A121 OPU-IVP and ET

Adjustment of the use of milrinone in bovine *in vitro* embryo production

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Keywords: *in vitro* maturation, meiotic arrest, phosphodiesterase inhibitor.

In the present study the effect of three concentrations of milrinone (0, 50 and 100 μM), a phosphodiesterase inhibitor which can delay nuclear maturation to sincronize it with citoplasmic maturation, in oocytes of different grades [1+2: n=1252, 3: n=1245 and 4: (denuded): n=1040] were evaluated during *in vitro* maturation (IVM). Oocytes recovered from slaughterhouse ovaries were selected and washed in TCM-199 medium buffered with HEPES, pyruvate, amikacin and 10% fetal bovine serum (FBS). Then they were separated by grades and transferred to drops of maturation medium (TCM-199 NaHCO3 added with pyruvate, amikacin, 10% FBS, FSH and estradiol), containing the milrinone concentration for each group, (n=10-15 oocytes/ drop of 50 μL), totaling 9 groups. To adjust the protocol, three experiments were conducted. In Exp.1 (n=1497) and Exp. 2 (n=1184), oocytes were matured without LH, according to protocols proposed by Thomas et al. (Dev Biol, 244, 215-25, 2002) and Thomas et al. (Biol Reprod, 71, 1142-9, 2004), during 28 h and 24 h, respectively. In Exp.3 (n=856) maturation was done with LH for 24 h. In vitro fertilization (IVF) was performed for 22 h (Exp.1) or 18 h (Exp.2 and 3). At the end of these periods, presumptive zygotes were denuded, placed in culture medium (SOF) and incubated at 38.5°C with high O2 tension. On day 3 after IVF (D3) both feeding and evaluation of the cleavage rate (cleaved structures / oocytes in IVC * 100) were performed. On the 7th (D7) and 9th days (D9) blastocyst (blastocyst / oocyte in IVC * 100) and hatching rates (hatched embryos in D9 / blastocysts in D7 * 100), were evaluated, respectively. Data was analysed in SAS (ANOVA and Duncan test). In Exp.1, treatment effect was observed on: cleavage rate (0: 78.6a±2.3%; 50: 74.2ab±2.5%; 100 μM: 70.7b±2.6%; P=0.03); number of blastocysts (0: 0.6b±0.1; 50: 1.2a±0.2; 100 μM: 0.8ab±0.2; P=0.02) and blastocyst rate (0: 4.1b±1.1%; 50: 8.2a±1.2%; 100 μM: 5.4ab±1.2%; P=0.02). An effect of oocyte quality was observed on: number of cleaved embryos (1+2: 10.5b±0.3; 3: 11.6a±0.3; 4: 8.8c±0.4; P<0.01) and cleavage rate (1+2: 77.4a±2.1%; 3: 82.6a±2.0%; 4: 63.6b±2.3%; P<0.01). In Exp.2, there was a treatment effect on: cleavage rate (0: 56.3a±4.8%; 50: 50.2ab±4.0%; 100 μM: 46.6b±4.6%; P=0.04) and an effect of oocyte quality on: number of cleaved embryos (1+2: 7.1a±0.6; 3: 7.5a±0.7; 4: 5.6b±0.5; P<0.01). In Exp.3, there was only an effect of oocyte quality on: number of cleaved embryos (1+2: 10.8a±0.5; 3: 11.0a±0.6; 4: 6.8b±0.4; P<0.01), number of blastocysts (1+2: 2.2b±0.4; 3: 3.1a±0.4; 4: 2.0b±0.3; P=0.04) and blastocyst rate (1+2: 15.5b±3.0%; 3: 22.3a±3.1%; 4: 25a±4.4%; P=0.05). We conclude that milrinone seems to play greater influence in the dose of 50 μM when extending the period of maturation, and in the absence of LH; oocyte quality is important for IVEP; and although the experiments were not tested simultaneously, the use of LH seems to improve the efficiency of IVEP.

A122 OPU-IVP and ET

Effect of *in vitro* culture in the expression of genes related to epigenetic events in bovine embryo on D14 of development

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Keywords: bovine embryos, methylation, transcripts.

In mammals, correct DNA reprogramming is essential for gametogenesis and normal preimplantation embryonic development. Epigenetic events such as methylation of DNA and histone post-translation modifications have an essential role in reprogramming. The main epigenetic modifications may be affected by environmental factors, being their increase associated with assisted reproductive techniques. This fact may be due to in vitro culture and excessive manipulation. Therefore, abnormal methylation pattern may be responsible by the lower pregnancy rates observed in the IVP embryos compared to the in vivo produced. Considering that epigenetic modifications are catalyzed by enzymes such as DNA methyl (cytosine-5-) transferases (DMNTs), histone acetyltransferase (HAT), histone deacetylase (HDAC) and histone methyl transferases (HMTs), their expression can indicate epigenetic modifications that can compromise embryo development and maintenance of pregnancy. This study aimed to evaluate if IVP can affect expression of genes related maintenance (DNMT1) and establishment of DNA methylation (DNMT3B) and histone methylation (SUV39H1), in bovine embryos at a later stage of development(D14) For IVP, oocytes obtained from slaughterhouse ovaries were matured, fertilized (D0) and cultured in vitro to D7 On D7 of culture grade 1 blastocysts were selected and transferred in number of 10 to the uterine horn of recipient previously synchronized (Group vitro/vivo). As a control, embryos collected at day 7 post-insemination of superstimulated donor were used. After uterine flushing grade one blastocysts were transferred in number of 10 to the uteruses of synchronized recipients. Embryos from both groups (vitro/vivo) were collected in D14, and a biopsy of the trophoblast was collected and stored individually. The biopsies were grouped to form four pools of each group and RNA extraction was performed (RNeasy Mini Kit plus Qiagen). Level of expression of target genes was assessed by real time PCR, being cyclophilin used as endogenous control. The relative values of gene expression were obtained by the corrected amplification efficiency for each gene (Pfaffl equation) $\Delta\Delta$Ct method. Data were submitted to analysis of variance using Prophet 5.0 software. The mean of mRNA levels of target genes were compared using the Tukey test ($P < 0.05$). No difference was detected on transcript abundance of the evaluated genes between in vivo and in vitro produced embryos at D14 of development. These results suggest that in vitro culture did not affect the posterior expression of genes responsible for epigenetic changes and that probably these embryos possess the epigenetic pattern similar to those produced in vivo.

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A123 OPU-IVP and ET

Pre-ovum pick up (OPU) dominant follicle (df) ablation: effect on *in vitro* gyr (*Bos taurus indicus*) embryo production (IVP)

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Keywords: body condition score, embryos, milking cows.

The objective was to evaluate the effect of ablation of dominant follicle three days before ovum pick up (OPU) in lactating Gyr donors, considering oocyte yield and vitro embryo production (IVP). We also evaluated period of the year, body condition score and days in lactation (DEL). OPU procedures were performed 2 - 6 times, since 14 days post-partum at 21-day consecutive intervals. The trial was conducted at the EPAMIG research unit, Uberaba-MG. Twenty seven multiparous and 22 primiparous Gyr donors were randomly allocated in (DF-ablated, n=70) or not (control, n=128). Three days before OPU, cows were submitted to the ablation of DF as well as all follicles > 6 mm in diameter. From 198 OPU sessions, 6034 oocytes were obtained, being 3884 viable, resulting in 1114 blastocysts. Body condition scores (BCS) were attributed to donors on a scale from 1- very thin to 9- extremely obese. The mean number of viable oocytes recovered and blastocyst obtained after IVP per OPU session were 20.0 ± 10.6 and 5.70 ± 4.9, respectively. No effect on IVP yield traits due to parity, OPU session order or to the day post-partum the OPU was performed were detected. A one point increase in BCS resulted in an increase of 1.65 viable oocyte recovered at each OPU. Overall mean BCS was 4.35 ± 1.18 (3.0 – 7.0). Total and viable oocyte production were similar between DF-ablated (23.93±4.72 and 18.02±2.18) and control group (25.82±3.27 and 17.39±2.10), respectively. However, blastocyst production after IVP was superior ($p<0.05$) for the DF-ablated (5.65±1.02) compared to control (3.78±0.97). We conclude that DF ablation previous to OPU increases IVP efficiency. Also, lactating Gyr donors may be submitted to OPU as early as 14 d post-partum with 21 days of interval.

A124 OPU-IVP and ET

Dry and rainy seasonal effects on *in vitro* production program of Sindhi (*Bos taurus indicus)* embryos

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Keywords: bovine, IVP, seasonality.

This study aimed to evaluate the effect of the seasons on the cleavage, blastocyst and conception rates of recipients transferred with in vitro produced Sindhi embryos. The study was conducted based on data from successive sessions of follicular aspiration (OPU) in commercial programs from an IVP central of bovine embryos (Tecgene Technology and Animal Health) located in São Jose do Rio Preto, Sao Paulo, Brazil. The OPU sessions were performed in 154 Sindhi breed donors that belonged to a farm located in Novo Horizonte, Sao Paulo, between January of 2008 and June of 2010. Data obtained from 17 OPU sessions (eight during the rainy season and nine during the dry season) were grouped and evaluated according to the season to which they belonged; the rainy season included the months from November to April and the dry season months from May to September. From a total of 4,485 cumulus-oocyte complexes (COCs) recovered through OPU, 3,092 were matured and submitted to in vitro fertilization with conventional and sexed semen from bulls of the same breed and known fertility. The averages of selected COCs per donor were compared by ANOVA, whereas the data regarding to cleavage, blastocyst and conception rates were compared by Chi-square, adopting 5% of error probability in all analyzes. The proportion of the analyzed parameters obtained during the rainy and dry seasons were respectively: averages of selected COCs per donor of 20.9 and 25.8; cleavage rate of 80.3% (1439/1792) and 84.0% (1456/1733); blastocyst rate of 38.4% (596/1552) and 39.3% (606/1540); conception rate of 28.3% (140/495) and 28% (148/528). There was no statistical difference for any of the parameters ($p>0.05$). This study did not evaluate the interaction between the conventional or sexed semen and the blastocyst rate. However, Seidel Jr et al. (Theriogenology, 52, 1407-1420, 1999) demonstrated that the use of sexed semen decreased the blastocyst rate of in vitro produced embryos when compared to conventional semen. Therefore, it can be concluded that the variables analyzed were not affected by the rainy and dry seasons in an in vitro production program.

A125 OPU-IVP and ET

Prepubertal Holstein heifers have low efficiency when submitted to ovum pick-up and *in vitro* embryo production

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Keywords: Holstein, in vitro embryo production, prepubertal.

The present study evaluated the in vivo ovum pick-up (OPU), in vitro embryo production and the conception rate of embryos from prepubertal Holstein donors. The study was performed at Santa Rita farm, with a completely randomized experimental design, performed in six consecutive replicates with different animals. A total of 128 donors of four animal categories: prepubertal heifers (PP, n=32), pubertal heifers (PU, n=32), lactating cows (LC, n=32) and non-lactating cows (NLC, n=32) were submitted to OPU without previous synchronization, concomitantly. Immediately before the OPU, all follicles were quantified and classified according to their diameter [small (SF ≤ 6mm), medium (MF = 6 to 10mm) and large (LF ≥10 mm) follicles]. Subsequently, all visible follicles (≥2mm) were punctured and the total recovered structures, as well as the quantity and quality grade of viable oocytes were registered. All viable oocytes were submitted to the in vitro embryo production and their development (cleavage and blastocyst rate) was evaluated. The same bull and semen batch were used for the oocytes fertilization of all donor categories. The embryos produced were transferred in crossbred recipients (*Bos taurus* x *Bos indicus*). Variables were analyzed by the GLIMMIX procedure of SAS®. No difference (P=0.08) between the experimental groups were observed in the total number of follicles aspirated (PP: 17.7±2.0; PU: 16.8±1.2; LC: 14.3±0.95; NLC: 19.9±1.6). However, it was found that the PP donors showed higher (P<0.0001) proportion of small follicles when compared to the other categories (PP: 58.6a, PU: 46.4bc; LC: 44.5c; NLC: 53.5%b). Despite the similar (P=0.13) total number of recovered oocytes (PP: 13.5 ± 2.1; PU: 12.5 ± 1.1; LC: 9.8 ± 1.0; NLC: 14.9 ± 1.6), the amount of viable oocytes was higher (P=0.007) in NLC (11.8 ± 1.4a) compared to LC (6.2 ± 0.86b), and the heifers maintained intermediate (PP: 10.0 ± 1.7ab, PU: 7.9 ± 0.8ab). However, it has been found that viable oocytes rate (PP: 74.1a; PU: 63.0b; LC: 62.7b; NLC: 78.9% a P=<0.0001) was similar (P>0.05) between PP and NLC. Moreover, PP donors showed lower (P<0001) cleavage rate (PP: 68.7a, PU: 98.4b, LC: 89.3b; NLC: 88.6%b, P<0.0001), lower (P<0001) blastocyst rate (PP: 5.0a, PU: 12.5b, LC: 17.9b; NLC: 33.1% c, P<0.0001) and fewer (P<0001) produced embryos (PP: 0.5 ± 0.2a, PU: 1.0 ± 0.5b, LC: 1.1 ± 0.6b , NLC: 3.9 ± 0.6c, P<0.0001) per OPU session compared to other categories. Finally, no difference (P=0.13) was observed in the conception rate between embryos from the different categories (PP: 0.0 [0/15]; PU: 9.7 [3/28]; LC: 28.6 [10/25]; NLC: 32.7% [36/74]). In conclusion, prepubertal Holstein donors have lower efficiency at in vitro embryo production in comparison to the other categories. Besides that, non-lactating cows showed to be the most efficient category for in vitro embryo production.

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A126 OPU-IVP and ET

Effect of primer progesterone in oocyte quality and *in vitro* embryo production of Nelore cattle

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Keywords: embryo, IVF, pre-pubertal heifers.

The objectives of this study were to characterize follicle population, quality and number of oocytes recovered and in vitro embryo production in pre-pubertal Nelore heifers previously exposed to progesterone (P4). The experimental design included 42 Nelore females, distributed into 4 experimental groups, as follows: PP-P4-24d- pre-pubertal heifers undergoing (OPU) previously exposed to P4 (CIDR®, Zoetis) for 24 days n=11); PP-P4-7d– pre-pubertal heifers undergoing OPU previously exposed to P4 for 7 days (n=11); PP – pre-pubertal heifers undergoing OPU without previous P4 priming (Negative Control, n=10); and VC – mature cycling cows undergoing OPU previously exposed to P4 for 7 days (n=10). On D0, heifers in PP-P4-24d received a CIDR®, which was removed on D7 of the protocol. These same animals received another P4 device on Day 14. Animals in groups PP-P4-7 d and VC received a P4 device for 7 days (Day 24 to 31). All females received intramuscularly 2.0 mg of estradiol benzoate (BE; Gonadiol, Schering Plough) on day 24 of the protocol. All devices were removed previously follicular aspiration (Day 31). At the time of OPU, ovaries were examined by ultrasonography to estimate follicular population. Data was analyzed with the procedure GLIMMIX of SAS and shown as means ± SEM. Means were assumed different when $P<0.05$. The number of recruited follicles (43.3±2.0 vs 58.9±2.4 vs 58.1±2.4 vs 58.2±2.7, $P<0.001$), number of total oocytes (25.9±1.5 vs 37.1±1.9 vs 41.4±2.3 vs 33.1±2.0, $P<0.001$), and number of viable oocytes (15.9±1.2 vs 26.1±1.6 vs 30.8±1.8 vs 22.5±1.7, $P<0.001$) was less in PP-P4-24d than in PP-P4-7d, PP, and VC, respectively. However, there were no differences among groups in terms of total embryos produced per OPU session (4.7±0.7 vs 4.4±0.7 vs 5.1±0.7 vs 4±0.7, respectively for PP-P4-24d, PP-P4-7d, PP, and VC, $P=0.7$). In addition, the embryo rate (number of embryos/number of viable oocytes) was lower ($P<0,001$) in PP-P4-7d (18.1%) and PP (15.3%) in comparison to PP-P4-24d (29.7%) and VC (25.9%) groups. As a conclusion, prepubertal Nelore heifers exposed to 24 days of progesterone previously to the OPU presented lower number of follicles and recovered oocytes but higher in vitro embryo production rates.

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A127 OPU-IVP and ET

Pregnancy establishment in Holstein cows after transferring *in vivo* or *in vitro* produced embryo

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Keywords: dairy cows, embryo production, pregnancy.

This study aimed to evaluate the conception rate at 30 and 60 days of pregnancy and the pregnancy loss of Holstein recipients after embryo transfer of embryos produced in vivo (superstimulation and uterine flush, SOV-UF) or in vitro (ovum pick-up and in vitro production, OPU-IVP). The database were obtained from a commercial dairy farm (Agrindus S/A, Descalvado-SP) performed during the year 2013. A total of 933 fresh embryos transfer was evaluated; 624 embryos were obtained from the SOV-UF procedure with non-sorted sperm and 309 embryos from the OPU-IVP with sex-sorted sperm. All the donors were Holstein cows. The SOV-UF procedures were performed weekly and OPU-IVP twice a month throughout the year. The recipients were 286.1 ± 2.8 (± SD) days in milk, 4.0 ± 2.8 prior services and 65.5% (611/933) of the donors were multiparous. All recipients were maintained in free-stall facilities receiving diet sufficient to maintain or exceed the requirements of the category according to the NRC 2001. The pregnancy diagnosis was performed by ultrasonography exams at 30 days of gestation and 30 days later the pregnancy was confirmed by rectal palpation. Data were analyzed using the GLIMMIX procedure of SAS 9.3 ®. The recipients that received embryos derived from in vivo production showed higher conception rate at 30 [SOV-UF: 43.8% (273/624) vs. OPU-IVP: 26.5% (82/309); P< 0.0001] and 60 days of gestation [SOV-UF: 34.4% (213/620) vs. OPU-IVP: 20.4% (63/ 309); P< 0.0001] when compared to in vitro embryos produced. However, no difference was observed for pregnancy loss between the two groups [SOV-UF: 20.8% (56/ 269) vs. OPU-IVP 23.2% (19/82); P= 0.79]. In conclusion, embryos of Holstein donors produced in vivo have greater ability to establish pregnancy after embryo transfer into lactating Holstein recipient compared to embryos produced in vitro. However, there was no difference in pregnancy loss.

A128 OPU-IVP and ET

Cushion fluid media in the selection of bovine sperm for IVF

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Keywords: bovine semen, centrifugation, cushion fluid.

Centrifugation on discontinuous Percoll gradients is one of the most used procedures for sperm selection in IVP. However centrifugation may affect irreversibly sperm cells and impair rates of in vitro fertilization. Aiming to minimize potential cell damage caused by centrifugation, Cushion Fluid medium (Minitüb; Tiefenbach, Germany) has been used to process equine and boar semen. In that regard, no studies using Cushion fluid medium for separation of bovine semen have been reported. The aim of present study was to determine the influence of use of Cushion Fluid medium for sperm separation on sperm recovery rate and viability of bovine spermatozoa. A pool of semen from two *Bos taurus taurus* bulls was used (5 replicates). Sperm selection was carried out by mini Percoll discontinuous gradient 30, 60 and 90% method (Folchini et al., Rev. Bras. Repr.Anim., v.36, p.239-44,2012). Samples were centrifuged at 2200 x g for 5', and the pellet formed was diluted in 300 ul of TALP-FERT medium in combination with Cushion Fluid medium according to following experimental groups: no Cushion Fluid (T1), or 150 uL of Cushion Fluid (T2), being immediately centrifuged at 2200 x g for 1'. Semen samples were assessed for motility, vigor, plasma membrane integrity, sperm cell recovery rate, production of reactive oxygen species (ROS), levels of glutathione (GSH) and activity of superoxide dismutase enzyme (SOD). Statistical analysis was carried out using the ANOVA test ($p<0.05$). T1 showed a higher motility (78 ± 4.5%) compared to T2 (68% ± 8.4). No difference was observed in sperm recovery rate between T1 (42.0 ± 4.9%) and T2 (38.6% ± 3.6) groups, as well as in other variables analyzed post-sperm selection. Cushion Fluid medium did not influence sperm recovery rate, membrane integrity, vigor, ROS production, levels of glutathione (GSH) and superoxide dismutase enzyme (SOD) activity, but decreased sperm motility. Further studies should be conducted using the Cushion Fluid medium in the sperm selection process in bovine to verify the effects of the medium on fertilizing capacity and embryonic development.

A129 OPU-IVP and ET

The use of Folltropin® diluted in a slow release carrier (MAP5®) previously to the follicular aspiration increases the efficiency of the in vitro embryo production programs in Holstein donors

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Keywords: hyaluronic acid, *in vitro* embryo, superstimulation.

The study evaluated the effect of the donor superstimulation prior to the ovum pick-up (OPU) with different FSH-P (Folltropin®; Tecnopec) doses diluted in a slow release carrier (hyaluronic acid, MAP5®, Bioniche) in the in vitro embryo production (IVP). A total of 90 non-lactating Holstein donors was distributed into four different groups: Control (CON; n=22); Folltropin 200 mg (FOLL; n=23); MAP5/Folltropin 200 mg (M200, n=22) and MAP5/Folltropin 300 mg (M300, n=23). On a random day of the estrous cycle (Day 0) all cows received an intravaginal progesterone device (P4; Primer®, Tecnopec) and 2mg of estradiol benzoate (BE, RIC-BE®, Tecnopec), intramuscular (IM). On Day 4 and Day 5, the FOLL group received four decreasing doses of Folltropin IM; the MAP5 groups (40mg/mL) received a single dose (IM) on Day 4 AM. The CON group received same protocol, however, with no superstimulatory treatment. The P4 devices were removed on D7 AM and cows were submitted to OPU. Immediately before the OPU, all visible follicles were quantified and classified according to their diameter [small (<6mm), medium (6 to 10mm) and large (>10 mm) follicles]. Data were analyzed by orthogonal contrast using the GLIMMIX procedure of SAS. The contrasts established were: C1 (Superstimulation effect): CON vs. (FOLL+M200+M300); C2 (MAP5 effect): FOLL vs (M200+M300); C3 (dose effect): M200 vs. M300. A greater (C1: P<0.0001) proportion of medium follicles was observed in animals submitted to superstimulation [FOLL: 64.3 (301/468); M200: 67.9 (347/511); M300: 66.5% (300/451)] compared to CON group (19.2%, 68/355). Also, greater number of aspirated follicles (CON: 16.1 ± 1.1; FOLL: 20.4 ± 1.4; M200: 23.2 ± 2.3; M300: 19.6 ± 1.6; C1: P = 0.01), total oocytes retrieved (CON: 13.1 ± 1.0; FOLL: 16.5 ± 1.2; M200: 19.5 ± 2.1; M300: 15.4 ± 1, 4: C1: P = 0.01), viable oocytes (CON: 9.3 ± 0.7; FOLL: 12.2 ± 1.2; M200: 15.6 ± 1.7, M300: 11.4 ± 1.2; C1: P = 0.02), cleavage rate [CON 75.6 (155/205); FOLL: 85.1 (239/281); M200: 79.6 (273/343); M300: 79.4% (210/263); C1: P=0.002] and blastocysts per OPU session (CON: 2.4 ± 0.5; FOLL: 3.7 ± 0.7; M200: 4.7 ± 0.7; M300: 3.1 ± 0.6; C1: P = 0.06) were observed in females submitted to the superstimulation treatment compared to the CON group. Additionally, cows of the M200 group obtained greater recovery rate [M200: 84.0 (429/511) vs. M300: 78.7% (355/451); C3: P = 0.009), higher number of viable oocytes (M200: 15.6 ± 1.7 vs. M300: 11.4 ± 1.2 C3: P = 0.04) and blastocysts per OPU session (M200 4.7 ± 0.7 vs M300: 3.1 ± 0.6, C3: P = 0.06) compared to animals of the M300 group. In conclusion, the administration of Folltropin, associated or not with MAP5®, previously to OPU increases the efficiency of in vitro embryo programs in non-lactating Holstein cows. Additionally, the use of 200mg of Folltropin associated with MAP5 was sufficient to achieve superior results in in vitro embryo programs in non-lactating Holstein cows.

A130 OPU-IVP and ET

Evaluation of different protocols of epidural anesthesia for relaxing cervical in Santa Inês sheep

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Keywords: embryo collection, embryo transfer, small ruminant.

The aim of this study was to promote analgesia and dilation in the cervix of Santa Ines ewes, allowing the passage of a stainless steel rod into the uterus for embryo collection by the nonsurgical method. Thirty primiparous ewes were studied. Each animal underwent four epidural treatments described below with minimum intervals of three weeks between the procedures. G1: NaCl 0.9% (saline - 1mL for each 7.5 kg); G2: ketamine (2.0 mg.kg-1 Cetamin®, Syntec, Cotia, Brazil); G3: ketamine (2.0 mg.kg-1) and morphine (0.1 mg.kg-1 Dimorf ® Cristalia, Itapira, Brazil) and G4: ketamine (2.0 mg.kg-1) and xylazine (0.05 mg.kg-1 kensol®, König, Buenos Aires, Argentina). For G2, G3 and G4 volume was completed with saline solution until the concentration of 1ml for each 7.5kg. For estrus synchronization, the ewes were injected with two doses of synthetic prostaglandin (0.5mL Prolise, Tecnopec®, São Paulo, Brazil) with an interval of 11 days Nine days after the second injection, the animals were sedated with acepromazine (0.1 mg.kg–1 Acepran®, Vetnil, Louveira, Brazil) and diazepam (0.2 mg.kg-1 Diazepam, Teuto, Anápolis, Brazil) duct IV. Ten minutes after sedation of epidural space was punctured with a Tuohy needle and the animals received the epidural injections. Ten minutes later, a Collins speculum was inserted into the vagina, the cervix was clamped and tractioned until the vulvar commissure using an Allis tweezer and was fixed using two Pozzi clamps. The attempts to passage the cervix using a Hegar dilator were performed at 10, 20 and 40 minutes after epidural analgesia and each trial lasted five minutes. When cervix was traversed, a urinary catheter was inserted into the vagina using a mandrel and 20 to 40 ml of saline solution was injected into the uterus to confirm that the catheter was correctly positioned and thus allowing uterine flushing. The following parameters were analyzed using a scoring system (Rafael DeRossi, Small Rum Res 83, 74-8, 2009.): Relaxation of the vulva and vagina, anal relaxation, analgesia and cervical dilation. Data were submitted to ANOVA and Friedman test, and the comparison whether the uterus could be entered among groups was analyzed by the Chi-square test. Regarding analgesia and anal relaxation, there was no difference between G2, G3 and G4 ($P>0.05$) and all of them promoted adequate analgesia and anus relaxation, but just the G1 ($P<0.05$) no followed that. Regarding the transcervical passage rate, for G1 it was 50.0% (15/30), G2 53.3% (16/30), G3 46.6% (14 /30) and G4 53.3% (16/30). These results lead us to conclude that the protocols of epidurals used did not induced dilation of the cervix in ewes, although G2, G3 and G4 have promoted adequate analgesia.

A131 OPU-IVP and ET

Effect of Butirolactone I on the gene expression of PTGS2, GREM1 and PFKP of the cumulus cells in bovine in vitro matured oocytes

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Keywords: butirolactone, cumulus cells, gene expression.

A small proportion of bovine in vitro matured oocytes (IVM) has the potential to develop to embryo. Aiming to improve oocyte competence, some inhibitors of cyclin-dependent kinases, such as butirolactone I (BL), have been used to synchronize the nuclear and cytoplasmic maturation of bovine oocytes. Moreover, the cumulus cells (CCs) also play an important role for the acquisition of in vitro oocyte competence. Therefore, the aim of this study was to investigate the gene expression of PTGS2, GREM1 and PFKP in the CCs of in vitro or in vivo matured (IVOM) bovine oocytes. Ovaries were collected from a local slaughterhouse, and the oocytes from the follicles were obtained. Oocytes were randomly divided into 5 groups: I (immature oocytes); II (IVOM); III (IVM); IV (meiosis block, 12h; reversal, 12h); and V (meiosis block, 24h; reversal, 24h). All reagents were purchased from Sigma-Aldrich (St. Louis, USA), unless otherwise specified. The cumulus-oocytes complexes (COCs) were washed and selected in PBS medium plus 10% FCS (Nutricell®, Campinas, Brazil). The medium 199, supplemented with sodium bicarbonate, sodium pyruvate, penicillin, FSH and LH (Bioniche Inc., Canada), estradiol, cisteamine and 10% FCS was used to IVM. The IVM medium, supplemented with 10 μM of BL, without hormones and FCS, was used to meiosis block. For the IVOM, the estrus of 10 Nelore donor cows were synchronized with progesterone (Primer®, Tecnopec, Brazil), estradiol benzoate (Sincrocio®, Ourofino, Brazil) and cloprostenol sodium (Ciosin®, MSD, Brazil). For super stimulation of the ovaries, FSH was injected (six decreasing doses), and final follicle maturation was induced with gonadorelin (Fertagyl®, Intervet, Brazil). The COCs were obtained through OPU, 19-20 h after gonadorelin injection. The CCs were removed by several pipetting in PBS medium with 0.1% hyaluronidase. Then, droplets containing only CCs were centrifuged (3,355 x g/10 min) and frozen in total RNA extraction medium. Gene expression was investigated by quantitative RT-PCR, normalized by GAPDH constitutive gene. The results were analyzed using ANOVA and Tukey's test, with $P<0.05$ taken as significant. There was no statistical difference among the groups for PTGS2. However, the abundance of transcripts for GREM1 was higher ($P<0.05$) in the CCs from oocytes from group II than from the other groups. The CCs from group II showed higher ($P<0.05$) abundance of transcripts for PFKP compared to groups I and IV, with similar results for groups I and IV. In conclusion, the meiosis block with BL did not affect the abundance of transcripts of any gene investigated, however, as a higher GREM1 expression was observed in the CCs from IVOM oocytes, further researches using different compounds for IVM medium may identify pivotal factors that are related with the expression of these genes in CCs.

Financial support: FAPESP.

A132 OPU-IVP and ET

Apoptosis in embryos exposed to the HSP90 inhibitor during *in vitro* maturation of bovine oocytes

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Keywords: 17AAG, heat shock, *in vitro* maturation.

The heat shock protein 90kda (HSP90) is a cytoprotective chaperone and its inhibition with 17-(allylamino)-17-demethoxygeldanamycin (17AAG, Sigma, St. Louis, USA) during IVM reduces oocyte competence, decreasing embryo production rate (Souza et al., 2013. Anim Reprod, 10:515). To assess possible damage in the embryos after exposure to different 17AAG concentrations during IVM, this study aimed to determine the total number of embryonic cells, apoptotic index of the inner cell mass (ICM) and the trophoblast (TE) of blastocysts. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in a factorial experiment design with three 17AAG concentrations (0, 1 and 2μM) and two-exposure time (12 and 24h) during IVM at 38.5ºC under 5% CO2 and saturated humidity. Oocytes were *in vitro* fertilized (IVF) for 20h and incubated under the same maturation conditions. After IVF, the presumptive zygotes were denuded in a solution of PBS plus 0.1% hialuronidase and then cultured in CR2aa medium supplemented with 2.5% FCS (Nutricell, Campinas, Brazil) in a incubator at 38.5ºC under 5% CO2, 5% O2 and 90% N2, and saturated humidity for 8 days. Cleavage was evaluated at day three and blastocysts were evaluated at day seven and day eight post-fertilization. Expanded blastocysts with 192h post-fertilizationfrom different treatments (0μM=42; 1μM=47; 2μM=39 and 12h=60; 24h=68) were fixed in 4% paraformaldehyde and available by TUNEL assay (DeadEndTMFlorimetric TUNEL System-Promega). Data from each treatment were analyzed by Generalized Linear Model procedure of SAS software (version 9.1) considering effect of exposure time, 17AAG concentration and interaction, and means were compared by Student Newman Keuls test. Values are shown as mean±SEM. There was no difference in the total number of cells, number of apoptotic cells and apoptotic index of cells analyzed embryos derived from treatments with different concentrations or time of exposure to the inhibitor, nor interaction between concentration and exposure time. However, there was a decrease in the number of cells of the ICM of embryos from oocytes treated with 1μM and 2μM of 17AAG compared to 0μM (36.36±1.26, 39.68±1.68 and 43.42±1.95, respectively). The data also showed differences ($P < 0.05$) in the number of apoptotic cells of TE for 0μM, 1μM and 2μM (15.97±2.8a, 12.05±1.54a,b and 7.23±0.84b, respectively) and in the TE apoptotic index among embryos from oocytes exposed to a higher concentration of the 17AAG in maturation medium (2μM - 8.81±0.80) compared to the concentrations of 0μM and 1μM (15.51±1.78, 13.64±1.56, respectively). The use of 17AAG during IVM does not interfere on blastocyst total cell number or general apoptotic index, but it can reduce ICM cell number and TE apoptotic index, resulting in lower embryo developmental ability, as already reported in previous study.

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A133 OPU-IVP and ET

Effect of holding medium and temperature on bovine oocytes on maturation and subsequent embryonic development

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Keywords: bovine, *in vitro,* oocyte.

Holding immature oocytes before maturation simplifies their transport and helps in scheduling later manipulations for in vitro production of bovine embryos. The objective of this study was to evaluate the effect of holding bovine oocytes in maturation medium (MIV, Vitrogen®, Brazil) or H-199 medium (H-199, Vitrogen®, Brazil) for 5 hours at different temperatures (38ºC or 5ºC) on subsequent embryonic development after in vitro fertilization. Bovine ovaries were collected at an abattoir and transported during 2 hours to the laboratory. Cumulus–oocyte–complexes (COCs) were recovered by follicle aspiration with an 18G needle and syringe, using PBS supplemented with 70ug/ml of gentamicin and 0.1% of PVA, this solution was used for holding oocytes during the recovery procedure. The COCs recovered were divided into four groups (G1: MIV at 38ºC; G2: MIV at 5ºC; G3: H-199 at 38ºC and G4: H-199 at 5ºC). Subsequently, the COCs in each treatment group were matured for 22–24 h before fertilization and in vitro culture. Oocytes were fertilized using frozen–thawed semen that was used before in other in vitro fertilization procedures with good blastocyst production (control bull). A selection of motile spermatozoa was carried out by gradient centrifugation with 90/45% discontinuous Percoll density. Fertilization was performed using 10ul of sperm (final concentration 1 x 10^6 spermatozoa/ml) in 60ul of fertilization medium containing oocytes. The gametes were incubated for 18 to 22 h at 38.5ºC under 5% CO_2 in 100% humidified air. Immediately, zygotes were washed and transferred to culture medium into 70ul microdrop covered with mineral oil. On day 7 (D7) of culture (day 0; D0=IVF day), blastocyst production was evaluated identifying the blastocele in the embryos. A total of 208 oocytes were analyzed in this experiment. Differences in blastocyst rates between the experimental groups were analyzed as a completely randomized design with 4 treatments and 4 replicates for each analysis (G1: MIV at 38ºC; G2: MIV at 5ºC; G3: H-199 at 38ºC and G4: H-199 at 5ºC) and the mean comparisons were made with LSMEANS and Duncan test with significance defined at $P<0.05$. Statistical evaluations were carried out using the SAS Software, PROC GLM. The blastocyst development rate was superior in G1 (32.1%) and G3 (26.4%) at 38ºC compared to G2 and G4 in which oocytes were kept at 5ºC (3.9% and 3.9%, respectively). The number of blastocysts developed at 38ºC was significantly different ($P<0.005$) from those kept at 5ºC before going to the microdrops of maturation. These results indicate that the temperature at which oocytes are held has a significant effect on embryonic development when comparing holding media and that the phase of oocyte transportation to the laboratory after the ovum pick up procedure must be well controlled and monitored in order to obtain successful in vitro production of bovine embryos at commercial level.

A134 OPU-IVP and ET

Effect of sustained treatment with a GNRH agonist on the follicular population and *in vitro* embryo production from *Bos indicus* donors: preliminary results

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Keywords: follicular population, Nelore, OPU.

The objective of this study was to evaluate the antral follicle population, the in vivo ovum pick up procedure (OPU) and the following in vitro production (IVP) of embryos from Nelore cows (*Bos indicus*) treated during 120 days with a GnRH agonist (Deslorelin; Suprelorin 12®; Virbac). A total of 20 cycling cows (CL presence) was divided in two experimental groups: Deslorelin Group (n=10; cows receiving an ear implant loaded with the GnRH agonist); and Control Group (n=10; cows that received a blank ear implant without the GnRH agonist). All animals underwent an OPU session (Day -14) before applying the ear implants (Day 0). Immediately before the application of the ear implants (Day 0) all females underwent their second OPU session, followed by 4 consecutive OPU sessions spaced 30 days apart (Day 30 to Day 120). The same semen batch from a single previously tested sire was used in all IVP procedures. Repeated measures analyzes were performed with the procedure GLIMMIX of SAS 9.3, taking into account the effects of treatment, time and one-way interaction between treatment and time, with "cow" kept in all models as a random effect. There were no treatment-by-time interactions for most variables (P>0.05), except by proportion of embryos produced (P=0.02). The Deslorelin Group presented a greater number of visualized follicles (40.9±4.4 vs 33.1±3.8; P=0.09), total recovered oocytes (20.6 ± 2.5 vs 16.0 ±2.0; P=0.07), viable oocytes (16.0 ± 2.2 vs 11.8 ±1.8; P=0.07), proportion of viable oocytes (76.5±2.7 vs 69.1±2.6; P=0.03), cleaved embryos (10.0 ± 1.6 vs 6.9 ± 1.3; P=0.07) and proportion of cleaved embryos (62.1% vs 59.1%; P=0.3) compared to Control Group. In contrast, there was no effect of treatment in terms of number of produced embryos per OPU session (3.44 vs 3.28; P=0.44). In addition, there was a significant effect of time for number of visualized follicles (P=0.01), total recovered oocytes (P=0.05), viable oocytes (P=0.08), proportion of viable oocytes (P=0.006), cleaved embryos (P=0.07) and proportion of cleaved embryos (P<0.0001). However, there was no difference (P=0.18) in number of embryos produced per OPU session throughout the time. Interestingly, cows in Control Group had greater (P=0.02) proportion of produced embryos at Day 90 (57.5%) and Day 120 (31.9%) compared to cows in Deslorelin Group (90d: 22.0 and 120d: 11.2). In conclusion, these results suggest that the sustained use of a GnRH agonist can increase the number of visualized follicles in the ovaries as well as the total amount of retrieved oocytes and viable oocytes per OPU session. Nevertheless, long-term use of a GnRH agonist may reduce the proportion of viable embryos produced.

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A135 OPU-IVP and ET

Effect of the ovarian cyclicity on quality and nuclear competence of bovine oocytes submitted to *in vitro* maturation

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Keywords: cyclicity, nuclear maturation, oocytes.

The efficiency of in vitro production of bovine embryos (PIV) depends, largely, of the quality of the gametes used to carry out the process (De Wit et al. 2000, J Anim Sci, 78, 1277-283). On each session of follicular aspiration (OPU), a heterogeneous population of oocytes, with different developmental capacity after in vitro fertilization (IVF), is obtained. It is postulated that the quality of the oocytes depends of the quality of follicles in which they are developed (Lonergan and Fair, 2008, Theriogenology, 69, 17-22). However, the full identity of the factors that regulate the follicular and/or oocyte quality is not yet known. This study aimed to evaluate the effect of the ovarian ciclicity of cows on quality and nuclear competence of oocytes submitted to in vitro maturation. Ovaries were obtained from local slaughterhouses in pairs belonging to the same animal and classified into three experimental groups: 1) with corpus luteum (CL+); 2) without corpus luteum from cows with cyclic sexual activity (CL-); 3) ovaries from cows in anestrus (NCL). After follicular aspiration, the cumulus-oocytes complex (COCs) were classified into four quality grades, depending on their morphological characteristics (Leifried and First, 1979, J Anim Sci, 48, 76-83). Afterwards, groups of 20 oocytes (360/experimental group), classified as grade 1 and 2, were cultivated for 24 hours at 38.5 ° C and 5 % CO2, in 100 µL of maturation medium (TCM-199/SFB). Nuclear maturation was assessed by orcein 2 % at 7, 14 and 24 h of culture. Results were assessed by t-test and means were compared using Tukey test at 5 % probability. No differences ($P>0.05$) were observed in the quality of oocytes retrieved in the different grades of classification evaluated (21.6±2.6, 23.3 ± 2.5 and 24.3±3.5 in grade 1; 22.3±1.7, 27.7±2.4 and 24.7±3.8 in grade 2; 25.9±2.8, 25.6±2.0 and 21.9±2.7 in grade 3; 30.1±2.7, 23.2±3.2 and 28.3±4.5 in grade 4, for CL+, CL- and NCL, respectively). At 14 h of culture there was a greater ($P<0.05$) rate of oocytes at MI stage in the group CL+ in relation to NCL (73.3±5.4 vs 56.2 ± 9.6). However, the proportion of oocytes that reached the MII stage at 24 h of culture was lower ($P<0.05$) in CL+ group compared to the CL- group (45.6 ± 2.3 vs 73.2±6.4). In conclusion, these results suggest that the ovarian activity of cows has no effect on the morphological quality of COCs retrieved. The presence of the corpus luteum decreases the ability of immature oocytes to reach the MII stage at 24 h of in vitro culture.

A136 OPU-IVP and ET

Influence of follicular diameter and time of cleavage in *Bos indicus in vitro* produced embryos

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Keywords: early cleavage, follicular size, late cleavage.

In the in vitro production (IVP) of embryos, the diameter of the follicles from which oocytes are obtained and the time of the onset of the first cleavage possibly affect oocyte competence and early embryonic development. The aim of the present study was to investigate the developmental potential of early-, intermediate- and late-cleaving bovine blastocysts after fertilization of oocytes from follicles up to 2 mm and from 4 to 8 mm diameter. Ovaries (n = 991) from *Bos indicus* cows were obtained from a local slaughterhouse. From the selected oocytes, 699 from the ≤ 2 mm Group and 639 from the 4-8 mm Group were subjected to in vitro maturation and fertilization. The embryos were cultured and the onset of the rate of cleavage was evaluated, originating the early- (≤ 28 h after IVF), intermediate- (28-34h after IVF) and late-cleavage groups (34-54 h after IVF). The blastocyst rate was also assessed. Cleavage and blastocyst rates were compared by a logistic regression test, with a significance level of 5%. Cleavage rates were higher ($P < 0.05$) in the late cleavage groups when compared to the early cleavage ones, for both 4-8 mm follicles (4-8 mm late: 30% vs. 4-8 mm early: 19%), and ≤ 2 mm follicles (≤ 2 mm late: 33.8% vs. ≤ 2 mm early: 16.6%). Cleavage rate of the intermediate cleavage group was higher ($P < 0.05$) in embryos derived from follicles of 4-8 mm (28.4%) than ≤ 2 mm follicles (21.5%). The blastocyst rates of the 4-8 mm follicles (36.3%; 161/444) were higher than ≤ 2 mm follicles (22.9%; 111/485; $P < 0.05$) and the rates of the groups of early and intermediate cleavage (45.3%; 100/221; and 38.3%; 118/308; vs. 13.5%; 54/400; of the late cleavage group, $P < 0.05$). In conclusion, higher rates of blastocyst were obtained from embryos that cleave up to 34 h post-fertilization and from embryos derived from follicles of 4-8 mm of diameter, indicating a higher development competence of the embryo pre-implantation stage.

A137 OPU-IVP and ET

Hatching rate of PIV bovine embryos vitrified using ops with Ethylene glycol (EG) + Dimethyl sulfoxide (DMSO) at different concentrations

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Keywords: embryo, OPS, vitrification.

The bovine embryos produced *in vitro* (IVP) are more sensitive to freezing, due in part to the accumulation of lipid droplets in the cytoplasm of embryonic cells. Moreover, the success of embryo cryopreservation PIV is fundamental to the development of marketing of genetic material in domestic and foreign markets. In this study, two experiments were conducted: in the first, the aim was to evaluate the hatching rate of bovine embryos vitrified IVP in the expanded blastocyst (Bx) stage using the methodology OPS ("open pulled straw") with the use of ethylene glycol solutions (EG) + Dimethyl Sulfoxide (DMSO), at increasing concentrations, Treatment 1: 5% and 10%, Treatment 2: 10%and 20% and Treatment 3: 15% and 25%, associated with 1M glucose. The second experiment was aimed to determine the hatching rate of bovine IVP embryos, vitrified BX on stage with the use of solutions with increasing concentrations of ethylene glycol (EG) + Dimethyl sulfoxide (DMSO), Treatment 1, 5% and 10%, and 10% Treatment 2, and Treatment 3, 20% 15% and 25 %, associated with 1M of sucrose. In experiment 1, the hatching rate of the embryos unglazed group (G3) was 72% higher ($P<0.05$) for vitrified groups, which showed hatching rates of 0% (Treatment 1), 0% (Treatment 2) and 23% (Treatment 3). In experiment 2, the hatching rate of the embryos unglazed group was 76% higher ($P<0.05$) for vitrified groups, which showed hatching rates of 0% (Treatment 1), 0% (Treatment 2) and 70% (Treatment 3). Among the vitrified groups, embryos from Experiment 2 Treatment 3 using the combination of 15% and 25% EG and 1M Sucrose DMSO had higher hatching rate ($P<0.05$). Therefore, it is concluded that EG + DMSO solutions at higher concentrations associated with 1M solution of sucrose showed good results in vitrification and can thus improve cryotolerance of embryos.

A138 OPU-IVP and ET

Gene expression of GREM1, PTGS2 and PFKP in bovine cumulus cells from *in vivo* or *in vitro* matured oocytes supplemented with different macromolecules

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Keywords: cumulus cells, gene expression, *in vitro* maturation.

Gene expression of GREM1, PTGS2 and PFKP in cumulus cells (CCs) can be identified in oocytes with high potential for in vitro development. Therefore, this study was designed to quantify the transcripts of these genes from bovine oocytes in vivo matured (IVOM) or cultured in in vitro maturation (IVM) medium, supplemented with different macromolecules. Ovaries obtained at slaughterhouse had their follicles (2 to 7 mm in diameter) punctured and the cumulus-oocyte complexes (COCs) divided in two groups: control group (immature oocytes) and IVM group. All reagents were purchased from Sigma-Aldrich (St. Louis, USA), unless otherwise specified. The COCs were washed and selected in PBS medium plus 10% FCS (Nutricell®, Campinas, Brazil). For the IVM, groups of 20-25 oocytes were cultured in Medium 199, supplemented with sodium bicarbonate, sodium pyruvate, penicillin, FSH and LH (Lutropin®, Bioniche Inc., Canada), estradiol, cisteamine and 10% FCS, 4 mg/ml BSA or 1 mg/ml PVA, at 38.8º C, under humidify atmosphere, 5% CO2, in air, for 24 h. For the IVOM, ten Nelore donor cows were synchronized (D0) using intravaginal progesterone device (Primer®, Tecnopec, Brazil) and estradiol benzoate (Sincrocio®, Ourofino, Brazil). On the days 4, 5 and 6 the animals received 180 mg of FSH (Folltropin-V®, Bioniche Inc., Canada), in six decreasing doses, and on D6 cloprostenol sodium (Ciosin®, MSD, Brazil) was administered. On D7 the progesterone device was removed and ovulation was induced with gonadorelin (Fertagyl®, Intervet, Brazil). The COCs were obtained through OPU, 19-20 h after receiving gonadorelin. The CCs were removed by several pipetting in PBS medium with 0.1% hyaluronidase. Then, droplets containing only CCs were centrifuged (3,355 x g/10 min) and frozen in total RNA extraction medium. Gene expression was investigated by quantitative RT-PCR, normalized by GAPDH constitutive gene. The results were analyzed using ANOVA and Tukey's test, with $P<0.05$ taken as significant. The amount of transcripts for the gene GREM1 was higher ($P<0.05$) in CCs obtained from IVOM than for immature oocytes or IVM groups. The CCs of immature oocytes or IVM + PVA showed lower abundance of transcripts for PTGS2, when compared with CCs recovered from IVOM. There was no statistical difference among the IVM groups for the gene PFKP, however, lower expression was found in CCs obtained from immature oocytes in comparison with oocytes derived from IVOM. Thus, it be concluded, that gene expression of GREM1, PTGS2 and PFKP in CCs, under the experimental conditions, was not altered by the addition of different macromolecules in IVM medium, however, a higher expression of GREM1 in CCs recovered from IVOM suggests further researches addressed to establish more efficient medium and/or IVM conditions to improve oocyte competence.

A139 OPU-IVP and ET

Cervix dilation and trasncervical embryo recovery in cervical Santa Inês sheep

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Keywords: cervical relaxation, non- embryo transfer, sheep.

The objective of this study was to evaluate cervix relaxation with or without estradiol aiming to collect embryo by non-surgical technique in Santa Inês ewes. A total of 24 pluriparous ewes had estrus induced by intravaginal sponges (60mg MAP, Progespon®, Syntex, Buenos Aires, Argentina) for six day plus 200IU eCG (Novormon 5000®, Syntex, Buenos Aires, Argentina) i.m. and 37.5µg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina) latero-vulvar, 24h before sponge removal. The ewes were monitored for estrus detection from sponge removal at 12h interval end bred with fertile rams. Twelve hours after estrous onset, with the aid of Allis forceps, cervix were immobilized for traction and a HEGAR dilator was inserted into cervical ostiun to check the facility of transposing. After this, ewes were equally shared between to treatments according to body condition score and cervical transposing to be subjected to transcervical uterine flushing seven days after estrous onset. In Group (n=12; 6 ewes with cervical transposing), ewes received 37.5 µg d-cloprostenol latero-vulvar and 1mg estradiol benzoate (Estrogin®, São Paulo, Brazil) i.m. 16h before uterine flushing and 50 IU oxytocin (Ocitocina Forte UCB®, São Paulo, Brazil) i.v. 20min before uterine flushing. In Group 2 (n-12; 5 ewes with cervical transposition at estrus), ewes received the same treatment of the G1 but changing estradiol for 1 mL saline. Qualitative variables were analyzed by qui-square and the quantitative analysis of variance to check differences between treatments with means tested by t-test (5%, SAEG®). Parameters evaluated were similar for both groups (P>0.05). Estrous response was 100%. The interval from sponge removal to estrous onset and duration of estrus were 41.5±8.6 and 36.0±10.6 h, respectively. Cervical transposing at estrus was 46% (11/24). Successful uterine flushing was performed in 33% G1 (4/12) and 25% G2 (3/12) ewes. In successful and non-successful uterine flushed ewes 6.0±1.1 and 3.3±1.6 cervical rings were transposed, respectively. All G2 flushed had cervical transposing at estrus. Two successful uterine flushed G1 ewes had no cervical transposing at estrus. The efficiency of uterine flushing (liquid injected / recovered) was 94%. Embryo recovery varies from 0 to 3 structures (1.0±1.1) per flushing. With this study it is concluded that the technique of transcervical collection closed loop is possible in sheep because has satisfactory rate of washing performance. Studies with other agents for cervical relaxation are encouraged.

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A140 OPU-IVP and ET

Influence of rubber piston syringe used in recovery of oocytes in the development of sheep embryos produced *in vitro*

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Keywords: granulosa cells, heat inactivated estrus sheep serum, *in vitro* fertilization.

This research evaluates the influence of the rubber piston syringe used in follicular puncture for production and survival of embryos produced by in vitro fertilization (IVF). The oocytes were obtained from sheep ovaries from slaughterhouses. In this case 195 follicles> 1 mm and <2mm of 65 ovaries were aspirated. The ovaries (n = 30) with punctured rubber piston syringe form the group 1 and the remainder (n = 35) was aspirated with a plastic syringe plunger, characterized the group 2. Oocytes were classified according to the presence of the cumulus oophorus cells (CCO) in: a) totally surrounded by CCO, b) partially surrounded by CCO, or c) undressed. A total of 42 oocytes was obtained, with an average of 0.69 oocytes per ovary, or 0.23 oocytes by puncturing. Only 32 oocytes classified as a) and b) were used for IVF. Was used for the maturation TCM199 medium (GIBCO, USA) with the addition of 20% sheep serum in heat inactivated (HISS) and antibiotics (penicillin, streptomycin, amphotericin, Cat 5245AE-600 (GIBCO, USA) in an atmosphere with 5% CO2 in air 38.5° C. The Semen was trained in Ovidutal Synthetic Fluid (SOF Tervit, HJRF'72, 30, 493-497), supplemented with 20% HISS 5 hours in a sealed tube at 38 ° C. From the Group 1) were in total 15 oocytes, 11 type a) and type b 4). In Group 2) a total of 17 oocytes, 16 of the type a) and type 1 b) were obtained. The techniques and methods used for maturation, fertilization and culture were similar to group 1) and Group 2). After fertilization presumptive zygotes were cultured for 96 hours in an oven, under the same conditions of IVM. After 48h of IVF the group 1) showed 4 morulae and 5 two-cell embryos, and 6 unfertilized oocytes; Group 2) produced 5 morulae, 3 two-cell embryos and 9 unfertilized oocytes. After 96 hours post-IVF the Group 1) showed 2 blastocysts, 2 morulae and 5 embryos at the two-cell stage. However, the Group 2) did not develop the embryos and 5 morulae and 3 embryos in two cell stage showed signs of cytoplasmic degeneration and retraction of blastomeres. The results were analyzed by the method of differences between proportions, that for the production of embryos in the first 48 hours post-fertilization there were no significant differences between the two methods, however, there were differences with respect to embryo survival after 96 h after IVF (P = 0.08). Thus, there was no difference in embryonic development until 48 hours after IVF, comparing the use of syringe plunger with plastic or rubber for the recovery of sheep oocytes. However, 96 hours after IVF embryos produced from oocytes retrieved puncture using syringe plunger stop the development and showed signs of degeneration of blastomeres.

A141 OPU-IVP and ET

Oocyte recovery and blastocysts production of Nelore donors on OPU-IVP program in commercial production of embryo

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Keywords: *Bos indicus*, fertilization, follicle.

The present study aimed to determine the influence of donor age and weather season on oocyte retrieval and blastocyst production using sexed or conventional semen. Data were obtained from a commercial program of Nelore embryos. A total of 12 Nelore donors (*Bos taurus indicus*), aged 3-17 years was used in 198 sessions of follicular aspiration, being on average 16 OPU per cow with 57 days of interval. The overall average of blastocyst by aspirated oocytes was 38.3% per OPU. To determine the influence of age on oocytes and blastocysts we performed Pearson correlation analysis. To determine the influence of seasons (autumn-winter or spring-summer) and type of semen used (sexed and non-sexed) we used the unpaired t test. For all comparisons, we adopted a 5% level of significance. The age of the donor interfered significantly on the number of oocytes and blastocysts. There was a negative correlation ($p < 0.05$) between age and number of oocytes ($r = -0.7535$) and number of blastocysts ($r = -0.6943$). Perhaps the decrease of blastocyst production with advancing age of the donor is due to lower oocyte recovery and not by the lower competence of oocytes, because there was no correlation ($p > 0.05$) between age and oocyte competence (% blastocysts). A higher ($p < 0.05$) blastocyst production in spring-summer (42.58 ± 13.47) was observed, in comparison to autumn-winter (33.75 ± 9.10). The use of sexed semen in IVF reduced ($p < 0.05$) the rate of blastocyst production when compared to the non-sexed semen (26.08 ± 17.44 vs 39.91 ± 10.92, respectively). In summary, we observed a lower oocyte recovery with increasing age of the donor. The seasons and the type of semen influenced blastocyst production. The best results occurred during spring-summer using non-sexed semen.

A142 OPU-IVP and ET

Reduction of intracytoplasmic lipid accumulation in bovine oocytes matured with linolenic acid did not affect the nuclear maturation and acquisition of competence for embryonic development

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Keywords: in vitro maturation, intracytoplasmic lipid accumulation, linolenic acid.

This study was conducted to evaluate the effects of supplementation with different concentrations of linolenic acid (ALA) during IVM of bovine oocytes, in the presence of FCS or BSA, on the intracytoplasmic lipid accumulation, nuclear maturation and subsequent embryo development in vitro. COC's were IVM during 22h at 38.5 °C and 5% CO2 in air, in TCM-199 medium with bicarbonate, hormones and macromolecules (10% FCS or 0.6% BSA, Control FCS and Control BSA groups, respectively). According to the treatments, the medium was supplemented with 10 (ALA 10 FCS or BSA), 50 (ALA 50 FCS or BSA) or 100 μM ALA (ALA 100 FCS or BSA). For determination of the relative amount of lipid content, immature oocytes (n=232) and IVM (n=656) were stained with Sudan Black B, a lipophilic dye, and examined by light microscopy. After grayscale conversion, oocytes were delimited to determine the area and the average intensity (pixels) were analyzed by Q-Capture Pro image software. For determination of the nuclear maturation rate (n=1061), oocytes were stained with Hoechst 33342 and evaluated under a fluorescence microscope (404nm excitation and 526nm emission) to determine the percentage of mature oocytes (metaphase II). A sample of COC's (n=1332), IVM in the same conditions above described, was subjected to IVF and the zygotes were IVC in SOFaa media at 38.5 °C and 5% CO2 in air, for 7 days. Development rates until blastocyst stage (Bl) were evaluated at 168 hpi. The statistical model was a complete factorial, including the effect of the culture media supplement, ALA concentration and interactions. The averages were compared by Tukey's test ($P<0.05$). As there was no significant interactions ($P>0.05$) in the nuclear maturation and embryonic development analysis, only the main effects were presented as least squares means (LSM) ± standard error mean. The relative lipid content was 178.4±3.3 in immature oocytes, which was increased ($P<0.05$) after IVM in groups Control FCS (218.0±4.8), ALA 10 FCS (198.8±3.9), ALA 50 SCB (199.7±5.5), ALA 10 BSA (201.3±3.7) and ALA 50 BSA (205.1±3.9), but remained similar to immature oocytes ($P>0.05$) in the groups ALA 100 FCS (186.6±4.9), Control BSA (197.7±4.5) and ALA 100 BSA (187.8±4.6). Nuclear maturation rates were not affected by the macromolecule (BSA: 72.2±2.5 and FCS: 74.8±2.5) or by ALA concentrations (ALA 0: 75.5±3.5, ALA 10: 73.4±3.5, ALA 50: 75.2±3.5 and ALA 100: 70.0±3.5). Bl production rates were affected ($P<0.05$) by the macromolecule (BSA: 29.0±2.6 and FCS: 39.3±2.6), but were not affected ($P>0.05$) by ALA concentration (ALA 0: 32.3±3.7, ALA 10: 33.0±3.7, ALA 50: 32.2±3.7 and ALA 100: 39.2±3.7). The supplementation with 100 μM ALA in IVM medium supplemented with FCS or BSA resulted in reduction of the intracytoplasmic lipid accumulation in bovine oocytes. However, this supplementation did not influence the oocyte nuclear maturation and the subsequent embryonic development.

A143 OPU-IVP and ET

Influence of breed composition of crossbred cows between Gir and Holstein breeds in performance as oocyte donors

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Keywords: *in vitro* embryo production, oocyte quality, ovum pick-up.

The aim of this study was to investigate the effect of the breed composition on the quality of oocytes obtained from Gyr and Holstein crossbred donors used in embryo IVP system. Crossbred cows were assigned to two groups: Gh (lower Holstein composition, between 40.2 and 46.6%; n=10) and Hg (higher Holstein composition, between 71.4 and 87.5%; n=10). Donors were subjected to ultrasound guided ovum pick up (n=10 OPU sessions per group) performed by the same technician in which cumulus-oocyte complexes were collected and classified morphologically (according to number of layers of cumulus cells and homogeneous cytoplasm) in Grades I, II, III and nonviable. The evaluated parameters compared between groups were: mean number of GI, GII, GIII, and total oocytes obtained per procedure, viable oocyte rate, oocyte recovered rate, mean number of follicles between 2 and 8 mm detected and number of follicles detected at right and left ovary. Means were compared between groups using Student T test and the rate of viable and recovered oocytes by Chi-square test. Statistical analysis was performed using GraphPad Instat 3 software, considering the significance level of 5%. 474 COCs (294 for the Gh group and 180 for the Hg group) were collected. No significant differences ($p>0.05$) between groups Gh and Hg, respectively, were observed for the mean number of GI (0.1 ± 0.32 vs. 0.1 ± 0.32), GII (1.9 ± 1.73 vs. 0.9 ± 1.60), and GIII (7.2 ± 4.52 vs. 4 ± 3.83) oocytes, mean viable oocytes (9.2 ± 5.92 vs. 5 ± 5.08), rate of viable oocytes (31.94 vs. 27.78%), mean of visualized follicles in the right ovary (16.9 ± 5.30 vs. 13.3 ± 4.69) and left ovary (12.5 ± 6.15 vs. 11.5 ± 6.08), and recovered oocyte rate (128.41% vs. 109.27%). Regarding the number of 2-8mm detected follicles, a difference ($p<0.05$) between Gh (22.6 ± 6.22) and Hg (15.4 ± 5.42) groups was observed. We conclude that no differences were detected in most characteristics, except on the average of 2-8mm follicles, which was increased in donors with lower Holstein composition. It is possible that the high rate of recovered oocytes for both Hg and Gh groups are due to puncture of follicles smaller than 2 mm, which ultrasonographic visualization is difficult due to technical limitations. Therefore, the results suggest that the degree of Holstein breed do not affect the quality or retrieval of oocytes from crossbred donors, considering the parameters and breed compositions evaluated, however, we observed a positive effect of Gyr breed on visualized follicular population.

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A144 OPU-IVP and ET

Can use of FSH during synchronization of follicular wave emergence of Holstein donors improve the efficiency of IFV programs?

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Keywords: follicular aspiration, FSH IVEP.

Several groups have reported the occurrence of a positive effect of FSH stimulation prior to OPU on the quality of oocytes recovered and developmental competence of oocytes. However, there are still some questions regarding the best protocol using FSH for this purpose, because there is huge variation in the number and time of FSH applications, and the interval between the last application of FSH and the OPU (Coasting period) . In this context, it is worth noting that in addition to stimulating the growth of a group of follicles to the diameter of > 5 mm, the coasting period appears to be essential for the acquisition of oocyte competence (BLONDIN et al., Theriogenology, v.48, p.803–813, 1997). The hypothesis of this study is that the use of 200 mg FSH split into 4 or 6 doses in non-lactating Holstein cows with synchronized follicular wave emergence, increases the number of recovered and viable oocytes, and the number of embryos IVP. Thirty six Holstein cows used as oocyte donors were homogenously allocated to one of three treatment groups in a 3x3 Latin square design: Control (C); 4 doses of FHS (F4); 6 doses of FSH (F6). All cows were synchronized using the same protocol for synchronization of follicular wave emergence, except for the administration and number of doses of FSH as previously described. At random days of the estrous cycle (D0), all cows received an intravaginal P4 device (Primer®, Tecnopec-Agener União, São Paulo, Brazil) and 2mg estradiol benzoate (Ric-BE®, Tecnopec-Agener União). Three days after (D3), all cows received 0.150 mg D-Cloprostenol (Prolise®, Tecnopec-Agener União). Cow from Control group received no additional treatment. Cows form group F4 were treated with 200 mg FSH split in 4 doses of similar concentration given approximately 12 h apart, starting on D4 AM. Cows form group F6 were treated with 200 mg FSH split in 6 doses of similar concentration given approximately 12 h apart, starting on D3 AM On D7, the device was removed and OPU was done concomitant with antral follicle count in each ovary. The oocytes considered as viable were sent to IVEP. Data was analyzed Shapiro Wilk test and Analysis of Variance was undertaken using the PROC MIXED of SAS 9.3, using orthogonal contrasts C1 (C x Treatment with FSH) and C2 (F4 x F6). There was no difference between groups in the embryo conversion rate ($\mu = 25.01 \pm 1.92$; $P = 0.50$) and the number of viable embryos ($\mu = 32.23 \pm 2.76$, $P = 0.89$). However, when the cows were treated with F4 greater number of oocytes were retrieved (19.22 ± 1.98 vs 16.44 ± 1.58; $P = 0.04$), and greater number of viable oocytes (13.36 ± 1.49 vs 10.97 ± 1.29, $P = 0.03$) and oocytes sent for in vitro culture (15.72 ± 1.64 vs 13.25 ± 1.46; $P = 0.04$) were observed compared to F6. Thus, the use of 200 mg FSH split in 4 doses may increase the number of oocytes retrieved from OPU and sent for in vitro culture.

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A145 OPU-IVP and ET

Effect of paraoxonase 1 (PON1) during oocyte maturation and subsequent development of IVP bovine embryos

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Keywords: antioxidant, bovine embryos, PON1.

Some studies suggest that the enzyme paraoxonase 1 (PON1) may exert an antioxidant effect on cell membranes. Therefore, it could improve oocyte competence by minimizing oxidative stress. The aim of this study was to evaluate the effect of different levels of PON1 added to the IVM medium on both cleavage and blastocyst rates at D2 and D7 post IVF. Biotecnologia Animal® provided the media used for IVP (Brazilia, DF, Brazil). In 8 replicates, a total of 1600 cumulus oocyte complexes (COCs) were collected from bovine abattoir-derived ovaries and randomly divided into four groups of 50: T0 = 0.0 mg/mL, T1 = 0.02 mg/mL, T2 = 0.04 mg/mL and T3= 0.08 mg/mL of recombinant PON1 (Chesapeake PERL Inc., Savage, USA). Maturation of COCs took place in an incubator with 5% CO2 at 39˚C for 24 hours. To analyze the activity of PON1 at IVM, 15 µL of media were collected at times 0 and 24 h of maturation. Sperm were recovered by minipercoll density gradient centrifugation and IVF wells were inseminated at a concentration of 1x106 sperm/mL. After 18 hours, presumptive zygotes were cultured for 7 days in SOFaa with FCS under controlled conditions (5% CO2, 5 % O2 and 90% N2 at 39˚C). Statistical analysis was performed using the GLM procedure of SAS software (SAS Institute, Cary, NC, USA) to account for possible linear, quadratic and cubic effects of exogenous PON1 on enzyme activity, cleavage and blastocyst rates. Any effect between level of PON1 activity and the blastocyst rate was accounted by Pearson correlation test. There was a linear effect for average activity of PON1 in IVM medium, which was 2.1 ± 2, 15.2 ± 2, 28.5 ± 2 and 52.4 ± 2 U/mL for T0, T1, T2 and T3, respectively ($P<0.0001$). There was no effect of PON1 on cleavage rate (81.7%, 85.1%, 81.0%, 82.5 %, for T0, T1, T2 and T3 respectively; $P > 0.05$). However, adding PON1 to IVM medium improved linearly embryo development by D7 as shown: 22.1% (T0), 29.2% (T1), 32.6% (T3) and 34.4% (T4) ($P < 0.0001$). Moreover the latter effect was supported by a positive correlation between the level of PON1 in the IVM media and blastocyst rate at D7 ($r = 0.35$ and $P = 0.04$). In sum, PON1 supplementation has a positive effect on the bovine embryo development, possible via improved in vitro maturation.

A146 OPU-IVP and ET

Effect of follicular wave synchronization on *in vitro* embryo production in Nelore cows

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Keywords: embryo, Nelore, synchronization.

In vitro embryo production is an important technology and many studies have been made to improve its efficiency. The aim of this study was to analyze the influence of follicular wave synchronization on embryo production. The experiment was conducted at Estancia Olho D' agua in Mandaguaçu - PR from January and November 2012. Twenty Nelore cows (approximately 450 kg) were randomly assigned into one of two treatments: T1 - Control (n=10) and T2 - Synchronization of follicular wave (n=10). Animals in T2 received an ear implant containing 3.0 mg norgestomet (Crestar) on Day 0, 2.0 mg of estradiol benzoate (Estrogin) and 500 μg of PGF2α (Ciosin®). Five days later, follicles were aspirated using an ultrasound (Mindray DP2200) and a 5MHz convex probe coupled to a 18G (Jelcor) needle and vacuum pump (cook - 75 mm Hg). After each aspiration, oocytes were selected according to their morphology and immediately sent to the laboratory where they were submitted to in vitro maturation (IVM), fertilization (IVF) and culture (IVC). For data analysis, the SAS software was used, applying the procedure for generalized linear models (PROC GENMOD) with Poisson distribution and identity link function. Eight follicular aspirations in each animal were performed every 21 days. After each section, the animals were allocated to the other treatment in the following procedure. Thus a total of 160 follicular aspirations was performed. The total number of oocytes (T1: 18.46 ± 0.46, T2: 20.85 ± 0.49, $p < 0.0005$), viable oocytes (T1: 12.10 ± 0.37, T2: 13.14 ± 0.39, $p < 0.06$) and rate of embryo development (T1: 39.08 ± 0.68 %, T2: 43.25 ± 0.71 %, $p < 0.0001$) were higher in animals submitted to follicular wave synchronization before follicular aspiration, compared to those who were aspirated without synchronization. In conclusion, follicular wave synchronization is a viable alternative to increase embryo production in Nelore cows.

A147 OPU-IVP and ET

Use of FSH in protocols for synchronization of follicular wave emergence for OPU in dairy cattle

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Keywords: dairy cattle,follicular aspiration, FSH.

Biotechnologies as OPU-IVEP have been widely used to achieve faster genetic improvement in herds, diminishing the generation intervals. However, the OPU-IVEP is still poorly efficient in high-producing dairy cattle, especially because of their reduced follicular population. Several studies have shown a positive effect of FSH in dairy and beef cattle when given 48 to 72 h before OPU. In this basis, the hypothesis of this study is that the use of 200mg FSH divided in 4 or 6 doses in non-lactating Holstein cows with synchronized follicular wave emergence increases the number of follicles, the recovery rate and the number of embryos produced in vitro. Thirty six non lactating Holstein cows used as oocyte donors were homogenously allocated to one of three treatment groups in a 3x3 Latin square design: Control (C); 4 doses of FHS (F4); 6 doses of FSH (F6). All cows were synchronized using the same protocol for synchronization of follicular wave emergence, except for the administration and number of doses of FSH as previously described. At random days of the estrous cycle known as D0, all cows received an intravaginal P4 device (Primer®, Tecnopec-Agener União, São Paulo, SP, Brazil) and 2 mg estradiol benzoate (Ric-BE®, Tecnopec-Agener União, São Paulo, SP, Brazil). Three days after (D3), all cows received 0.530 mg Sodium Cloprostenol (Cioprostin®, Innovare Biotecnologia e Saúde Animal Ltda, Monte Aprazível, SP, Brazil). Induction of luteolysis was performed in order to afford more stroma space for follicular growth and facilitate the visualization of follicles during the OPU. Cow from Control group received no additional treatment. Cows form group F4 were treated with 200 mg FSH (Folltropin®, Bioniche Animal Health, Belleville, Ontário, Canada) divided in 4 doses of similar concentration given approximately 12 h apart, starting on D4 AM. Cows form group F6 were treated with 200 mg FSH divided in 6 doses of similar concentration given approximately 12 h apart, starting on D3 AM On D7, the device was removed and OPU was done concomitant with antral follicle count in each ovary. The oocytes considered as viable were in vitro fertilized with sex-sorted frozen-thawed semen from Holstein bulls. Data was analyzed Shapiro Wilk test and Analysis of Variance was undertaken using the PROC MIXED of SAS 9.3, using orthogonal contrasts C1 (C x Treatment with FSH) and C2 (F4 x F6). Greater number of antral follicles were observed at OPU when cows were treated with F4 (58.8 ± 6.0) compared to F6 (50.8 ± 4.3; P = 0.02). However, there was no effect of FSH treatment on oocyte recovery rate (C = 35.1% vs FSH = 32.9%; P = 0.43) and number of embryos produced in vitro (C = 3.8 ± 0.6 vs FSH = 3.4 ± 0.5; P = 0.47). Thus, the use of 200 mg FSH divided in 4 doses may improve the number of ovarian follicles for OPU, and should be better studied.

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A148 OPU-IVP and ET

Gene expression in co-cultured granulosa cells from taurine and zebu cattle with high or low rates of *in vitro* embryo production

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Keywords: Gir, granulosa cells, Holstein.

In vitro embryo production (IVEP) is characterized by a large variation of results among donors. The absolute efficiency of IVEP is directly related to the variation in the production of cumulus-oocyte complexes (COC), as evidenced in the comparison between donors taurine and zebu breeds (Viana et al., Acta Sci Vet 39(1): 409, 2011). However, individual differences in embryo production rates are more complex and difficult to predict. The aim of this study was to evaluate the potential of the gene expression analysis of granulosa cells in co-culture as an indirect and noninvasive approach to explain IVEP results. Granulosa cells (GC) co-cultured with oocytes collected from Gir (*Bos indicus*) and Holstein (*Bos taurus*) donors were recovered and evaluated. The cell samples were allocated into groups according to breed (Gir or Holstein) and to blastocyst production rate (high: >50%, low: <20%), including at last six donors per group. The GC were recovered on the seventh day of culture and stored at -80°C with RNA later. RNA extraction and cDNA synthesis were performed using a commercial kit, RNeasy Micro Kit (Qiagen, Germany) and SuperScript III First-Stand Synthesis Supermix (Invitrogen, USA), respectively. The relative quantification of cDNA was performed by Real-Time PCR using the commercial kit Power SYBR Green PCR Master Mix (Applied Biosystems). The genes IGFR1, BAX, StAR, INHA, LHR and PRDX1 were evaluated using β-actin gene as an endogenous control. The results were analyzed by ANOVA and differences between groups were compared by Tukey's test. Results are presented as mean ± SEM. There was an interaction between breed and blastocyst production rate for the PRDX1 gene, which was over-expressed in the high production Holstein group ($P<0.05$). There was no difference ($P>0.05$) between expression values of other genes among groups. The PRDX1 gene is normally expressed in response to oxidative stress, and the results suggest a greater potential for adaptation to culture conditions for COC from Holstein donors with higher embryo production rates. The in vitro culture of GC, however, may have modulated other potential differences in the expression of other genes, limiting the use of the model proposed here.

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A149 OPU-IVP and ET

Comparison of phospholipids presents in MII bovine oocytes from different maturation systems

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Keywords: mass spectrometry, oocyte maturation, phospholipids.

The present study aimed to compare the phospholipids profile in bovine oocytes matured in different systems. For the experiment four groups were used: 1) immature oocytes aspirated from slaughterhouse ovaries (CON); 2) immature oocytes obtained by OPU in unstimulated heifers (IMA); 3) immature oocytes obtained by OPU in stimulated heifers (FSH group), and 4) in vivo matured oocytes (MII). In all the groups with OPU, the ablation of follicles larger than 5 mm was performed, and a progesterone implant (Primer®) was also inserted on the eighth day, after D0. All animals remained with the implant for six days. Two days after, in FSH and MII groups, superovulation was initiated with 80 mg i.m. of FSH (Folltropin-V), given in decreasing doses over a four days period. On D12, 0.15 mg of PGF2a i.m. (Veteglan®) was injected. For IMA and FSH groups the OPU was performed at D13 and D14, and for the MII group, 24 hours after GnRH administration (Gestran®). Immature oocytes from the three immature groups CON, IMA and FSH underwet IVM in TCM -199 media supplemented with FCS 10% and FSH. In MII group only those COC's with expanded cumulus cells and/or the presence of the first polar body were considered. Matured oocytes were denuded and stored in methanol at -80 °C. To the phospholipid profile determination the spectrum were obtained by mass spectrometry MALDI -TOF. Spectra with the ions peaks were acquired in positive/reflected mode mass spectrometry Auto Flex Speed MALDI-TOF/TOF (BrukerDaltonics, Germany). A total of 10-15 oocytes per group was used and the oocytes were placed individually in the MALDI plate. In each spectrum, after isotopic peaks exclusion, the most intense ions were considered as the starting point to search for the lipids corresponding values m/z. Only the m/z values that were clearly distinct from the noise level were included in the principal component analysis (PCA). After identifying in the PCA the ions responsible for most variation between treatments, the relative intensities of these molecular components were submitted to an ANOVA and the means were compared by Tukey test ($P<0.05$). The PCA analysis identified clusters of ions 640.6; 760.6 and 782.6 m/z as the most dispersed ($P<0.05$). However, in the ANOVA, only the cluster of 760.6 m/z corresponding ion, probably a phosphatidylcholine [PC (34:1) + H] + was more abundant ($P<0.05$) in oocytes of FSH group, compared to oocytes from MII group. Despite this punctual difference observed, the results indicate that the different maturation systems do not alters the oocyte phospholipids profile.

A150 OPU-IVP and ET

Long distance transportation and culture of bovine embryos in portable incubators for 24, 48 and 72 hours prior to transfer

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Keywords: aspiration, portable incubator, sexed semen.

The aim of this study was to evaluate the viability of in vitro produced embryos, transported for a long distance (1600 km), and cultivated for 24, 48 and 72 hours in portable incubators. This methodology makes IVP viable in places far way from laboratories and also when recipient and donors are far from each other. Seventy Gir embryo donors had their oocytes recovered by OPU, and Nelore and Nelore/Caracu crossed heifers were used as recipient. Following the selection, oocytes were stored in loked identified cryotubes, maintened in oocytes transporters with temperature control at 38.0ºC and taken to the IVF lab. Embryos were transferred to recipient on day seven postfertilization. In that way, they were transported at days 6, 5 and 4 from fertilization. All oocytes were fertilized with sexed semen. Embryos were placed in cryotubes, identified with the number of donar and transported into portable incubators, having control of CO2, temperature of 38.8ºC and gas exchanges occurring every 4 hours. Pregnancy diagnosis was performed at days 30 and 60 after embryo transfer. In the statistical analysis, a test of nonparametric binomial test of two proportions was carried out, with 5% significance, in order to determine the occurrence of differences in blastocyst production rates and pregnancy rates of embryos transported in cultivation for 24, 48 and 72 hours. Blastocysts production rate was 18.19%, 22.28% and 22.72% in cultivations for 24, 48 and 72 hours, respectively. 24-hour cultivation statisticaly reduce blastocyst formation ($P < 0.05$). Results of conception rate at 30 and 60 days were similar in all days of embryo culture, with rates of 44.51%, 42.82% and 44.66% at 30 days after embryo transfer, for 24, 48 and 72 hours of culture, respectively, and 41.12%, 40.99% and 42.42% at 60 days after embryo transfer, for the 24, 48 and 72 hours of culture, respectively. The pregnancy loss at 60 days in the 24, 48 and 72 hours of cultivation was the 7.62%, 4.27% and 5.03%, respectively. Long distance transportation and embryo cultivation for 24, 48 or 27 hours prior to transfer, in portable incubators, is viable considering the conception rates. However, the blastocyst production rate was lower when the culture occurred for 24 hours.

A151 OPU-IVP and ET

Assessment of cortisol and P4 in reproductive activities in recipient cows in Acre

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Keywords: homone, receptor, TETF.

The purpose of the present experiment was to establish in recipients of bovine embryos (n = 235), if the correlation between plasma concentrations of cortisol and P4 (mean ± standard deviation) has an influence on reproductive performance. Based on the simplification of the P36 protocol for TETF, protocols were developed as follows: on a random day of the estrous cycle (D0), each cow received 1 g of P4 trough a P4 intravaginal release device and 2.5 mg estradiol benzoate (BE) intramuscularly (IM). At D8 the P4 device was withdraw and 150µg D-cloprostenol (PGF2a), 400 IU of eCG and 1mg of BE were intramuscularly administered. On D16 blood samples were collected by venipuncture of the coccygeal vein and each cow received a transferred embryo (blastocyst, grade 1 or 2) after CL localization by U.S. (Aloka SSD 500, Aloka, Japan). At D41, diagnosis of gestation was made (DG) and repeated on D71, for confirmation of pregnancy or embryonic loss. Reproductive activity conditions adopted were: "respond to the hormonal synchronization protocol (Resp. P.)" and "did not respond to hormonal synchronization protocol (N.resp. P.)". Still, in the group (Resp. P.), the subdivisions "respond and are not pregnant (Resp. PNE.)" and "respond and are pregnant (Resp. PE.)" were settled. The absence of CL in the D16 classified the recipient as "did not respond to hormonal synchronization protocol (N.resp. P)". Plasma levels of cortisol and progesterone were estimated by radioimmunoassay (RIA) in solid phase. For correlations we used the Pearson correlation at a significance level of 5%. The means were analyzed by Student's t test at a significance level of 5%. The respective plasma concentrations of cortisol and P4 did not differ statistically ($p > 0.05$) between pregnant and/or non pregnant cows, but a positive correlation ($p < 0.05$) was observed between them. The "N.Resp. P." group, because of the absence of CL, presented lower plasma P4 concentration ($p < 0.05$) compared to the "Resp. P." group. A similar response was observed regarding the behavior of cortisol, which kept the difference among their means ($p < 0.05$) between pregnant and non-pregnant recipients. So the correlation between cortisol and P4 was not observed in group "N.Resp". The plasma concentrations of cortisol observed, presumably interfered with the responsiveness of the cows to the TETF protocols. It was found, therefore, a correlation between cortisol and P4 for the animals that responded to the protocol, which was kept in groups Resp. PNE. and Resp. PE.

A152 OPU-IVP and ET

Gene expression in sheep oocytes meiotically inhibited by roscovitine

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Keywords: mRNA, ovine, roscovitine.

All the mRNA mobilized during oocyte maturation, fertilization and early embryogenesis is transcribed in oocytes kept at germinal vesicle (GV). With the meiotic resumption, which occurs spontaneously in COCs destined to IVP, this transcriptional activity is interrupted. So, this study aimed to determine if the arrest of sheep oocytes at GV, using roscovitine, favors the transcription of essential genes to competence acquisition. For this, COCs grade 1 and 2 were cultured, for 6 hours, in a maturation medium consisting of TCM199, cysteamine, pyruvate, penicillin and fetal bovine serum (control) plus 75μM roscovitine (treatment). The culture was performed in an incubator at 38.5°C and 5% CO2. After 6 hours, 100 oocytes, in each experimental group, were stained with Hoechst 33342 and evaluated under fluorescence microscope. For evaluation of gene expression before (0 hours) and after 6 hours of culture, four pools of 25 denuded oocytes were stored in lysis buffer (Qiagen RNeasy Mini Kit) at -196°C. The mRNA was extracted with RNeasy® kit (Qiagen) and the reverse transcription was performed using the Sensiscript RT Kit (Qiagen) with poly T primer. The expression of NLRP5, ZAR1, BMP15, GDF9, SOD1, BCL2 and BAX genes was assessed by qPCR using Power SYBR® Green PCR Master Mix (Applied Biosystems). CYC-A gene was used as housekeeping gene. The relative expression of each gene was calculated using the ΔΔCT method with efficiency correction by Pfaffl method. Data were subjected to analysis of variance according to completely randomized design and means were compared by Tukey test at 5% probability. The rate of oocytes at GV in roscovitine treatment was significantly higher (87%) compared with the control (48%), evidencing the meiotic arrest. At the end of 6 hours, the level of BAX in treatment was lower than that observed in control, while the BCL-2 was highly expressed in both groups. This relation suggests that the culture conditions were adequate and that roscovitine 75 μM did not affect the cell viability. The relative mRNA amount of NLRP5 and SOD1, after 6 hours in the presence of roscovitine, was significantly lower than that observed at 0 hours and in control. For the other genes it was not detected any change in the expression profile between groups and times. It appears, therefore, that the meiotic arrest for 6 hours did not improve the expression of genes studied. This result, however, does not discard the possibility of increase of these and other genes in different times of inhibition.

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A153 OPU-IVP and ET

The number of oocytes recovered from Holstein donors has no effect on the pregnancy establishment of *in vitro* embryo programs

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Keywords: embryo, non-lactating cow, OPU.

The present study aimed to evaluate the effect of the number of recovered oocytes from non-lactating Holstein (*Bos taurus*) donor on the in vitro embryo production and the possible effect on the pregnancy establishment after transferring these embryos to crossbred (*Bos taurus* x *Bos indicus*) recipients. The retrospective study comprised a database from a commercial dairy farm (Fazenda Bela Vista, Tapiratiba-SP) performed during May and October of 2013. At a random day of the estrus cycle, a total of 264 non-lactating females was submitted to ovum pick-up procedure (OPU). The oocytes were classified and only viable oocytes were submitted to the in vitro embryo production. The embryonic development (cleavage and blastocyst rate) was evaluated and the embryos were transferred in crossbred recipients. The pregnancy diagnosis was performed by rectal palpation 50 to 60 days after embryo transfer. The data were analyzed on the GLIMMIX procedure of SAS 9.3®. The donors were classified into three categories according to the total number of oocytes recovered: 1. Low (14.6 ± 0.4), n = 88; 2. Medium (24.2 ± 0.3), n=88; and 3. High (42.3 ± 1.8), n=88. It was found that females with high number of recovered oocytes presented higher number of viable oocytes (High: 28.1 ± 1.2a; Medium: 17.9 ± 0.4b; Low: 10.8 ± 0.4c; P<0.0001), cleaved embryos (High: 22.0 ± 1.1a; Medium: 14.4 ± 0.5b; Low: 8.1 ± 0.4c; P<0.0001) and blastocysts (High: 5.1 ± 0.4a; Medium: 4.2 ± 0.3b; Low: 1.8 ± 0.2c; P<0.0001) when compared to cows with medium and low number of recovered oocytes. However, similar viable oocytes rate (High: 67.6 ± 1.6%; Medium: 74.0 ± 1.7%; Low: 73.6 ± 2.1%; P = 0.07), cleavage rate (High: 77.7 ± 2.0%; Medium: 79.6 ± 1.5%; Low: 74.1 ± 2.4%; P = 0.10) and blastocyst rate (High: 18.7 ± 1.3%; Medium: 23.1 ± 1.8%; Low: 17.2 ± 2.3%; P = 0.21) were observed among categories. Finally, similar (P = 0.66) conception rate was observed after embryo transfer from donor with high (43.5%, 107/246), medium (46.2%, 85/184) and low (38.3%, 31/81) number of recovered oocytes after OPU. Therefore, the total number of oocytes recovered from non-lactating Holstein donor per OPU session affects the in vitro embryo production; however, the produced embryos had no difference on the ability to establish the pregnancy.

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A154 OPU-IVP and ET

Use of ACTH as an alternative for hormonal supplementation on *in vitro* maturation of bovine oocytes

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Keywords: ACTH, FSH, IVM.

Routinely FSH is used to stimulate follicular development in protocols for ovarian hyperstimulation. In vitro, this hormone is used as a promoter of maturation and inducer of cumulus cells expansion. Although its effects are widely reported, there is great variation in the superovulatory response when different products are used. In order to evaluate possible differences in the molecular profiles of different products, Folltropin-V (84 % FSH + 16 % LH, extracted from porcine pituitary, 400 mg, Bioniche) and Sigma FSH (extracted from porcine pituitary, 50 IU, Sigma) were compared. The samples were hydrolyzed with trypsin were analyzed by high performance liquid chromatography ultra-fast reverse phase (HPLC FAST-RP) in order to separate the molecular components. After separation, mass spectrometry MALDI - TOF (MS) of the fractions obtained was performed to evaluate the samples composition by detecting ions of peptides derived from those proteins. Later we performed MALDI-TOF/TOF (MS/MS) in order to elucidate the primary structures of some of its components (De novo sequencing). A fragment detected in both products matched to the pre-prohormone proopiomelanocortin, which is converted to Adrenocorticotropin Hormone (ACTH). Based on these findings, we used the Sigma FSH (used in IVM) and ACTH (synthesized and purified in laboratory) to evaluate the action of that hormone in the IVM of bovine oocytes. CCOs obtained from slaughterhouse ovaries were selected and divided into two groups: FSH (n=73), considered the control group, and ACTH (n=89). The IVM medium was modified to evaluate the isolated action of each hormone, consisting of TCM199 supplemented with PVP360 - 0.8 mg/ml, L-glutamine - 10 mg/mL and Amicacin 250 mg/mL. And, according to the group, was added 0.01 IU/ml of FSH or 0.007 mg/ml of ACTH. The CCOs were matured for 24 hours at 38.8°C and 5% CO2. After maturation they were in vitro fertilized and cultured until D8 of development. Cleavage (D2), blastocysts (D7), and hatch (D8) rates were evaluated. Four replicates were performed. Preliminar data were analyzed by Chi-square test ($P<0.05$). Differences were detected in cleavage and blastocyst (D7) rates between treatments (FSH: 68.49% and 20.54%; ACTH: 42.69% and 8.98%, respectively). However, blastocysts (23.27% - FSH 13.48% - ACTH) and hatching rates (41.17 % - FSH, 25.0 % - ACTH) on D8 were similar between groups. During the experimental period, the average rate of blastocysts using the standard laboratory IVM medium was 42.5% on D7. The low rate of embryo production from FSH group may be due to the use of PVP as a substitute for FCS, which is an undefined component and could mask the action of the hormones tested. Although blastocyst hatching rates on D8 did not differ from the group with FSH, ACTH showed lower results in all evaluations performed.

A155 OPU-IVP and ET

Retrospective study of factors that affect the fertility of bovine embryo recipients

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Keywords: biotechnology, embryo, recipients.

The aim of this study was to identify critical points with negative impact on fertility of bovine embryo recipients. In the experiment we used 6527 reproductive data from embryo recipients (Number of embryos transferred per recipient, stage of development of embryos transferred, corpus luteum quality, type of embryo produced, season of the transfer, synchrony between donor and recipient, and which side the embryos transferred are placed into the uterus) of a farm located in the state of São Paulo during the years 2000-2004. Data were analyzed using the Statistical Analysis System for Windows (SAS, 2001), by multivariate logistic regression using the LOGISTIC procedure of SAS. Variables were removed by reverse elimination, according to Wald's statistical criteria with stipulated value P> 0.20. The final statistical model included the variables of quality of corpus luteum, embryo development, embryo quality, biotechnology's type used (TE-SOV or OPU- IVF) and number of transfer [repeat breeders (> 3 embryos transferred) or not repeat breeders]. After this initial analysis, the variables were analyzed for the binomial distribution using the GLIMMIX procedure of SAS. There was no interaction between these variables (P>0.05). In the study, it was found that the pregnancy rate was influenced by the number of embryos transferred [not repeat breeders (35.7%, 2019/5650) and repeat breeders (32.0%, 281/877); P=0.001], by the stage of embryonic development [morula (42.0%, 748/1782)a, by the early blastocyst (31.7%, 292/921)b, blastocyst (32.0%, 402/1224) and expanded blastocyst (32.8%, 790/2412)b; P=0.01], the quality of the corpus luteum [CL1 (35.7% (1631/4571); CL2 (36.2%, 458/1266) and CL3 (31.3%, 174/556), P=0.01] and type of embryo production [fresh embryos produced *in vivo* (47.4%, 509/1074)a, frozen embryos produced *in vivo* (38.8%, 590/1520)b, and embryos produced *in vitro* (30.3%, 1175/3875)c, P=0.001]. However, pregnancy rate was not influenced by season [summer - November to February (35.8%, 596/1665) and winter - April to July (33.4% (945/2830), P=0.85] by sync donor/recipient [D-2 (39.7%, 418/1053), D-1 (34.5%, 1100/3189), D0 (35.5%, 639/1798), D+1 (29.4%, 143/487); P=0.13], or by the side of transfer [right side (35.0%, 1398/3991) and left side (35.6%, 901/2534), P=0.98]. We concluded that fertility in embryo recipients is influenced by multiple factors in which there was lower fertility in repeat breeders, in embryos up to the morula stage, quality of the corpus luteum 2 and 3, and in frozen or *in vitro* produced embryos.

A156 OPU-IVP and ET

In vitro production of Gyr (*Bos taurus indicus*) embryos and pregnancy rates: of donor age and sire influence

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Keywords: longevity, sexed-sorted semen, zebu.

In vitro production of bovine embryos (IVP) is a technique of great economic interest, which, associated with the use of sex-sorted semen allows obtaining females of superior genetics values. The aim of this study was to evaluate the donor age and sire effect on IVP and pregnancy rates. Gyr donors (85) were submitted to 363 ovum pick-up (OPU) sessions at an EPAMIG research unit (Fazenda Experimental da EPAMIG at Uberaba – MG). Sex-sorted semen (X) from 18 Gyr bulls was used. For evaluation of the donor age effect, the cows were classified into three groups: AC1: 2-6, AC2: 7-10, and AC3: >11 years old. All OPU sessions resulted in 6084 oocytes, 2537 embryos, which produced 1105 pregnancies. The pregnancy rate values of 41.7% and 39.5% were observed at 30 and 60 d after embryo transfer, respectively. The mean number of viable oocytes per OPU (15.8 ± 10.7) was influenced ($P<0.05$) by donor age, in which, a reduction of 0.9 structures for each additional year of age were observed. The mean number of viable oocytes/OPU was greater ($P<0.05$) in AC1 compared with other groups (AC1: 23.8±1.4a, AC2: 18.9±1.1b, AC3: 14.0±1.6b). The mean number of produced embryos per donor was 6.9±5.3, ranging from 0 to 32 between donors. The mean number of pregnancies per OPU session was greater ($P<0.05$) in the cows up to 10 years old (3.4±0.8) compared with donors with more than 11 years old (1.8±0.9). A higher ($P< 0.05$) number of embryos per OPU session were produced by AC1 donors compared with other groups (AC1: 9.1±1.5a, AC2: 7.1±1.5b, AC3: 4.1±1.6b). The average oocyte to embryo conversion rate was 41.7%, ranging from 2.6 to 57.1 %, and the oocyte to embryo conversion rate of the two worst bulls (2.6 and 5.1%) differed ($P<0.05$) from the remaining, which had conversion rates varying between 18.8 to 57.1%. The present findings agree with the literature, in which older cows produced fewer oocytes, had lower oocyte to embryo conversion rate and yielded greater amount of lower quality embryos, which reduced the pregnancy rates. Therefore, Gyr donors up to 10 years old are more efficient on IVP programs and there is great variability between bulls regarding sex-sorted semen fertility in the in vitro embryo production.

A157 OPU-IVP and ET

Reproductive cycle in mules: ovarian structures and use as embryo recipient

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Keywords: mules, ovaries, recipient.

Mules are generally ignored in the reproductive context due to the classic concept of infertility and absence of ovarian activity. Therefore, currently few studies are conducted on this topic. The aim of this study was investigate the ciclicity in ovaries of mules and report the birth of an asinine foal from a mule. Ovaries (n= 72) from mules were obtained at a local slaughterhouse, ovarian capsules were removed with scissors and scalpel blade. Ovaries were measured and externally inspected for presence of corpora lutea and follicles. The visualized follicles were aspirated with a 20 mL syringe containing heparin solution and needle 40x12 (18G1 ½) for oocyte retrieval. It was possible to verify that 45.8% (33/72) of the ovaries presented corpora lutea and 15.3% (11/72) had follicles > 10mm. It was possible to obtain an oocyte of all follicles aspirated. From all oocytes (n= 11) recovered, three were intact with expanded cumulus cells, while all the others were atretic. Considering the presence of follicles, corpora lutea and expanded cumulus oocytes in the ovaries of mules, we consider the use of this category as recipient for asinine embryos. After identifying regular cycles in a mule, the follicular control was performed after detection of estrus of the donor donkey. When the donkey had a 38 mm follicle and uterine edema grade 3 (scale 1-3), the ovulation was induced with human chorionic gonadotropin (hCG) (Vetecor®, Hertape-Calier, Spain) (2500 IU) IM. On the following day, the donor was covered by a donkey male. The ovulation occurred within 48 hours, detected by ultrasonography. At D8 just five days post ovulation the embryo was collected by uterine flushing via transcervical with a double system with 1 L of lactated ringer's solution. After identification, the embryo was washed in 10 drops Holding plus (Embriolife®, Vitrocell, Brazil) and transferred to the recipient mule, which had signs of estrus two days later after donor. The recipient had ovulation induced (hCG 2500 IU IM) after the detection of 37 mm follicle and uterine edema grade 3. Ovulation was confirmed by ultrasonography. On the day of transfer (D5), the mule had a rigid uterine tone and corpus luteum of approximately 30 mm. The embryo transfer was made at uterine body with transrectal palpation, due to the difficulty for passing the cervix because of its small diameter. After five days the pregnancy diagnosis was performed, with confirmation at 60 days post-transfer, both by transrectal ultrasonography. The eutocic birth of the foal donkey occurred after 372 days gestation. We highlighted that the occurrence of regular cycles in mules may be more frequent than commonly expected. Furthermore, the use of this cyclicality can be considered for use in reproductive biotechnologies such as embryo transfer.

A158 OPU-IVP and ET

Reduction on discontinuous Percoll® gradient volume improves recovery rate and plasmatic membrane integrity of bovine sperm

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Keywords: bovine semen, Percoll volumes, sperm selection.

Discontinuous Percoll® gradient is one of the most utilized methods for sperm selection. Aiming to improve sperm cell recovery rates, numerous variables, such as different forces and centrifugation time, composition and volume of gradients, which are comprehended in the process of sperm selection, have been investigated. The aim of this study was to compare the influence of different discontinuous Percoll® gradient volumes on recovery rate, motility, vigor and integrity of the plasma membrane of bovine sperm. Semen samples from two *Bos taurus taurus* bulls were thawed at 35ºC in a water bath and homogenized to form a pool, which was used for further analyses. Experiments were replicated five times. Sperm selection was performed by Percoll® gradients 30, 60 and 90% (Folchini et al., Rev. Bras. Reprod. Anim., v.36, p.239-244, 2012), being the volumes of each layer adjusted according to experimental groups: Control: 300µl at 90%, 300µl at 60% and 300µl at 30%; Treatment 1 (T1): 100µl and Treatment 2 (T2): 200µl of each layer. Samples were first centrifuged at 2200 x g of force for 5' and, subsequently, centrifugation for washing on FERT-TALP medium was performed at 2200 x g for 1'. Post-Percoll samples were immediately evaluated for motility, vigor and integrity of the plasma membrane. Recovery rate was obtained using a formula that considers initial and final volumes as well as initial and final concentrations (Machado et al., Theriogenology, v.71, p.1289-97, 2009). Integrity of the plasma membrane evaluation was performed after incubation with the probes propidium Iodide (PI) and Carboxyfluorescein Diacetate (CFDA). For this purpose, 200 cells were counted per treatment for each replicate. Data were analyzed by Chi-square Test ($P<0.05$). No difference was observed for motility, vigor, and recovery rate among Control (73.3%; 3.0; 29.0%), T1 (80.8%; 3.7; 39.3), and T2 (79.2%; 3.5; 36.2) experimental groups. However, samples from T2 group showed (76%) percentage of sperm cells with intact plasma membrane than T1 (54%) and Control (61%) groups. These results showed that it is possible to obtain higher recovery rate of sperm cells with high membrane integrity rates using Percoll gradient with 200µl volume.

A159 OPU-IVP and ET

Effect of cumulus cells biopsy from cumulus –oocyte- complex and individual culture system on bovine *in vitro* embryos production

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Keywords: biopsy, oocyte competence, RNA.

To date, morphological parameters have been used to select oocytes for assisted reproduction techniques. However, these parameters are insufficient to distinguish more competent from less competent oocytes, whereas the other methods available are invasive preventing the oocyte to be used. Considering that cumulus cells have a bi-directional communication with the oocyte and that they play an important role in the growth and maturation they can be used to indicate oocyte quality in a noninvasive way. Several studies have identified candidate genes that may be associated with oocyte competence and can be used as markers for oocyte quality in cumulus cells. The best way to prove the efficiency of markers in the cumulus cells would be to monitor individual development of each COC to the formation of the blastocyst after taken a sample of the cell. This study was performed aiming to assess the amount of RNA that can be obtained in a COC biopsy, and the effect of cumulus cells biopsy and individual culture in embryonic development. COC were obtained from slaughterhouse ovaries and after selection they were placed in a 60µl drop of PBS. With the aid of an ophthalmic blade Straight 15 ° Accutome®, a small fragment of COC was removed and stored individually. To assess the amount of RNA in the biopsies, five groups were evaluated in three replicates. The groups comprised the number of biopsies obtained from each COC, used to form the pool, which were 1, 5, 10, 15 and 20 biopsies. RNA was extracted using the RNeasy Plus® kit and was quantified on Nanodrop. Initially the effect of individual culture was evaluated, WOW system in which IVM, IVF and IVC were performed was compared to control group in which cultured was performed in groups in a 200µl drop in a total of three replicates. Subsequently, to test the effect of the biopsy, the blastocyst rate from biopsied COC cultured in WOW up to D7 was compared to that of the control group in a four replicate experiment. The RNA data were analyzed by Kruskal -Wallis test ($P<0.05$) and those of embryo development by Chi-square test ($P<0.05$). RNA abundance was not different among groups of 1, 5, 10, 15 or 20 fragments (5.36 ± 3.39, 8.52 ± 7.51, 5.59 ± 11.63; 10.45 ± 1.98, 11.95 ± 6.32), however a great variation among replicates for all groups was observed. No difference in blastocyst rate was also detected in D7 between individual culture in WOW (33.9 % vs. 40.4 %). However, individual culture associated with biopsy affected the blastocyst rate which was higher than that in the control (22.1 % vs. 48.7 %). This reduction in blastocyst rate may be due to the longer handling time of the COC for the biopsy and manipulation in the WON plate. In conclusion, individual culture associated with COC biopsy reduce blastocyst production, nevertheless they are important tools to be used to obtain RNA for studies of markers validation.

A160 OPU-IVP and ET

Nuclear and cytoplasmic maturation of bovine oocytes treated with different inhibitors of meiosis during transportation

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Keywords: bovine oocyte, *in vitro* maturation, meiotic inhibitors.

The aim of this study was to evaluate nuclear and cytoplasm maturation of bovine oocytes treated with different meiotic inhibitors during transport. Oocytes (n=1910) were transported in 100µl of TCM199 medium with 0.3%BSA, antibiotics and hormones (Control), supplemented with physiological [100% follicular fluid (FF)] or chemical inhibitors [100µM butirolactone-I (BL), 100mM milrinone (MR) or 100µM forskolin associated with 500µM IBMX (FIBMX)]. The transport was made in a portable incubator (Minitub) for 6h at 38.5°C. As control, oocytes were cultured in 5% CO2 in air (C1) or in the portable incubator (C2). After follicular removal (0h) and transport (6h), oocytes were evaluated for meiotic progression (1µg/mL Hoechst 33342), microfilaments distribution (1µg/mL phalloidin), mitochondrial distribution and potential (ψ) (500nM MitoTrackerRed), production of reactive oxygen species (ROS; 5µM H2DCFDA) and apoptosis (TUNEL). Meiotic progression and apoptosis were compared by $\chi 2$ test. Data in % of microfilaments and mitochondrial distribution was transformed in arc sen$\sqrt{}$% and analyzed by ANOVA, after that the means were compared bu Tukey's test. The ψ and ROS were analyzed by ANOVA followed by Tukey's test (P>0.05). The percentage of oocytes at germinal vesicle stage was similar (P>0.05) to C1, BL, FIBMX, MR e FF (46.3 to 54.9%), which were higher than C2 (31.5%; P<0.05) and lower than 0h (84.9%; P<0.05). The percentage of oocytes at germinal vesicle breakdown stage was similar (P>0.05) to C1, BL, FIBMX, MR e FF (45.0 to 52.7%), which were lower than C2 (67.6%; P<0.05) and higher than 0h (15.1%; P<0.05). Microfilaments distribution was similar (P>0.05) between groups for Normal category (12.8±10.4 a 46.1±7.2), Discontinuous (45.0±8.8 to 61.8±8.2) and Absent (1.8±1.8 to 7.9±6.2). In Diffuse category, MR (37.4±4.6) was higher (P<0.05) than 0h (6.4±3.5) and FF (8.1±4.6), but similar (P>0.05) to C1 (16.7±5.1), C2 (8.9±5.3), BL (19.8±4.4) and FIBMX (27.2±10.8), which were similar (P>0.05). Mitochondrial membrane potential (measured in arbitrary fluorescence units – AFU) was similar (P>0.05) between treatments (148.4±13.5 to 168.1±13.1), as well as mitochondrial distribution which were predominantly peripheral (47.8±12.8 to 77.8±5.6). The amounts of ROS, measured in AFU, were higher (P<0.05) in MR (160.8±5.8) than BL (141.9±2.9), but similar (P>0.05) to other treatments (153.7±3.9 to 145.8±4.9). Apoptosis was higher (P<0.05) in C2 (12.0%) and MR (13.8%) than for 0h (1.5%) and C1 (1.4%), but similar (P>0.05) to other treatments (7.8% to 7.1%). Oocytes transported in the presence of MR showed more ROS production and apoptosis was higher, although it did not interfere in nuclear maturation process. The other inhibitors did not promoted detrimental alterations. In conclusion, the inhibitors used in the present study, with the exception of MR, allowed the maturational progress in a similar way to the control groups.

A161 OPU-IVP and ET

Comparative analysis of GNRH or LH administration to induce ovulation in multiple ovulation programs in Holstein donors

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Keywords: dairy cows, *in vivo* embryo, multiple ovulation.

The present study evaluated the efficiency and in vivo embryo production of multiple ovulation (MO) protocols using GnRH or LH as ovulation inducers in Holstein donors inseminate at fixed time. A total of 31 lactating and 30 non-lactating donor cows was submitted to two MO treatments in a crossover design, reaching a total of 122 uterine flushing processes. Cows received one of two ovulation inducers in the MO protocols: GnRH agonist (Licerelin, Gestran®; GEST; n=61) or LH (Lutropin®; LUTR; n=61). On a random day of the estrous cycle (Day 0; AM), cows received an intravaginal progesterone device (P4; Primer®, Tecnopec) and 2.0 mg of estradiol benzoate (RIC-BE®, Tecnopec) intramuscular (IM). On Day 4, donors received 300 mg (non-lactating cows) or 400 mg (lactating cows) of Folltropin® administrated in six decreasing doses, every 12 hours. On Day 6 (AM and PM) 0.150 mg of cloprostenol (Estron®, Tecnopec) was administered. Furthermore, all females received 400 IU of eCG (Folligon ®, MSD) during the morning of Day 7. The P4 devices were removed on Day 7 PM. On Day 8 AM non-lactating cows of GEST group received 125 µg IM of lecirelin (Gestran ®, Tecnopec) and LUTR group received 25 mg IM of LHp (Gestran®, Tecnopec) whereas lactating cows received the same treatments except for the moment that was on Day 8 PM. The non-lactating females were inseminated at fixed time on D8 PM and D9 AM, and the lactating cows were inseminated at fixed time on D9 AM and PM. Uterine flushing and embryo recovery was performed on D15 in both categories, non-lactating during the morning and lactating cows in the afternoon. Immediately before uterine flushing, the number of corpora lutea (CL) was evaluated and recorded. The same mating was maintained in both MO protocols for each female. The statistical analysis was performed using the Glimmix procedures of SAS®. There was no interaction between category and treatment for any of the response variables analyzed (P=0.20). Additionally, there was no difference between the tested ovulation inducers for total number of CL (GEST: 11.1±1.2 vs. LUTR: 11.4±1.2; P=0.85), total number of recovered structures (GEST: 7.3±0.8 vs. LUTR: 8.4±0.8; P=0.14) and number of viable embryos (GEST: 1.9±0.4 vs LUTR: 2.7±0.6; P=0.19). However, differences were verified on recovery rate (GEST: 61.1% [406/675] vs. LUTR: 66.4% [460/693]; P=0.02), fertilization rate (GEST: 43.8% [178/406] vs. LUTR: 50.9% [234/460]; P=0.01) and rate of viable embryos (GEST: 25.6% [104/406] vs. LUTR: 32.6% [150/460]; P=0.01) between experimental groups. In conclusion, despite the similar viable embryo production per flushing between groups, females from the LUTR group presented higher recovery rates, fertilization rates and rates of viable embryos.

A162 OPU-IVP and ET

Effect of extended omega 6 fatty acids supplementation on embryo production and oocyte quality in dairy cows

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Keywords: early lactation, transition period, whole raw soybeans.

The folliculogenesis can be divided into two phases: pre-antral and antral growth (Ireland, 1987, Reproduction 34, 39-54). The studies related with lipids supplementation in dairy cows, aim to evaluate the effect of diet on the development of antral follicles, however few studies are related with folliculogenesis from pre-antral phase. The oocyte quality is directly associated with fatty acids composition, particularly, the membrane phospholipids content, which plays an essential role during and post-fertilization development (Santos et al., 2008, Reproduction 43, 23-30). The objective of this study was to determine the effects of omega 6 (ω6) fatty acids supplementation during transitional period on embryo production and oocyte quality in dairy cows. Twenty-nine Holstein cows were used in four experimental groups in a randomized design. The animals were separated into 4 treatments to receive the following diet: 1) Group 0 – control, not supplemented with ω6 fatty acids source, animals were fed the control diet (whole raw soybeans) from ninety days before calving to parturition; 2) Group 30 – supplemented with ω6 acid source (12% of whole raw soybeans addition in dry matter basis) from thirty days before calving to parturition; 3) Group 60 – supplemented with ω6 fatty acid diet similarly as Group 30, however, from sixty days before calving to parturition; and 4) Group 90 – supplemented with ω6 fatty acid diet similarly as Group 30, however, from ninety days before calving to parturition. After calving all cows were given the same diet during ninety days, with 12% of whole raw soybeans inclusion in DM basis. The pre- and post-partum diets supplemented with whole raw soybeans were formulated to achieve 4.8% of ether extract (EE), approximately, whereas the diet without inclusion of whole raw soybeans was formulated to achieve 2.8% of EE. The ovum pick-up procedure (OPU) was performed on days 21 ± 3 DIM (OPU1), 42 ± 7 DIM (OPU2), 63 ± 7 DIM (OPU3) and 90 ± 7 DIM (OPU4). After OPU procedure, selected oocytes were classified in grade I, II and III. Only oocytes with grade I, II and III were subjected to in vitro fertilization (IVF). Data were analyzed using PROC MIXED of SAS 9.1, with the effect of diet, OPU and interaction as fixed effects, and animal as random effect. The data were analyzed by polynomial regression. Treatment effect ($P<0.05$) was observed for oocytes of grade II. There was OPU effect ($P<0.05$) for total oocytes, oocytes of grade III and cleaved embryos. Interaction, linear and quadratic effects were not observed. In conclusion, omega 6 fatty acids supplementation did not affect the embryo production and oocyte quality in post-partum period in dairy cows.

A163 OPU-IVP and ET

Bovine oviductal epithelial cells and oviduct fluid modulate differently the incidence of polyspermy during porcine *in vitro* fertilization

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Keywords: oviductal fluid, sperm-oocyte interaction, zona pellucida maturation.

Although the prevalence polyspermy under natural conditions is moderate, in IVF systems polyspermy remains as major obstacle to successful development of viable embryos in several species, mainly in porcine. Thus, the present study was designed to examine the response of bovine oviduct epithelial cells (BOEC) cultured in vitro for seven days and oviductal fluid (OF) in the incidence of polyspermy during porcine IVF. Cumulus-oocyte complexes (COCs) obtained from abattoir ovaries by aspiration of the follicles 3–5 mm were matured in vitro for 44 h in TCM199 medium supplemented with 10% FBS, cysteamine (570 mM), EGF (10 ng/mL), and FSH (400 ng/mL). After the IVM, COCs were denuded and randomly allocated to one of three treatments: i) Control (n = 98) – IVF in tris-buffered medium (TBM), ii) OF (n = 99) – IVF in TBM medium supplemented with 10% OF coming from cows oviducts classified as late follicular phase, based on the appearance of the ovaries, and iii) BOEC (n = 98) – IVF in TBM medium in the presence of BOEC on the seventh day of culture. Co-culture with spermatozoa (5×104 cell/mL) was performed for 20 h. Putative zygotes were fixed (alcohol-chloroform-acetic acid, 80: 10: 10: v/v), stained (1% lacmoid), and examined under phase contrast microscopy. Penetration and monospermy rate and pronuclear formation were assessed in each putative zygote. The results of five repeated were tested for normality using the Kolmogorov-Smirnov test, which indicated the normality of the data. Therefore, the parameters were compared using one-way ANOVA followed by Tukey's test. Data are expressed as the mean ± SD. The penetration rate (%) was similar between the control (51.0 ± 4.9) and OF (31.3 ± 4.0) group, but both were lower than ($P < 0.05$) BOEC (71.5 ± 11.6). Differently, the monospermic rate of control (48.5 ± 13.0) and BOEC (54.9 ± 8.3) groups were similar, while both were lower than ($P < 0.05$) OF (93.3 ± 7.8) groups. Nevertheless, BOEC and OF (38.8 ± 5.7 and 29.3 ± 5.0, respectively) groups were equally effective ($P > 0.05$) in the production of monospermic zygotes, when considering the total number of oocytes used for IVF. However, the OF group did not differ from the control group (24.5 ± 5.5), while BOEC was higher ($P < 0.05$). The average pronucleus per oocyte was similar among groups: 1.21 ± 0.13 (control), 1.83 ± 0.50 (BOEC) and 1.34 ± 0.42 (OF). In conclusion, the OF used in porcine IVF exerts beneficial effect on oocytes by controlling the penetration and hence the incidence of polyspermy, while BOEC exerts the effect in sperm increasing penetration, but without the modular polyspermy.

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A164 OPU-IVP and ET

Effect of seasonality on oocytes production of Sindhi breed bovine females (*Bos taurus indicus*)

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Keywords: dry season, follicle aspiration, recovered structures.

The identification of oocytes competence from *Bos taurus indicus* donors in generating embryos after in vitro fertilization in the different climatic conditions is important to establish a strategy for embryo production in several regions of Brazil (Camargo et al., Theriogenology, 68, 626-638, 2007). Therefore, the aim of this study was to compare the performance of oocytes production in Sindhi breed bovine females in two different climatic conditions in Southeastern Brazil (rainy and dry seasons). Data were obtained from an IVP commercial center of bovine embryos (Sexing Technologies of Brazil, São Paulo, Brazil), regarding to the OPU sessions performed between March 2011 and March 2013 in Sindhi donors from a farm located at Sao Paulo State. The sessions were performed in the months from October to March (rainy season) and from April to September (dry season). Data consisted of results from 15 OPU sessions (eight sessions during the rainy season and seven during the dry season) performed in 85 donors, being 35 animals aspirated more than once, either in the rainy or dry season. Data regarding to the number of recovered, viable and degenerated structures were subjected to analysis of variance, being the oocytes recovery rates compared by Chi-square test, and all analyzes was adopted $\alpha = 5\%$. The mean responses per OPU session in rainy and dry seasons, respectively, were: average number of recovered oocytes (23.12 ± 4.1 and 23.57 ± 7.6), average number of viable oocytes (15.62 ± 3.3 and 17.00 ± 5.6), average number of degenerated oocytes (7.25 ± 1.4 and 7.28 ± 2.9) and oocytes recovery rate (67.3 and 73.6%). There was no significant difference ($P>0.05$) between the rainy and dry seasons for any of the variables analyzed. Studies have showed that Zebu females produce a higher number of total and viable structures during the rainy season, where environmental management and feeding conditions are more favorable (Fernandes et al., Brazilian Journal of Veterinary Research and Animal Science, 38, 131-135, 2001). However, the similar results we found in rainy and dry seasons may be explained due to optimal conditions at the farm, including nutrition and facilities. We conclude that Sindhi donors were able to produce the same average number of recovered and viable oocytes structures, both in the rainy and dry season, being favorable for the implementation of in vitro production programs in commercial herds.

A165 OPU-IVP and ET

Use of commercial or handmade well of well (wow) dishes for *in vitro* production of bovine embryos

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Keywords: embryo viability, *in vitro* production of embryos, microwell embryo culture.

The Well of Well (WOW) embryo culture system was developed as an alternative for culturing individual embryos in a single drop. It is used in the handmade cloning technique in which the removal of the zona pellucida (ZP) is required. The individual culture of several embryos in a single drop is also useful for transfection or transduction of zygotes where ZP removal is required. Well of well embryo culture dishes can be acquired on the commercial market or handmade. The aim of this study was to evaluate the efficiency of commercial or handmade WOW dishes in the production of embryos without ZP. Oocytes from ovaries collected at slaughterhouse were selected and in vitro matured in TCM 199 medium (Invitrogen, California, USA) supplemented with 10% of estrus cow serum and 20ug of FSH mL-1 in atmosphere with saturated humidity and 5% of CO_2 in air and 38.5ºC for 24h. The oocytes were in vitro fertilized in FERT-TALP medium with 2x106 spermatozoa mL-1 for 20-22h in the same conditions of in vitro maturation. Following in vitro fertilization, the presumptive zygotes were denuded with hyaluronidase at 0.1% m/v (Sigma, St Louis, USA) by vortexing for 5 minutes, and were randomly divided into three groups. G1 (n=244): cultured in conventional dishes, without ZP removal; G2 (n=132): cultured in commercial WOW dishes, with ZP removal; G3 (n=144): cultured in handmade WOW dishes, with ZP removal. In all groups, we used CR2aa medium supplemented with 2.5% SFB in atmosphere with 5% CO_2, 5% O_2 and 90% N_2. In G2 and G3 groups, ZP was removed with pronase 2mg ml-1 (Sigma). In the G3, the microwells were produced in 35x10mm petri dishes without the culture medium using a glass microneedle with the edge preheated. It was left the smallest space as possible among the microwell, they were covered with CR2 medium and any formed air bubbles inside the microwell were removed. In all groups, 25-30 presumptive zygotes per dish and the medium was covered by mineral oil. For the statistical analysis, data was submitted to the qui-square test ($P<0.05$). There was no difference ($P>0.05$) in cleavage rate between G2 and G3 treatments (91.5 and 88.0%, respectively), but both were higher ($P<0.05$) than G1 (62.5%). The blastocysts rate on day 7 did not differ ($P>0.05$) among groups (24.5, 16.8 and 19.7%, for G1, G2 and G3, respectively). Blastocysts rate on day 8 in G2 (18.4%) was lower ($P<0.05$) than G1 (31.5%), but not different ($P>0.05$) from G3 (24.3%). The percentage of cleaved embryos that developed to blastocyst until day 8 was lower ($P<0.05$) in G2 (19.8%) and G3 (25.6%) compared with G1 (50.5%). We conclude that commercial and handmade WOW dishes allow bovine embryos development without ZP, although WOW system has reduced the proportion of embryos developing toward blastocyst stage compared to conventional embryo culture system.

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A166 OPU-IVP and ET

Production evaluation of *in vitro* of bovine embryos after addition of blood plasma submitted of platelet lysis to the middle of maturation increased or not of fetal bovine serum

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Keywords: *in vitro* maturation, oocyte, platelet-rich plasma.

Platelet has several growth factors (GF), which have mitogenic and angiogenic activity. Therefore, this study was carried out to investigate the possible effects of blood plasma, after platelet lysis (BPPL), added either alone or in combination with FCS to the IVM medium of bovine oocytes, as evidenced by the subsequent embryonic development. Blood of one heifer, obtained by vein puncture in tube containing anticoagulant, was centrifuged (120 x g and 555 x g, both for 7 min) for obtaining the PRP. Then, PRP was divided in too aliquots, being one of them filtered (Milllex®; 0.22 μm), and frozen, resulting in BPPL/FFr, while the other one was frozen (-86º C/24 hours), thawed and filtered, referred here as BPPL/FrF. Ovarian follicles (3 to 7 mm in diameter) were punctured, and oocytes were washed and selected in PBS medium plus 10% FCS. Then, 20-25 oocytes were transferred to droplets of IVM medium, consisting of Medium 199 (Sigma-Aldrich®, M-5017, USA), supplemented with FSH, LH, pyruvic acid, sodium bicarbonate and gentamicin sulfate, according to the experimental design: group I, IVM medium + 10% FCS; group II, IVM medium + 10% BPPL/FFr); group III, IVM medium + 10% BPPL/FrF); group IV, IVM medium + 10% BPPL/FrF + 10% FCS. IVM was performed at 38.7º C, under humid atmosphere of 5% CO2, in air, for 24 h. For IVF, one semen sample was thawed and subjected to sperm selection through Percoll® gradient, followed by dilution in TALP-IVF medium. Gametes were incubated for 20 h and presumptive zygotes denuded and transferred to droplets of m-SOF medium. IVF and IVC were performed under the same conditions mentioned for IVM. The results (n/%) of oocytes that cleaved and reached blastocyst (B)/expanded blastocyst (EB) stages were monitored at 72 and 168 h post-insemination, respectively. Data were analyzed with ANOVA and t test of Bonferroni, with $P<0.05$ taken as significant. Higher percentage ($P<0.05$) of cleaved oocytes was observed for group I (79.8%, 95/119) than for the others, with similar results (Group II 65.0%, 78/120; Group III 51.6%, 63/122; and Group IV 61.7%, 74/120). Similarly, difference ($P<0.05$) was found among groups I (27.7%, 33/119) and others, II (7.5%, 9/120), III (4.9%, 6/122) and IV (6.7%, 8/120) for B and EB production. In conclusion, BPPL, either with or without FCS addition, did not have positive effect on the in vitro embryo production, probably due to the lack and/or inefficiency to obtain the GF through the present protocol.