



A091 OPU-IVP and ET

Seasonality effect on *in vitro* production of embryos in cattle and buffaloes

E.C.M. Pereira¹, E. Oba¹, M.V.P. Silva², V.B. Andrade², Á.M. Borges², E.B. Magalhães², J.G.V. de Grázia²,
D.C. Antonino³, N.V. Sollecito²

¹UNESP, Botucatu; ²UFMG, Belo Horizonte; ³UFU, Uberlândia.

Keywords: *in vitro* production of embryos, bovine, buffalo.

We evaluated the effect of the reduction of the natural photoperiod in the state of Minas Gerais (Latitude: 19.93 S, Longitude: 43.93 O) on the rate of *in vitro* embryo production (IVP) (cleavage and blastocyst) in cattle and buffaloes. According to the length of days, the months of the year were grouped into: unfavorable period for reproduction (October to February), favorable (March to June) and transition (July to September). The oocytes were obtained from slaughterhouse ovaries. Altogether, 153 buffalo oocytes and 662 bovine oocytes were distributed similarly between periods. Oocytes were placed in wash medium (TCM 199, 10% of fetal bovine serum (FBS), 22 µg/mL of sodium pyruvate, 83 µg/mL of amikacin sulfate). They were matured in an incubator (38.5°C, 5% CO₂, 95% humidity) for 24 hours in maturation medium (TCM 199 + 10% FCS + 22 µg/mL of pyruvate + 5 IU/mL of LH + 0.05 mg/mL of FSH + 1 µg/mL of estradiol + 83.4 µg/mL of amikacin and 50 mcg/mL of cysteamine) and were then fertilized for 18 to 22 hours. The reagents used were from Sigma-Aldrich, St. Louis, USA. The zygotes were denuded and then cultured in SOF medium (synthetic oviduct fluid) + 2.5% FBS for 7 days. The cleavage rate of embryos cattle and buffaloes was assessed between 48 (D2) and 72 (D3) hours after IVF and blastocyst rates were observed 168 (D7) and 192 (D8) after IVF. Statistical analysis was performed using the ANOVA/Duncan or Kruskal-Wallis tests and the level of significance was $P < 0.05$. Regarding the different seasons (favorable, transition and unfavorable) in IVP, buffaloes noticed an improvement in cleavage (63.36 ± 7.00 , 35.92 ± 3.39 and 38.66 ± 3.5) and blastocyst (47.96 ± 5.01 , 20.18 ± 3.76 and 28.80 ± 2.99) rates during favorable breeding season ($P < 0.05$), but no difference was found in bovine (66.39 ± 2.23 , 56.18 ± 2.60 and 67.34 ± 2.14) ($P > 0.05$). Comparing the two species at different times, it was found that during favorable season for reproduction, blastocyst rate of buffaloes (47.96 ± 5.01) was higher than that found for bovine (28.91 ± 2.09) ($P < 0.05$) under the same culture conditions. However, in other periods of the year (transition and unfavorable), it was observed an increasing in cleavage in bovine (56.18 ± 2.60 and 67.34 ± 2.14) compared to buffaloes (20.18 ± 3.76 and 28.80 ± 2.99) ($P < 0.05$). The total production of blastocysts for buffaloes (25.07 ± 6.34) and bovine (27.92 ± 3.54), not considering the time of year, did not differ ($P > 0.05$). Thus, it is concluded that reducing the photoperiod positively influences IVP in buffaloes; however, at other times of the year production is similar to that of bovine.



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Swine oocytes submitted to negative pressure stress-induced improves the *in vitro* embryo production

G. Santos, C.F. Brogni, C.C. Ferreira, L.G. Silva, J.C. Mezzalira, L.U. Ohlweiler, J. Cristani, A. Mezzalira

UDESC, Lages.

Keywords: controlled stress, nitrocooler, pig IVF.

Many studies have shown that the subjection to controlled stress can increase the viability of cells and mammalian gametes. Our group has been evaluating the effect of controlled negative pressure stress-induced, obtained by a handmade equipment (Nitrocooler). This study aimed to evaluate the *in vitro* embryonic development of pig oocytes subjected to 500 mBar negative pressure during 3 minutes. Ovaries from pre pubertal swine females were obtained from a slaughterhouse, and the follicles of 3 to 6 mm diameter were aspirated. After the search, oocytes were classified and only those of excellent quality were selected for the experiment. Two experimental groups were conducted: untreated control group (n = 709) and negative pressure group (n = 696), which was subjected to 500 mBar negative pressure during 3 minutes. Maturation was performed in two steps, being the first in TCM-199 medium supplemented with 26.19 mM NaHCO₃, 25% Follicular Fluid, 0.1 mg/mL L-Cysteine, 10 ng/mL EGF, 100 IU/mL Penicillin G, 0.1 mg/mL Streptomycin, 0.5 mg/mL LH, 0.01 IU/mL FSH and 1mM dbcAMP, for 22 hours. In the second step oocytes were matured for 19 hours in medium similar to the first one, with the exclusion of LH, FSH and dbcAMP. *In vitro* fertilization was performed in medium M-TBM, supplemented with 0.4 mg/mL caffeine and 2 mg/mL BSA. It was used fresh semen obtained from one male tested for IVF. The spermatozoa were selected by mini percoll gradient (90% and 45% respectively). The selected sperm were incubated with oocytes for 3 hours at a concentration of 62,500 sperm/mL. After, presumptive zygotes were mechanically denuded and then subjected to 7 days of culture in PZM-3 medium supplemented with 3 mg/mL BSA. All steps were performed in an incubator at 38.8°C with 5% CO₂, and saturated humidity. Cleavage rate was evaluated on day 2, and blastocyst rate on day 7 of culture. The experiment was performed in 11 replications, and data was compared by Chi-squared test ($P \leq 0.05$). Cleavage rates from Control group (68.9%) and negative pressure group (70.2%) were not statistically different ($P > 0.05$). However, the blastocyst rate of Control group (18.7%) was lower ($P < 0.05$) when compared to the negative pressure group (24.1%). Data shows that the 500 mBar negative pressure induced-stress during 3 minutes improves the viability of immature pig oocytes, resulting in an increase in the blastocyst rate. Still, the nitrocooler equipment proved to be of quick and easy application.



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Adaptation of SPOM system improves bovine *in vitro* embryos production

G.R. Leal¹, M.A.G. Graciosa², R. Pasolini², C.A.S. Monteiro¹, A.J.R. Camargo², C.S. Oliveira¹, R.V. Serapião²

¹EMBRAPA Gado de Leite - CESM, Valença; ²PESAGRO-RIO, Niterói.

Keywords: Forskolin, IBMX, Cilostamide.

Since it was established, the *in vitro* maturation (IVM) system "Simulated Physiological Oocyte Maturation" (SPOM) has attracted the attention of several embryologist worldwide (Albuz, Hum Reprod, v25, p12, 2010). This system mimics the physiological events of maturation by using cAMP modulators that promote increased of oocyte competence through maintenance of meiotic arrest. It involves a pre IVM (100µM Forskolin and 500µM IBMX) lasting for 2 hours followed by an extended IVM period (20µM Cilostamide) of 28h. Recent results obtained by our group demonstrated a reduction in the embryo production rates of SPOM group compared to the CONTROL and no difference was found in nuclear maturation rate between 24 and 28h of IVM (data not published). Therefore, the aim of this study was to evaluate the effect of adapted-SPOM system (SPOMadapted) on *in vitro* production of bovine embryos. Five replicates were performed in which the oocytes were obtained from slaughterhouse ovaries, selected and randomly distributed into two groups: CONTROL (n = 243) and SPOMadapted (n = 301). In the control group (TCM 199), oocytes were matured for 24 hours at 38.5°C and 5% CO₂ in atmospheric air and high humidity. In the SPOMadapted group, oocytes remained for 2h on the pre IVM (TCM 199 with 100µM and 500µM IBMX Forskolin) followed by 24 hours of IVM (TCM 199 + 20µM of cilostamide) under the same conditions as the control. After IVM, oocytes were *in vitro* fertilized with semen from a single Holstein bull, and were subsequently transferred to culture droplets, where they remained for 7 days. The cleavage and blastocyst rates were compared between groups by Fisher exact test using GraphPad INSTAT program, at 5% significance level. Cleavage rate showed no difference (P > 0.05) between CONTROL (69.1%) and SPOMadapted (68.50%) groups. However, the blastocyst rate was higher (P < 0.005) in SPOMadapted group compared to the control group (33.9% vs 24.9%). It was concluded that the SPOMadapted system, which had a reduction of 4 hours in the IVM period, positively influenced the *in vitro* production of bovine blastocysts.

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Regulation of protein kinase B (Akt) influences the *in vitro* maturation of bovine oocytes

A.G. Curcio, T.I.S. Ribeiro, R.C. Maia, C.S. Paes de Carvalho, C.R. Quirino, B.L. Dias, A.J.B. Dias

UENF, Campos dos Goytacazes, RJ.

Keywords: Pi3K/Akt pathway, embryo in vitro production, triciribine.

The oocyte maturation involves a series of events that lead to female gamete capacitation for fertilization and embryo development. Around 80% of in vitro matured oocytes reach nuclear maturation, but only 60% complete cytoplasmic maturation. This lack of synchronization between nuclear and cytoplasmic maturation can affect early embryonic development and in vitro embryo production. Works developed by our group have shown that modulation of PI3K/Akt pathway increase bovine in vitro production. The aim of this study was to evaluate the effect of different concentrations of Triciribine® (Merck, Darmstadt, Germany), a selective Akt inhibitor, on in vitro maturation of bovine oocytes. COCs (grade I and II) were matured in vitro in TCM 199 supplemented with 10% FCS, FSH 10 g/mL, LH 5µg/mL and 1% penicillin/streptomycin. This maturation medium was supplemented with 0nM (control-C), 1 nM (Group I-GI), 5 nM (GII) or 10 nM (GIII) of the inhibitor. IVM was performed in 100 µL drop (20 COCs/drop), submerged in mineral oil and maintained in a humidified atmosphere containing 5% CO₂ in air, at 38.5°C, for 22 hours. Nuclear maturation evaluation was made after acetic orcein staining. The oocytes were considered matured when showed metaphase plate observed under optic microscope. The cytoplasmic maturation analysis was performed by active mitochondria distribution stained with MitoTracker® Red CMXRos (Molecular Probes®, Invitrogen, Eugene, OR, USA), observed by fluorescence microscopy and classified into three categories: peripheral (mitochondria located at periphery - immature oocytes), transition (incomplete mitochondrial distribution) or scattered (mitochondria homogeneously distributed in the cytoplasm - matured oocytes). Different concentrations of Akt inhibitor used in this study did not significantly alter nuclear maturation of bovine oocytes matured in vitro (SNK test, $P > 0.05$) (C: 75.72 ± 4.55 ; GI: 84.50 ± 13.43 ; GII: 75.69 ± 2.84 ; GIII: 69.92 ± 2.12). On the other hand, the use of inhibitor significantly reduced the number of oocytes with peripheral mitochondria distribution (C: 37.82 ± 12.11 ; GI: 17.34 ± 4.98 ; GII: 23.69 ± 11.59 ; GIII: 8.40 ± 6.57) and increased the number of oocytes that exhibits mitochondria transition configuration (C: 31.49 ± 17.22 ; GI: 54.82 ± 8.19 ; GII: 39.84 ± 7.47 ; GIII: 49.29 ± 6.31). The treatment did not significantly change number of oocytes that exhibits mitochondria dispersed configuration (C: 30.68 ± 6.57 ; GI: 32.0 ± 6.02 ; GII: 36.47 ± 8.34 ; GIII: 42.31 ± 3.86). The Triciribine® concentrations used in this study did not affect nuclear maturation, but the results show for the first time the participation of PI3K/Akt pathway in mitochondrial migration and cytoplasmic maturation of in vitro-matured bovine oocytes. The effect of the inhibitor should be further investigated on in vitro production of bovine embryos.



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Morphologic evaluation and differential staining of ICM and TE cells of in vitro-produced bovine embryos with the addition of different supplements in maturation and culture media

E.C.S. Recalde¹, T.L. Ikeda¹, E.C. Galhardo², M.J. Sudano³, F.C. Landim-Alvarenga¹

¹FMVZ-UNESP, Botucatu; ²IBB/FMVZ-UNESP, Botucatu; ³UNIPAMPA, Bage.

Keywords: inner cell mass, trophectoderm, propidium iodide.

This study aimed to compare the effects of the supplementation with fetal calf serum (FCS), polyvinyl alcohol (PVA) or insulin like growth factor type 1 (IGF-1) during in vitro maturation (IVM) and in vitro culture (IVC) on embryonic development to the blastocyst stage and the percentage of the inner cell mass (ICM) and trophectoderm (TE) cells. COCs (n = 20/drop) grades I and II, obtained from slaughterhouse ovaries, were selected and matured in vitro in TCM-199 (supplemented with pyruvate, hormones and antibiotics) with the addition of 10% of FCS (FCS), 3 mg/mL of PVA (PVA) or 100 ng/mL IGF-1 (IGF). IVM was performed in 90 µL droplets, covered with mineral oil, in 5% CO₂ in air and 38.5°C for 22 to 24 hours. Matured oocytes were fertilized with 2 x 10⁶ spermatozoa/mL and incubated up to 18 hours. Putative zygotes were IVC in SOFaa (2.7 mM myoinositol, 0.2 mM pyruvate, 5 mg/mL BSA, 100 µg/mL streptomycin, 100 UI/mL penicillin, and 85 µg/mL amikacin), with the respective addition of 2,5% of FCS (FCS), 3 mg/mL of PVA (PVA) and 100 ng/mL of IGF-1 (IGF), during seven days in 5% CO₂ in air and 38.5°C. Cleavage and blastocyst rates were analyzed at 48 and 168 hours post-insemination (hpi), respectively. Blastocysts, expanded blastocysts and hatched blastocysts (n = 210) were submitted to simplified technique for differential staining of ICM and TE cells (Thouas, G. Reproductive BioMedicine Online webpaper, v. 3, n. 1, p. 2529, 2001) and analyzed under inverted epifluorescence microscope. Red fluorescent nuclei were considered from TE and blue from ICM. The experimental design was a factorial 3x3 (three supplements in IVM and three in IVC), in a total of 9 groups (n = 120 oocytes/group). Three replicates were performed. Data were analyzed by analysis of variance (ANOVA), using PROC GLIMMIX model from SAS software (SAS Inst. Inc., Cary, NC, USA). Tukey's test was used to compare the means (P < 0.05). Cleavage rate was similar for all groups (P > 0.05). FCS-FCS group (46.74% ± 3.28) presented the highest blastocyst rate compared to other groups, followed by PVA-FCS (32.56% ± 3.28), IGF-FCS (25.23% ± 3.28) and FCS-IGF (20.13% ± 3.28). FCS-PVA, PVA-IGF, IGF-IGF, PVA-PVA and IGF-PVA groups presented the lowest embryo production (<12% of blastocyst). For differential staining analysis, PVA-PVA group (54.26% ± 6.19) presented the highest percentage of ICM cells compared to IGF-FCS, PVA-IGF, PVA-FCS, FCS-PVA and FCS-FCS groups (32.02% ± 4.8 to 37.17% ± 3.28 cells). IGF-PVA, IGF-IGF and FCS-IGF groups obtained intermediate values (39.55% ± 3.71 to 45.82% ± 7.58). Furthermore, PVA-PVA group (45.73% ± 6.19) obtained the lowest percentage of TE cells compared to IGF-FCS, PVA-IGF, PVA-FCS, FCS-IGF, FCS-PVA and FCS-FCS groups (60.44% ± 3.71 to 67.97% ± 4.8). IGF-IGF and IGF-PVA groups obtained 55.75% ± 5.87 e 54.14% ± 7.5, respectively. The absence of FCS, particularly during IVC, was deleterious to blastocyst production, and the supplementation with PVA or IGF-1 did not reverse this negative effect.



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Electrophoresis profile of bovine cumulus cells as related to oocyte quality and *in vitro* maturation

M.M. Ramirez Hernandez¹, I.C. Velez², P.R. Villamil¹, A.A.A. Moura¹

¹Universidade Federal do Ceará, Fortaleza, ²Universidad Nacional, Bogota.

Keywords: electrophoresis; cumulus cells; proteins.

Cumulus cells and oocyte quality are essential for *in vitro* embryo development. Hence, methods to evaluate oocyte viability are crucial to improve embryo production. However, most of these methods are invasive and unable to predict the blastocyst yield. The present study was conducted to evaluate the proteome of bovine cumulus cells from cumulus-oocytes complexes (COCs) of different qualities. Ovaries were collected at an abattoir and COCs were aspirated from 3-8 mm follicles. COCs were grouped in four categories based on morphological characteristics and time of maturation: We selected immature COCs Grade I (GI): 4 \geq layers of compact cumulus cells and homogenous ooplasm; Grade II (GII): 4 \geq layers of slightly expanded cumulus cells and less homogenous ooplasm; Grade III (GIII): 4 \geq layers of expanded cumulus cells and non-homogenous ooplasm. Also, there was a group formed by Grade I COCs that were matured *in vitro* for 24 h (GI-24h). Cumulus cells from ten oocytes were mechanically separated and stored at -20°C. For protein extraction, cumulus cells were placed in a solution containing 1% triton X-100 and sonicated at 4°C for 15 minutes. Then, proteins were subjected to 1-D SDS-PAGE (GE Healthcare, USA), using 30 μ g total protein/lane. Gel was stained with Coomassie Blue R-350 and gel image was recorded as TIFF format file and Quantity One software 4,5 (Bio Rad, USA) was used to analyze the gel. A minimum of tenfold background criteria was used to determine presence/absence of the bands and the intensities of the established bands for each group were compared. Eight bands were detected in gels made with proteins obtained from GI COCs. Ten, three and two bands were detected in gels made with proteins obtained from for GI-24 h, GII and GIII, respectively. Based on gel analysis, 43% of all bands had between 61.9 and 78.5 kDa, 39% between 81.8 and 99.5 kDa and only 17% of bands had kDa values from 118.3 to 202.3. A band of 78.6 kDa was found in all groups, with greater intensity in cumulus cells from GIII COCs. Also, there was a 99.5-kDa band present in GI and GI-24h, but with greater in the later. In conclusion, identification of proteins expressed in cumulus cells will allow the definition of molecular markers of oocyte quality. Moreover, this study contributes for understanding of how cumulus cell proteins modulate oocyte competence and relate to early embryo development.



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Intra-follicular immature oocyte transfer (IFIOT) derived embryos result in pregnancies in cattle

**J.F.W. Sprícigo¹, S.B. Sena Netto¹, C.V. Muterlle¹, F.M.C. Caixeta¹, S.A.D. Rodrigues¹,
I. Pivato¹, M.A.N. Dode²**

¹Universidade de Brasília, Brasília; ²Embrapa Recursos Genéticos e Biotecnologia, Brasília.

Keywords: oocyte injection, *in vivo* embryo, pregnancy.

The use of a technique that associates the advantages of *in vivo* and *in vitro* systems would be the best option for animal multiplication. The technique that fulfills these requirements is the immature oocytes intra-follicular transfer (IFIOT) in which immature oocytes obtained by OPU are injected into a dominant follicle from a synchronized recipient. The aim of this study was to evaluate the potential of IFIOT-derived embryos in establishing pregnancy after transfer. For that, 3 replicates were performed, and in each one 5 Gir cows were submitted to OPU and 6 Nellore heifers were synchronized to be used as oocyte recipients (ovulators). For synchronization of ovulators a conventional TAI protocol was used. After the P4 removal and the administration of 2 ml of prostaglandin at D-2 and at D-1, 1 mL EB, D0 was considered the day of injection. After OPU of Gir cows, graded I and II oocytes were loaded into 60 µL of follicular fluid in groups of 10-25 and were injected into the dominant follicle. For the selection of ovulators, besides the diameter, the follicular irrigation was taken into account, and only animals with a single follicle >10mm and with "initial" irrigation between 20-40% (as measured by ColorDoppler) were used. The oocyte injection was performed approximately 54 hours after P4 withdrawal. An OPU guide (WTA®) equipped with a 7.5 MHz probe, and a modified vacuum system. Briefly, the system was filled with PBS and by negative pressure the oocytes were loaded with the follicular fluid into the needle end (27G X 90mm). Just after IFIOT, a single dose of frozen/thawed semen from a Gir bull was used for artificial insemination (AI), immediately followed by the administration of 2ml of GnRH. Eight days after IFIOT, embryos were collected by uterine flushing and CL was evaluated. The CL was confirmed in the same ovary that the injection was performed in 6 (100%) animals. From the total of 108 injected COCs, 28 (26%) structures were recovered and 11 (39%) of those structures were viable embryos. In only one heifer, it was not recovered any structure. Whereas in each uterine flushing probably there was a structure derived from the ovulators, after deleting these 5 structures (one per cow), 23 (21%) were recovered, of which 6 (26%) were viable embryos. After transfer of 11 embryos, 4 (36%) pregnancies were confirmed, at 60 and 90 days. From one recipient, 5 embryos were recovered and after transfer, 3 pregnancies were obtained and therefore we may assume that at least two pregnancies were derived from IFIOT. This is the first report of pregnancies using embryos obtained from IFIOT. We conclude that IFIOT, although has low efficiency, is able to produce viable embryos that can result pregnancies after ET.



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Impact of injectable P4 and eCG protocol before OPU on in vitro production of bovine embryos

F. Sonnewend¹, J.P.N. Andrade¹, R.A. Rezende², A.N. Borges², A.L. Silva², M.R.B. Mello¹

¹Universidade Federal Rural do Rio de Janeiro, São José dos Campos; ²Girolando São Rafael, Andrelândia.

Keywords: ovum pick up, donor cow, progesterone.

The objective of the present study was to evaluate the impact of a synchronization protocol previously to OPU session on in vitro production of bovine embryos. Eleven donors were used, being nine Gir, one Holstein and two crossbred (Holstein x Gir), managed on pasture of Brachiaria MG5 (*Brachiaria brizantha* cv mg5 vitória) and mineral supplementation ad libitum. The study was carried out in a crossover experimental design, so that all donors were submitted to the two treatments. Females were divided into two groups: control (CON, n = 11) and experimental (EXP; n = 11) groups. The CON donor cows were aspirated at random stage of the estrous cycle. EXP females, in random day of the estrous cycle, received 150mg im injectable progesterone (SincroGest injetável®-Ouro Fino, Sao Paulo, Brazil), 2 mg of estradiol benzoate (Estrogin®, Agroline, Sao Paulo, Brazil) and 150µg cloprostenol (CIOSIN®, MSD, Sao Paulo, Brazil), which was considered day 0 (D0). At D3, 400 IU of eCG were applied (SincroeCG-Ouro Fino, Sao Paulo, Brazil) and females submitted to ovum pick up (OPU) in D6. The OPUs were performed with ultrasound assistance (Mindray DP-2200 Vet) and a vacuum pump (BV-003D-WTA Brazil) coupled to a suction guide, always by the same technician. The following variables were assessed: total number of oocytes (TO), number of viable oocytes (VO), percentage of cows with corpus luteum on the day of OPU (CL) and blastocyst rate (BR). The parameters were statistically analyzed by t test for continuous variables and by chi-squared test for binomial, both with 5% significance. The CON and EXP groups showed 249 and 310 TO; 119 and 151 VO; 46.42 and 31.57% for BR, respectively, and there were no significant differences between these evaluated parameters. However, differences were found between the percentage of donors that had CL on the day of OPU (68 and 18% for CON and EXP, respectively). It was also studied the relationship between the presence of CL and percentage of viable oocytes (VO), regardless of treatment. The cows with a CL on the day of OPU had higher proportion of viable oocytes ($P < 0.05$) than cows which had not CL in OPU (50.4 and 44% for animals with and without CL, respectively). We conclude that the hormonal protocol used prior to OPU session was able to reduce the number of CL on the day of aspiration, but did not improve the quantity and quality of oocytes produced. The presence of CL on the day of OPU session resulted in a higher percentage of viable oocytes.



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Color Doppler ultrasound as a substitute to laparoscopy for the CL count in superovulated sheep

P.H.N. Pinto¹, G.M. Bragança¹, C.R. Leite¹, G.B. Santos¹, R.M. Rosa¹, V.A.P. Alfradique¹, L.H.S. Gonçalves¹, M.F.A. Balaro¹, J.F. Fonseca², F.Z. Brandao¹

¹UFF, Niterói; ²EMBRAPA, Coronel Pacheco.

Keywords: superovulation, corpus luteum, doppler ultrasound.

This study aimed to evaluate the Color Doppler ultrasound as a substitute for laparoscopy for counting of corpora lutea (CL) in superovulated sheep. Twenty-five nulliparous Santa Ines ewes (11.9 ± 1.1 months old, BCS: 2.8 ± 0.3) were superovulated using the Day 0 protocol concept. For previous wave synchronization, intravaginal progestagen sponges (Progespon®, Zoetis, Campinas-SP, Brazil) were kept for 6 days and on Day 5, 300 IU eCG (Novormon®, Schering Plough, São Paulo, Brazil) and 0.24mg cloprostenol (Estron®, Tecnopec, Sao Paulo, Brazil) were given intramuscularly (IM). Thirty-six hours after sponge removal, 25 µg licerelin was administered IM. The superovulation started 80 hours after sponge removal by the use of 200 mg of FSH/ per ewe (Folltropin-V®, Bioniche Animal Health, Ontario, Canada) in six declining doses, every 12 hours (50/50, 30/30, 20/20 mg), IM. At the first FSH dose, a new sponge (Progespon®, Zoetis, Campinas-SP, Brazil) was inserted and removed at the time of the fifth dose. At the last FSH dose, 0.24 mg of cloprostenol (Estron®, Agener Union, Sao Paulo, Brazil) and, 24 hours later, 25 µg of leirelin (Gestran Plus®, Tecnopec, Sao Paulo-SP, Brazil) were administered IM. Ewes were mated every twelve hours from the last FSH dose to the end of estrus. Twelve hours before embryo collection, Color Doppler ultrasound exams were performed using Sonoscape S6® equipment (Sonoscape, Yizhe Building, Yuquan Road, Shenzhen, China), coupled to a 7.5 MHz linear transducer (transrectal) to predict the number of corpora lutea (CL-DOPPLER). Previous to embryos collection, the number of CLs was determined again by laparoscopy (CL-LAPARO). The CL-DOPPLER and CL-LAPARO were compared through the Pearson's correlation coefficient, Simple Linear Regression Analysis and Intraclass Correlation Coefficient (ICC). For all tests, $P < 0.01$ was considered as statistically significant. A high correlation between the number of CL-DOPPLER and CL-LAPARO ($r = 0.92$; $r^2 = 0.85$; $P < 0.01$) and an excellent ICC (0.93; $P < 0.01$) were obtained. In conclusion, the Color Doppler ultrasonography is highly efficient to estimate the number of CLs in superovulated ewes. This represents an important advance because it replaces invasive laparoscopic procedure, avoids fasting, drugs use and unnecessary handling in animals that did not respond to the treatment. Therefore, the Color Doppler ultrasound can replace the laparoscopy for the assessment of superovulated sheep.



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Follicular wave synchronization on *in vivo* embryo production in Santa Ines sheep

**R.M. Rosa¹, M.F.A. Balaro¹, P.H.N. Pinto¹, H.F.R.A. Saraiva¹, R.I.T.P. Batista¹, V.A.P. Alfradique¹,
L.S. Ribeiro¹, J.F. Fonseca², R. Ungerfeld³, F.Z. Brandao¹**

¹Faculdade de Veterinária - Universidade Federal Fluminense, Niteroi; ²EMBRAPA Caprinos e Ovinos, Coronel Pacheco; ³Universidad de La República Uruguaniteroiy, Montevideu.

Keywords: embryo, superovulation, ovine.

This study aimed to evaluate two hormonal protocols for synchronization of follicular wave emergence on *in vivo* embryo production in Santa Ines sheep under tropical conditions. Twenty two nulliparous Santa Ines sheep, kept on intensive system, were used. Group one (GT, n = 10) received an intravaginal implant containing 0.33 g of progesterone (Eazi-Breed CIDR® Sheep and Goats, Zoetis Ltda, São Paulo, Brazil) for all period of hormonal protocol and 0.24 mg of cloprostenol (Estron®, Agener Union, São Paulo, Brazil) i.m.. Group two (GEm; n = 12) received the synchronization of the follicular wave emergence as proposed by Balaro et al. (2016). The superovulation started after 56 and 80 hours, from GT and GEm, respectively. For both groups, 200 mg of FSHp/per animal were administered (Folltropin®, National Pharmaceutical Chemistry Union S/A, São Paulo, Brazil) in six decreasing doses (25%/25%, 15%/15% and 10%/10%) every 12 hours. In GEm, at the first FSHp dose, an intravaginal sponge impregnated with 60 mg of medroxyprogesterone acetate (Progespon®, Schering Plough, São Paulo, Brazil) was inserted and at the fifth dose, ewes also received 0.24 mg of cloprostenol (Estron®, Agener Union, São Paulo, Brazil). The intravaginal implant (GT) or sponge (GEm) was taken at the last FSHp dose. Subsequently, estrus detection and mating occurred every 12 hours. Seven days after the last FSH dose, embryos were collected by surgical uterine flushing. An ultrasound (Sonoscape S6®, Sonoscape, Yizhe Building, Yuquan Road Shenzhen, China) equipment coupled to a 7.5 MHz linear transducer (transrectal) was used to assess the follicular population on the progestagen insertion (D0), in the first superovulatory dose and for CL count immediately before embryo collection. Furthermore, regardless of the occurrence of premature regression of CL (PRCL), all ewes were collected. Normal and non-normal parametric data were evaluated by t-test and Mann-Whitney, respectively. Frequency data were evaluated by Fisher's exact test. It was considered as significant when $P < 0.05$. No differences ($P > 0.05$) were detected, between GT and GEm, in the follicular population at the day of the progestagen insertion (7.8 ± 2.6 vs. 6.0 ± 1.6) and the first FSHp dose (8.1 ± 2.6 vs. 8.9 ± 2.3). The number of CL were also similar ($P > 0.05$) between G1 (6.9 ± 5.1) and G2 (7.1 ± 3.1). The number of animals with PRCL in GT was 60% (6/10), greater than the 8.3% (1/12) found in GEm ($P < 0.05$). The number of collected structures (6.6 ± 4.3 vs. 0.6 ± 0.7 ; $P < 0.01$) and viable embryos (4.6 ± 3.9 vs. 0.3 ± 0.5 ; $P < 0.01$) were greater in GEm compared to GT. The recovery rate also differed between groups, being greater in GEm than GT (75.6% vs. 8.1%; $P < 0.01$). The greater PRCL rate in GT probably contributed to the smaller number of viable embryos. Thus, it is suggested the appliance indicated the GEm protocol for *in vivo* embryo production in Santa Ines sheep under tropical conditions.

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A101 OPU-IVP and ET

***In vitro* maturation of bovine oocytes alters expression of miRNAs**

M. Del Collado¹, J.C. Silveira¹, T.H.C. de Bem², M.L.F. Oliveira¹, B.M.S.M. Alves¹, A. Castro Netto³, L.A. Silva¹, F.V. Meirelles¹, F. Perecin¹

¹FZEA-USP, Pirassununga; ²FMRP-USP, Ribeirão Preto; ³Fininvitro Reprodução Animal, Bauru.

Keywords: *in vitro* maturation, *in vivo* maturation, miRNAs.

Oocyte maturation occurs concomitantly with oocyte chromatin condensation and transcriptional decrease. However, gene expression control can occur in these cells post-transcriptionally, by miRNA-mediated mechanisms. The miRNA expression profiles of bovine oocytes during early and late steps of maturation are poorly known. It is also unknown the repercussion of the exposure of bovine oocytes to *in vitro* culture conditions during maturation on miRNAs expression. This study aimed to determine miRNA expression in immature bovine oocytes and in mature bovine oocytes undergoing *in vivo* and *in vitro* maturation, to identify miRNAs differentially expressed among these groups and the pathways regulated by them. For that, pools (3 per group) of 20 denuded-oocytes obtained from immature and *in vitro*- and *in vivo*-matured COCs were used for miRNA extraction using a combination of TRizol reagent (Invitrogen) and the miRNeasy kit (Qiagen). Reverse transcription was carried out using the miScript PCR System (Qiagen). For RT-qPCR analysis of the 348 bovine miRNAs, a total of 100 ng of cDNA was used per sample. Data were normalized with the geometric mean of 3 endogenous control genes (RNT43 snoRNA, Hm/Ms/Rt T1 snRNA and bta-miR-99b). Expression levels were calculated using the 2- Δ Ct method and data were tested by ANOVA and compared by Tukey's test at 5%. A total of 299 miRNAs were found expressed in the oocytes, with 23 exclusively detected in the immature group, 8 in the *in vitro*-matured oocytes and 7 in *in vivo*-matured. Of the miRNAs found in all three groups, 18 were differentially expressed, with 4 of them higher (bta-miR-106b, bta-miR-17-5p, bta-miR-155 and bta-miR-21-5p) and 2 of them lower (bta-miR-451 and bta-miR-764) in the *in vitro* group when compared to *in vitro* counterparts. We used DIANA to determine enriched pathways regulated by these miRNAs. The elevated miRNAs in the *in vitro* group regulated genes from the TGF-beta (12 genes), p53 (17 genes), focal adhesion (17 genes), PI3K-Akt (47 genes), RNA transportation (26 genes) and cell cycle (24 genes) pathways. Furthermore, bta-miR-451 and bta-miR-764, that were lower in the *in vitro* group, regulate genes from mTOR (1 gene), ubiquitin mediated proteolysis (1 gene), glycosphingolipids biosynthesis (2 genes), TGF-beta (3 genes) and glycine, serine and threonine metabolism (2 genes) pathways. Some Individual miRNAs or miRNA-regulated pathways found in this study were previously associated with oocyte competence (Sohel et al., PLoS One, e78505, 2013). In conclusion, IVM induced modifications in the expression of miRNAs associated with oocyte maturation, with potential repercussions on transcriptional control and quality of the oocytes and embryos.

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A102 OPU-IVP and ET

Utilization of MAP-5 in P-36 superstimulatory protocol for Angus cows (*Bos taurus*)

R.M. Bonotto¹, F.S. Rosa¹, A.L.M. Bonotto², B. Mion³, F.D. Mozzaquatro⁴

¹FAI-Faculdade de Itapiranga, Itapiranga; ²Central ABN Agropecuária, Santiago; ³Universidade Federal de Pelotas, Pelotas; ⁴UNIPAMPA Campus Uruguiana, Uruguiana.

Keywords: embryo transfer, hialuronic acid (MAP-5), pFSH (folltropin).

Embryo transfer is a biotechnology widely used in *Bos taurus* cows. This principle involves a superovulation protocol for inducing multiple ovulations. The P-36 protocol is one of the most used for this purpose as it allows artificial insemination at fixed time (TAI) facilitating the management of donor and recipient cows. The superovulation is made with pFSH (eight decreasing doses every 12 hours), but studies show that the administration pFSH with a slow absorption decreases the number of applications, maintaining similar embryos production. Therefore, the aim of this study was to compare superovulation protocol for Angus cows, using the traditional P-36 protocol or with reduction of FSH injections. It was used 24 Angus cows as donors, randomly distributed into 2 groups: control and MAP-5 (hyaluronic acid), in a crossover design. At random stage of the estrous cycle (D0), donors received an intravaginal device containing 1.0 g progesterone (DIV) and estradiol benzoate (3 mg, IM). In the control group, the animals were superovulated with pFSH (via IM, total dose = 200 mg) twice daily in decreasing doses from D4 to D7, while the MAP-5 group were performed only two administrations of pFSH diluted in MAP 5 (5 mg/ml hyaluronic acid) on D4 (75% of dose) and D6 (25% of dose). The embryo collections were performed at D15. Data were analyzed in Proc Mixed of SAS with a significance level 5%, considering the variables: number of follicles on the day of ovulation induction, number of corpus luteum at the time of embryo collection, number of total structures collected, fertilized number of structures and the number of viable embryos. The control group showed higher the number of follicles (10.08 ± 0.61 vs. 8.25 ± 0.61 ; $P = 0.0275$) and corpus luteum (7.25 ± 0.59 vs. 3.25 ± 0.59 ; $P < 0.0001$) than MAP-5 group, which resulted in a 71.90% ovulation rate for the control group and 39.39% for the MAP-5 group. The total number of structures (8.29 ± 0.98 vs. 3.08 ± 0.98 ; $P = 0.0002$), fertilized structures (6.50 ± 0.90 vs. 2.50 ± 0.90 ; $P = 0.0015$) and viable embryos (4.21 ± 0.72 vs. 2.00 ± 0.72 ; $P = 0.0164$) was also higher in control group when compared to MAP-5 group. Thus, it is concluded that the superovulated protocol using pFSH diluted in MAP 5 was not effective for the Angus breed donors.



A103 OPU-IVP and ET

Effect of maturation period with different oxygen tension and type of semen in cleavage rates, blastocyst yield and pregnancy outcome in Nelore (*Bos indicus*) cattle

**N.Z. Chacha¹, R.Z. Puelker², K.R. Coelho¹, C.A. Zanenga¹, M.C. Caldas-Bussiere³,
V.L. Maciel³, C.R. Quirino³**

¹Embriza, Campo Grande; ²PROGEST Biotecnologia Em Reprodução e Saúde Animal, Botucatu; ³UENF, Campos dos Goytacazes.

Keywords: IVP, effect, Nelore.

This study aimed to evaluate the incubation conditions established during in vitro maturation (IVM) of oocytes (duration and oxygen tension), the type of semen (conventional (C), n = 12.177; and sexed (S), n = 3.630) and the possible correlations with cleavage, blastocyst and pregnancy rates. Selected COCs were transported in 2 mL cryotubes (1 oocyte /13.3 µL medium) containing 400µL of IVM medium TCM-199 (supplemented with 0.2 mM pyruvate, 10% FCS and gonadotropins) and 300 µL of oil silicone at 38.5°C and atmosphere of 5% CO₂, 5% O₂ and 90% N₂. After the transport period, the cryotubes were transferred to incubator with 100% of humidity at 38.5°C with an atmosphere of 5% CO₂ (\cong 20% O₂), with a total time ranging from 20-29 h of IVM (24 h in average). The timing of fertilization was set according to the type of semen, i.e., 18 to 20 h for the conventional semen and 8 to 10 h for the sexed-semen. Presumptive zygotes were denuded and cultured in SOFaa supplemented with 2.5% FCS up to 7 days. The cleavage and blastocyst rates were evaluated at 72 and 168 hours post-insemination (hpi), respectively, and the procedures were performed at Embriza Laboratory, Campo Grande, Mato Grosso do Sul, Brazil. Rates of cleavage, blastocyst and pregnancy were analyzed by ANOVA, means were compared by SNK test ($P < 0.05$) and the variables were analyzed by Pearson's correlation. Higher cleavage and blastocyst rates were observed when using conventional semen (78.1% \pm 22.79 and 36.0% \pm 21.28) compared to sexed semen (58.3% \pm 27.80 and 29.3% \pm 20.39), respectively ($P < 0.05$). The pregnancy rates was higher ($P < 0.05$) when using sexed semen (65.5% \pm 25.99) compared to conventional semen (56.7% \pm 24.94). When evaluating the correlation between the variables, it was observed that an increased IVM period during transport (atmosphere 5% O₂) was correlated with a decrease in cleavage rates ($r = -0.083$, $P = 0.0183$). However, increasing the duration of IVM in the laboratory (atmosphere 20% O₂) rised the cleavage rates ($r = 0.094$, $P = 0.0074$), but such increase was not correlated with blastocyst and pregnancy rates. The total IVM duration had no correlation ($P > 0.05$) with the variables studied. Therefore, it can be concluded that: 1) changing the period of IVM and the gaseous atmosphere in the transport of oocytes had no influence in the blastocyst and pregnancy rates, and this use can be indicated for transportation period and 2) the pregnancy rate was higher when using sexed-semen with a shorter fertilization period.



A104 OPU-IVP and ET

Post-implantation development of Holstein-Gyr biopsied embryos

**B.A.F. Barros¹, C. Freitas¹, C.C.R. Quintão², A.J.R. Camargo³, P.M.S. Rosa¹, R.V. Serapião³,
C.A.S. Monteiro¹, L.S.A. Camargo², C.S. Oliveira¹**

¹EMBRAPA Gado de Leite LRA-CESM, Valença; ²EMBRAPA Gado de Leite, Juiz de Fora; ³PESAGRO Rio, Niterói.

Keywords: embryo biopsy, IVPE, biotechnology.

Embryo biopsy associated with genotyping allows to obtain genomic informations even before the birth of the animals. The aim of this study was to evaluate the post-implantation development of Holstein-Gyr crossbred biopsied embryos to prove the safety of this biotechnology. Therefore, viable oocytes were obtained by Ovum Pick Up (OPU) of Gyr and crossbred $\frac{1}{4}$ Holstein – $\frac{3}{4}$ Gyr, matured and fertilized with sexed semen (female) of Holstein bull. It was used incubation system of 38.5° C, 5% CO₂ in atmospheric and high humidity air, and IVM, IVF and IVC culture media were purchase from Bioklone® (Jaboticabal, Brazil). Approximately 155h after IVF, blastocysts were assessed and placed into individual droplets, and, with the embryo splitting blade (Micromanipulator The Microscope Company®, USA) approximately 20% of the embryo was sectioned, opposite the internal cell mass. After 3-5 h, in medium HSOE (Bioklone®, Jaboticabal, Brazil) at 37°C (hotplate), re-expanded embryos were transferred to recipients with synchronized estrus (n = 247 embryos; Control: 118, Biopsy: 129), of which 64 pregnancies (C: 29, B: 35) have been completed and are described in this study. The pregnancy percentage (30 days), pregnancy loss (30 to 90 days, after 90 days and stillbirths) and males were compared between groups using Fisher's exact test (P = 0.05). The average period of gestation and birth weight were compared by t Test (P = 0.05) in GraphPad INSTAT program. Pregnancy rate was reduced (P = 0.03) in the biopsied embryos (C: 55.1% vs B: 41.1%) at 30 days after transfer. However, higher pregnancy loss between 30-90 days was observed in the control group (B: 0% vs C: 13.8%; P = 0.004). Thus, the percentage of pregnancies at 90 days did not differ (P = 0.37) between groups (C: 47.5%; B: 41.1%). Therefore, it is suggested that the biopsy promotes a selection of embryos with the greatest potential for development, which does not occur in the control group, in which the embryos with low potential implant but have higher death rates up to 90 days. Pregnancy loss in middle and final gestation (C: 3.4%; B: 8.6%, P = 0.62) and at birth and losses at birth (C: 6.9; B: 5.7, P = 1.00) did not differ between groups. The gestation period (C: 280.6 ± 3.83; B: 280.7 ± 25.4 - P = 0.92; Mean days ± SD), birth weight (C: 33.0 ± 4.49 kg; B: 34.46 ± 4.95 kg - P = 0.25), and the percentage of males (C: 8.3%; B: 20% - P = 12.41) did not differ between groups. We conclude that the embryo biopsy technique is safe and does not affect the production rates of the Holstein-Gyr crossbred calves.

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A105 OPU-IVP and ET

Bovine breeds performance submitted to OPU and IVP in the central Chaco region in Paraguay

G.F. Soria¹, R.F. Soria¹, M.F. Soria¹, F.P. Lopes¹, D.S. Romero², E. Friesen³

¹Fecunda Biotecnologia, Pedro Juan Caballero; ²Reprogen, Asuncion; ³Cooperativa Multiactiva Neuland Ltda., Colonia Neuland.

Keywords: *in vitro* fertilization, donor, biotechnology.

The female performance of the hybrid breeds Brahman and Santa Gertrudis and of Gelbvieh breed submitted to OPU and IVP was evaluated, and the pregnancy rate of their embryos in semi-arid environment of Central Chaco, Paraguay. The donors and recipients are raised on a property located at coordinates S 22 ° 46'38 "W 60 ° 26'28 ", 50 Km to the west of Cologne Neuland in Boqueron department, in Gatton Panic (*Panicum maximum*) a mega-thermal pasture adapted to the region with annual rainfall records between 800 to 1,000 mm, and has its climate characterized as Mega-thermal Semi-Arid according to Thornthwaite. The animals are part of a herd selected for dams and sires production for use primarily in that Paraguayan Chaco region. Donor Brahman, Santa Gertrudis and Gelbvieh underwent OPU sessions and IVP in the years 2014-2015, with repetitions of sessions ranging from one to seven per individual; the work was performed each year from March to November. Total average of retrieved oocytes from each donor, total average of transferred IVP embryos from each donor and the pregnancy rate for each breed, in 2014 was: Brahman breed (n = 16) 15.04 (EP ± 3.76), 3.82 (EP ± 0.96) and 35.48% (77 pregnancies / 217 ET); Santa Gertrudis (n = 15): 14.15 (EP ± 3.65), 3.57 (EP ± 0.92) and 46.53% (67 pregnancies / 144 ET) and Gelbvieh (n = 2): 61.5 (EP ± 43.49), 32 (EP ± 22.63) and 56.25% (36 pregnancies / 64 ET). In 2015, the total average for retrieved oocytes, transferred IVP embryos and the pregnancy rate for the Brahman breed (n = 11) was: 22.9 (EP ± 6.90), 5.87 (EP ± 1.77) and 52.23% (105 pregnancies / 201 ET); and for the Santa Gertrudis breed (n = 12): 22.76 (EP ± 6.57), 4.8 (EP ± 1.38) and 55.26% (105 pregnancies / 190 TE). It is noteworthy that only in 2014, in a single session, two females of Gelbvieh breed underwent OPU and had difference in total oocytes retrieved: eight oocytes vs. 115 oocytes; and ET, one transferable embryo vs. 63 transferable embryos with pregnancies registered for both donors. Regarding performance on OPU and IVP there was no statistical difference when comparing Brahman and Santa Gertrudis in the same year by the Student t test (P < 0.1); however, there was a higher oocyte retrieval (P < 0.1) in 2015 compared to 2014 for the Brahman and Santa Gertrudis breeds. No statistical difference was registered for transferred IVP embryos; so this parameter, like the pregnancy rate in 2015, was numerically higher compared to 2014, demonstrating the expectation of economic gain for producers of the Paraguayan Chaco region by the use of reproductive biotechnologies. The pregnancy rate (%) was higher among the embryos of Santa Gertrudis compared to the Brahman breed in both periods. The use of assisted reproductive biotechnologies (OPU, IVP and ET) showed satisfactory results between breeds selected in this region and can be used successfully in animal breeding programs.



A106 OPU-IVP and ET

***In vitro* production of bovine embryos: partial replacement of fetal calf serum by bovine serum albumin on *in vitro* culture and addition of folic acid on oocytes maturation**

C. Gennari Verruma¹, A. Mioranza², C.L. Miranda Furtado³, R.A. Vila³, E.S. Ramos³, R.B. Lôbo³

¹Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto; ²Biovita, Cascavel;

³Faculdade de Medicina de Ribeirão Preto, Ribeirão Preto.

Keywords: bovine embryo, phospholipid, folic acid.

The bovine IVP faces many obstacles that interfere negatively in the production and survival of embryo, like oocytes with morphological differences (grade I, II and III) and composition of IVM, IVF and IVC medium. The components present in the culture medium, as well as those stored by the oocyte during maturation process, are associated to genetics and epigenetics alterations, influencing the embryo quality and the success of bovine embryo IVP. The FCS seems to influence the embryo production and has been related with the abnormal accumulation of lipids that affect the process of cryopreservation. (Rumpf. Rev. Bra. de Zoo. 36, 229-233. 2007). The folic acid (FA), in turn, seems to help the IVP of embryos, correcting possible epigenetics alterations related to assisted reproduction biotechnologies (Kim et al. Journal of Nutritional Biochemistry. 20, 917-926. 2009). The aim of this work was to evaluate the effect of partial replacement of FCS for BSA in the embryonic lipid composition and, posteriorly, evaluate the influence of FA in the IVM of grades I and III oocytes, aiming to increase the production of embryos derived from grade III oocytes. The study was performed in two experiments, both in triplicates. In the first experiment COCs (n = 297) were matured in IVM medium (TCM199 with Earles' salt, glutamin, FCS, FSH, LH, amikacin and estradiol). Embryos were cultured in two different mediums: CR2 control (10% FCS, 0.03 g BSA V, alanine, glycine and amikacin in 10 mL of medium) and CR2 modified (5% of FCS, 0.03 g of BSA V FAF, 0.03 g BSA V, alanine, glycine and amikacin in 10mL of medium). Embryo production was evaluated using Student's t test ($P < 0.05$) and the lipid composition of embryos, classified as expanded blastocysts on day 7 (D7), was determined by Ultra High Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS). The reduction of FCS did not influence ($P = 0.35$) embryonic production on D7 on control group ($51.5\% \pm 6.1$) and modified ($44.9\% \pm 6.3$). The lipid analysis revealed that the embryonic culture in modified CR2 medium reduced the concentration of three phospholipids classes, the dioleoil phosphatidylcholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3- [phospho-rac-(1-glycerol)] (POPG) and sphingomyelin (SM2) ($P = 0.04$, $P = 0.05$ and $P = 0.01$, respectively). This result corroborates with the hypothesis that the embryo can absorb lipids from FCS. On the second experiment, 360 oocytes (180 grade I and 180 grade III) were matured on IVM medium and 350 oocytes (180 grade I and 170 grade III) were matured on IVM medium (supplemented with 10 mM FA in final volume of 10 mL). The embryonic culture was performed in modified CR2 medium. The embryonic production was evaluated using Student's t test ($P < 0.05$). The addition of FA on IVM medium increased ($P = 0.02$) the embryo production derived from grade III oocytes, with a total of 56 embryos ($32.6\% \pm 1.6$) without FA and 64 embryos ($42.3\% \pm 3.0$) with FA. There was no influence ($P = 0.29$) of FA supplementation for grade I oocytes, presenting, on D7, 88 embryos ($52.7\% \pm 6.9$) without FA and 84 embryos ($45.4\% \pm 4.6$) with FA. However, more studies should be performed to determine the effects of FA supplementation during IVM on gene expression in oocytes with different morphological qualities.



A107 OPU-IVP and ET

Improvement on development of equine preantral follicles after six days of *in vitro* culture with FSH

N.C. da Silva, M.C. Max, C.B. da Silva, S.M. Gonzalez, A.G. Lindquist, I. Búfalo, M.M. Seneda

Universidade Estadual de Londrina, Londrina.

Keywords: equine, FSH, preantral follicles.

The aim of this study was to evaluate the effects of different concentrations of follicle stimulating hormone (FSH) in minimal essential medium supplemented (MEM+) on developing equine pre-antral follicles cultured *in vitro*. Ovaries were collected at a local slaughterhouse, from 5 mares in seasonal anestrus. Eleven ovarian tissue fragments of approximately 3x3x1 were obtained from one ovary of each animal. One fragment was selected randomly and immediately fixed and processed for histological analysis (Control group; Day 0). Remaining fragments were placed in PBS supplemented with penicillin (200 IU/mL) and streptomycin (200 mg/mL) at 4° C for 1 hour (transport time to the laboratory). The fragments were cultured *in situ* for 2 days (D2) or 6 days (D6) in MEM+ (ITS (6.25 mg/ml insulin, 6.25 mg/ml transferrin, and 6.25 ng/ml selenium), pyruvate (0.23 mM), glutamine (2 mM), hypoxantina (2 mM), bovine serum albumin (BSA; 1.25 mg/ml), 20 IU/ml penicillin and 200 mg/ml streptomycin), supplemented with crescent concentrations (10, 50, 100 e 200 mg/mL) of FSH (Folltropin®, Bioniche Canada Inc, Ontario, Canada), according with the experimental groups: Control (D0), MEM+ (D2), MEM+ (D6), MEM+ 10 ng/mL FSH (D2), MEM+ 10 ng/mL FSH (D6), MEM+ 50 ng/mL FSH (D2), MEM+ 50 ng/mL FSH (D6), MEM+ 100 ng/mL FSH (D2), MEM+ 100 ng/mL FSH (D6), MEM+ 200 ng/mL FSH (D2) and MEM+ 200 ng/mL FSH (D6). After culture, the fragments were fixed in Bouin and processed for classic histology. The preantral follicles were classified according with the developmental stage (primordial or in development: primary + secondary) and on the morphology (intact or degenerated). The statistical test used was Fisher's Exact Test ($P < 0.05$). The slides ($n = 1,187$) with 4,018 histologic sections were evaluated with optic microscopy. The follicles were observed in only 9.7% (388 of 4,018) of the histological sections. From 861 evaluated follicles, 488 were primordial and 373 were under development at different stages (primary and secondary). Moreover, 59.7% were morphologically intact. After 2 days of culture, cultured groups with 200 ng/mL FSH (61/94, 64.9%), as well as the concentration of 10 ng/mL FSH cultured for 6 days (21/29, 72.4%) showed the best development rates, compared to the other treatments. Regarding the integrity of equine preantral follicles, the concentration of 100 ng/mL (75.6%) on culture for 2 days and the concentrations of 50, 100 and 200 ng/mL (75.0%, 92.9% and 95.4%, respectively) on culture for 6 days showed best results compared to the rest of the groups. Based on follicular development and the presence of morphologically intact follicles, we can conclude that there was a positive effect of the addition of FSH in the *in vitro* culture. However, the positive effects varied according to the concentration at 2 and 6 days of culture.



A108 OPU-IVP and ET

Validation of an experimental model for evaluation of the final growth of the dominant follicle from prepubertal heifers treated with equine Chorionic Gonadotropin (eCG)

M.M. Soares¹, G.A.M. do Nascimento², G.B. Magenis², V.G. Rizzi², L.M.S. Lima², C.C. Barbosa², L.H.S. Doi², P.S. Baruselli³, G.G. Macedo¹

¹UFU, Uberlândia; ²Ourofino, Cravinhos; ³Universidade de São Paulo, São Paulo.

Keywords: follicular wave, OPU, bovine.

The aim of this study was to validate a model for studying the follicular wave of pre-pubertal zebu heifers, as well as the effects of eCG administration on the final development of the dominant follicle (DF). For this study 20 Nellore heifers was used, aging from 12 to 16 months, weighting 237 ± 11 kg and with a body condition score of 3.04 ± 0.17 . At the beginning of experiment (D0) the animals were subjected to two ultrasonography evaluations with a 10 days interval to select the heifers in anestrous, followed by OPU process witch eliminate all visible follicles of the ovaries, thus stimulating a new follicular wave onset. In D5, the animals were scanned by US to map the ovary and measure the follicles diameter. In D6, the animals were divided according to the size of their dominant follicles and weights, into two treatments: IM injection of 1.5 ml of 0.5% NaCl solution (control, n = 10) or 300 IU eCG (Folligon®, MSD, São Paulo, Brazil; n = 10). The ovaries were evaluated daily for 12 days. Analyses were performed by proc GLIMMIX SAS. Data were presented as mean \pm standard error and a significance was considered when $P < 0.05$. All heifers showed the onset of a new follicular wave after OPU, proving that the model was efficient. At the moment of treatment, the animals presented the same DF diameter (Control = 8.1 ± 0.4 mm; eCG = 8.4 ± 0.3 mm; $P > 0.05$). There was no effect of eCG in the DF diameter (control = 9.8 ± 0.5 mm; eCG = 10.3 ± 0.7 mm; $P > 0.05$) and follicular growth rate until the follicle regression (control = 1.6 ± 0.1 mm / day; eCG = 1.9 ± 0.2 mm / day; $P > 0.05$). The emergence of a second wave occurs faster in the eCG group (2.6 ± 0.2 days) than in the control group (3.9 ± 0.3 days; $P < 0.05$). The follicular wave was longer in the eCG group (5.1 ± 0.5 days) than in the control (4.5 ± 0.2 days; $P < 0.05$). Even though in this study eCG did not act as an inducer of ovulation, three cows from eCG group ovulated indicating its role in the DF growth and in increasing E2 production, thus, stimulating pre-ovulatory surge of LH. The study describes the follicular behavior of prepubertal zebu heifers and demonstrates a model for studying the follicular dynamics in anestrous cows, since its proved efficiency in prepubertal heifers that also have low LH pulsatility.



A109 OPU-IVP and ET

Characterization of prepubertal Gyr oocytes: oocyte size, morphology, and metabolic activity

P.M.S. Rosa¹, B.A.F. Barros¹, R. Pasolini², A.J.R. Camargo², R.V. Serapião², C.S. Oliveira¹

¹EMBRAPA Gado de Leite LRA-CESM, Valença; ²PESAGRO-RIO, Niterói.

Keywords: oocyte quality, OPU, *Bos indicus*.

Gyr breed has significant relevance in Brazil dairy systems. However, this breed presents late puberty onset, which delays their reproductive life. A possible approach to overcome this delay is the use of prepubertal animals as oocyte donors for IVF. The aim of this study was to assess, using three approaches (oocyte size; oocyte cytoplasm/cumulus cells quality – morphological assessment; and Brilliant Blue Cresil (BBC) staining), the oocyte quality of prepubertal Gyr donors. For that, 10 prepubertal animals underwent hormonal treatment during 18 days (D0- P4 implant / 1g, D12- removal of the implant and administration of 0.5 mg ECP and 200 IU eCG, D18- ovum pick up (OPU)). The animals were evaluated at D18, regarding their response to hormonal treatment, and grouped as: animals that did not ovulate (PPN, n = 6) or animals that ovulated (PPO, n = 4). Cows (C, n = 4) were used as controls for this analysis, and were not subjected to hormonal treatment. Following OPU, oocytes were selected and exposed to BBC staining (Manjunatha et al, Theriogenology, v.68,p.1299-1304, 2007). Images of each structure were captured for analysis using ImageJ software, regarding oocyte size; morphological assessment based on cumulus cells and oocyte cytoplasm (G1, G2, G3 – viable; and G4 – non-viable: denuded, degenerated oocytes); and BBC staining (BBC-: growing oocytes, with increased G6PDH metabolism; BBC+: mature oocytes, with low G6PDH metabolism). This study was approved by the local ethics committee (CEUA-EGL protocol 24.2015). Averages of oocyte size and number of oocytes per animal were compared by ANOVA, and morphological assessment and BBC percentages were compared by Chi-square, among C, PPN and PPO groups (P = 0.05, Minitab Software). The average oocyte number (C= 8 ± 2.94 , PPN= 18.3 ± 13.70 , PPO= 15.2 ± 2.21) and the average oocyte size (C= $136.1 \mu\text{M} \pm 79.6$, PPN= $132.3 \mu\text{M} \pm 46.3$, PPO= $138.9 \mu\text{M} \pm 48.7$) did not differ among the categories. The percentage of BBC positive oocytes was also similar among groups (C= 78.1%, PPN = 73.6%, PPO= 68.8%). Regarding morphological quality, no difference was detected among groups in each class (G1: C= 15.6%, PPN= 13.6%, PPO= 9.8%; G2: C= 9.3%, PPN= 14.5%, PPO= 8.1%; G3: C= 21.8%, PPN= 20%, PPO= 19.6; G4: C= 0%, PPN= 2.7%, PPO= 1.6%). No difference was detected for percentage of viable oocytes (G1, G2 and G3) among groups (C= 46.8%, PPN= 48.1%, PPO= 37.7%). Therefore, in all three analysis, no difference was detected among oocyte quality of Gyr cows and prepubertal animals subjected to hormonal treatment, ovulating or not. This result suggests that prepubertal Gyr animals can be used as oocyte donors for IVF embryo production, accelerating their reproductive life.



A110 OPU-IVP and ET

The cushioned centrifugation during sperm selection increases fertilization, cleavage and blastocyst rates on bovine IVP

C.L.L.U.F. Machado, F.G. Leivas, D. Missio, K. de Mattos, D.S. Brum

Universidade Federal do Pampa, Uruguaiana.

Keywords: bull sperm, percoll gradients, IVF.

The centrifugation in sperm separation techniques aims to remove the seminal plasma and diluents, besides increasing the concentration of viable sperm. However, this step causes several damages in the spermatid cells. To reduce these injuries, cushioning methods have been used, with success prior to the freezing of equine semen. This study was conducted to evaluate the protective effect of cushioned centrifugation during sperm selection by Discontinuous Percoll® Gradients (DPG) for spermatozoa intended for bovine PIV, by fertilization rate and embryonic development. The cushioning system was carried out by adding 150µl of a colloid solution (CushionFluid®; Minitube, Tiefenbach, Germany) under the Percoll® gradient. Five replicates were performed, where straws of two *Bos taurus* bulls were thawed and divided into two treatment groups: Control (C) with only the DPG (Guimarães, A. G., *Anim Reprod Sci*, v. 146, p. 103-10, 2014); and treatment, Cushioned Centrifugation (CC), which was the DPG method plus the cushioned system. After the selection process, a dose of 2x10⁶ spermatozoa/mL of each group was utilized for IVF of previously matured oocytes. A total of 200 presumptive zygotes / treatment were stripped and incubated in a Hoechst 33342 solution (10mg / ml) for evaluation of the fertilization rate, being considered fertilized zygotes with two or more pro nuclei or fused nuclei. The other presumptive zygotes (85/treatment) were individually cultured for seven days in SOFaaci medium + 10% MES (mare in estrus serum) and BSA, in an embryonic monitoring system (Primo Vision, Cryo Mangement Ltd., Hungary). Embryonic development was assessed by cleavage on day two (D2) and embryo production on day seven (D7), and also the determination of the time of the first cleavage. Data were analyzed by chi-square (X²; P < 0.05). The treated group (CC) had higher fertilization and cleavage rate (56.1 and 80.0%), when compared to the control (45.9 and 64.7%). Though the time of the first cleavage has been similar among the treatments, it was observed that embryos treated with cushion solution had a greater rate of cleavage prior to 28h post-insemination (hpi) compared to the control group (32.5 and 18.8%, respectively), suggesting that these embryos have more capacity to reach the blastocyst stage (Barreta, M. H., *Experimental Cell Research*, v. 318, p. 10, 2012). In relation to the embryos produced in D7, the blastocyst rate (Bl) was higher in the CC group (14.0%) than in C group (2.2.%). The results of this experiment suggest that the cushioned centrifugation protects the sperm cells, increasing fertilization, cleavage and blastocyst rates in IVP of bovine embryos.



A111 OPU-IVP and ET

Effect of the transfer with fresh or frozen embryos and embryo quality on the pregnancy rate in Angus cattle

B. Mion¹, R.M. Bonotto², A.L.M. Bonotto³, M.L.G. Pinto⁴, F.S. Rosa²

¹Universidade Federal de Pelotas, Pelotas; ²FAI-Faculdades de Itapiranga, Itapiranga; ³Central Abn Agropecuária, Santiago; ⁴EPAGRI Empresa de Pesquisa Agropecuária de Santa Catarina, Lages.

Keywords: embryo, ET, cryopreservation.

The success of embryo transfer depends on factors associated with the embryo, the recipient, the transfer technique, or the interaction between these factors. Among them, the embryo quality has an important influence on pregnancy rates, since good or excellent embryos quality are more likely to establish pregnancy. It is known that cryopreservation may decrease embryo quality in a way that the result obtained after the embryo transfer can be altered. The aim of this study was to evaluate the effect of the transfer of fresh or frozen embryos and embryo quality on the pregnancy rate in Angus cattle. A retrospective analysis was performed using data of 3,211 anovulations held in Central ABN Agropecuária, located in the city of Santiago / RS, between 2002 and 2015. Two thousand three hundred and fifty-eight anovulations were performed with fresh embryos and eight hundred fifty-three with frozen embryos, quality 1 and 2, classified in accordance with the rules of the IETS. The recipients were previously evaluated and the embryos transferred after seven days of estrus (± 24 hours). Ultrasonographic evaluation was performed after 30 days, aiming the pregnancy diagnosis. Statistical analysis was performed using the chi-square test, thorough SAS software (SAS Institute Inc., Cary, NC, USA). The result obtained with the transfer of fresh embryos (61.64%) and frozen (60.23%) were similar ($P = 0.2373$). However, pregnancy rate obtained after the transfer of quality 1 embryos was greater than that obtained with quality 2 embryos, achieving a result of 64.81% and 57.94%, respectively ($P = 0.0014$). Therefore, it is possible to conclude that cryopreservation did not affect pregnancy rates. However, the results obtained with the transfer of embryos of different quality confirm what has already been described, better quality of embryos provide improved results in TE.



A112 OPU-IVP and ET

Effect of reducing the time of oocyte and sperm interaction on *in vitro* production of bovine embryos

A.C. Leite, B. Vanelli Rocha Couto, N.V. Sollecito, T. Mata Martins, P. Pimenta Nunes, E.B. Magalhães, Á.M. Borges

UFMG, Belo Horizonte.

Keywords: *in vitro* fertilization, oocyte-sperm interaction, *in vitro* produced embryos.

The *in vitro* culture conditions for bovine embryos have a fundamental role in the cleavage, embryonic genome activation, differentiation and embryo viability (Moore et al., Theriogenology, v.68, p.1316, 2007). Moreover, the female and male gametes co-incubation time has a key role in *in vitro* fertilization (IVF) systems. It's well established that incubation of COCs with $1-1,5 \times 10^6$ spermatozoa/mL sperm concentration, for 18 to 22 hours, produces acceptable embryo produced rates. However, it has also been reported that the gamete incubation time reduction of 18-24 to 10 hours, with low concentration of spermatozoa, is able to produce similar embryonic development rates. Long IVF periods, as well as high sperm concentrations, increase the polyspermy incidence and reduce embryo production (Berland et al., Vet. Science, p.1, 2011). The aim of this study was to evaluate the effect of reducing the time of co-incubation duration at fertilization, on bovine embryo development potential. *In vitro* matured oocytes were subjected to fertilization with Holstein bull semen, previously tested, using the sperm concentration of $0,3 \times 10^6$ spermatozoa/mL, for 10 or 18 hours of incubation time. The presumptive zygotes were cultured in SOF medium plus BSA and FBS, for seven days. We evaluated the number of matured oocytes pre-insemination, cleavage rate at 72 hours and the blastocysts production in the eighth day after fertilization. To evaluate the oocytes maturation, embryo cleavage and blastocyst production, the contingency analysis by Fisher's exact test was performed. 1343 oocytes were divided into two incubation times: 10 hours (n = 533) and 18h (n = 810). The COCs maturation rate was 96.6% and 92.2% for the groups 10h and 18h fertilization, respectively (P > 0.05). The cleaved percentage for 10h of incubation group (86.1%) was higher than 18h (79.1%) (P < 0.05). The production of embryos in relation to the total number of cleaved structures was 80.4% and 51.3% for 10h and 18h, respectively (P < 0.05). The blastocysts production rate in relation to the total COCs subjected to *in vitro* maturation was also superior in 10h of incubation (69.2%) compared with 18h (40.6%) (P < 0.05). It was observed that is possible to obtain higher blastocyst rates using 10 hours of co-incubation duration at *in vitro* fertilization of bovine oocytes.



A113 OPU-IVP and ET

Effect of the supplementation of Pantaneira breed cows with linseed (*Linum usitatissimum* L.) on the *in vitro* production of embryos - preliminary results

**A.C.B. de Lima, C.J. Tavares Cardoso, G.L. Ortiz, L.S. Paes, P.G. Loesia Lima, R.F. da Silva,
W.A. Leite da Silva, M.G.C.R. Ferreira, M.B. Souza, F.A. Melo Sterza**

UEMS, Aquidauana.

Keywords: supplementation, embryos, bovine.

The aim of this study was to evaluate the effect of supplementation with linseed on the production of cattle embryos obtained by OPU. Six Pantaneira breed cows were used, with body condition score 3.5 to 4.0 (scale of 1-5), managed in a rotational grazing on grass Mombasa (*Panicum Maximum*) and supplemented with 2 kg of concentrated feed (22% CP and 70% TDN) per day. The animals were randomly divided into 2 groups: control group (CTRL, n = 3) and linseed group (LINH, n = 3), differing only by the supply of 0,800kg / animal / day of linseed in the diet of LINH group. The supplementation period was 92 days that were conducted 3 OPU sessions guided by ultrasound (OPU) with a mean interval of 30 days between each other. 313 Complex Cumulus oocytes were obtained (COCs) and only COC's of degrees 1 and 2 were considered viable and submitted to *in vitro* maturation (IVM) for 24 hours in TCM 199 supplemented with 10% FCS, 22 / mL pyruvate, 0.5 mg / ml of follicle stimulating hormone and 50 ug / ml luteinizing hormone. After IVM, COCs were placed in 100 uL drops of Tyrodes medium supplemented with 0.6% bovine serum albumin (BSA), gentamicin 22 ug / ml pyruvate and 10 g / mL heparin. It was used semen of Pantaneira breed with proven fertility at fertilization *in vitro* (IVP) of the same bull and same match on all collections, which was prepared in Percoll® gradient. The insemination dose was 10⁶ living sperm / mL. IVF was performed in TALP for 18-22 h and culture *in vitro* in SOF containing 8 mg / ml fatty acid free BSA, 1 mM glutamine and 5% FBS, where presumptive zygotes were kept for 8 days (D8). All steps of IVP were performed with Sigma Aldrich® products, in an incubator at 38.5°C, 5% CO₂ and maximal humidity. It conducted a randomized design in factorial 2 x 5 (2 treatments x 5 repetitions). There was no effect (P > 0.05) of lipid supplementation on the cleavage rate (67.62% vs 72.0% control group and linseed, respectively) and the blastocyst rate (30.98% vs CONTR LINH 30.11%). It is concluded that supplementation with 0.800 kg of linseed in the diet of Pantaneira breed cows did not affect the quality of oocytes and embryos produced *in vitro*.

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A104 OPU-IVP and ET

Effect of animal category (prepubertal, pubertal and pregnant) on *in vitro* embryo production in Holstein heifers

B.M. Bayeux¹, L.M. Carvalho², Y.F. Watanabe¹, R.D. Mingoti³, A.S. Oliveira³, M.O. Chiba³, A.J. Azrak⁴, P.M.N. Castro⁵, A.H. Souza⁶, P.S. Baruselli¹

¹Departamento de Reprodução Animal, VRA-FMVZ-USP, São Paulo; ²Bovigênese Biotecnologia da Reprodução, Bofete; ³Watanabe Tecnologia Aplicada(WTA), Cravinhos; ⁴Pectec Manejo e Assessoria, Ribeirão Preto; ⁵Médico Veterinário Autônomo, Ribeirão Preto; ⁶Ceva Sante Animale, Paulínia.

Keywords: heifers, IVPE, *Bos taurus*.

The aim of this study was to evaluate the *in vitro* embryo production of Holstein heifers (*Bos taurus*). A total of 179 animals of 3 categories: prepubertal heifers (PP, 8 to 10 months old; n = 60); pubertal heifers (PU, 10 to 12 months old; n = 60) and pregnant heifers (PR, 14 to 18 months old; n = 59) were submitted to ovum pickup (OPU), at random stages of the estrous cycle. Animals were classified as cyclic (pubertal) or non-cyclic (prepubertal) based on the presence or absence of a corpus luteum during ultrasound evaluations (Mindray® DP 2200) performed 14 days prior to and immediately before the OPU procedure. Six replicates with 10 animals of each category were carried out, resulting in 30 donors per replicate. In each replicate, all follicles ≥ 2 mm were aspirated and the total of recovered structures, quantity and quality of viable oocytes were recorded. Recovered viable oocytes were submitted to *in vitro* production and the embryonic development (cleavage rate and blastocyst rate) was evaluated. On day 7 of *in vitro* production the embryos were vitrified for later transfer into recipients. All oocytes were fertilized by sexed semen from the same sire of Holstein breed (*Bos taurus*) and semen batch. Data were analyzed with the GLIMMIX procedure using the SAS® software. Pubertal heifers had a greater number of recovered oocytes (PU = 15.6 ± 1.4^a vs PP = 9.8 ± 1.3^b vs PR = 9.8 ± 1.6^b ; P < 0.001) as well as viable oocytes (PU = 9.1 ± 0.95^a vs PP = 4.6 ± 0.6^b vs PR = 5.6 ± 1.1^b ; P < 0.001) compared to other categories. In contrast, cleavage rate was similar between pubertal and pregnant heifers (PU = $56\%^a$ vs PR = $78\%^a$ vs PP = $31\%^b$; P < 0.001). Interestingly, pregnant heifers had a greater number of embryos produced per OPU (PR: 1.76 ± 0.3^a vs PU: 0.9 ± 0.2^b vs PP: 0.13 ± 0.1^c ; P < 0.001) and ultimately greater blastocyst rate (PR = $37\%^a$ vs PU = $4\%^b$ vs PP = $2\%^c$; P < 0.001) when compared to other heifer-categories. In conclusion, pregnant heifers were more efficient in terms of *in vitro* embryo production compared to prepubertal and pubertal Holstein heifers (*Bos taurus*).



A115 OPU-IVP and ET

Kisspeptin: effects on bovine oocytes *in vitro* maturation

M. Oliveira¹, K.A. Alves², L.R. Peixoto¹, T.S. Maia¹, E.C.M. Pereira¹, J.O. Jacomini¹, M.E. Belleti¹, R.M. Santos¹, G.G. Macedo¹

¹Universidade Federal de Uberlândia, Uberlândia; ²Universidade Estadual do Ceará, Itaperi.

Keywords: oocyte competence, cows, kisspeptin.

This study aimed to evaluate different concentrations of kisspeptin, as well as the interaction of kisspeptin and FSH/LH in *in vitro* maturation and oocyte competence in cattle. The Experiment 1 aimed to determine the minimum concentration of Kisspeptin (Kp) to be used, and Experiment 2 aimed to evaluate Kp interaction with FSH and LH. The oocytes were collected in a commercial slaughterhouse and only Grade I oocytes were utilized. For Experiment 1 it was used as a basis the addition of 10⁻⁶M of Kp-10, as reported for *in vitro* maturation of porcine oocytes (Saadeldin, I.M. *Reproduction, Fertility and Development*, v.24(5), p.656-668, 2012). The oocytes were cultured in TCM-199 bicarbonate-buffered supplemented with 10% FBS, sodium pyruvate (22 µg/mL), amikacin (83 mg/mL), FSH (0.5 µg/mL), and different concentrations of Kp. The treatments were: T1.1: FSH + 0M Kp-10; T1.2: FSH + 10⁻⁷M Kp-10, T1.3: FSH + 10⁻⁶M Kp-10; T1.4: FSH + 10⁻⁵M Kp-10. In Experiment 2, the best concentration of Kp determined in Experiment 1 was employed. The treatments were: T2.1: no hormones; T2.2: FSH; T2.3: FSH + Kp-10; T2.4: FSH + LH; T2.5: FSH, LH + Kp-10; T2.6: Kp-10. The oocyte competence was determined by nuclear maturation, mitochondrial distribution, MitoTracker® Orange CMTMRos fluorescence intensity and DCF. The evaluation of nuclear maturation was made after 24 hours of incubation and the oocytes were stained with DAPI to determine the nuclear stage (Germinal Vesicle-GV, Metaphase I-MI and Metaphase II-MII). The mitochondrial distribution, classified as peripheral/semiperipheral and diffuse in clusters/granules, was evaluated after staining with the MitoTracker® Orange CMTMRos, as well as its fluorescence intensity. To determine the intensity of ROS oocytes were stained with DCF. The statistical analyses were performed using SAS GLIMMIX PROC. In Experiment 1 oocytes matured only with the FSH reached a smaller nuclear maturation when compared to those who were matured with Kisspeptin at different concentrations (T1.1:13/33; T1.2:28/35; T1.3:30/34; T1.4:28/32; P = 0.0001). There was no statistical difference in mitochondrial distribution between treatments (P > 0.05). The fluorescence intensity of MitoTracker did not differ among treatments (P > 0.05). The DCF fluorescence intensity was lower when the concentration of Kp was increased in the medium (T1.1:12177726.1; T1.2:10945982.83; T1.3:9820536.53; T1.4:9147016.38; P < 0.0001). Based on Experiment 1 results, the concentration of Kp was determined in 10⁻⁷M. In Experiment 2 the mitochondrial distribution was different between treatments, because oocytes matured with only Kp or FSH+LH, had higher oocyte competence than those matured with only FSH or without hormone addition (T2.1:66.66%; T2.2:66.66%; T2.3:75.86%; T2.4:91.17%; T2.5:82.85%; T2.6:91.17%; P < 0.05). The T2.1 resulted in lower nuclear maturation than the other treatments (T2.1:5/18; T2.2:18/32; T2.3:22/29; T2.4:26/33; T2.5:26/34; T2.6:25/34; P = 0.0094). The fluorescence intensity of probes MitoTracker and DCF was lower when Kp was added to the maturation medium (T2.1:1228363/540069; T2.2:2307984/1395751; T2.3:1941890/1114948; T2.4:2502145/1722376; T2.5:2286173/1467782; T2.6:1859411/979325; P < 0.0001). So this is the first study that shows that Kisspeptin stimulates oocyte maturation without the presence of gonadotropins in the maturation medium.



A116 OPU-IVP and ET

In vitro* production of bovine embryos in culture medium containing different concentrations of antioxidant extracted from *Lippia oil origanoides

N.V. Sollecito¹, E.C.M. Pereira², J.G.V. de Grázia¹, A.C. Leite¹, B. Vanelli Rocha Couto¹, V.B. Andrade¹, E.B. Magalhães¹, Á.M. Borges¹

¹Universidade Federal de Minas Gerais, Belo Horizonte; ²Universidade Federal de Uberlândia, Uberlândia.

Keywords: *in vitro* production, antioxidants, *Lippia origanoides*.

In vitro production of embryos has sought alternatives to increase the blastocyst rate, including the addition of antioxidants to the culture media. This study aimed to evaluate the rate of cleavage and *in vitro* production of bovine blastocysts, using maturation and culture media containing cysteamine and antioxidants extracted from *Lippia origanoides* oil. Ovaries were used slaughterhouse cattle, transported in saline 0.9% NaCl and there 38.0°C to the laboratory. The follicular aspiration was performed with a needle (12Gx40) coupled to a 5ml disposable syringe, and follicular fluid was placed in 15mL tubes for 10 minutes for sedimentation of oocytes. The supernatant was removed and the excess was washed in TCM-HEPES 199. Only degree 1 and 2 oocytes were selected, according to the analysis of complex *cumulus oophorus* cells and quality cytoplasm. The oocytes were washed in: TCM bicarbonate supplemented with 10% fetal bovine serum, 22mg/ml sodium pyruvate, 50ug/ml gentamicin sulfate, 5µg/ml of LH, 1µg/ml of FSH, 10ug/ml estradiol and 2.5mg/ml of *Lippia origanoides* antioxidant and then were incubated in atmosphere with 5%CO₂, 38.5°C for 22 to 24 hours. Then, the oocytes were inseminated with 1x10⁶ sperm *in vitro* fertilization medium and incubated in a humidified atmosphere with 5% CO₂, 38.5°C for 18 to 20 hours. After this period, the oocytes were denuded and directed to five treatments containing m-SOF (modified) without adding antioxidant (T1), medium supplemented with 50uM/ml cysteamine (T2) and medium supplemented with 2.5; 5.0 and 1ug/ml of antioxidant *Lippia origanoides*(T3, T4 and T5, respectively). The Shapiro-Wilk test was used to assess the normality of continuous variables. Statistical analysis was performed using the ANOVA (post hoc Tukey) and Kruskal-Wallis. The significance level was set at P < 0.05. Cleavage rates did not differ between treatments (P > 0.05) and were 74.5; 72.5; 66.7; 67.3 and 64.2%, respectively for T1, T2, T3, T4 and T5. Blastocyst production rates were 40.4; 28.6; 24.1; 30.7 and 33.8 % for T1, T2, T3, T4 and T5, respectively. The production rate of blastocyst in T1 was higher (P < 0.05) than T5. Already T2, T3 and T4 do not differ (P > 0.05). Despite the treatments supplemented with antioxidant *Lippia origanoides* present satisfactory cleavage rates, the final production of blastocysts in the groups supplemented with antioxidants was lower than that found in the control group. Thus, in this study, the use of *Lippia origanoides* oil did not show good results for blastocyst rate on *in vitro* embryos.



A117 OPU-IVP and ET

Effect of FSH treatment prior to OPU on ovarian vascularization in Holstein oocyte donor cows

J.C.B. Silva, R.M. Ferreira, B.M. Oliveira, K.M. Lemes, M. Maturana Filho, J.R. Naves, T. Santin, G. Pugliesi, E.H. Madureira

USP, Pirassununga.

Keywords: Doppler, FSH, OPU.

The aim of this study was to evaluate the effect of different hormonal treatments on ovarian vascularization score of oocyte donor cows. The experiment was conducted at Fazenda Santa Maria, Pouso Alegre - MG. Thirty-six non-lactating and not pregnant Holstein cows were evenly split into 3 groups: control, treated with 4 (4FSH) and 6 (6FSH) FSH applications. To allow an equitable distribution of cows between treatment groups, Body Condition Score (BCS) and historical production of oocytes and embryos from donors were considered because these same had participated in a monthly trade program IVP. All cows were kept in pasture pickets with water ad libitum and mineral mix, and supplemented with 2 kg of concentrate containing 12% protein. At random stage of the estrous cycle, defined as D0, all cows received an intravaginal device = 1 g of P4 (Primer®, Tecnopec, São Paulo, Brazil), associated with 2 mg of estradiol benzoate i.m. (Ric BE®, Tecnopec, São Paulo, Brazil). The cows of the control group (n = 12) did not receive FSH application. However, the cows in the 4FSH group (n = 12) received 200 mg of FSH on day D4 and D5 split into four equal doses of 50 mg each in 12 h intervals. On the days D3, D4 and D5, the cows in the 6FSH group (n = 12) received the same FSH dose (200 mg, Folltropin, Tecnopec, Brazil), but split into six equal doses of 33.3 mg administered at 12 hour intervals. All the cows of the three groups were treated with PGF2 α (Cioprostinn®, Innovare Biotechnology and Animal Health Ltda, São Paulo, Brazil) on D3 morning. On D7, P4 device was removed and then the ovarian vascularization was evaluated using a Doppler ultrasound device with linear transrectal probe of 6.5 MHz (M5vet, Mindray®, China). The color Doppler mode was used for the subjective assessment of the vascularization of the ovaries. The images from both ovaries were followed for 10 to 15 sec in each ovary, recorded and analyzed by two different evaluators. A classification with scores from 1 to 4 was performed in accordance with the film obtained from each ovary, being that classification 1 (one) means the minimum vascularization and 4 (four) the maximum vascularization, adapted from Silva e Ginther (2010, J. Reproduction v. 139, n. 2, p. 453–463). All the cows have gone through all the treatments in crossover design. Data was analyzed using the Glimmix procedure of SAS 9.3, with orthogonal contrasts. The average of the BCS of the cows was 3.4 ± 0.1 on a scale of 1 to 5 and did not differ between groups. The ovarian vascularization score was similar ($P = 0.43$) in cows treated (1.9 ± 0.1) or not (1.7 ± 0.1) with FSH. No effect was observed of the FSH dose ($P = 0.67$) on the ovarian vascularization (4FSH = 1.8 ± 0.1 and 6FSH = 1.9 ± 0.1). Therefore, it can be concluded that the treatment with FSH, regardless its administration in doses fractionated into 4 or 6 times, did not influence the ovarian hemodynamic evaluated by color Doppler.

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A118 OPU-IVP and ET

Catosal® B12 effects on animal reproduction: oocyte quality and embryo production

E. Siqueira Filho¹, A.A. Fidelis², A.F. Silva¹, L.C. Feliciano¹, M.E. Abadia¹, A.S. Carvalhêdo¹

¹Embriotec Reprodução Animal, Anapolis; ²UPIS, Brasília.

Keywords: Catosal, in vitro fertilization, bovine.

Taurine breeds produce lower in vitro embryo rates and one of the main reasons why this happens is the low quality and quantity of oocytes when compared to Zebu breeds. The use of Catosal® B12 has been described as responsible for the animal energy metabolism improvement. This project aimed to evaluate the effect of using Catosal® B12 in Holstein females treated previously to the in vitro embryo production process. A total of 57 ovum pick up were performed. Two groups were formed: 1 - control (19 cows) without using Catosal® B12; 2 - treated (38 cows) with two applications of Catosal® B12 before follicular aspiration. The following design was applied for the Catosal® B12 treatment: D0 - 1st Catosal® B12 application - 25 ml; D3- 2nd Catosal® B12 application - 25 ml; D6- follicular aspiration. The aspirated oocytes were placed in drops with maturation medium for 22-24 hours. After the maturation period, they were transferred to fertilization medium drops and inseminated with female sexed semen. Sperm and oocytes were co-incubated for 15-18 hours. The presumptive zygotes were transferred to culture medium, evaluated in D3 to observe the cleavage rate and in D7, the blastocyst rate. After evaluation, they were transferred to previously synchronized recipient cows. After 30 days of transfer, recipients were evaluated by ultrasound to investigate pregnancy rate. Results were subjected to data normality test and the variance test elected for all parameters was the Mann - Whitney test. The percentage of viable oocytes (79% control and 72,2% treated) and pregnancy (52,6% control and 42,5% treated) did not differ between the groups. However, the cleavage and embryo production in vitro rates had a better result ($P < 0.01$) on treated group when compared to the control one (89.4% vs. 52.8% and 62.4% vs. 27.6 %, respectively). According to the results obtained in this study, the use of Catosal® B12 can be complementary within the OPU protocol, achieving an improvement on embryo production results for Holstein donors.



A119 OPU-IVP and ET

Differential expression of members of the IGF system in OPU-derived COCS from Gir (*Bos indicus*), Holstein (*Bos taurus*) and 1/2 Holstein x 1/2 Gir (*Bos taurus/indicus*) cows

A.C. Lopes¹, T.M. Miyauchi¹, M.P. Palhão¹, R.B. Silva², M.J. Sudano³, C.A.C. Fernandes¹,
A.C.S. Castilho⁴, E.S. Caixeta⁵

¹Departamento de Medicina Veterinária, Laboratório de Reprodução Animal, UNIFENAS, Alfenas; ²Curso de Medicina Veterinária, Faculdade Sudoeste Paulista, Avaré; ³Unipampa, Uruguaiana; ⁴Pró-reitoria de Pesquisa e Pós Graduação, Universidade do Oeste Paulista, Presidente Prudente; ⁵Departamento de Medicina Veterinária, Laboratório de Reprodução Animal, UNIFENAS / Unifal, Alfenas.

Keywords: dairy cattle, oocyte quality, gene expression.

In last years, it has been noted increasing utilization of in vitro embryo production (IVEP) in dairy breeds production. In this context, is known that differences in the number and development potential of oocytes between European (*Bos taurus*) and zebu (*Bos indicus*) cows affect the efficiency and economic viability of IVEP. Since, insulin-like growth factor (IGF) system is related to quality of oocytes, the aim of this study was to quantify mRNA abundance of IGF system members in cumulus oocytes complex (COCs) from Gir, Holstein and 1/2 Holstein x 1/2 Gir cows. Pools of 20 immature COCs from Gir (n = 4 pools), Holstein (n = 4 pools) and 1/2 Holstein x 1/2 Gir cows (n = 4 pools) were obtained by ovum pick-up on farms in Alfenas and Três Pontas in the southern region of Minas Gerais state, Brazil. Oocytes and cumulus cells (CC) were mechanically separated and stored in liquid nitrogen. Only the COCs with homogeneous cytoplasm and surrounded by, at least, three layers of compact cumulus cells were selected for the experiment. Total RNA was extracted from pools of 20 oocytes and their respective CC using the RNeasy® kit (Qiagen). The investigation of target genes (*IGF1* and *IGF2*, their receptors - *IGFR1* and *IGFR2*, as well as IGF binding proteins - *IGFBP2*, *IGFBP4*, and pregnancy-associated plasma protein-A (*PAPPA*) was assessed by real time RT-PCR using Power SYBR® green master mix (Applied Biosystems) and normalized by Cyclophilin (*CYC-A*). Relative quantification of mRNA abundance was determined using the $\Delta\Delta C_t$ method with correction by Pfaffl's equation. Effects of breeds on the expression of target genes in oocytes and CC were tested by ANOVA and means were compared by Tukey-Kramer HSD test. Differences were considered significant when $P < 0.05$. The relative mRNA abundance of *IGF2*, *IGFR2* and *IGFBP4* was higher in the CC of Holstein donor compared to Gir and cows. In oocytes, mRNA encoding *IGFR1* was higher in Gir compared to Holstein and 1/2 Holstein x 1/2 Gir animals. The mRNA abundance of *PAPPA* was higher in CC and oocytes in Gir and 1/2 Holstein x 1/2 Gir cows compared to the Holstein donors. No differences on mRNA abundance of *IGF1*, *IGFR1* and *IGFBP2* in CC and *IGF1*, *IGF2*, *IGFR2*, *IGFBP2* and *IGFBP4* in oocytes were demonstrated among different breeds. In conclusion, the higher *PAPPA* mRNA abundance in oocyte and CC from Gir and 1/2 Holstein x 1/2 Gir donors associated with low expression of *IGFBP4* in CC of these breeds suggest more efficient degradation of IGFBPs which results in greater bioavailability of IGF in Gir and crossbred (1/2 Holstein x 1/2 Gir) COCs when compared to the Holstein immature COCs.

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Surgical treatment of partial prolapse of vaginal mucosa in Nelore cows submitted to follicular aspiration guided by ultrasound - technical report

G.A.M. do Nascimento, I.A.S. Mello, R.R. Corrêa

Ouro Fino Saúde Animal, Cravinhos.

Keywords: prolapse of the vaginal mucosa, partial vaginectomy, follicular aspiration guided by ultrasound.

The partial prolapse of the vaginal mucosa is a disease of the reproductive tract commonly reported in cows aged over 24 months, pregnant or not, managed in intensive system, overfed and submitted to follicular aspiration. The objective of this study was to report the cases of partial prolapse of the vaginal mucosa and their treatment in six adult Nelore cows managed in the same property, located in the state of São Paulo. The partial prolapse of the vaginal mucosa often impossible follicular aspiration of these animals and thus compromised the reproductive activity of these within the herd. For treatment of animals was chosen partial vaginectomy technique of the prolapsed mucosa. For the surgical procedure was used epidural low anesthesia and anesthetic block of the prolapsed area. Cleaning of the perineum and vulva it was performed and then the prolapsed portion was manually pulled with the use of sterile gauze and obstetrical forceps. A probe was inserted into the urethra of animals and then the anus was sutured temporarily using the standard tobacco pouch, all this to avoid contamination of the operative field. To this procedure were used stainless steel forceps produced for this purpose, responsible for traction, fixation, stability and delimitation of the portion to be sectioned. In the previous portion to that fixed forceps, the suture was made with simple points separated using absorbable thread, and for added security a new parallel suture plan the first was made. After the suture was performed amputation of the prolapsed portion. Immediately after surgery the animals were treated with antibiotics and anti-inflammatory, both as a single dose by intramuscular route, 3 animals received combination Penicillin G Benzathine 5.000.000 IU + Penicillin G procaine 5.000.000 IU + dihydrostreptomycin 10 g (Penfort® PPU Ourofino Animal Health, Cravinhos, Brazil) and 20 mg Dexamethasone (Cortiflan, Ourofino Animal Health, Cravinhos, Brazil) and the other 3 animals received the association between Oxytetracycline 10 g and sodium Diclofenac 0.5g (Ourotetra Plus LA, Ourofino Animal Health, Cravinhos, Brazil). After surgery one animal had bleeding, however, it has been controlled and the animals don't showed complications. Another animal had new prolapse, however, less intense, 15 days after the initial procedure due to insufficient primary resection, and a new surgical interference occurred 55 days after the first. All animals fully recovered and did not develop new prolapse of the vaginal mucosa and followed in regular reproductive activity, demonstrating the effectiveness of the surgical procedure.



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Ability of the ovine corpus luteum formed after follicle aspiration in the establishment and maintenance of pregnancy obtained from intrafallopian oocyte transfer

R. Denadai, L.F.S. Scagion, T.L. Ikeda, F.C. Landim-Alvarenga, E. Oba, S.D. Bicudo

FMVZ-UNESP-Botucatu, Botucatu.

Keywords: sheep, corpus luteum, follicle aspiration.

Aiming to evaluate the capability of sheep develop pregnancy after oocytes intra-fallopian transfer (OIFT), with corpus luteum (CL) formed after aspiration of the pre-ovulatory follicles, we used five adult ewes, without determined breed, after ovarian cyclicity confirmed by ultrasound. The females were submitted to estrus synchronization treatment, by inserting intravaginal device of slow release of natural progesterone (EZI-BREED CIDR OVINOS E CAPRINOS®, InterAg, New Zealand), being intramuscularly administrated 5 mg of dinoprost (Lutalyse®, Pfizer Laboratory Ltda., Brazil) on the fifth day from the beginning of the treatment. The vaginal device was removed on the fourteenth day and 500 IU of eCG was administered (Novormon®, Syntex Industries Biochemistry & Pharmaceuticals SA, Argentina). After 48 hours from the use of gonadotropin, an ultrasound of the ovaries was performed to verify the presence of at least one follicle with a diameter equal to or greater than 5 mm, indicative of being pre-ovulatory. Thereafter, all visible follicles were aspirated guided by laparoscopy. The material aspirated from the follicles was evaluated in a stereomicroscope for the identification of complex cumulus oophorus (COCs), and the classification was performed with basis on the characteristics of ooplasm and cumulus cells. During the laparoscopic procedure, an artificial insemination with fresh diluted semen was conducted, depositing 100 x 10⁶ of spermatozoa in each uterine horn. In three sheep, the OIFT was performed in the ipsilateral greater pre-ovulatory follicle of the ovary of only one autologous COCs, which had homogeneous cytoplasm and expanded cumulus. This procedure was not performed in two sheep, since they did not present the appropriate COCs to the established criteria. The number of recovered COCs on the three recipients was identical to the aspired follicles, demonstrating the impossibility of the occurrence of spontaneous ovulations or the presence of residuals oocytes after the LOPU. The ultrasonographic monitoring of the ovaries was performed for the LCs confirmation, and the number of luteal structures formed corresponded to the pre-ovulatory follicles vacuumed in each sheep. An ultrasonographic diagnosis of pregnancy was performed after 35 days of the OIFT, and two from the three recipients were diagnosed as pregnant, with the fetus and fetal wraps presenting characteristics consistent with gestational age in relation to the date of OIFT. The parturition occurred 151 days post-transfer in one of the females and 153 days in the other, with the birth of a lamb for each recipient, without clinical changes during the immediate postpartum period. Thus, it was shown that it is possible the establishment and development of normal gestational after OIFT in recipients with CL formed after aspiration of follicles guided by laparoscopy. Further studies are needed to better understand the potential of these biotechnological procedures.



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Synchronization effect of follicular wave growth in the production of buffalo oocytes

E. Oba¹, B.V. Rodrigues Rizoto de Mattos², Á.M. Borges³, M.R.J.M. Henry³, A.C. Leite³, V.B. Andrade³, M.V.P. Silva³, J.G.V. de Grázia³, E.C.M. Pereira²

¹Universidade Estadual Paulista, Botucatu; ²Universidade Federal de Uberlândia, Uberlândia; ³Universidade Federal de Minas Gerais, Belo Horizonte.

Keywords: buffalos, protocol, ovary.

This study aims to investigate the effect of different protocols of follicular wave synchronization of bubaline oocytes donor regarding the number of follicles, the recovery rate, the number and quality of oocytes produced. 12 adult buffalo were used under different protocols that resulted in the aspiration of follicles guided by ultrasound (OPU) 3 to 4 days after the emergence of follicular wave. Each animal was tested at least three times for each treatment. The treatments were: Treatment 1 (T1): animals not fixed with OPU held in random day of the estrous cycle; Treatment 2 (T2): follicular wave reset prior to aspiration of all ovarian follicles, performing the OPU 3-4 days later; Treatment 3 (T3): placement of intravaginal progesterone device for 6 days, together with 2 mg of estradiol benzoate, 50 mg of progesterone and 150µg of prostaglandin, the OPU done at the implant removal; Treatment 4 (T4): similar to T3, however, T4 was applied, 100mg of FSH, 36-48 hours prior to OPU. At the OPU procedure, animals were evaluated for the number and size of follicles, classifying them into small (2-5mm), medium (6-9mm) and large (≥ 9 mm). After collection, the oocytes were processed and classified according to morphological characteristics: Grade A, Grade B, Grade C, Grade D, expanded or naked. Statistical analysis was performed under ANOVA / Duncan test or Kruskal-Wallis test and the significance level was $P < 0.05$. The observed number of follicles per animal in T2 ($7.50 \pm 0.63a$) did not differ from the number T1 ($9.39 \pm 0.66ab$ - $P > 0.05$); but it was lower than T3 ($10.44 \pm 0.92b$) and T4 (11.39 ± 0.67 $P < 0.05$). Regarding quality, the number of oocytes grade A in T3 it was superior to the other treatments (T1 = 0.78 ± 0.22 ; T2 = 0.36 ± 0.20 ; T3 = 2.00 ± 0.50 ; T4 = 0.82 ± 0.32). The recovery rate, the number of oocytes retrieved and the number of oocytes grade B, C, D, naked and expanded did not change ($P > 0.05$) between treatments. The synchronization treatments using hormones (T3, T4) or not (T2) did not alter the average number of follicles, as they did not differ from T1 ($P > 0.05$). There was no difference between the total number of follicles observed at the moment of OPU and between different treatments ($P > 0.05$). However, comparing the number of follicles between the same treatments, we noticed a higher number of small follicles in relation to medium and large follicles ($P < 0.05$). It was concluded that the proposed protocols were not efficient to improve the number and quality of the OPU recovered structures.



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Influence of integrated-crop-livestock system in the *in vitro* production of cattle embryos

**F.A. Melo Sterza¹, W.A. Leite da Silva¹, M.G.C.R. Ferreira¹, H. Kischel¹, G.L. Ortiz¹, M.B. Souza¹,
A.C.B. de Lima¹, E.D.S. Arruda¹, R.F. da Silva¹, R. Poehland²**

¹Universidade Estadual do Mato Grosso do Sul, Aquidauana; ²Leibniz Institute For Farm Animal Biology, Dummerstorf.

Keywords: heat stress, reproduction, Nelore.

The negative influence of heat stress on embryogenesis is known. The environmental management, through natural or artificial shade, can mitigate thermal stress and may favor the animal homeothermic control and, consequently, improve their reproductive performance. The temperature and humidity index (THI) is used to evaluate the thermal comfort. When THI exceeds the value 72, it is considered that cow is in heat stress. When is up 83, it is considered severe stress and may compromise important body functions. In this sense, the objective of the study was to evaluate production of bovine embryos *in vitro* from 16 Nelore donors managed in integrated crop-livestock-forest system (ICLF) or not. For this experiment Nelore breed animals were used, with average weight of 350 kg and 2 years old. They were divided into two experimental areas: the shadow group, managed in ICLF system (n = 8), and the ICL group (integrated-crop-livestock), with little or no natural shade (n = 8). They were installed weather mini-stations in both experimental fields to measure and control environmental data to calculate THI. The animals underwent 3 OPU sessions in the period between December and March 2016. Viable COC's (grades 1 and 2) were matured *in vitro* (IVM) for 24 hours in TCM 199. It was used semen of an *in vitro* fertilization (IVF) proved Nelore bull, which was prepared in Percoll® gradient. The insemination dose was 10⁶ living sperm / mL. IVF was performed in Talp medium for 18-22 hours. After then, we performed the *in vitro* culture (IVC) in SOF medium, where presumptive zygotes were kept for 8 days (D8). All steps of IVP were performed in an incubator at 38.5°C, 5% CO₂ and maximal humidity. The THI demonstrated that both groups were in heat stress condition, however the ICL group was close to the severe stress situation (THI ICLF = 77.70 and ICL= 82.21; P < 0.05), demonstrating that the system ICLF is capable to minimize thermal stress. The COC and cleavage viability rates did not differ between groups (viability rate 55.8 and 53.65; cleavage rate 49.60% and 29.40% for ICLF and ICL respectively.). However, the blastocyst rate was significantly higher in ICLF group (31.7% vs. 16.1%). The results of this study demonstrate that the ICLF system is able to minimize heat stress with direct consequences on the production of bovine embryos.

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Decrease of reactive oxygen species in porcine oocytes by using recombinant oviduct-specific protein in the IVM medium

T.S. Kawamoto¹, L.S. Amorim², L.L. de Oliveira³, P.S. Lopes⁴, S.E.F. Guimarães⁴, J.D. Guimaraes⁵

¹Universidade Federal de Uberlândia, Uberlândia; ²BIOTRAN Ltda, Alfenas; ³Departamento de Biologia UFV, Viçosa; ⁴Departamento de Zootecnia da UFV, Viçosa; ⁵Departamento de Veterinária UFV, Viçosa.

Keywords: *in vitro* maturation, recombinant protein, reactive oxygen species.

The porcine *in vitro* embryo production is noteworthy due to the support given by the culture techniques to cloning and transgenic biotechnologies that increasingly gain importance in the biomedical field. However, *in vitro* embryo production of porcine exhibit barrier linked with high rates of polyspermy and because of the damage that reactive oxygen species (ROS) cause in oocytes and embryos. Thus, this study aimed the production of recombinant oviduct specific protein (pOSP) for the use in maturation medium of *in vitro* porcine oocytes. We evaluated the effect of pOSP (10 µg/mL) in the last four hours of *in vitro* maturation on the concentration of ROS in porcine oocytes. For this, ovaries from slaughterhouse were aspirated to obtain oocytes grade I and II. Subsequently, these oocytes were placed to mature in the medium North Carolina State University 23 (NCSU 23) supplemented with 10% porcine follicular fluid (v:v), 10 mg/mL EGF (sigma), 10 IU/ml of hCG (Vetecor®, Hertape Calier, Brasil), 10 IU/mL eCG (Folligon®, MSD Saúde Animal, Brasil) and 0.1 mg/mL of cysteine (sigma) in the first 22 hours. In the following 22 hours, the oocytes were placed in medium NCSU 23 without hormone supplementation. Four repetitions were performed, with 80 oocytes observed and distributed into two groups. For the production of the oviduct specific protein was obtained a plasmid, company Gen Script®. This plasmid was inserted into competent *E. coli* cells, then the oviduct specific protein was obtained, making use therefore of transgenic techniques, induction of expression, purification and determination of protein levels. The evaluation of ROS was made by staining with dichlorohydrofluorescein diacetate (DCFDA) and then the data were analyzed by ANOVA and means compared by Duncan test with 5% significance. To evaluate the pOSP production, polyacrylamide gel electrophoresis was performed and the dosage was obtained by the Bradford method. Regarding ROS dosage, there were differences between the control group and the group treated with the addition of the recombinant protein ($26.4 \pm 10.9 \times 16.6 \pm 10.5$ %; $P < 0.05$), whereas the treated group showed satisfactory results in reducing reactive oxygen species in porcine oocytes. In conclusion, the technique for the production of recombinant pOSP was satisfactory and this was effective in reducing the amount of ROS, allowing the pOSP to be considered a proteic antioxidant.