancy rate [number of pregnancies/number of transferred embryos; (Q1=34.0%, 152/447; Q2=31.0%. 220/708; Q3=44.0%, 497/1129; Q4=37.0%, 805/2178; P=0.13)] did not differ within groups. The cleavage rate increased according to the number of recovered oocytes per donor (P=0.0008; R2=0.09436). The blastocysts production rate decreased according to the number of recovered oocytes per donor (P<0.0001; R2=-0.22642). There was no difference on the probability of pregnancy according to the amount of recovered oocytes per donor (P=0.1; R2=0.03608). It is possible to conclude that donors with greater number of oocytes have lower blastocyst production rate. Furthermore, the amount of recovered oocytes per donor does not interfere on pregnancy rate.
Acknowledgments: Sexing Technologies.

A115 OPU - IVF and ET

Effect of number of oocytes recovered per OPU on *in vitro* embryo production and pregnancy rate of Nelore (*Bos Indicus*) cows

Yeda Fumie Watanabe[*1], Osnir Watanabe[2], André Dayan[2], Rodolfo Daniel Mingoti[3], Laísa da Garcia da Silva[3], Flávia Morag Elliff[3], Roberta Machado Ferreira[3], Pietro Sampaio Baruselli[3]

[1]Vitrogen – YVF Biotech, Cravinhos, SP; [2]WTA – Watanabe Tecnologia Aplicada, Cravinhos, SP; [3]Departamento de Reprodução Animal, FMVZ/USP, São Paulo, SP.

The aim of the present study was to evaluate the relationship between the number of oocytes recovered per OPU section of Nelore cows with IVEP efficiency, as well as with field fertility (pregnancy after embryo transfer; ET). For that, the analysis of 9,470 follicular aspirations (OPU) performed by nine veterinarians in 1,658 Nelore oocyte donors for in vitro embryo production (IVEP) during the period of 2001 to 2010 in 16 commercial farms was conducted. Semen from 178 Nelore sires was used for the in vitro fertilization. For data analysis, donors were divided according to the amount of oocytes recovered on the moment of OPU into Group 1 (Q1) – lower quartile (n = 13,246); Group 2 (Q2) – intermediate lower quartile (n = 25,376); Group 3 (Q3) – intermediate higher quartile (n = 40,119) and Group 4 (Q4) – higher quartile (n = 75,645). Data were analyzed using PROC GLIMMIX of SAS (9.4 version). "Interactive data analyses" of SAS was used to calculate probabilities. There were differences within groups relative to the number of recovered oocytes per OPU (Q1 = 9.4; Q2 = 18,1; Q3 = 28.6 and Q4 = 53.9), number of viable oocytes per OPU (Q1 = 8.4; Q2 = 15.9; Q3 = 25.4 and Q4 = 47.7) and blastocyst production per OPU (Q1 = 2.6; Q2 = 4.9; Q3 = 7.8 and Q4 = 13.5). However, there was no difference between groups regarding the cleavage rate [number of cleaved oocytes/number of recovered oocytes; Q1 = 68.8% (8,089/11,756); Q2 = 66.7% (14,900/22,337); Q3 = 66.2% (23,582/35,634); Q4 = 63.9% (42,714/66,860; P = 0.08)] and the blastocyst rate [Q1 = 44.9% (3,634/8,089); Q2 = 45.9% (6,841/14,900); Q3 = 46.4% (10,948/23,582); Q4 = 44.5% (19,001/42,714); P = 0.23]. The pregnancy rate was higher for the lower quartile (Q1 = 44.1%, 187/424a) when compared to the other groups [Q2 = 40.3% (302/750)b; Q3 = 39.9% (n/899)b; Q4 = 38.6% (387/1,003)b; P = 0.001). The probability of cleavage (R2 = -0.084; P < 0.001), probability of blastocyst production (R2 = -0.034; P = 0.01), and probability of pregnancy (R2 = -0.072; P < 0.0001) decreased as the number of recovered oocytes per donor increased. In conclusion, both the efficiency of IVEP (cleavage and blastocyst production) and the pregnancy rate are negatively influenced by the increase of the number of recovered oocytes per OPU in Nelore cows.
Credits: Fapesp 2012/50533-2 (GIFT), and CNPq 152030/2016-6.

A116 OPU - IVF and ET

Efficiency of OPU (Ovum Pick-Up) in females bubalinas (*Bubalus Bubalis*) dairy, in management of pasture

Gustavo Alighiere Lopes Silva[1], Anelise Sarges Ramos[1], Vanessa Cunha Brito[2], Wilton Figueiredo Lima[1], Álvaro Chaves Neto[1], Nathália Nogueira da Costa[2], Henry Daniel Manrique Ayala[1], Sebastião Tavares Rolim Filho[1], Haroldo Francisco Lobato Ribeiro[1], Otávio Mitio Ohashi[2]

[1]UFRA, Belem, PA; [2]UFPA, Belem, PA.

The objective of this study was to evaluate the efficiency of OPU (Ovum Pick-Up) performed for in vitro embryo production in buffaloes (Bubalusbubalis), through the rate of aspiration follicles, rate of oocytes recovered, rate of oocytes for FIV. The experiment occurred on a farm specialized in the production of milk of buffaloes, located in the municipality of Bujaru 45 km from the capital of Belém. The property presents a herd bubalino consisting of mestizo animals and pure blood (Murrah and Mediterranean) destined for the production of milk to pasture. Used 47 buffaloes multiparous with dairy production above 7 litres in a single milking daily, previously selected through ultrasound examination for determination of ovarian size and follicular population and with body condition score around 3,0 (1 = very lean, 5 = very fat), which originates from producers in the States of Pará, Ceará, Bahia, Rio Grande do Norte and São Paulo. The females were subjected to a total of 43 follicular aspiration sessions (OPU; 18G; 1,7 mm Teflon suction line of internal diameter and 80 cm long; 50 mmHg). Visible follicles were vacuumed (≥ 2mm ≤ 8mm) through ultrasound (DP 4100VET). The aspirations were carried out weekly, each animal being aspired in 14-day intervals, with 7 to 14 buffaloes per session. The data was submitted to ANOVA and the averages compared to the Tukey test ($P < 0,05$). They were vacuumed a total of 5,186 follicles, 2,845 oocytes recovered and 1,800 oocytes for FIV, obtaining a recovery rate of oocytes of 54.86%. The averages obtained by animal and by section of OPU were 12.64 ± 5.87 of aspirated follicles; 6.93 ± 5.55 of oocytes recovered and 4.40 ± 3.89 of oocytes for IVF, higher than that found by other authors, who have achieved averages of 9.1 (aspiration follicles), 3.5 (oocytes recovered) and recovery rate of 38.4% (Baruselli, et al. Revista Brasileira de Reprodução Animal, 31, 285-292, 2005), demonstrating good development of opu in the females bubalinas in this study. However, numerically, the follicular population in buffalo is much lower when compared to the bovine Zebuíno (Gimenes et al., Reproduction in Domestic Ruminants, 1, 357-375, 2010), causing FIV in Bubalino to become much more expensive than in bovine (Ohashi et al., RevBrasReprodAnim, 41, 195-200, 2017). A large individual variation has occurred since donors are from varied backgrounds. Other authors also relara that low results in OPU are coming from individual and race variation (Boni et al. Proceedings of 5th World Buffalo Congress, 5, 787-792, 1997). Therefore, the OPU sessions in the Buffaloes used have shown good efficiency, presenting a good amount of suction follicles, oocytes recovered, oocytes for FIV, in addition to a satisfactory oocitária recovery fee.

A117 OPU - IVF and ET

Extracts from cerrado plants as antioxidant agents on *in vitro* embryo production

Andrei Antonioni Fidelis[1], Francislete Rodrigues Melo[1], Fernanda Mulinari[1], Victoria Wetzel Oliveira Lima[1], Barbara Elias Reis Hodecker[1], Margot Alves Nunes Dode[2]

[1]UPIS, Brasilia, DF; [2]EMBRAPA, Brasilia, DF.

The present study evaluated the effect of ethanolic extracts of plants from Cerrado- Brazil, containing high levels of polyphenols (antioxidant), on in vitro embryo production (IVEP) in cattle. Ovaries from slaughterhouse were used to collect grade I and II cumulus-oocyte-complexes (COC), which were submitted to in vitro maturation, fertilization (D0) and culture. Different concentrations (0; 1mg/mL; 01mg/mL and 0.01mg/mL) of cagaita (Eugenia dysenterica) and murici (Byirsonima crassifolia) extracts were added to the culture medium during embryo development. The parameters analyzed were: cleavage rate on D3, blastocyst rate in D6, D7 and total and apoptotic cells number by TUNEL method. The ability of those extracts to request free radicals from the culture medium was analyzed by ABTS colorimetric method. To do that an aliquot of the culture media was collected from each treatment drop at two different time points (D0 and D7). Data were analyzed by analysis of variance - ANOVA and the means were compared by TUKEY, with a significance level of 5%.The results of embryo production did not differ between the control group: cleavage 80.5% (136/169), blastocyst rate D6: 30.2% (51/169) and D7: 41% (69/169) and the groups treated with murici 0.1mg: 81.9% (149/182), 23.6% (43/182) and 35.2% (64/182) and 0.01mg: 78% (127/163), 32.% (52/163), 38.7 % (63/163). The total embryonic cells and the proportion of apoptotic cell in expanded blastocyst (BX) in D7 were similar among the groups (P=NS). Except for the 1mg group, that showed high toxicity and death already on cleavage stage evaluation. Regarding the capacity of polyphenols to remove free radical, no differences (P>0.05) between those same groups (control, 0.1 and 0.01mg). The only difference detected ($p < 0.05$) was also for the 1mg/ml group, which showed an increase on the amount of free radicals. However, the cagaita extract showed a similar behavior for cleavage rates in control group: 80.6% (179/222); 1mg: 78.3% (177/226); 0.1mg: 81% (187/231); 0.01mg: 82.5% (184/223). Yet, when evaluating the blastocyst rate at D6, a lower rate ($p<0.05$) was observed for the 1mg group 27/226 (12%) compared to the to control group 57/222 (25.7%), 0.1mg 59/231 (25.5%) and 0.01mg 76/223 (34%) groups. The same profile was observed at D7, with 45.5% of embryos in the control (101/222); 35% in the 1mg group (79/226 $p < 0.05$); 42% in 0.1mg (98/231) and 50% in the 0.01mg (112/223) groups. The number of BX cells was similar among all groups. However, the proportion of apoptotic cells was lower ($p < 0.01$) in the group with 0.01mg cagaita (2.8%) than the others (control: 8.33%, 1mg: 5% and 0.1mg: 5.4%). The ABTS results for cagaita were similar for all groups. The results showed that extracts of the tested plants were toxic at concentration of 1mg/mL in However, when they were diluted thousand times, it was possible to observe a decrease in apoptotic cells using 0.01 mg of cagaita extract (Eugenia dysenterica). This same dilution of the murici extract did not affected any of the evaluated parameters. It can be concluded, that the cagaita extract (0,01mg/mL) is an alternative to be use as a coadjuvant for the reduction of the oxidative stress induced by the adverse conditions of IVP.

A118 OPU - IVF and ET

Serum FSH, AMH related to number and morphology of oocytes in superovulated 4 to 7 months old Nelore breed females (*Bos Taurus Indicus*)

Taynan Stonoga Kawamoto[1], Thaís Abritta Zacarias[1], Ana Luiza Silva Guimarães[2], João Ricardo Scaliante[1], Sarah de Andrade Dias Rodrigues[3], Ricardo Alamino Figueiredo

[1]Universidade Federal de Uberlândia, Uberlandia, MG; [2]Universidade de Brasília, Brasilia, DF; [3]Universidade Estadual de Maringá, Maringá, PR; [4]Embrapa Recursos Genéticos e Biotecnologia, Brasilia, DF.

The inclusion of prepubertal bovine females in reproductive management can make herd's genetic gain faster by shortening generation interval. However, these female oocytes have lower competence, blastocyst and pregnancy rates, when compared to those obtained from postpubertal animals. So, this study aimed to evaluate serum FSH and AMH concentrations in superovulated (TG-Treated Group) Nelore females of 4 to 7 months old, as in control group (CG) and their respective oocyte retrieval and quality by OPU (Storz Xenon300W Laparoscope, Tuttlingen, Germany). Nine females (cross-over design) were allocated at random to two groups: The CG (n=9), which the greatest follicle ablation was performed on D2 (5 days before OPU) with the aid of transrectal ultrasonography (MyLab 30VetGold, Esaote, 5-7.5MHz transducer, Genova-Italy). And to the TG (n=9), in which D0 represented the protocol beginning with intravaginal device Progesterone insertion (P4, 0.33g. Eazi-Breed-CIDR, Pfizer Animal Health, Brazil) plus 2mg Estradiol Benzoate injection (im Ric-BE, Tecnopec-Brazil). From D4 on, 6 FSH injections were given during 3 days (im, 12/12h: 1x40mg + 5x20mg = 140mg; Folltropin, Bioniche Animal Health, Belleville-Ontario, Canada). At the last FSH injection, LH (2.5 mg) was administered (Lutropin, Bioniche Animal Health, Belleville-Ontario, Canada). Then, the OPU was performed 20-24h after the last FSH injection (D7) and the P4 devices were removed thereafter. The follicles were counted and aspirated COCs were classified. Blood sample collections for FSH measuring were performed 2 days before, at the day and 1 day after the OPU procedure, as for the AMH measuring, it was performed at D5 and at D8. Data were analyzed by Kruskal-Wallis, ANOVA, T-test and Chi-square test. The TG had higher serum FSH concentrations ($p<0.05$) on days 5 (1.16 ± 0.31 ng/ml), 6 (1.21 ± 0.45 ng/ml) and 7 (0.95 ± 0.26 ng/ml) than the CG (0.56 ± 0.17 ng/ml at D5, 0.60 ± 0.25 ng/ml at D6 and, 0.60 ± 0.14 ng/ml at D7). In addition, a greater number of aspirated follicles (152 vs. 95) and higher numbers of oocytes grades I and II (59% vs. 25%) were observed in the TG compared to the CG, respectively ($p <0.05$). However, GC presented more grade III and IV oocytes when compared to TG (53.3% vs 37.1%), whereas the mean AMH concentration (1.48 ± 0.37 ng / ml) was not different between TG and CG nor between the days of collection ($p>0.05$). Thus, this superovulation protocol led to higher serum FSH concentrations, which possibly had a role to a greater quantity and better quality of the retrieved oocytes, without changing the serum AMH levels in the animals.
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A119 OPU - IVF and ET

Influence of climatological conditions in reproductive variables of Nelore race females

Suellen Neves[*1], Fábio Luiz Bim Cavalieri[2], Josmar Mazucheli[3], Daniela Bóboo Moreski[1], Isabele Picada Emanuelli[2]

[1]UNICESUMAR, Maringa, PR; 2.UNICESUMAR/ICETI, Maringá, PR; [3]UEM, Maringá, PR.

An extrinsic factor that can interfere in the reproductive variables is the climatic changes occurring throughout the year. This factor has a determinant role in the quality of the pastures that can interfere in the embryo production. This study aimed to evaluate the effect of the seasons of the year and the climatic variables of temperature and precipitation on reproductive variables in Nelore cows. Eighteen cows, with at least 7 aspirations / cows, all from the same genetic lineage were housed 40 km from the city of Maringá, with a Brachiaria grass diet at will and 2 kg of concentrate / day. The reproductive variables evaluated were the production of total aspirated oocytes, viable oocytes and PIV embryos. In vitro embryo production was performed with viable oocytes maturation for 22-24 hours in TCM 199 medium (10% FCS, FSH 0.1µg / mL and LH 50µg / mL); the in vitro fertilization in TALP-FIV medium for 22-24 hours with a dose of 1x106 spermatozoa / mL. The probable zygotes were farmed in SOF (Synthetic Oviduct Fluid) supplemented with 2.5% FCS and BSA (5mg / ml) in an incubator (38.3 °C, 5% CO2 and maximum humidity). On the seventh day of cultivation the viable embryos for transfer were evaluated. The climatological data were obtained at the National Institute of Meteorology (INMET) throughout the year 2012, through the automatic monitoring station located in Maringá region (latitude -23.4º, longitude -51.91º and altitude 542m), and for both the Temperature and precipitation the average of the 10-day period was effectuated. For the data analysis, the logistic regression models (viable oocytes and embryo production) and Poisson regression (total oocytes) were used. Analyzing the climatic data, it was observed that the climate was typical throughout the year (rainy summer and dry winter), without extreme events related to temperature and precipitation. The average winter temperatures were 25 °C in summer, in autumn and in spring 22 °C and 18 °C in winter. The average precipitation of the deciduous was 75mm in the summer and 10mm in the winter. There was no effect of the season of the year with the analyzed reproductive variables having as minimum and maximum mean of: 17 to 28 total oocytes; 10 to 17 viable oocytes; and from 3.5 to 6.5 viable embryos by aspiration. Relating the total oocytes aspirated with precipitation and temperature, no significant difference was found between climatic variations and aspirated oocytes, as did oocytes with viable oocytes and embryo production. The data analyzed show that, in Nelore cows with good nutritional management, there is no influence of precipitation and temperature climatological variables on the reproductive variables of oocyte production and viability, and embryo production in vitro.

A120 OPU - IVF and ET

Influence of elapsed time from loading to transfer with *in vitro* bovine embryo on pregnancy rates in the State of Mato Grosso do Sul

Natali Freire Zanenga Chacha[1], Carlos Alberto Zanenga[1], Merlison Figueiredo Pedroso[1], Luiz Carlos Cesar da Costa Filho[2], Eliane Vianna da Costa e Silva[2], Rodrigo Daneluzzi Fioravante Fiorani[3], Daniely Coelho de Menezes[1]

[1]Embriza Biotecnologia, Campo Grande, MS; [2]Grupo de Estudos em Reprodução Animal do Estado de Mato Grosso do Sul (GERA-MS)-FAMEZ/UFMS, Campo Grande, MS; [3]Cenatte Embriões, Pedro Leopoldo, MG.

Aiming to facilitate the logistics of commercial laboratories regarding the elapsed time from loading to transfer, periods of up to 10 hours and longer periods of up to 24 hours were compared utilizing the "Synthetic Oviduct Fluid" medium with buffered HEPES (HSOF) in order to measure their influence on the pregnancy rates of recipients at 60 days. Selected cumulus-oocyte complexes (COC) were transported in 2 mL cryotubes (1 oocyte/13.3µL of medium), containing 400µL of IVM medium TCM-199 (supplemented with 0.2 mM ofpyruvate, 10% of FCS and gonadotropins) and 300µL of silicone oil, at a controlled temperature of 38.7°C and atmosphere of 5% of CO_2, 5% of O_2 and 90% of N_2. After the transport period, the cryotubes were transferred to incubators with 100% of humidity at 38.7°C, with an atmosphere of 5% of CO_2 (≅20% O_2), with a total time ranging from 20-25 hours of IVM (24 h on average). The period of fertilization was from 8 to 10 hours, under the same conditions as described for the IVM. Presumptive zygotes were denuded and cultured in SOFaa, supplemented with 5% of FCS for up to 7 days. Cleavage and blastocyst rates were evaluated at 48 and 168 hours post-insemination (hpi), respectively. The procedures were performed at Embriza laboratory, located in Campo Grande, MatoGrosso do Sul, Brazil, and the media produced by the Cenatte Embriões laboratory, located in Pedro Leopoldo, Minas Gerais, Brazil. 1,944 transfers were carried out in the state of Mato Grosso do Sul in the period from July 2016 to January 2017 and were divided into 4 groups: G1 (>10h; n=600), G2 (>10-14 h; n=432), G3 (>14-17 h; n=507) and G4 (>17-24 h; n=405). The analyses of frequency dispersion were performed by a X^2 test considering the effects of the technique compared to each other with a level of significance $P<0.05$. The detected pregnancy rates were 44.9%a (G1), 51.16%a (G2), 44.97%a (G3) and 48.28%a (G4). There was no significant difference between the studied groups, keeping for the period from loading to the end of the transfer in the HSOF medium to be of up to 24 hours without any loss in the pregnancy rates.

A121 OPU - IVF and ET

Lipid content of blastocysts produced *in vitro* from oocytes of small and large follicles – preliminary results

Diego Borba Muller[1], Kelly Annes[2], Jorge Abrão P. Vilela[1], Roniele Santana Valente[1], Diana Pedroso Caetano[1], Jeferson Bueno Fripp[1], Marcella Pecora Milazzotto[2], Fernando Silveira Mesquita[1], Mateus José Sudano[1]

[1]Universidade Federal do Pampa, Uruguaiana, RS; [2]Universidade Federal do ABC, Santo André, SP.

In vitro produced (IVP) embryos have higher content of cytoplasmic lipid droplets compared with the in vivo counterparts. The reasons for the increased lipid deposit in IVP embryos are still lacking. In previous studies, it was observed that oocyte recovered from large follicles had higher cytoplasmic lipid content when compared with the oocytes from smaller follicle sizes. The aim of this study was to assess cleavage, blastocyst rate and the cytoplasmic lipid content of blastocysts produced in vitro from oocytes of small and large follicles. Slaughterhouse cow ovaries were used for the recovery of oocytes from small (≤5 mm, n= 322) and large (≥6mm, n= 93) follicles. The diameters of follicles were carefully determined with caliper device followed by volume monitoring. Only oocytes with homogeneous cytoplasm and with more than three layers of cumulus cells were submitted to in vitro maturation, fertilization and culture, as previously described. Cleavage and blastocyst production were recorded at day 3 and day 8 after fertilization, respectively. Expanded blastocysts at day seven after fertilization (Day7Ex, n= 22), and embryos that were not expanded blastocyst at day seven but became expanded blastocysts at day eight (Day8Ex, n= 23) were collected for semi-quantitative lipid content evaluation using the Sudan Black B staining. Expanded blastocysts were prepared following a previously established protocol. ImageJ software was used to convert the Sudan Black B-stained blastocysts images to gray scale and to determine the lipid content per embryo expressed as gray intensity. The data were analyzed by ANOVA using the PROC GLIMMIX of SAS. Cleavage (74.8 ± 3.6 vs 51.9 ± 4.4) and blastocyst (26.4 ± 9.0 vs 13.8 ± 1.7) rates (%) were higher ($P<0.05$) on embryos derived from oocytes of large follicles compared with those from small follicles, respectively. Day7Ex from oocytes recovered from large follicles had increased ($P<0.05$) cytoplasmic lipid content compared with the blastocysts derived from oocytes of small follicles (5.3 ± 0.3 vs 4.3 ± 0.4 of gray intensity, respectively). Day8Ex derived from oocytes of large and small follicles had similar ($P>0.05$) cytoplasmic lipid content (7.6 ± 0.5 vs 7.7 ± 0.4 of gray intensity, respectively). However, Day8Ex had increased ($P<0.05$) lipid deposit when compared with Day7Ex. Therefore, the preliminary findings of this study reveal the following: i) Oocytes recovered from large follicles reached increased cleavage and blastocyst rate; ii) Day 7 expanded blastocyst derived from oocytes of large follicles had increased lipid content compared with blastocyst derived from oocyte of small follicle size; iii) Lipid deposit did not vary among follicle sizes at Day 8 blastocysts; iv) Blastocysts with delayed expansion of blastocoel (Day 8) had higher lipid content compared with blastocysts with early expansion (Day 7). Acknowledgements: CNPq, FAPESP, FAPERGS and CAPES.

A122 OPU - IVF and ET

Nuclear maturation of bovine oocytes submitted to the *in vivo* maturation using intrafollicular transfer of immature oocytes (IFIOT) system

Luzia Renata Oliveira Dias[1], Otavio Augusto Faria[2], Felippe Manoel Costa Caixeta[1], Sarah de Andrade Dias Rodrigues[1], José Felipe Warmling Sprícigo[3], Margot Alves Nunes Dode[3]

[1]Universidade de Brasília, Brasília, DF; [2]Médico Veterinário Colaborador, Brasília, DF; [3]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF.

This study aimed to evaluate the kinetics of nuclear maturation of bovine oocytes submitted to the in vivo maturation using intrafollicular transfer of immature oocytes (IFIOT) system. To do that, ovulatory cows were previously synchronized. On day 0 (D0) the animals received an intravaginal progesterone-releasing device (1 g), and an injection (i.m.) of 2 mg Estradiol Benzoate. On D8, the progesterone implants were removed and an injection of Prostaglandin F2α analog (0.150 mg d-Cloprostenol) was made (i.m.). On D9 a 1 mg of Estradiol Benzoate (i.m.) was administered and on day 10 oocytes were injected into a ≥10 mm diameter follicle together with an injection of a gonadotrophin releasing hormone (GnRH) analogue - Buserelin. A total of 890 grade 1 and 2 oocytes obtained from slaughterhouse ovaries were used, being 417 for TIFOI and the remainder for Control. In the control group the oocytes were placed in IVM and removed at 0, 8, 12 and 16h. For TIFOI, 30 oocytes per ovulatory cow were used, which at 8, 12 and 16h post-injection were recovered by ovum pick up (OPU). Treatments and number of oocytes evaluated by treatment were: Control 0h (n=51); 8h Control (n=60); Control 12h (n=60); Control 16h (n=38); TIFOI 8h (n=79); TIFOI 12h (n=88); TIFOI 16h (n=7). Oocytes from all groups were denuded by and are fixed for further evaluation of nuclear maturation by lacmoid stain. Oocytes were classified according to meiotic stage in: germinal vesicle (GV), germinal vesicle break (GVB), metaphase I (MI), anaphase I (AI), telophase I (TI), metaphase II (MII) and abnormal. Data were analyzed by Chi-square test ($P<0.05$). At 0h, before maturation or injection, 96.1% of the oocytes were found at GV stage. At 8 h, most of the oocytes of the Control group were in MI stage (76.6%), while TIFOI 8h group presented a greater percentage ($P<0.05$) of oocytes in stage (97.5%). The percentage of oocytes in MI at 12h was similar ($P>0.05$) between the Control (81.7%) and TIFOI 12h (73.9%) and both presented oocytes at more advanced stages of meiosis (Control=TI 13.3%, MII 1.7%, TIFOI=AI 4.5%, TI 7.9%). Control group 16h had oocytes abnormal (2.6%), in MI (52.6%), AI (18.4%), TI (21.1%) and MII (5.3%). In the TIFOI 16h group at the time of aspiration the majority of the animals had already ovulated and, therefore, a small number of oocytes were recovered (n=7), being classified as abnormal (14.3%), MI (57.1%), TI (14.3%) and MII (14.3%). The results suggest that the in vivo maturation system using the TIFOI method was adequate, since the oocytes exposed to this system presented kinetics of maturation similar to the in vitro, being even more homogeneous than in vitro with 8 h of maturation.

A123 OPU - IVF and ET

Glucose metabolism of bovine cumulus oocyte complexes matured *in vitro* with the addition of different suplements

Elena Carolina Serrano Recalde[1], Lais do Nascimento Cintra[1], Mateus José Sudano[2], Fernanda Cruz Landim Alvarenga[1]

[1]FMVZ/UNESP, Botucatu, SP; [2]UNIPAMPA, Uruguaiana, RS.

Cumulus-oocyte complex (COC) is formed by the oocyte and cumulus cells, and between them there exists a paracrine and gap junction bidirectional communication fundamental to determine oocyte viability. COCs preferentially use glucose as substrate, which is converted in pyruvate and lactate through glycolysis. Glucose metabolism during oocyte maturation is involved in meiotic progression regulation, ooplasm maturation, oxidative stress reduction, and it is related to cumulus cells expansion after FSH stimulous. This study aimed to compare the effects of the addition of fetal bovine serum (FBS), polyvinyl alcohol (PVA) or insulin-like growth factor -1 (IGF-I), during in vitro maturation (IVM) over COCs glucose metabolism. Grade I and II COCs (n=20/drop) obtained from ovaries from slaughterhouse were selected in Dulbecco's modified PBS containing 3 mg/mL of PVA and transferred to TCM HEPES. COCs were matured in vitro with TCM199 (supplemented with 0.2 mM pyruvate, 1 µg/mL FSH, 50 µg/mL LH, 100 µg/mL streptomycin, 100 UI/mL penicillin, and 85 µg/mL amikacin) with the respective addition of 10% of FBS (FBS), 3 mg/mL of PVA (PVA) or PVA + 100 ng/mL IGF-1 (IGF). IVM was performed in petri dishes with 90 µL droplets, covered with mineral oil at 38.5ºC and 5% CO_2 in humidified air for 22 to 24 hours. Glucose and lactate concentrations were determined in spent maturation media (including media without cells). After IVM, spent media were collected, snap frozen, and stored at -80°C. Samples of five experimental replicates were analyzed by a Hitachi 912 chemical analyzer (F. Hoffmann-La Roche Ltd.). To determine glucose uptake, concentration of glucose of media cultured without cells was taken as reference. Glucose uptake and lactate production were expressed as pmol/COC per h. Data were analyzed by analysis of variance (ANOVA) from PROC GLIMMIX model from SAS software (SAS Inst. Inc., Cary, NC, USA). Tukey test was used to compare the means. PVA (835.53± 7.38) and IGF (824.17± 7.38) groups showed a higher ($p < 0.05$) glucose uptake compared to FBS (769.68 ± 7.38) group. However, it has to be considered that FBS medium had initially lower concentration of glucose than other groups. This may have happened because addition of 10% of serum leads to a dilution of initial concentration of glucose of TCM199 media. IGF group (1908.41± 28.63) had higher lactate synthesis compared to group FBS (1879.77± 28.63), which produced more lactate than PVA (1793.86± 28.63) group. With the presented results it is possible to conclude that the addition of IGF-I in oocyte maturation leads to a higher efficiency in glucose utilization as a substrate and produce higher quantities of metabolites as lactate through the glycolytic pathway.

A124 OPU - IVF and ET

Live birth of domestic cat by *in vitro* fertilization using oocytes recovered after mild follicular stimulation with equine chorionic gonadotropin

Daniel Veraguas, Paula Fernanda Gallegos, Diana Echeverry, Sandra Rocio Cuevas, Darling Sáez-Ruiz, Fidel Ovidio Castro, Lleretny Rodriguez-Alvarez

Department of Animal Science, Faculty of Veterinary Sciences, University of Concepcion, Chillán.

The domestic cat is a valuable model for the generation of assisted reproductive techniques that might be used in the conservation of endangered wild felids. However, the *in vitro* embryo production systems in the domestic cat still have a low efficiency. The oocyte competence is an important factor that determine the successful in the *in vitro* embryo production systems. In humans, the mild follicular stimulation with gonadotropins (or priming) prior to the *in vitro* maturation (IVM), has been used to increase the oocyte maturation and blastocyst formation rates. The objective of this research was to evaluate the mild follicular stimulation with eCG in the *in vitro* fertilization system (IVF) in the domestic cat. For this purpose, nine domestic cat were treated with a subcutaneous dose of 200 IU of eCG and were subjected to ovariohysterectomy 4 days later for ovaries recovery and cumulus-oocyte complexes (COCs) collection. Additionally, others two cats were synchronized for embryo transfer procedure with 200 IU of eCG and an intramuscular dose of 100 IU of hCG 4 days later. Each cat correspond to an individual biological replicate, for this reason, the COCs recovered from each cat were matured, fertilized and cultured separately. For IVM, only grade I and II COCs were selected and matured in TCM-199 Earle's salts medium supplemented with 4 mg/mL BSA, 0.1 IU FSH-LH (Pluset), 0.36 mM sodium pyruvate, 2 mM glutamine, 2,2 mM calcium lactate, 1 µg/mL 17-β estradiol, 20 µg/mL EGF and 50 µg/mL gentamycin for 28-30 hours in a 5% CO_2, 5% O_2 and 90% N_2 humidified atmosphere to 38.5ºC. The IVF was realized using epididymal cat sperm which was refrigerated to 4ºC for 24 hours, 1.5 – 2.5 x 10^6 spermatozoa /mL were incubated with 20-30 COCs in TALP medium supplemented with 6 mg/mL BSA, 0.36 mM sodium pyruvate, 1 mM glutamine, 2.2 mM calcium lactate, 1% MEM non essential amino acids (NEAA), 0.01 mg/mL heparin sodium salt and 50 µg/mL de gentamycin for 18 hours in a 5% CO_2 humidified atmosphere to 38.5ºC. The presumed zygotes were cultured in SOF medium in a 5% CO_2, 5% O_2 and 90% N_2 humidified atmosphere to 38.5ºC during 7-8 days. The cleavage, morula, blastocyst and hatching blastocyst rates were estimated. Once finished the culture, the blastocysts and hatching blastocysts were fixed and stained with Hoechst for total cell counting. Additionally, a total of 23 blastocysts were transferred into the uterine horn of the two previously synchronized cats (15 and 8 blastocysts per cat, respectively). The descriptive statistic was realized using the statistical software Infostat. Regarding to *in vitro* embryo production, the results of this research demonstrated that the domestic cat oocytes recovered after eCG priming are capable to develop *in vitro* after IVF until the blastocyst stage. Cleavage rate was 155/239 (64.9%), morula rate 115/155 (74.2%), total blastocyst rate 51/155 (32.9%) and hatching blastocyst rate 15/155 (9.7%). Furthermore, the embryo staining revealed the total cell number (mean ± standard deviation) of the blastocysts (182.8 ± 76.9) and hatching blastocysts (420.2 ± 106.1) generated. Finally, one gestational vesicle was detected at the 25 days of gestation in the cat that received 15 blastocysts and a healthy female kitten born after 64 days of gestation. No implantation was detected in the cat that received 8 blastocysts. In conclusion, the mild follicular stimulation might be a useful alternative for the *in vitro* and *in vivo* embryo development in the domestic cat and could be applicable in wild felid species.

A125 OPU - IVF and ET

Propilenoglycol treatment increases blastocyst production rate on Holstein cows on lactation PEAK

Romulo Germano Rezende[1], Rodolfo Daniel Mingoti[1], Roberta Machado Ferreira[1], Marcos Henrique Alcantara Colli[1], Flávia Morag Elliff[1], Yeda Fumie Watanabe[2], Tiago de Oliveira Carneiro[3], Rodrigo Selitto Belli[1], Ana Elisa Negrão Pereira Barreto[4], Pietro Sampaio Baruselli[1]

[1]Departamento de Reprodução Animal, VRA/USP, São Paulo, SP; [2]WAT FIV - Vitrogen, Cravinhos, SP; [3]CattleVitro, São João da Boa Vista, SP; [4]Fazenda J-ida, Águas da prata, SP.

This study aimed to evaluate the effect of propilenoglycol (PPG) suplementation on IVEP of Holstein (Bos taurus) cows. A total of 84 lactating cows, of those, 45 on lactation peak [(PEAK) DIM < 100 days] and 39 repeat-breeder cows [(RB) DIM > 200 days, not pregnant], were used. On Day 0 all cows were summited to OPU, for follicular ablation on a random phase of the estrous cycle, then distributed in a factorial design 2 x 2 in order to evaluate the treatment and category effect: Control group (CTL; without treatment, n=23 cows PEAK), group RB (without treatment, n=21 cows RB); group PPG-PEAK (treatment with 500mL of intra-ruminal infusion with propilenoglycol twice a day, during 5 days, n=22 cows PEAK) and group PPG-RB (treatment with 500mL of intra-ruminal infusion with propilenoglycol twice a day, during 5 days, n=18 cows RB). On day 5 all cows were submitted to a second OPU. Five replicas were used with animal from both categories and treatments. On each replica, all follicles ≥ 2 mm were picked-up and the amount and quality of the oocytes were registered. Oocytes were submitted to IVP and embryo development (cleavage and blastocyst rate) were evaluated. On day 7of in vitro production, embryos were vitrified for later transfer. Oocytes were fertilized with sexed semen from the same Holstein (Bos taurus) sire and same ejaculate. Data were analyzed using GLIMMIX procedure of SAS®. There was no significant difference (P= 0.52) within categories and treatment for: total number of oocytes, CTL-PEAK (5.09 ± 0.95), PPG-PEAK (3.27 ± 0.67), CTL-RB (6.14 ± 1.11) and PPG-RB (5.38 ±0.76); number of viable oocytes (P=0.847): CTL-PEAK (2.7 ± 0.51), PPG-PEAK (1.82 ± 0.46), CTL-RB (3.33 ± 0.72) and PPG-RB (2.67 ± 0.62); cultivated oocytes number (P= 0.3416): CTL-PEAK (4.39 ± 0.81), PPG-PEAK (2.82 ± 0.65), CTL-RB (5.1 ± 1.03) and PPG-RB (4.89 ± 0.69). The blastocyst number per OPU was similar between the analyzed treatments and categories: CTL-PEAK (0.87 ± 0.28), PPG-PEAK (1.05 ± 0.23), CTL-RBB (2.00±0.51) and PPG-RB (1.56 ± 0.5). There was interaction between treatment and category (P= 0.0031) on cleavage rate of cultivated oocytes, that was superior on group CTL-RB (70%a) when compared to the CTL-PEAK group (36%b) and did not differ from groups PPG-PEAK (58%ab) and PPG-RB (55%ab). There was interaction between treatment and category (P= 0.0118) on blastocyst rate of cultivated oocytes, that was superior (P=0.0118) on cows from group PPG-PEAK (42%a) and CTL-RB (40%a), when compared to CTL-PEAK group (18%b) and did not differ from PPG-RB group (31%ab). The treatment with propilenoglycol for five days increased the cleavage rate and blastocyst rate on lactating Holstein cows on lactation peak.

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A126 OPU - IVF and ET

In vitro embryo production in Canchim primiparous cows (3/8 *Bos indicus and* 5/8 *Bos taurus*) maintained in grazing area with or without shade presence: preliminary results

Amanda Prudêncio Lemes[1], Alexandre Rossetto Garcia[2], Yeda Fumie Watanabe[3], Marina Okano Chiba[3], Anelise Carla Camplesi[1], Andre Faria Pedroso[2], Alessandro Giro[4], Daniela Botta[4], Narian Romanello[4], Lindsay Unno Gimenes[1]

[1]UNESP, Jaboticabal, SP; [2]Embrapa Pecuaria Sudeste, Sao Carlos, SP; [3]Vitrogen - YVF Biotech, Cravinhos, SP; [4]UFPA, Belém, PA.

The aim was to identify the influence of natural shade in grazing system on in vitro embryo production (IVEP) of suckling primiparous Canchim cows. Were used 18 donors, previously selected for follicular population, with 385.0±10.24 kg and 26.6±3.50 days post-partum at onset of experiment, while grazing pastures with shade provided by in a silvopastoral system (n=10, PRA, presence of eucalyptus trees with 15x2 m spacing) or pastures without shade (n=8, PR), at Embrapa Southeast Livestock. The Temperature and Humidity Index (THI) and Black Globe Humidity Index (BGHI) were measured during all experimental period in both experimental areas. All pastures were intensively managed in a rotational system. To IVEP, 4 OPU sessions were performed 4 OPU sessions, once a month, from January to April 2017, simultaneously, were measured the rectal temperature (oC). The aspirated follicles (AF) was counted to calculate recovery rate (Rr) and then, were performed the counting and morphological evaluation of cumulus oophorus-oocytes complex (COC). To IVF, semen with fertility recognize of the same bull was used. Cleavage rates (Cr) on D3, hatched blastocysts on Day 7 (HrD7), on Day 8 (HrD8) and on Day 9 (HrD9) rates were evaluated. Those classified in Grade I to III were put in maturation medium and carry to Vitrogen Laboratory (Cravinhos, SP, Brazil) to proceed IVF, IVC, and evaluation of cleavage rates (Cr) on D3, hatched blastocysts on D7 (HrD7), on D8 (HrD8) and on D9 (HrD9) rates. The data were analyzed as repeated measures (PROC MIXED, SAS®) and the results showed as least square means±SE. THI (70.4±0.03 and 70.2±0.02, P<0.001) and BGHI (73.3±0.04 and 72.8 ± 0,04, P<0.0001) values were higher in PR than PRA, respectively and were reducing by month during the experimental period in PR and PRA (P<0.0001). There were no interaction between replica and grazing system for any of the variables, as well there were no differences between cows maintained on PRA or PR, respectively, to RT (38.3±0.11 and 38.9±0.12oC, P=0.78), AF (25.6±2.32 and 28.2±2.64, P=0.46), Rr (83.3±8.69 and 75.2±9.89, P=0.54) Cr (90.2±4.68 and 83.8±5.30, P=0.37), HrD7 (37.7±4.64 and 30.8±5.13, P=0.32), HrD8 (30.8±4.81 and 21.2±5.33, P=0.19), or HrD9 (18.8±3.41 and 15.7±3.67, P=0.54). Contrary to expectations, the number of viable oocytes were higher in January (13.0±2.13) and March sessions than in February (9.8±2.05) and April (8.7±2.10) (P=0.03), coincidentally with higher RT in January (39.4±0.17) and March (39.2±0.16), compare to February (38.4±0.16) and April (38.5±0.16) (P<0.0001). The HrD9 was higher in March (2.7±3.40) when compared to February (1.6±0.41) and April (1.2±0.41) (P=0.04). These results allow us to conclude that animals maintained in PR and PRA were not showed body temperatures during morning that determine thermal stress. Therefore, the IVEP was similar between donors maintained in the same for PR or and PRA grazing system.

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A127 OPU - IVF and ET

In vitro embryo production in buffalo: comparison between calves, prepubertal Heifers and lactating cows

Júlio César Barbosa Silva[1], Romulo Germano Rezende[2], Marcos Henrique Alcantara Colli[2], Bernardo Marcozzi Bayeux[2], Rodolfo Daniel Mingoti[2], Oscar Alejandro Ojeda-Rojas[3], Andrea Cristina Basso[4], Julianne de Resende Naves[2], Pietro Sampaio Baruselli[2]

[1]Embryo Sys Reprodução Animal, Ouro Fino, MG; [2]Departamento de Reprodução Animal, VRA/USP, São Paulo, SP; [3]Departamento de Nutrição Animal - VNP - USP, Pirassununga, SP; [4]In Vitro Brasil, Mogi Mirim, SP.

The aim of this study was to compare the production of embryos of calves in relation to prepubertal heifers and lactating cows of bovines species. The experiment was carried out at Paineiras do Ingaí farm, Alambari – São Paulo. For this purpose, 30 bovines females of three animal categories were divided according to the following quantities: 10 calves with 2 to 4 months of age, 10 prepubertal heifers with 13 to 15 months of age and 10 lactating adult cows. On random day, the calves received intravaginal device (Cidr Ovinos, Zoetis) considered as day zero (D0). In the D5 and D6, the calves received 140mg of FSH (Folltropin, Tecnopec, Brazil) divided into 4 decreasing doses at 12-hour intervals and were aspirated, posteriorly, by laparoscopy (LOPU - Laparoscopy Ovum Pick Up) on D7. On random day of estrus cycle, prepubertal heifers and adult lactating cows were submitted to follicular aspiration (OPU). Both LOPU and OPU were performed on the same day. Two of the ten heifers that had been submitted could not be aspirated because they had their bladder filled at the time of laparoscopic intervention. The oocytes produced were selected for morphological appearance, inserted in tubes containing maturation medium, covered with a layer of mineral oil. A gaseous mixture containing 90% N2, 5% CO2 and 5% O2 was placed inside the tube for 15-20 seconds and then maintained on the oocyte carrier (WTA, Cravinhos, Brazil) after capping, at 38°C of temperature until the arrival at the laboratory. All cumulus oocyte complexes recovered (TO - viable + nude + with irregular cytoplasm) were sent to the laboratory. The in vitro fertilization (IVF) occurred between 22 and 26 hours after start in vitro maturation (IVM). Single bull semen was used, keeping the same fertilization match for all oocytes. After 18 hours of the moment fertilization, in vitro culture (IVC) was started and the blastocysts were vitrified six days after IVF. Cleavage and blastocyst rates were done three and six days after of IVM, respectively. The data were analyzed by PROC GLM of SAS 9.3, using Tukey's Test to detect the differences between the groups. Among the animal categories, there was no significant statistical difference in the number of OV (calves = 7.63 ± 2.69, heifers = 6.20 ± 1.55, cows = 3.20 ± 0.90, P=0.1033), cleaved structures (calves = 2.75±0.86; heifers = 3.10±0.67; cows = 2.10±0.43, P = 0.5492) and embryos produced (calves= 1.00±0.57; heifers = 1.50±0.34; cows = 1.10±0.38, P = 0.3621). In contrast, a significant statistical difference was observed in the TO (calves = 10.88±3.25; heifers = 15.50±2.07; cows = 5.80±1.29, P = 0.0129) and in structures conducted for IVC (calves = 10.38±3.06; heifers = 15.30±2.06; cows = 5.70±1.30, P = 0.0110). All the embryos produced were vitrified and only five blastocysts of the calves category were transferred to synchronized recipients at the São Paulo University (Campus Fernando Costa; Pirassununga/SP). Two pregnancies were diagnosed (conception rate = 40%) at the 30 and 60 days of age and two healthy calves were born.

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A128 OPU - IVF and ET

In vitro production of bovine embryos in the different Zebu breeds

Mariana Antonia Pedroso[1], Monica Zuchelli Jaguszeski[1], Adalgiza Pinto-Neto[1], Monica Zuchelli Jagunszeski[2], Jennifer T. Ferreira Gregianini[2], Fernando Skoniesky[3], Hugo Franciscon[1], Antônio Campanha Martinez[4], Marcelo Falci Mota[1], Luiz Sérgio Merlini[5]

[1]Universidade Federal da Fronteira Sul, Realeza, PR; [2]In Vitro Acre, Rio Branco, AC; [3]Universidade Tecnológica Federal do Paraná, Dois Vizinhos, PR; [4]Universidade Estadual de Maringá, Umuarama, PR; [5]Universidade Paranaense, Umuarama, PR.

The improvement of the systems involved in the in vitro production (IVP) of embryos in cattle has been pivotal for the study and understanding of several factors and biological mechanisms that may influence the success of the technique, from the collection and maturation of COCs, embryo cultivation, to the development of the embryo for transference. Studies with IVP in different cattle donor breeds reveal different effects in the number of COCs, in the embryo development and in the pregnancy rate. With the purpose of analyzing the effect of different cattle breeds in IVP of embryos, this study analyzed the in vitro production of embryos of Nelore, Brahman and Gir, originated from OPUs performed in two farms by a private company (In Vitro Acre) located in the city of Rio Branco in the state of Acre during 2015 and 2016. Data were submitted to ANOVA and the means were tested by Tukey's test, with a 5% significance rate, using SAS. From a total of 187 OPUs, 39.57% (74/187) were performed in Nelore, 35.29% (66/187) in Brahman and 25.13% (47/187) in Gir donors, resulting in 62.33 ± 13.87 OPUs per each breed. It could be noticed that donors from the Nelore breed presented higher mean results when compared to the Brahman and Gir donors, which presented similar results between themselves ($p>0.05$), in the amount of Grade I COCs and Grade II aspirated and cultivated COCs, cleaved embryos, blastocysts and expanded blastocysts, packaged embryos, embryos lost after packaging, embryos transfered to the recipients and pregnancy total ($p<0.05$). The means of Grade III COCs was higher in Nelore, followed by Gir, which was higher than Brahman ($p<0.05$). The means of denuded oocytes was similar between Nelore and Brahman ($p>0.05$), Nelore and Gir ($p>0.05$), and higher in Brahman donors when compared to Gir ($p<0.05$). The pregnancy rate means by aspirated COCs and by transfered embryos was similar between Nelore and Gir donors ($p>0.05$), between Brahman and Gir donors ($p>0.05$), and higher in Nelore donors when compared to Brahman ($p<0.05$). Brahman donors presented higher morula means than Nelore and Gir ($p<0.05$), which presented similar means ($p>0.05$), and lower related to initial blastocysts than the other breeds studied ($p<0.05$), that presented similar means ($p>0.05$). The means of degenerated COCs, ecloded blastocyst, cleavage rate (% - total cleaved embryos/total aspirated COCs) and the pregnancy rate by cleaved embryo (%) were similar among the breeds ($p>0.05$). In these study conditions, it can be concluded that the donor breed influences most of the parameters of IVF of embryos, without actually interfering on the means of degenerated COCs, ecloded blastocysts, cleaved rate and pregnancy rate by cleaved embryo.

A129 OPU - IVF and ET

In vitro production of Nelore embryos in different farms

Monica Zuchelli Jaguszeski[1], Mariana Antonia Pedroso[1], Adalgiza Pinto-Neto[1], Monica Zuchelli Jagunszeski[2], Jennifer T. Ferreira Gregianini[2], Fernando Skoniesky[3], Hugo Franciscon[1], Antônio Campanha Martinez[4], Marcelo Falci Mota[1], Luiz Sérgio Merlini[5]

[1]Universidade Federal da Fronteira Sul, Realeza, PR; [2]In Vitro Acre, Rio Branco, AC; [3]Universidade Tecnológica Federal do Paraná, Dois Vizinhos, PR; [4]Universidade Estadual de Maringá, Umuarama, PR; [5]Universidade Paranaense, Umuarama, PR.

The in vitro production (IVP) of embryos has been studied and used for the genetic improvement of the cattle herd. There is a growing interest for this technique, allowing the recovery of oocytes from donors through follicular aspiration guided by ultrasound (OPU) and the production of embryos with high genetic value, which can be used without major apparent changes in the reproductive tract of females, regardless of their estrous cycle phase. However, this technique is not vastly used in commercial cattle herds due to its operational cost and the variation in results, possibly due to the variation in the technique and in the handling of donors and recipients, which are characteristic of each property, as well as embryo losses and birth of large calves. The objective was to analyze PIVE in Nelore cows in different farms. Within this context, the IVF of embryos in Nelore cows originated from OPUs performed in five farms was analyzed. Three of the farms were located in the state of Acre, one in the state of Rondônia in Brazil and one in Bolivia). The IVF was performed by a private company (In Vitro Acre), which is located in the city of Rio Branco, Acre, in 2015 and 2016. Data were submitted to ANOVA and the means were tested using the Tukey's Test, considering a 5% significance rate, using the R Statistical Program. A total of 465 OPUs were performed, with means of 93 ± 31.63 OPUs per farm, using on average 65.49 ± 16.08% different donors. All variables studied were influenced by the farm ($p<0.05$), and the mean values by OPU ranged from 1.72 ± 3.62 to 0.60 ± 1.43 Grade I COCs; 4.64 ± 4.92 to 2.34 ±3.15 Grade II; 11.53 ± 10.50 to 20.11 ± 13.40 Grade III; 2.12 ± 3.70 to 0.79 ± 1.90 denuded oocytes; 6.86 ± 5.60 to 4.05 ± 3.34 degenerated COCs; 33.20 ± 20.66 to 23.80 ± 14.30 aspired COCs; 29.70 ± 19.33 to 19.82 ± 12.47 cultivated COCs; 22.84 ± 15.55 to 14.24 ± 10.28 cleaved embryos; 1.17 ± 2.45 to 0.14 ± 0.62 morulas; 2.57 ± 3.00 to 0.80 ± 1.51 initial blastocysts; 2.04 ± 3.08 to 1.29 ± 1.92 blastocysts; 7.07 ± 8.70 to 2.50 ± 3.60 expanded blastocysts; 0.15 ± 0.85 ecloded blastocysts; 11.50 ± 10.30 to 6.56 ± 5.61 packaged embryos; 3.32 ± 6.50 to 0.72 ± 2.04 embryos lost after packaging; 8.55 ± 8.75 to 3.94 ± 4.73 transfered embryos; 80.20% ± 55.09 to 61.71% ± 24.49 cleavage rate (total cleaved embryos/total aspired COCs); 3.60 ± 3.84 to 1.64 ± 2.43 pregnancies; 11.80% ± 10.64 to 8.76% ± 9.68 pregnancy rate/aspired COCs; 19.53% ± 17.51 to 9.53% ± 13.75 pregnancy/cleaved embryo rate and 39.14% ± 30.14 to 25.49% to 31.77 pregnancy/transfered embryo rate. In the study conditions, it can be concluded that typical management of each farm influences all parameters of the in vitro production of embryos in Nelore cows.

A130 OPU - IVF and ET

In vitro production of Nelore embryos in different times of the year

Adalgiza Pinto-Neto[1], Mariana Antonia Pedroso[1], Monica Zuchelli Jagunszeski[1], Helton Aparecido Garcia Gregianini[2], Jennifer T. Ferreira Gregianini[2], Fernando Skoniesky[3], Hugo Franciscon[1], Antônio Campanha Martinez[4], Marcelo Falci Mota[1], Luiz Sérgio Merlini[5]

[1]Universidade Federal da Fronteira Sul, Realeza, PR; [2]In Vitro Acre, Rio Branco, RO; [3]Universidade Tecnológica Federal do Paraná, dois Vizinhos, PR; [4]Universidade Estadual de Maringá, Umuarama, PR; [5]Universidade Paranaense, Umuarama, PR.

The IVP programs allow an accelerated genetic growth by means of multiplication of animals with differentiated genetics, thus benefiting the entire animal production system. However, it still presents a few hindrances that compromise their results due to aspects inherent to the embryo, the receiving animal and the environment. Among the factors related to the environment, weather variations affect the reproductive performance of the animals and may affect the quality of the oocyst, the fertilizing capability of the spermatozoa, the quality of the embryo and the uterine environment of the receiving animal for the implementation, thus resulting in the compromising of the pregnancy rate. Therefore, the purpose of this study was to assess the performance of Nelore donors in IVP programs in five farms assisted by a private company (In Vitro Acre) located in the city of Rio Branco, in Acre, in 2015 and 2016, in the dry (May to September) and wet (October to April) seasons. Data were submitted to ANOVA, and the means were tested by the Tukey's test, considering a 5% significance rate using the R statistic program. From the 539 OPUs performed, 38.03% (205/539) were performed during the dry season and 61.97% (334/539) during the wet season. It could be observed that, during the dry season, the means of denuded oocytes, morula, blastocysts and ecloded blastocysts were higher than the ones observed in the wet season ($p<0.05$), of 1.70 ± 3.02 and 083 ± 1.84; 0.90 ± 2.00 and 0.17 ± 0.69; 1.99 ± 2.48 and 1.20 ± 2.31; 0.04 ± 0.48 and 0.17 ± 0.82, respectively. The Grade I COCs (1.62 ± 2.98 e 1.36 ± 2.40), Grade II COCs (3.66 ± 3.92 e 3.77 ± 4.64), Grade III COCs (16.00 ± 12.00 e 16.00 ± 12.00), degenerated (5.03 ± 4.27 e 5.41 ± 5.33), aspired (29.20 ± 19.24 e 27.84 ± 18.32), cultivated (25.76 ± 17.90 e 24.64 ± 17.73), cleaved embryos (19.56 ± 14.27 e 18.81 ± 14.80), blastocysts (1.64 ± 2.02 e 1.69 ± 3.04), expanded blastocysts (4.46 ± 5.82 e 5.40 ± 7.02), packaged (9.05 ± 7.82 e 8.62 ± 8.56), lost after packaging (2.09 ± 3.79 e 2.08 ± 5.45), transfered (6.96 ± 7.45 e 6.55 ± 6.48) and pregnancy (3.04 ± 3.57 e 2.80 ± 3.05) means per OPU were similar among dry and wet seasons, respectively ($p>0.05$). In the conditions of this study, it can be concluded that the dry and wet seasons only influence the means of the denuded oocytes, morula, blastocyst and ecloded blastocysts, and does not interfere in the total pregnancy by OPU, as well as other variables, possibly since this study was performed in a location where the seasons of the year are not very evident, only characterized by the amount of rainfall.

A131 OPU - IVF and ET

Synchronization protocols of oocyte population for OPU/IVM purpose in cattle

Ana Caroline Silva Soares[1], Jhessica Naomi Sakoda[1], Bernardo Marcozzi Bayeux[2], Valentina Lodde[3], Alberto Maria Luciano[3], Jose Buratini[1]

[1]UNESP, Botucatu, SP; [2]USP, Pirassununga, SP; [3]UNIMI - University of Milan, Milão.

Oocytes isolated post mortem from bovine antral follicles are heterogeneous in chromatin configuration, gap-junction functionality between oocyte and cumulus cells, transcriptional activity and developmental competence. This heterogeneity could account for the low efficiency of current OPU/IVM strategies. We aimed to characterize the oocyte population obtained by OPU at a random day and to develop a protocol to homogenize the oocyte population destined to IVM/IVF. In experiment 1, 10 lactating Holstein cows were used in a crossover design. Treatments were: 1) OPU at a random day (Control); 2) aspiration of all visible follicles at a random day (D0), two IM injections of FSH (Folltropin; 56mg) 12h apart on D2, OPU from follicles >2mm on D4 (ASP-FSH/D4). In Experiment 2, 4 lactating Holstein/Girolanda cows were subjected to ASP-FSH with OPU on D5 (ASP-FSH/D5) and oocytes obtained on D0 were used as Control. Oocytes were fixed in 60% methanol/PBS, stained with Hoechst 33342 and examined by fluorescence microscopy to be classified according with the chromatin configuration in the germinal vesicle as GV0, GV1, GV2, GV3 or as GVBD when resuming meiosis. Data were arcsine transformed and groups compared by paired T test. Control oocytes from experiment 1 (n=90); were in GV0 (8.87%), GV1 (21.94%), GV2 (38.8%), GV3 (19.77%), GVBD (1.25%) and degenerated (9.75%). The protocol ASP-FSH/D4 (n=69 oocytes) abolished GV0, GVBD and degenerated oocytes, increased GV1 to 46.55% (P=0.03), tended to decrease GV3 (13.36%; P<0.1), but did not alter GV2 (40.09%). The number of oocytes recovered was not significantly different but recovery rate was lower in ASP-FSH/D4 (34.5% vs. 54.55%; P=0.01). The protocol ASP-FSH/D5 (n=57 oocytes) increased GV2 (80.76% vs. 47.17%) and decreased GV3 (9.72% vs. 34.5%; P<0.05) in comparison with the Control (n=83). In conclusion, oocytes recovered by OPU at a random day are heterogeneous and strategies combining follicle aspiration with FSH treatment can promote a more homogeneous population of oocytes for OPU/IVM purpose.

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A132 OPU - IVF and ET

Oocyte quality and quantity differ between primiparous and multiparous *Bos indicus* cows

Paphael Evangelista Orlandi, Miguel Pizzolante Bottino, Luiz Manoel Souza Simoes, Ana Paula Castro, Ranon Coelho de Oliveira, Thiago Takiya Pontes, Urian Ribeiro Fernandes, José Nélio de Sousa Sales

Universidade Federal de Lavras, Lavras, MG.

The objective was to evaluate quantity and quality of oocyte in primiparous and multiparous *Bos indicus* cows on early post-partum. The study enrolled 48 lactating cows (24 primiparous and 24 multiparous), with post-partum between 30 and 45 days and body condition score of 2.59±0.03 (1 to 5 scale). In a random day of the estrus cycle (D0) all animals received 2 mg of estradiol benzoate (Sincrodiol®, Ourofino, Brazil) and an intravaginal progesterone device (Sincrogest®, Ourofino, Brazil). Five days after hormonal treatments (D5), counting and puncture of all follicles larger than 2 mm were performed. The obtained follicular fluid was transferred to 100 x 200 mm petri dishes containing DMPBS plus 1% PVA for classification and evaluation of cumulus-oophorus complexes (COCs) under stereomicroscope (Nikon®, SMZ645, Japan) according to IETS manual (IETS. Manual of the International Embryo Transfer Society. 4th edition. Illinois: IETS, 2009. 175p 2009). Oocyte quality was evaluated by the Oocyte Quality Index [OQI= (grade I*1+grade II*2+grade III*3+non viables*4)/total of oocytes]. All data were analyzed by GLIMMIX procedure of SAS and continuous variables were presented by mean ± standard error. It was verified that primiparous cows presented a lower number of follicles at D5 (18.0±1.9 for primiparous cows and 20.7±1.5 for multiparous cows; P=0.05). In addition, it was verified that primiparous cows showed a greater number of degenerated oocytes (1.9±0.7 for primiparous cows and 1.2±0.3 for multiparous cows; P=0.05). There was no difference between animal categories (primiparous and multiparous cows) for the number of score 1 oocytes (4.7±0.8 and 5.0±0.8; P=0.83), score 2 oocytes (4.1±1.0 and 3.5±0.4; P=0.23), score 3 oocytes (3.5±0.8 and 3.1±0.6; P=0.51), total number of oocytes recovered (14.2±1.9 and 12.8±1.2; P=0.14), oocyte quality index (2.1±0.1 and 2.1±0.1; P=0.93) and total viable oocytes (12.3±1.9 and 11.5±1.3; P=0.38). In conclusion, primiparous cows presented a lower number of ovarian follicles and a greater number of degenerated oocytes at an early post-partum period.
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A133 OPU - IVF and ET

Oocyte quality and *in vitro* embryo production of Taurine and Zebu cattle

Anita Soares Barbosa Guimaraes[1], Marcelo Siqueira El Azzi[2], Gabriela Mansano do Nascimento[3], Ana Lucia Almeida Santana[1], Carmo Emanuel Almeida Biscarde[4], Larissa Pires Barbosa[1]

[1]Universidade Federal do Reconcavo da Bahia, Cruz das Almas, BA; [2]Universidade Federal de Lavras, Lavras, MG; [3]Universidade Estadual de Campinas, Campinas, SP; [4]Universidade Federal da Bahia, Salvador, BA.

The aim of the present study was to compare the results of Simmental (*Bos taurus taurus*) and Brahman (*Bos indicus indicus*) donors, regarding oocyte recovery and quality and in vitro embryo production. A total of 243 follicular aspirations were realized from 95 Simmental (SIM) and 150 Brahman (BRA) donors cows between November 2015 and March 2017. The ovum pick-up (OPU) aspirations were performed by the same veterinarian using ultrasound equipped with intravaginal microconvex sector transducer (7.5 MHz). Oocytes collection was performed using PBS at 36 °C added heparin. Then oocytes were classified as grade I, II, III (GI, GII, GIII) or naked (N) according to morphological quality, packed in straws with maturation medium and sent to the laboratory on a carrier (atmosphere of 5% CO2; 5% O2 at 36°C). They were cultured in a maturation medium (9.0mL of TCM 199 Earles Salt; 1.0mL of FBS; 20µL of pyruvate; 10µL of FSH; 100µL of LH; 10µL of estradiol; 50µL of amikacin) for 22-24h at 38.7 °C, with 99% humidity and 5% CO2 in air. Following maturation, oocytes were submitted to fertilization (10mL of FERT TALP; 0.06g BSA-FAF; 20µL of pyruvate; 440µL of PHE; 110µL of heparina; 50µL of amikacin) between 18 and 22h. The zygotes were transferred to a culture medium (9.3 mL of CR-2; 0.05 g of BSA-FAF; 500 µL of FBS; 100 µL of alanine; 100 µL of glycine; 40 µL of amikacin), where remained for seven days. The data were processed by Statistical Package for Social Sciences (SPSS) software version 13.0 and evaluated by the Shapiro-Wilk test, not attending normality criteria. The Mann-Whitney test was used to compare the means, considering a level of significance lower than 0.05. Comparing the two breeds, BRA presented a greater oocyte recovery (16.93±1.23) compared to SIM (12.00±0.82) ($P<0.05$). There was a difference ($P<0.05$) in the morphological quality of cumulus oocyte complexes (COCs) (3.91±0.3 and 2.85±0.25 in GI, 4.85±0.49 and 3.06±0.32 in GIII, 0.46±0.08 and 0.21±0.06 in N), with Brahman breed superiority. There was no difference between breeds only for GII oocytes ($P>0.05$). Regarding the embryo production, the total mean of embryos differed ($P<0.05$), being greater in BRA and lower in SIM (6.73±0.55 and 2.38±0.32, respectively). The rate of morulae was higher in SIM than in BRA (0.17±0.59 and 0.01±0.01), although the inverse occured in the number of blastocysts and expanded blastocysts, which is higher in BRA (0.83±0.12 and 5.67±0.49) than in SIM (0.13±0.05 and 1.66±0.26) ($P<0.05$). Brahman cows presented a higher oocyte recovery and a greater number of viable oocytes than Simmental cows, as well as higher production of viable embryos.

A134 OPU - IVF and ET

Results from a commercial *in vitro* embryo production program in Dorper and white Dorper sheep

Letícia Alecho Requena[1], Pedro Nacib Jorge Neto[1], Hernan Baldassarre[2]

[1]Novagen Genética Ltda., Itapira, SP; [2]Mcgill University, Montreal.

In vitro embryo production (IVEP) was tested in a year-round program in a commercial flock of Dorper (DP) and White Dorper (WD) sheep. Donors and recipients were synchronized with intravaginal sponges containing 60 mg medroxyprogesterone acetate for 16 days. Donors received a total of 100 mg FSH (Folltropin®) in 3 injections at 12h intervals starting ~36h before laparoscopic Ovum Pick-Up (LOPU). Recipients received 500 IU of eCG (Novormon®) and 125 mg cloprostenol (Ciosin®) at the time of sponge removal and 50 µg GnRH (Fertagyl®) 36h after that. LOPU, IVM, IVF and IVC procedures were conducted as previously described (Baldassarre et al. 2012. Anim Reprod, 9 (3), 188-194). Briefly, the females were restrained on a laparoscopy table in a 45° angle and then, using a 5 mm laparoscope and an atraumatic grasping forceps to uncover the ovaries, all follicles ≥ 2 mm diameter were aspirated using a 20G needle connected to a vacuum line. IVM were performed in maturation medium under mineral oil, at 38.5°C in humidified atmosphere with 5% CO2 in air for 24 h. Fertilization was conducted in mSOF supplemented with 10% estrus sheep serum with Percoll-enriched frozen semen from 4 males (2 of each breed) at ~50,000 motile sperm per drop. After ~15h in IVF, the presumptive zygotes were cultured in mSOF for 6 days at 38.5°C in humidified atmosphere with 5% O2, 5% CO2 and 90%N2. Blastocyst-staged embryos were transferred into the uterus of synchronized recipients with a morphologically sound corpus luteum. Results were statistically tested for significance by Oneway Anova and t test at 95% confidence level. Overall, 89 LOPU were conducted, which resulted in a total of 1003 oocytes recovered (11.3±6/donor) of which 958 entered IVM (10.7±6/donor). Between breeds, the number of oocytes collected (11.8 vs. 10.8) and % cleavage (61.9 vs. 67.3) were not statistically different, however, the number of transferable embryos/donor was significantly higher in the WD compared with the DP breed (4.9 vs. 3.3, $P<0.05$). Similarly, when comparing results from the seasonal (fall-winter) vs. the non-seasonal (spring-summer) halves of the year, no statistical differences were observed for the number of oocytes recovered/donor (10.7 vs. 11.6) and % cleavage (68.1 vs. 62.9), but the % of transferable embryos was significantly higher during the breeding season (45.8 vs. 32.9, $P<0.05$). Significant differences were found between the 4 males used at the levels of cleavage rate (31.9^{c} vs. 56.4^{b} vs. 80.8^{a} vs. $81.6\%^{a}$, $P<0.05$) and transferable embryo yields (19.8^{c} vs. 30.9^{bc} vs. 42.8^{ab} vs. $65.7\%^{a}$, $P<0.05$). Notably, the best two males were one of each breed. In total, 308 embryos were transferred into 273 recipients and the pregnancy rate was 35.5%. Pregnancy rate was not statistically influenced by breed, donor age group, male or season. These results confirm that LOPU-IVEP is commercially ready for application in the propagation of valuable sheep in a year-round scheme.

A135 OPU - IVF and ET

Improvement of bovine *in vitro* embryo production by ovarian follicular wave synchronization prior to ovum pick-up

Fabio Morotti[1], Fábio Luiz Bim Cavalieri[2], Marcelo Marcondes Seneda[1], Antonio Hugo Bezerra Colombo[2], Maria Aparecida Andreazzi[2], Isabele Picada Emanuelli[2], Luiz Paulo Rigolon[3]

UEL, Londrina, PR; 2.Unicesumar, Maringá, PR; 3.UEM, Maringá, PR.

This study evaluated the effects of the synchronization of ovarian follicular wave emergence on the efficiency of bovine *in vitro* embryo production (IVP). *Bos indicus* cows (n = 20) with 3- to 10-year-old and weighing an average of 450 kg were divided into two groups (control *vs*. synchronization) to received repeated ovum pick-up (OPU) sessions and subsequent IVP. Cows in the control group (n = 10) were submitted to OPU procedures without any previous hormonal treatment. Animals in the synchronization group (n = 10) received a protocol-based progesterone implant (Crestar®, MSD Saúde Animal, Sao Paulo, Brazil), 2 mg of estradiol benzoate (Bioestrogen®, Biogenesis-Bagó, Garín, Argentina) and 150 µg of D-cloprostenol (Croniben®, Biogenesis-Bagó, Garín, Argentina) on a random day of the estrus cycle (Day 0). In this group the OPU was performed on Day 5. A total of eight aspiration procedures were performed in each group, with an interval of 21 days, and all animals of both groups received the two treatments. After IVP, embryos in blastocyst stage were transferred to recipients synchronized previously by fixed time and the diagnosis was performed 60 days later by transrectal ultrasound (5-MHz linear transducer, Mindray 2200, Shenzhen, China). Data were analyzed by ANOVA or Chi-square Test ($P \leq 0.05$) and are presented as mean ± standard deviation or proportion. The group that received the synchronization of ovarian follicular wave emergence pre-OPU showed a greater ($P < 0,05$) mean of embryo production (5.9 ± 0.5 *vs*. 4.5 ± 0.4), a higher proportion of embryos produced [45.8% (472/1030) *vs*. 38.5% (357/927)) and tendency ($P = 0,07$) to a greater number of conceptions per OPU session (2.2 ± 0.2 *vs*. 1.6 ± 0.2) in relation to the group that did not receive hormonal treatment. The total oocyte mean (17.8 ± 1.2 *vs*. 20.5 ± 1.3), the mean of viable oocytes (11.6 ± 1.0 *vs*. 12.9 ± 1.0), the proportion of viable oocytes [62.4% (927/1424) *vs*. 60.0% (1030/1639)] and the conception rate [37.0% (132/357) *vs*. 37.5% (177/472)] were similar ($P > 0.05$) between the control and synchronization groups, respectively. It was concluded that synchronization of ovarian follicular wave emergence prior to OPU results in a greater mean of embryo production, a higher embryo conversion rate and a tendency to a greater number of conceptions per OPU session, improving the efficiency of the IVP in cattle.

A136 OPU - IVF and ET

Suplementation with forskolin and 2, 4 dinitrophenol in *in vitro* culture improve bovine embryos rates

Emivaldo de Siqueira Filho[1], Alexandre Sardinha Carvalhêdo[1], Mara Emília Abadia[1], Luiz Antonio Abadia[1], Taynan Stonoga Kawamoto[2], Andrei Antonioni Fidelis[1]

[1]Embriotec, Anapolis, GO; [2]UFU, Uberlandia, MG.

Cryopreservation of embryos highlights for making viable the storage of biological material indefinitely, thus enabling the surplus storing and commercialization of these. However, an obstacle to greater dissemination of this technique is the sensitivity, to freezing, of the in vitro produced embryos. Thus, the present study aimed the use of forskolin (lipolytic agent) and 2, 4 dinitrophenol (oxidative phosphorylation uncoupler agent) during the in vitro culture of bovine embryos, with the intent of improving the quality and, consequently, the embryonic cryotolerance. For this, ovaries from slaughterhouse were aspirated to obtain grade I and II oocytes. Subsequently, these oocytes were submitted to maturation, in vitro fertilization and in vitro culture. In the D5 embryo culture, forskolin (treatment 1), 2, 4 dinitrophenol (treatment 2), forskolin associated with dinitrophenol (treatment 3) were added and a group with no adjuvants (control group) was maintained. In D7, the expanded blastocysts were cryopreserved by the conventional freezing technique (TK 1000 BR, Uberaba - Brazil) and 2 hours later, were thawed. We evaluated the effect of these coadjuvants on the blastocysts rates (D7) and the in vitro viability after vitrification/thawing at 24, 48 and 72 h as to survival rates and to hatching rates. Data on blastocyst rates were evaluated by ANOVA and survival and hatching rates were evaluated by Kruskal-Wallis, with a significance level of 5%. In relation to the blastocysts rates in D7, there was a difference ($p < 0.05$) between the control group (40.40%) and the group in which there was addition of forskolin in association with 2, 4 dinitrophenol (50.67%). The other groups did not differ among themselves. Regarding the survival rates at 24 hours (78.24%), 48 hours (64.97%) and 72 hours (65.21%), no differences were observed ($p>0.05$) among the groups. Concerning hatching rates at 24 hours (39.32%), 48 hours (53.28%) and 72 hours (58.40%), there were also no differences ($p>0.05$) among the groups. Thus, supplementation of the in vitro culture media of bovine embryos with forskolin in association with 2, 4 dinitrophenol was efficient in improving the embryonic development, observed through the higher blastocysts rates produced in D7 in this group; however such treatments did not result in embryo benefit after cryopreservation.

A137 OPU - IVF and ET

Pregnancy rate at 60 days of Nelore cattle recipients of embryos frozen through vitrification and ethylene glycol procedures in Mato Grosso do Sul State

Carlos Alberto Zanenga[1], Natali Freire Zanenga Chacha[1], Luiz Carlos Cesar da Costa Filho[2], Eliane Vianna da Costa e Silva[2], Rodrigo Daneluzzi Fioravante Fiorani[3], Merlison Figueiredo Pedroso[1], Katarine Rezende Coelho[1]

[1]Embriza Biotecnologia, Campo Grande, MS; [2]Grupo de Estudos de Reprodução Animal do Estado de Mato Grosso do Sul (GERA-MS) - FAMEZ/UFMS, Campo Grande, MS; [3]CENATTE Embriões, Pedro Leopoldo, MG.

Cryopreservation of bovine embryos provides a series of benefits, such as formation of gene banks, planning of the best time for the transfer and subsequent birth of animals, etc. The ethylene glycol does not only provide all the advantages of cryopreservation, but also brings an even greater ease of application in the field as it allows a direct transfer of embryos without any re-evaluation, eliminating the use of a stereomicroscope, rehydration media and embryo manipulation. Oocytes from 15 Nelore cattle donors were collected in the Arizona center located in the town of Dois Irmaos do Buriti in the state of Mato Grosso do Sul, Brazil, between March 2015 and December 2016. The selected cumulus-oocyte complexes (COC) were transported in cryotubes of 2mL (1 oocyte/13.3µL medium), containing 400µL of IVM medium TVM-199 (supplemented with 0.2 mM pyruvate, 10% FCS and gonadotropins) and 300 µL of silicone oil at 38.7°C and atmosphere of 5% CO_2, 5% O_2 and 90% N_2. After the transport period, the cryotubes were transferred to incubators with 100% of humidity at 38.7°C, with an atmosphere of 5% CO_2 (≅ 20% O_2), with a total time ranging from 20-25 h of IVM (24 h on average). The fertilization period was from 8 to 10 h, under the same conditions described for IVM. Presumptive zygotes were denuded and cultured in SOFaa supplemented with 5% FCS for up to 7 days. The cleavage and blastocyst rates were evaluated at 48 and 168 hours post-insemination (hpi), respectively. The procedures were performed at Embriza Laboratory, Campo Grande, Mato Grosso do Sul, Brazil and the media were produced by the Cenatte Embrioes laboratory, in Pedro Leopoldo, Minas Gerais, Brazil. This study aimed to compare the two cryopreservation techniques (vitrification and ethylene glycol) with a fresh transfer. A total of 671 transfers were carried out at the Recipient Center Arizona, and were divided into 3 groups: G1 (fresh transfers; n=490), G2 (vitrification; n=104) and G3 (ethylene glycol; n=77). The analyses of frequency dispersion were performed by a X^2 test considering the effects of the technique compared to each other with a level of significance $P<0.05$. The detected pregnancy rates were 47.86% a (G1), 42.31% ab (G2) and 35.06% b (G3). In regard to the groups, only G1 and G3 showed a significant difference. Both freezing techniques can be used since they do not present any statistical difference, however, application of the ethylene glycol technique brings further benefits due a greater ease of the transfer of the embryo.

A138 OPU - IVF and ET

Assisted reproduction technologies result in different gestation length and birth weights in Dorper and white Dorper sheep

Pedro Nacib Jorge Neto[1], Letícia Alecho Requena[2], Hernan Baldassarre[3]

[1]VRA-FMVZ/USP, São Paulo, SP; [2]Novagen Genética Ltda., Itapira, SP; [3]Mcgill University, Montreal.

We analyzed the impact of the type of assisted reproduction technologies on the the gestation length and birth weights, in 68 sheep pregnancies established by AI vs. in vivo (MOET) and in vitro (IVF) produced embryo transfers, at Cravinhos-SP. Protocols for estrus synchronization, AI, superovulation and flushing, in vitro embryo production and embryo transfer are described elsewhere (Baldassarre and Karatzas, Ani. Reprod. Sci. 2004. 82-83: 255–266). Conception day, i.e. gestation day 0, was established as the day of AI (for AI and in vivo embryo groups) or the day of IVF for the in vitro embryo group. Results were statistically tested for significance by Oneway Anova and t test at 95% confidence level. We found that the birth weight was not different between IVF and MOET pregnancies (5.11±1.4 and 4.7±0.9 Kg., respectively) but both were significantly higher than AI pregnancies (3.5±0.5 Kg., $P<0.05$). Interestingly, the gestation length of IVF pregnancies was significantly longer than that of MOET and AI pregnancies (148±3.0 vs. 145±1.6 vs. 146±2.0 days, respectively; $P<0.01$). No statistical differences were observed between breeds when comparing the birth weight (4.83±1.6 vs. 4.58±0.9 Kg) and gestation length (147±3.3 vs. 147±2.2 days) of pregnancies for Dorper and White Dorper. We also looked at the season at the time of conception as a source of variation and found that winter conception resulted in significantly lower birth weights compared with fall, spring and summer (3.7±0.4 vs. 4.9±1.0 vs. 4.9±1.9 vs. 4.9±0.9 Kg., respectively, $P<0.05$). As per gestation length, pregnancies from conception in the spring and summer were longer (149±3.3 and 148±1.9 days) than those from conception in the fall and winter (146±2.0 and 144±1.1, $P<0.05$). In summary, pregnancies from in vitro produced embryos have shown to last for longer and result in heavier lambs at birth which has the potential for increased incidence of dystocia and need for intervention. In that sense, induction of parturition around gestation day 146 may be a recommended management tool to minimize issues at lambing. The gestation length also seemed to be influenced by the season at conception, with longer gestations occurring when conceived out of season (spring-summer), which could very well be associated with seasonal variations in food availability when animals are in grazing conditions.

A139 OPU - IVF and ET

Use of quercetin as antioxidant on *in vitro* maturation of goat oocytes

Maria Naiara Pereira da Silva, Ana Arlete de Amorim Silva, Matheus de Jusus Sá Silva, Ricássio de Sousa Barberino, Bruna Bortoloni Gouveia, Mabel Freitas Cordeiro, Maria Helena Tavares de Matos, Edilson Soares Lopes Júnior

Universidade Federal do Vale do São Francisco, Petrolina, PE.

The objective of this study was to evaluate the effect of quercetin as an alternative antioxidant to cysteamine during *in vitro* maturation (IVM). Ovary goats were transported from the local slaughterhouse to the laboratory in 0.9% saline at 30 °C until three hours after slaughter. After collection, the oocytes were evaluated and divided into three groups: CIS Group, where the oocytes were immersed in MIV medium: TCM-199, supplemented with EGF (10 µg / mL), FSH / LH (10 µL / mL), Estrus sheep serum (100 µL / mL) and cysteamine (10 µL / mL); In Groups Q4 or Q8, oocytes were immersed in cysteamine-free base medium, supplemented with 4 µM or 8 µM quercetin, respectively. The IVM of the oocytes was performed at 38.5 °C in humidified atmosphere of 5% CO_2 in air for 24 hours. After IVM, DNA fragmentation of oocytes was evaluated by TUNNEL assay (Gouveia; Theriogenology, v. 86, p. 1275-1284, 2016) and GSH, ROS and mithocondrial activity levels were quantified as reported previously (Gouveia; Theriogenology, v. 89, p. 263-270, 2016). The data of maturation rate, cumulus cell expansion rate and percentage of DNA fragmentation were expressed as percentages and compared using Chi-Square test. Data from GSH levels, ROS and mitochondrial activity were evaluated by Kruskal–Wallis and Student Newman Keuls tests. The differences were considered significant when $P < 0.05$. The CIS and Q4 groups presented the same percentage of expanded cumulus cells (67.6% and 71.8%, respectively), but the Q8 (46.5%) group was significantly lower than the other groups ($P < 0.05$). The percentage of oocytes in metaphases II was higher in the Q4 group (57.1%) than in the CIS group ($P < 0.05$), but the CIS (25.0%) and Q8 (47.0%) groups were similar. Concerning percentage of oocytes presenting DNA fragmentation, there was a higher ($P < 0.05$) number of TUNEL-positive cells at CIS group (28.2%) than Q4 (0%) or Q8 (0%) group. Oocytes from the CIS and Q4 groups showed the same levels of reactive oxygen species (ROS) and glutathione (GSH). In addition, oocytes matured with 4 µM quercetin showed higher mitochondrial activity than mature oocytes in the CIS and Q8 groups ($P < 0.05$). In conclusion, 4 µM of quercetin can be used as an alternative to cysteamine in the *in vitro* maturation of goat oocytes, as it resulted in rates of oocyte maturation of goats larger than those obtained with cysteamine, maintaining constant levels of cell expansion of the cumulus, glutathione, ROS, in addition to elevating mitochondrial activity. However, the concentration of 8 µM led to the reduction of ROS levels, GSH and oocyte mitochondrial activity, demonstrating lower cell viability.

A140 OPU - IVF and ET

Variation in the number of follicles aspirated according to the quantity of OPU (Ovum Pick-Up) in dairy buffalo, bred to pasture

Anelise Sarges Ramos[1], Vanessa Cunha Brito[2], Gustavo Alighiere Lopes Silva[2], Wilton Figueiredo Lima[1], Henry Daniel Manrique Ayala[1], Nathália Nogueira da Costa[2], Marcela da Silva Cordeiro[3], Haroldo Francisco Lobato Ribeiro[1], Sebastião Tavares Rolim Filho[1], Otávio Mitio Ohashi[2]

[1]UFRA, Belem, PA; [2]UFPA, Belem, PA; [3]IFPA, Belem, PA.

The present study aimed to analyze the repeatability of the number of follicles aspirated in OPU sessions in dairy buffalo used as donors of oocytes, as well as checking to classify donors according to their follicular population, aiming to select high-population follicular cows. There were selected 47 buffaloes (Murrah and Mediterranean), of a total of 190 females (24,7%), through the ovarian size, above 2,5cm x 1,5cm (length × width) and the number of follicles, counted by tltrasound exam (DP 4100VET). 14 follicular aspirations (OPU) were carried out using the following protocol, 21G needle; 1,7 mm internal diameter Teflon suction line and 80 cm in length; and pressure 50 mmHg. The OPU were carried out in groups with 7 to 14 animals (aspirated follicles greater than or equal to 3mm) and the suction interval of 14 days by buffalo. The cows have been classified according to the number of follicles aspirated in four groups, G1: Very good cows, with average follicles available over 20; G2: Good cows, ranging from 15 to 19; G3: Intermediate cows, with about 10 to 14 and G4: Bad cows, varying from 5 to 9. The data was submitted to ANOVA and the averages compared by Tukey test ($P < 0,05$). The G1 Group ($n = 2$) presented an average of 20.0±4.2 aspirated follicles, ranging from 38.5±7.7 in the OPU1 and 17.5±0.7 in OPU 14, not occurring statistical difference ($p > 0,05$), between OPU; in the G2 Group ($n = 11$) The average was 16±5,4 follicles, 21,2±8,7 in the OPU1 and 9.7±6.1 in the OPU14 ($p > 0,05$); in the G3 Group ($n = 19$) obtained 10.9±3.5 follicles, ranging from 11.4±5.1 in OPU1 and 8.6±2.8 in OPU14 ($p > 0,05$) and in the G4 group ($n = 15$) presented an average of 8.9±2.1 follicles available 6.0±2.5 in the OPU1 and 9.5±0.7 in the OPU14 ($p > 0,05$). There was a difference ($p < 0,05$) between the number of follicles aspirated among the groups (G1, G2, G3 and G4), demonstrating high variability among the groups and high repeatability within each group, which was probably because the protocol of aspirations did not induce ovarian lesions, maintaining the follicle population constant until the last aspiration in each group. Studies using donor selection by the size of the ovaries and follicle count, above ten per ovary, in buffaloes, has demonstrated that such a process can contribute to increase the number of viable oocytes (Ohashi et al., RevBrasReprodAnim, 41, 195-200, 2017). Although the buffaloes present a low number of follicles and large individual variation, some authors indicate that there are animals that present the population of follicles above the average, indicating that the number of antral follicles have high repeatability in bovine animals, remaining constant 8 to 10 years of age (Burns et al., BiolReprod, 73, 54-62, 2005). Therefore, the selection of donors by ovarian size and number of follicles, presents good efficiency, can contribute to increasing the number of viable oocytes and thereby improving the rates of PIVE, and consequently, decrease the cost of the pregnancy.

A141 OPU - IVF and ET

Laparoscopic ovum pick-up is a safe procedure for the collection of oocytes for preservation efforts in Pumas (*Puma concolor*)

Hernan Baldassarre[1], Letícia Alecho Requena[2], Juliane Bayão Carelli[2], Jorge Aparecido Salomão Júnior[3], Márcia Gonçalves Rodrigues[4], Sérgio Aparecido de Paula Ferreira[3], Anneliese de Souza Traldi[5], Pedro Nacib Jorge Neto[2,3,5]

[1]MCGILL University, Montreal, Canada; [2]Novagen Genética Ltda., Itapira, SP, Brasil; Instituto Corredor das Onças (ICOON), Campinas – SP – Brasil; [4]ICMBIO/MMA, Campinas, SP, Brasil; [5]VRA-FMVZ/USP, São Paulo, SP, Brasil.

Laparoscopic Ovum Pick-Up (LOPU) has been proposed as an ideal method for the collection of quality oocytes for conservation efforts based on *in vitro* embryo production (IVEP) and somatic cell nuclear transfer (SCNT). Furthermore, the procedure is compatible with collecting immature or *in vivo* mature quality oocytes by simply including (or not) hCG in the hormonal stimulation regime. We report herein the results of LOPU conducted in 3 pumas. Follicle development was stimulated by means of 750 IU eCG 4.5 days prior to LOPU. In the first stimulation, 2 females were injected with 500 IU hCG 84h after eCG and 24-30h prior to LOPU to promote in vivo maturation. All injections were conducted using blow darting technique. Animals were deprived from food (24h) and water (12h) in preparation for surgery. The LOPU procedure was conducted as previously described (Baldassarre et al.; Anim. Reprod., v.12, n.3, p717, 2015). Briefly, the females were restrained on a laparoscopy table in a 45° angle and then, using a 5 mm laparoscope and an atraumatic grasping forceps to uncover the ovaries, all follicles ≥ 2 mm diameter were aspirated using a 20G needle mounted in a plastic pipette connected to a collection tube and vacuum line. Interestingly, when hCG was included in the hormonal stimulation, 41 of 42 oocytes recovered showed signs of in vivo maturation (expanded cumulus). Two females were collected twice with a 26-month interval and yielded 55 and 22 (April 2015) vs. 32 and 25 (June 2017) usable oocytes, respectively. No sequels from prior procedure (e.g. adhesions, ovarian scars, etc.) were observed and the results suggested that previous procedure didn't affect the ability of the animals to respond to treatment since they yielded a rather large number of usable oocytes. The third animal that was subjected to LOPU in 2015 and yielded 21 oocytes was released into the environment nine months after LOPU. Prior to release she was equipped with a GPS collar that allowed satellite tracking of movements. Through this monitoring system, it was possible to detect when and where this animal was killed ~80 days after release, an quickly react and conduct a necropsy that showed that she was pregnant with 2 fetuses which reinforces the notion that LOPU is a safe procedure and doesn't impact negatively on the fertility of the animals. We believe this is the first report in which LOPU was repeatedly conducted in pumas and reporting fertility after LOPU. Our results validate LOPU as a safe reproductive procedure for the multiplication of wild felines as part of conservation strategies, specifically pumas and jaguars which are considered vulnerable in Brazil.