A124 OPU-IVP and ET

Conception and calving rates and pregnancy loss of vitrified embryos produced *in vitro* of Nelore cows under different diets

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Keywords: conception,embryo, nutrition.

The aim of this study was to evaluate the influence of high or low dry matter intake (DMI) and/or energy on conception rate, calving rate and pregnancy loss of vitrified embryos produced *in vitro*. Non-lactating Nelore cows (N=33), aged between 4 and 10 years, mean weight of 489.5±11.3 kg and BCS 3.25 (scale from 1 to 5) were used. Cows were confined without access to pasture, with two animals per stall. Mineral salt was provided in the diet and water *ad libitum*. After 15 days on the adaptation diet, cows were blocked by initial weight and randomly allocated in four experimental groups. The maintenance group (M) received a diet of weight maintenance consuming 1.2% of DM per kg of body weight (BW). The restriction group (0.7M) received the equivalent of 70% of the group M diet consuming 0.84% of DM per kg of BW. High intake group (1.5M) received the equivalent of 150% of the M group. The energy group (E) received a diet with DM similar to M group, however, with energy level equivalent to 1.5M (TDN = 75%). The cows received all the diets in a latin-square design. There were four sessions of ovum pick-up (OPU), 30 days apart. The oocytes were classified and taken to *In Vitro* Brazil laboratory, where all procedures of IVP and vitrification were realized. Embryos were thawed, evaluated and transferred to synchronized recipients [D0: insert of progesterone (P4) device and 2 mg estradiol benzoate (EB); D8: 0.6 mg estradiol cypionate (ECP), 0.5 mg sodium cloprostenol (PGF2α) and 300 IU equine chorionic gonadotropin (eCG); D16 and D17: embryo transfer]. The embryos were transferred by two experienced technicians. A total of 543 embryos were transferred to recipients with CL. Pregnancy diagnosis was performed by US 23 and 53 days after embryo transfer. All data of oocyte and embryo production have been described by Prata *et al*. (2011, *Acta Sci Vet*, v.39:1, p.338). Data were analyzed by PROC GLIMMIX of SAS and the results are presented as least squares means ± SEM following the order of treatments M, 0.7M, 1.5M and E. There was no difference in conception rate (%) among treatments at 30 (30.7±6.8; 38.7±6.8; 31.3±6.8 and 34.7±6.6; *P*=0.49) and 60 (24.6±5.6; 33.5±5.5; 25.6±5.7 and 29.2±5.4; P=0.43) days of pregnancy. Calving rate (%) was also similar among groups (21.1±3.9; 26.4±3.4; 22.5±3.3 and 25.7±3.5; *P*=0.73). Pregnancy loss (%) between 30 and 60 days (20.0±6.3; 13.3±4.8; 18.0±5.2 and 16.0±5.2; *P*=0.84) and between 30 days and calving (31.4±3.2; 31.6±3.2; 28.0±3.0 and 26.0±3.3; *P*=0.89) did not differ among groups. Therefore, changes in DMI or energy for a 30 day-period in non-lactating Nelore cows did not influence the conception rates of their vitrified IVP embryos.

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Effect of oocyte density on commercial in vitro bovine embryo production

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Keywords: cleavage rate, efficiency, embryo production rate.

The efficiency of the *in vitro* bovine embryo production (IVEP) is the key to increase the profitability of commercial labs. Some factors may affect embryo development *in vitro,* such as oocyte quality, energy substrate, oxygen tension, and embryo density (Khurana et al., Theriogenology, 54, 741-766). In ovum pick up (OPU) procedures the number of the oocytes recovered can be variable and low and, in commercial labs, oocytes and embryos have to be cultured from each individual donor cow, regardless of the recovered number, becoming necessary to process them in small groups. It has been reported that optimum development is achieved when oocytes are cultured in groups of 20-40, in comparison with groups of 5 and 10 structures (O 'Doherty et al.,Theriogenology, 48, 161-169).Therefore, the aim of this study was to investigate the effect of oocyte density on IVEP efficiency, which was conducted in a commercial lab of *in vitro* fertilization (IVF). In the lab, cumulus–oocyte complexes (COCs), obtained from random donors, were assigned in six groups: 1-5 COCS per drop (group 1, n=37 drops), 6-10 COCs (group 2, n=91), 11-15 COCs (group 3, n=89), 16-20 COCs (group 4, n=57), 21-25 (group 5, n=45) e 26-30 COCs (group 6, n=27), where n is the number of replicates of each group. Oocyte maturation was performed for 24 hours in drops of 80 µL. After that period, COCs were fertilized with semen from different bulls, in drops of 70 µL for 22 hours. After IVF, presumptive zygotes were cultured for seven days, in drops of 60µL. The percentage of cleavage (third day of culture) and blastocysts (seventh day of culture) were calculated on the number of viable oocytes. All the steps of embryo production were performed with the same conditions in the six groups, including volume of media and the incubation at 38,8 °C in a 5% CO_2 in air with high humidity. Data were analyzed by the method of least squares using analysis of variance by proc GLM. Differences between means were compared by Tukey test with 5% significance. Differences in cleavage rate between groups were not observed (groups 1-6, respectively: 84.9%±32.6%, 82.5%±26.1%, 85.3±19.5%, 80.3%±22.4%, 77.6%±22.5% and 82.1%±21%, P>0.05). Embryo production rate also did not differ between groups (groups 1-6, respectively: 35.4%±42.9%, 35.5%±37.8%, 40.5%±31%, 28.7%±26.3%, 30.5%±25.2% and 39.4%±28.6%, P>0.05). In conclusion, there was no influence of oocyte density on the IVEP, which confirms the efficiency observed in commercial scale, in which the number of oocytes is variable. Besides, other factors - as donors and bulls - which were not the focus of analysis in this study, can be involved.

A126 OPU-IVP and ET

Reduction in superovulation response of female bovine superstimulated with FSH in split dose

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Keywords: CL, FSH, superovulation.

This experiment aimed to evaluate the superovulation of Gyr (zebu) females, using conventional protocol with 8 decreasing doses with an interval of 12 hours or protocol with fewer applications and similar dosage (split dose). Sixteen females (total of 32 superovulations), aging 17-42 months, with body condition score varying from 2,5 to 4 (scale 1-5) were used, in a cross-over design. At the beggining of the treatment (D0), animals received progesterone device (Primer ® - Tecnopec, Brazil) plus 2 ml of estradiol benzoate (Ric-Be ® - Tecnopec). The females of the conventional group received 250 IU of FSH / LH (Pluset ® - Hertape Calier - Brazil) divided into eight decreasing doses administered in 12-hour interval (FSH / LH in D4, D5, D6 and D7 in the morning and afternoon, with their respective strengths: 50 IU; 37.5 IU; 25 IU and 12.5 IU). On D7 (morning), females were treated with 2 ml of cloprostenol (Veteglan ® - Hertape Calier), and removal of progesterone device was done in the afternoon of D7. The females of the split group also received 250 IU FSH / LH. On D4, 62.5 IU FSH/ LH intramuscular and 125,0 IU subcutaneously were administered in the morning. Twenty-four hours later, 62.5 IU were administered subcutaneously in the morning, and on D7 progesterone device was removed and 2 ml of cloprostenol were administered. Females of both groups received 2 ml of GnRH (Gestran ® - Tecnopec) in the morning of D8, and they were inseminated 12 and 24 hours later. On D15 embryo recovery was performed in both treatments. Superovulation response was done counting the number of corpora lutea in each ovary, with the aid of ultrasound. There was a reduction in the number of CLs at the time of collection (8.12 ± 3.26 and 4.69 ± 3.46).

A127 OPU-IVP and ET

Use of the SPOM system for maturation of bovine oocytes used for IVP

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Keywords: oocyte competence, maturation, meiotic arrest.

One of the limitations of the assisted reproductive techniques (ATRs) is the availability of competent oocytes. Therefore, the development of alternatives to increase the competence of oocytes used in ATRs (Hyttel et al., 1997, Theriogenology, 47, 23-32) is needed. This study aimed to test the SPOM system (*Simulated physiological oocyte maturation*; Albuz et al., 2010, Human Reproduction, 25, 2999-3011) in IVM of bovine oocytes by changing the FSH source, gaseous atmosphere and protein supplement. SPOM involves two steps the first is a short culture period (2h) in the presence of cAMP modulators agents (forskolin 100 μM, 500 μM of IBMX, Sigma Sto. Louis, USA) and the second is an extended IVM (30h) in the presence of 20μM of cilostamide (Sigma Sto. Louis, USA), associated with a high concentration of FSH (0.1 IU / ml). COCs (n = 452) were aspirated from follicles of 3-8 mm and distributed into four groups: G1: control, oocytes submitted to IVM, IVF and IVC, previously described (Machado et al., 2012, Zygote, 20, 123 - 134); G2: SPOM FSHr +BSA, oocytes submitted to the SPOM with recombinant FSH (FSHr) and 4 mg/ml FAF-BSA, 5% O_2 during IVM; G3: SPOM with FSH + BSA, oocytes submitted to the SPOM with porcine FSH (FSHs) and 4 mg/ml FAF-BSA, 5% O_2; G4: SPOM with FSH+10% FCS, oocytes submitted to the SPOM with FSHs and 10% FCS in atmosphere of 5% CO_2 in air; G5: SPOM FSHr + 10% FCS, oocytes submitted to the SPOM with FSHr and 10% FCS in 5% CO_2 in air. After IVM, oocytes were *in vitro* fertilized and cultured until D8. At that time, blastocysts were measured and those greater than 160 μm were stained with Hoechst 33342 to assess total cell number. Data of embryonic development were analyzed using χ^2 test ($P < 0.05$) and embryo size and total cell number were compared using the Kruskal-Wallis test ($P < 0.05$). Cleavage rate was similar ($P > 0.05$) between G1 (77.5%), G2 (70.3%) and G3 (67.4%), but lower than ($P < 0.05$) G4 (54.5%) and G5 (37.7%). Blastocyst rate differed ($P < 0.05$) among all groups being higher in G1 (42%), intermediate in G2 (26.5%), G3 (33.3%) and G4 (12.0%) and lower in the G5 (5.7%). When SPOM was performed under low O_2 tension, the size and number of cells in G2 and G3 embryos were similar ($P > 0.05$) to G1. In G4 and G5, the size of embryos (163.6 ± 63.9 and 171.9 ± 41, respectively) and the number of cells (125 ± 41 and 96 ± 7.7, respectively) were lower than the other groups. Results suggest that SPOM system, regardless of the FSH source and O_2 tension, did not increase blastocyst production neither affect their quality. However, SPOM in the presence of FCS and high O_2 tension in IVM, in spite of FSH used, have a deleterious effect on *in vitro* embryo production.

A128 OPU-IVP and ET

Effect of bull in in vitro embryo production of Holstein cows with sexed or conventional semen

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Keywords: IVP, OPU, sexed-semen.

The aim of this study was to evaluate the bull effect and the type of semen (sexed or conventional) used in in vitro embryo production from Holstein females. In the study, 660 Holstein oocytes donors of five different commercial farms were used during 2010 and 2011. A total of 1,814 ovum pick-up (OPU) sessions were performed on random day of the estrous cycle. All laboratory procedures, in vitro maturation, fertilization and culture were performed by the same method and laboratory (In Vitro Brasil, Mogi Mirim, SP, Brazil). For in vitro fertilization (IVF) 40 bulls were used: 28 had sexed semen and 12 had conventional semen. The variable analyzed was embryo production rate by OPU session (number of viable embryos per number of viable oocytes). Data were analyzed using the GLIMMIX procedure of SAS. The embryo production rate by OPU session was lower when sexed semen was used for IVF [24.5% (3,117/12,739)], compared with the conventional semen [35.1% (1,162/3,370), P <0.0001]. In contrast, after selecting 1/3 of bulls with the highest in vitro embryo production performance with sexed semen (n=4) and conventional semen (n=9), similar embryo production rate was observed [43.9% (645/1,468)], compared with conventional semen [39.8% (454/1,140); P = 0.06]. In conclusion, in vitro embryo production is reduced when sexed semen is used. However, it is possible to achieve similar rate between conventional and sexed semen when bulls with higher performance for in vitro embryo production is used. The data indicate that there is a significant effect of bull on in vitro embryos production when sexed semen is used.

A129 OPU-IVP and ET

The overstimulation treatments using Folltropin or Pluset showed similar efficiencies in non-lactating Holstein donors

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Keywords: dairy herd, *in vivo* embryo, superovulation.

The present study aimed to evaluate the superovulatory response and *in vivo* embryo production in non-lactating Holstein donors treated with Folltropin (Tecnopec, Brazil) or Pluset (Hertape Calier, Brazil) protocols. The study was performed in a commercial dairy farm (Agrindus S/A, Descalvado - SP). A total of 31 donors were initially allocated into two experimental groups in a cross-over design, totalizing 62 superovulation protocols (SOV). Females received two different superovulation protocols using either Folltropin (FOLL; n=31) or Pluset (PLUS; n=31). At a random day of the estrous cycle (D0; AM), cows received an intravaginal progesterone device (P4; Primer, Tecnopec) and 2.0 mg intramuscular (IM) of estradiol benzoate (RIC-BE, Tecnopec). On D4, cows from FOLL group received 300 mg Folltropin diluted into 18 ml of saline solution and injected in 8 decreasing doses (4 ml, 4 ml, 3 ml, 3 ml, 2 ml and 2 ml), 12 h apart. Cows from PLUS group received 400 IU of Pluset® diluted in 18 ml of saline and injected in 6 decreasing doses (4 ml, 4 ml, 3 ml, 3 ml, 2 ml and 2 ml), 12 h apart. On D6 (AM and PM) two doses of 0.150 mg of cloprostenol (Estron, Tecnopec) were injected. Furthermore, all animals received 400 IU of eCG (Folligon, Intervet, Brazil) at D7 AM. All P4 devices were removed on D7 PM and 62.5 µg IM of lecirelin (Gestran, Tecnopec) were injected 12 h later (D8 AM). The females underwent timed artificial insemination on D8 PM and D9 AM and embryo recovery performed on D15. Immediately before uterine flushing, the number of corpus luteum (CL) was evaluated and recorded. The same sire was used in both superovulation protocols for each female. Statistical analysis was performed using the GLIMMIX procedures of SAS. There was no difference between experimental groups for superovulation rate (FOLL: 96.8% vs. PLUS 90.3%; P = 0.33), total number of recovered ova (FOLL: 10.4±1.5 vs. PLUS: 7.7±1.6; P=0.08) and number of viable embryos (FOLL: 3.2±0.7 vs. PLUS: 3.6±0.8; P=0.59). However, there were differences on the total of CL (FOLL: 13.2±1.8 vs. PLUS: 10.6±1.8, P=0.04), recovery rate (FOLL 78.6%; 311/396 vs. PLUS: 70.9%; 216/305; P=0.04) and unfertilized ova (FOLL: 6.0±1.4 vs. PLUS: 3.2±1.0; P=0.04). In conclusion, although the FOLL group presented highest superovulatory response, the recovery of viable embryos per flushing was similar to between treatments. Therefore, the superstimulatory treatments (300 mg of Folltropin and 400 IU of Pluset) showed similar efficiency in SOV program of non-lactating Holstein donors.

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A130 OPU-IVP and ET

The oxygen tension and oocyte density utilized on IVM affects *in vitro* fertilization rates of bovine oocytes

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Keywords: IVF, oocyte density, oxygen tension.

The *in vitro* maturation (IVM) of bovine oocytes, have an important role in the success of *in vitro* fertilization (IVF). Many factors can influence the IVM events, including oxygen tension and oocyte density by volume of medium. Inadequate combination of these factors can induce ROS generation, affecting the fertilization and embryo development. The aim of this study was to evaluate the influence of the association of oxygen tension (5% or 20%) with different oocyte density by volume of medium (1:10 or 1:20μL) on the rates of *in vitro* fertilization in cattle. Bovine oocytes (n=331) were obtained from slaughterhouse ovaries, and after selection, they were randomly allocated into 4 treatments: T1: 1:10 in 5% of O_2; T2: 1:10 in 20% of O_2; T3: 1:20 in 5% of O_2; T4 1:20 in 20% of O_2. The oocytes were matured in TCM 199 plus 10% of estrous mare serum, EGF, LH, FSH and piruvate for 22 to 24 h in 39ºC and saturated humidity. For IVF, the spermatozoa were sorted by Percoll gradients (90, 60, 30%), and co-incubated with oocytes for 18 h in 5% of CO_2 in air and saturated humidity, in FERT TALP medium. Before IVF, cumulus cells were removed by successive pipetting. The presumptive zygotes were stained with Hoescht 33342 and the fertilization rates were evaluated by pro nucleus formation and sperm penetration. The statistical analyses were performed by Z test, with 5% of significance. The rate of normal fertilization (NF) of group T2 (35.37%) was higher than T1 (18.18%; $P<0.05$) but similar to T3 (26.83%; $P>0.05$) and T4 (21.52%; $P>0.05$). The rate of normal penetration (NP) rates did not differ between treatments ($P>0.05$). When the rates of NF and NP were analyzed in association, the T2 (48.78%) was higher than T1 (29.55%; $P<0.05$)) and T4 (29.11%; $P<0.05$), but similar to T3 (40.24%; $P>0.05$). These data show that the system utilized on T2 (1:10 in 20% O_2) and T3 (1:20 in 5% of O_2) presented the best rates of IVF, suggesting that when an higher oxygen tension (20%) is used the oocyte density must be high (1:10). A lower oocyte density (1:20) requires a lower oxygen tension (5%). The rates of IVF are influenced by oxygen tension and oocyte density by volume of medium utilized on IVM.

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A131 OPU-IVP and ET

Differences in *in vitro* fertilizing capacity and its relation with oxidative stress in cattle

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Keywords: IVF, oxidative stress, sperm selection.

The variation among bulls in sperm characteristics, fertilization and embryonic development rates, as long as some factors that induce infertility in males, such as oxidative stress, are obstacles to commercial IVF (Silva *et al*., Theriogenology, v.67, p.609-619, 2007). This study aimed to determine the effect of the oxidative stress in sperm morphofunctional characteristics, *in vitro* fertilizing capacity and subsequent embryonic development of semen from different bulls. In 5 replicates semen samples from four *Bos taurus* bulls were thawed for 30" in a 35ºC water bath. The sperm was sorted by Percoll gradientes (Folchini *et al.,* Rev. Bras. Reprod. Anim., v.36, p.239-244, 2012). After that the samples were evaluated for vigor, motility, concentration, morphology, production of reactive oxygen species (ROS), membrane integrity (HOS+), lipid peroxidation, glutathione (GSH) levels and superoxide dismutase (SOD) activity. A dose of $2x10^6$ spermatozoa/mL of each bull was used to IVF. Presumptive zygotes (100/treatment) were denuded and incubated with Hoechst 33342 solution (10mg/mL) and evaluated for the presence of spermatozoa penetrated, formation of the pronuclei or nuclei fused. Some of the presumptive zygotes (10/treatment) were individually cultured for 48 h in SOFaaci + 10% ESS and BSA in petri dishes over an embryonic monitoring system (Primo Vision, Cryo Mangement Ltd., Hungary). Embryonic development was assessed by the cleavage rate, time of first cleavage, and average number of blastomeres at 48h. Data were analyzed by chi-square (X^2) and ANOVA and the means were compared by Tukey test at 5%. No difference was observed in morphofunctional characteristics. Bulls 2 and 4 (49.20 ± 7.20 and 54.80 ± 7.89, respectively) in relation to bulls 1 and 3 (35.20 ± 7.02 and 37.20 ± 6.62). ROS levels were increased for bull sperm 4 (76.01 ± 4.43) when compared to bull 1, bull 2 and bull 3 (55.28 ± 11.54, 63.78 ± 8.54 and 59.68 ± 12.22). GSH levels were reduced in bulls' sperm 2 and 4 (27.27 ± 3.82 and 27.97 ± 1.03) when compared to bulls 1 and 3 (31.77 ± 2.74 and 33.45 ± 2.75). SOD activity was increased in bull 1 (9.72 ± 2.50) in relation to bulls 2, 3 and 4 (6.79 ± 1.12, 6.58 ± 1.64 and 7.45 ± 1.35). There was no difference in the lipid peroxidation levels, cleavage rate and time of the first cleavage. The bull 1 showed an increased SOD activity and had lower cells number after 48 h (2.5) and total penetration and fertilization rates (63.0%) compared to the others (80.8, and 88.0%, respectively). The bull 4, which showed a high level of ROS, had higher penetration and normal fertilization rates (80.0%) and number of embryonic cells at 48 h after IVF suggesting that ROS showed a beneficial effect. These results show that there are variations in the fertilizing capacity between bulls related to oxidative stress.

A132 OPU-IVP and ET

Pregnancy after transfer of donkey embryo in cycling mule as recipient

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Keywords: embryo transfer, mule, recipient.

Mules (*E. asinus* X *Equus caballus*) are usually infertile animals. However, some mules have regular cycles and could be considered as recipients, especially for donkey embryos, since this kind of embryo usually has problems when transferred to mares. When using mares as recipients the embryo losses are high, due to the non-invasion of trophoblast cells in the endometrium, with insufficient formation of endometrial cups and no production of eCG. The aim of this report was to evaluate the feasibility of using a mule as an alternative recipient to donkey embryo. The control of the follicular wave was performed after detection of estrus of the donkey donor. When the donor presented a pre-ovulatory follicle of 38 mm and uterine edema grade 3 (scale 1-3), the ovulation was induced with hCG (2500UI) IM. One day later, the donor was submitted to the copulation with a male donkey of proven fertility and detection of ovulation occurred within 48 hours. At D8.5 post-ovulation the embryo collection was performed by non surgical method in dual system with approximately 1 L of ringer's lactate. After three flushes, the content was recovered in petri dishes for the embryo detection. After identification, the embryo was washed in 10 drops of Holding plus (Vitrocell / Embriolife ®) and transferred to the recipient mule. This recipient showed signs of estrus two days after the donor. After checking the presence of a follicle of 37 mm and uterine edema grade 3, the ovulation was induced with hCG (2500 UI) IM and the ovulation was detected by ultrasonography. On the day of transfer (D5), the mule had firm uterine tonus and corpus luteum of approximately 30 mm. The transfer of the embryo into the uterine body required also transrectal palpation because of the difficulty in passing the small cervix. After five days, the pregnancy diagnosis was done and it was confirmed at 60 days post-transfer, both by transrectal ultrasonography. We conclude that it is possible to use natural cycles of cyclicing mules as a viable alternative to achieving better pregnancy rates of donkey embryos, even without the use of exogenous progesterone to maintain pregnancy.

A133 OPU-IVP and ET

Effect of hormonal treatments pre-OPU on oocyte recovery and *in vitro* embryo production in Girolando cows (*Bos taurus x Bos indicus*)

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Keywords: dairy cows, OPU, synchrozation – FSH.

The Girolando (*Bos taurus* x *Bos indicus*) breed is widespread in Brazil due to its morphologic and physiologic characteristics being favorable for dairy production in the tropics. The success and efficiency of the *in vitro* embryo production mainly depend upon the quantity and quality of oocytes recovered in OPU. To enhance the efficiency of the technique, the use of hormonal treatments pre-OPU may be necessary. The objective of this study was to compare oocyte recovery in the presence of a CL or not at the moment of the OPU and blastocyst production of cows subjected to different hormonal protocols pre-OPU. Twelve cyclic and non-lactating Girolando cows were blocked by parity and genetic traits and were randomly assigned to three groups: G1 - OPU in a random day of the estrous cycle; G2 - follicular wave synchronization, in which cows received on D 0 an intravaginal device of progesterone (CIDR®, Zoetis, Auckland, New Zeland), 2 ml of estradiol benzoate I.M. (Gonadiol®, Schering-Plough, Sao Paulo, Brazil) and 2 ml of $PGF_{2\alpha}$ I.M. (Ciosin®, Schering-Plough, New Jersey, EUA), and on D 5 OPU was performed; G3 - similar to G2, adding I.M. injection of 40 mg of FSH (Folltropin®, Bioniche, Belleville, Canada) on D 3. The cows underwent a total of six OPUs in a cross-over design, in which all of them went through all the treatments twice, in 30 days apart (between one OPU session and the next one). During OPU, the number of aspirated follicles was recorded and the presence of a CL was verified. Oocytes retrieved were quantified and classified, as well as the number of blastocysts produced in each experimental group. Data were analyzed by ANOVA. In a total of 68 OPU sessions, 778 follicles were aspirated, resulting in 689 COCs recovered (88.6%; G1 - 260; G2 - 278; G3 – 240; $P>0.05$). The average of aspirated follicles, oocytes recovered and viable oocytes per experimental group were, respectively: G1 – 10.8 / 9.4 / 6.1; G2 – 12.1 / 11.8 / 8.0; G3 – 11.4 / 9.1 / 6.2; ($P>0.05$). The average of *in vitro* maturated COC, number of cleaved embryos and blastocysts produced per treatment were, respectively: G1 - 6.9 / 5.7 / 1.3; G2 – 6.8 / 5.2 / 1.3; G3 – 6.5 / 6.0 / 1.8 ($P>0.05$). The number of CL present at OPU was greater ($P<0.05$) in G1 (16 in 24 OPU) than in synchronized groups (G2 - 5 in 23 OPU; and G3 - 5 in 21 OPU). Hormonal protocols of follicular wave synchronization were effective to regress the CL, which can facilitate the OPU procedure. The FSH dose used did not influence the quantity and quality of oocytes recovered. Also, hormonal treatments to synchronize the follicular wave and the use of low dose of FSH aiming the efficiency of OPU did not improve embryo production. In summary, in Girolando breed, OPU performed every 30 days results in similar embryo production between synchronized and FSH-stimulated cows and those without a treatment previously to OPU.

A134 OPU-IVP and ET

The FSH stimulus prior to the ovum pick-up increases the success of *in vitro* embryo production programs in Holstein cows

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Keywords: dairy herd, *in vitro* embryo, superestimulation.

The study evaluated the effect of FSH treatment (Folltropin, Tecnopec) prior the Ovum Pick-Up (OPU) using the slow release diluent MAP5 (hyaluronic acid, Bioniche) on *in vitro* embryo production (IVP) program of lactating and non-lactating Holstein cows. A total of 30 cows were used (n=15 lactating and 15 non-lactating cows) in a *cross-over* experimental design, from Agrindus S/A dairy farm, Descalvado-SP. The females were randomly allocated into three groups: control group (CON; n=30); Folltropin 200 mg (FOLL; n=30); MAP5/Folltropin 200 mg (MAP/FOLL, n=30). At a random day of the estrous cycle (D0 AM) all cows received an intravaginal progesterone device (P4; Primer, Tecnopec) and 2.0 mg of estradiol benzoate (RIC-BE, Tecnopec), intramuscular (IM). On D4 and D5 (AM and PM), the FOLL group received four decreasing doses of FSH IM (D4 AM and PM = 4ml; D5 AM and PM = 3 ml). The MAP5/FOLL group received a single dose of 5 ml IM of MAP5 with Folltropin on D4 AM. The P4 devices were withdrawal on D7 AM and cows were submitted to OPU at same day. Immediately before the OPU, all visible follicles were quantified and classified according to their diameter [small (SF = <6mm), medium (MF = 6 to 10mm) and large (LF = >10 mm) follicles. The same mating was maintained for all IVP procedures during the experiment. Variables were analyzed by the GLIMMIX procedure of SAS. There was no difference among experimental groups on the number of follicles aspirated (CON: 17.1 ± 1.1; FOLL: 17.2 ± 1.3; MAP5/FOLL: 18.3 ± 1.5; P = 0.34), total oocytes recovered (CON: 12.0 ± 1.2; FOLL: 10.3 ± 1.0; MAP5/FOLL: 10.9 ± 1.3; P = 0.21), number of LF (CON: 2.1 ± 0.2; FOLL: 1.5 ± 0.3; MAP5/FOLL: 1.8 ± 0.3; P = 0.06) and viable oocytes (CON: 9.3 ± 1.0; FOLL: 8.6 ± 0.9; MAP5/FOLL: 8.7 ± 1.2; P = 0.73). However, cows from FOLL (9.5 ± 1.1) and MAP5/FOLL group (8.9 ± 1.2) had higher number of MF compared to cows from CON group (3.61 ± 0.6, P <0.0001). In contrast, fewer number SF was observed in FOLL (6.2 ± 1.0) and MAP5/FOLL (7.7 ± 1.0) compared to the CON (11.5 ± 1.0^{a}, P <0.0001). It was also observed lower recovery rate in the FOLL (60%; 310/517) and MAP5/FOLL (59.5%; 327/550) compared to the CON (69, 8%; 359/514; P < 0.0001). However, the FOLL (34.5%; 89/258) and MAP5/FOLL (25.3%; 70/277) groups had higher blastocyst rate than the CON group (19.8%; 55/278^{b}; P < 0.0009). The FOLL (3.0 ± 0.5) resulted in higher number (P = 0.03) of viable embryos per OPU session compared to the CON (1.8±0.4^{b}) and an intermediate value was obtained in MAP5/FOLL group (2.3 ± 0.5^{ab}). In conclusion, FSH treatment prior to the OPU, with or without MAP5, increased the success of IVP programs in Holstein cows.

A135 OPU-IVP and ET

Results of OPU-IVP of *Bos taurus* donors over the year in subtropical climate

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Keywords: bovine, embryos, oocytes.

Bos taurus taurus donors besides having lower efficiency in the process of genetic multiplication by *in vitro* embryo production (IVEP) may also changes in their results according to the weather, especially in tropical regions (Al-Katanani et al. 2002, J. Dairy Sci., 85, 396-403). This study aimed to compare the distribution of IVEP results over the months of the year in donors *Bos taurus taurus*, done in the south and southwest of the Minas Gerais state (Brazil) by the same veterinarian. The region has mesothemic climate of CW classification, according to Koepen. Results from 960 follicular aspirations (OPU) in Holsteins (476), Simental (264), Angus (142) e Jersey (78) donors, done for a period of 12 months, were used. In this period the total rainfall was 2.970 mm, the maximum temperature was 38.7°C and the minimum was 4.6ºC. The management of the animals was semi-confinement with supplementation of corn silage and commercial grain concentrated. For OPUs, an ultrasound device with intravaginal microconvex transducer of 7,5 MHz (Mindray DP 2200) was used. All follicles larger than 3 mm were identified and punctured. The cumulus oocytes complexes (COCs) recovered were counted and classified based on their morphological aspect. The viable oocytes were matured *in vitro* (TCM 199) for 22-24 hours after the start of OPU at a temperature of 38.5°C, 5% CO_2 and saturated humidity. Sorted sexed semen from different bulls, evaluated for motility and vigor, was used for *in vitro* fertilization (IVF). After IVF the presumable zygotes were transferred to *in vitro* culture (SOF), where they remained for seven days at a temperature of 38.5°C and controlled atmosphere (5% CO_2). The average production of oocytes and embryos conversion was compared between breeds and months of the year by Dunn's test, considering a 5% significance. There was no breed effect for any variable. The donors produced an average of 11.6±9.8 total oocytes, 6.9±6.4 viable oocytes, and 1.6±0.9 embryos per OPU. Unlike described in other studies (Edwards *et al*, 1996, Biol. Reprod.,55, 341-346; Camargo *et al*, 2007, Theriogenology, 68, 626-632) no differences were observed in the total production of oocytes and embryos in different months of the year. The conversion rates of oocytes to embryos was also similar in different months of the year ($P>0.05$). It is believed that the difference compared to other studies is due to management conditions that can be very different between breeding systems. We conclude that, in the studied region, *Bos taurus taurus* donors did not demonstrate effects of seasonal climate variation in the production of viable and total oocytes, as well in the conversion of oocytes to embryos.

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A136 OPU-IVP and ET

The use of FSH diluted with slow release carrier (MAP5) reduces the efficiency of *in vivo* embryo production protocols of Holstein donors

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Keywords: *in vivo* embryo, MAP5, superstimulation.

The present study evaluated the superovulatory response and *in vivo* embryo production in lactating and non-lactating Holstein donors, treated with a superstimulation protocol using a slow release (MAP5; hyaluronic acid, Bioniche). The study was performed in a commercial dairy farm (Agrindus S/A, Descalvado - SP). A total of 59 donors (29 lactating and 30 non-lactating cows) were initially allocated into two experimental groups in a cross-over design, in a total of 119 superovulations (SOV). At a random day of the estrous cycle (D0), the animals received an intravaginal progesterone device (P4; Primer, Tecnopec; non-lactating cows - 1 device / lactating cows - 2 devices) and 2.0 mg intramuscular (IM) of estradiol benzoate (RIC-BE, Tecnopec). From D4 on, the FOLL group received Folltropin (Tecnopec; n=59; non-lactating cows=300 mg; lactating cows=400 mg) diluted in 20 ml of saline and fractionated in 8 decreasing doses (4 ml, 4 ml, 3 ml, 3 ml, 2 ml, 2 ml, 1 ml and 1 ml), 12 h apart. The MAP/FOLL group (n=59) received the same dose of Folltropin, however, diluted in MAP5 and fractionated in 2 decreasing doses (lactating cows - 6.5 and 3.5 ml; non-lactating cows - 5.0 and 2.5 ml) on days D4 and D6 of protocol. On D6 (AM and PM) 0.150 mg of cloprostenol (Estron, Tecnopec) was injected. On D7 pm, the P4 implants were removed and 12 and 24 h later 62.5 µg IM of lecirelin (Gestran, Tecnopec) was injected in non-lactating and lactating cows, respectively. The non-lactating females were inseminated on D8 PM and D9 AM and the embryo flushing was performed on D15; the lactating females were inseminated on D9 AM and PM and the embryo flushing was performed on D16. Immediately before flushing, the number of corpus luteum (CL) was evaluated and recorded. The sire was the same in both superovulation protocols for each female. Statistical analysis was performed by the GLIMMIX procedures of SAS. There was no interaction between lactating and non-lactating categories for the response variable analyzed ($P>0.05$), therefore the data were grouped. It was found that animals submitted to the FOLL group had higher superovulation rate [number of females with two or more CL per number of SOV protocols (FOLL: 89.8% and MAP/FOLL: 45.8%; $P<0.0001$), total of CL at the day of the uterine flush (FOLL: 10.8 ± 1.1 and MAP/FOLL: 3.7 ± 0.7; $P<0.0001$), total number of recovered ova [FOLL: 7.9 ± 0.9 and MAP/FOLL: 1.8 ± 0.6; $P<0.0001$], recovery rate [FOLL: 66.8% (425/636) and MAP/FOLL: 48.9% (107/219); $P<0.0001$] and the number of viable embryos [FOLL: 3.3 ± 0.6 and MAP: 1.8 ± 0.6; $P<0.0001$] when compared to the animals of MAP/FOLL group. Thus, it was concluded that the injection of two doses of FSH diluted with MAP5 reduces the efficiency of *in vivo* embryos production program of Holstein donors when compared to the use of FSH diluted with saline and injected in 8 decreasing doses.

A137 OPU-IVP and ET

Centrifugation force reduction in Percoll gradients for sperm selection increases the fertilizing capacity *in vitro* in bovine

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Keywords: centrifugation, IVF, sperm selection.

High centrifugal forces have been routinely used for separation of spermatozoa by the Percoll gradient method, to obtain a high sperm concentration. These forces, however, can cause anything from minor damage to sperm, such as reduced motility and vigor, until serious consequences to zygote formation, cleavage, and embryo development. This study aimed to determine the influence of centrifugal forces during the selection of sperm by the Percoll method in sperm recovery, morphofunctional characteristics, fertilizing capacity *in vitro*, and embryo development within the first 48h. Five replicates of semen from four bulls Bos taurus were used. After thawing they were evaluated using a phase contrast microscope at 400x magnification for motility, vigor, and concentration, and at 1000x magnification for morphology. The membrane integrity was assessed by hypo-osmotic shock technique, with sodium citrate. The samples underwent centrifugation in Percoll discontinuous gradient (30%, 60% and 90%) with the following forces: F1 (9000 x G) or F2 (2200 x G). Oxidative stress was evaluated by measuring the production of ROS (reactive oxygen species), lipid peroxidation, glutathione (GSH), and activity of superoxide dismutase (SOD). ROS levels in semen were determined by a spectrofluorimetric method using 2', 7'-dichlorofluorescein diacetate (DCF-D). A dose of $2x10^6$ spermatozoa/ml of each bull was used to IVF. Presumptive zygotes (100/treatment) were denuded and incubated with Hoechst 33342 solution (10 mg/ml) and evaluated for the presence of spermatozoa penetrated, formation of the pronuclei or nuclei fused. Some of the presumptive zygotes (10/treatment) were individually cultured for 48 h in SOFaaci + 10% ESS and BSA in petri dishes over an embryonic monitoring system (Primo Vision, Cryo Mangement Ltd., Hungary). Embryonic development was assessed by the cleavage rate, time of first cleavage, and average number of blastomeres at 48 h. Data were analyzed by chi-square (X^2) and ANOVA and the means were compared by Tukey test at 5%. There were no significant differences between F1 and F2 in the morpho-functional characteristics, evaluation of oxidative stress, cleavage (87 and 78%, respectively), moment of first cleavage (31.9 and 31.7 h) or average number of blastomeres at 48 h (3.3 and 3.6 cells). However, the F1 produced penetration and normal fertilization rates (203/324, 63%) smaller than F2 (241/354, 68%, $p < 0.05$). These results show that the force of 2200 x G increases the penetration and fertilization rates without reducing the sperm recovery when compared to 9000 x G, that is routinely used in sperm selection by Percoll in cattle.

A138 OPU-IVP and ET

The effect of FSH stimulation prior to the ovum pick-up in *in vitro* embryo production of Gir cows

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Keywords: *in vitro* embrio, lactating Gir cow, superstimulation.

The study evaluated the effect of the FSH (Folltropin, Tecnopec) diluted in slow release solution MAP5 (hyaluronic acid, Bioniche®) prior to the Ovum Pick-Up (OPU) on *in vitro* embryo production (IVP) program of lactating Gir cows. A total of 12 Gir (*Bos indicus*) cows was used in a *cross-over* design. Females were randomly allocated into three groups: control group (CON; n=12); Folltropin 133 mg (FOLL; n=12); MAP5/Folltropin 133 mg (MAP5/FOLL, n=12). At a random day of the estrous cycle (D0 AM) all cows received an intravaginal progesterone device (P4; Primer, Tecnopec) and 2.0 mg I.M. of estradiol benzoate (RIC-BE, Tecnopec). On D4 and D5 (AM and PM), the FOLL group received four decreasing doses of FSH I.M. (D4 AM and PM = 4ml; D5 AM and PM = 3 ml). The MAP5/FOLL group received a single dose of 3 ml I.M. of MAP5/Folltropin on D4 AM. The P4 devices were removed at D7 AM and cows were submitted to OPU at same day. The same mating was maintained for all IVP procedures during the experiment. The *In vitro* produced embryos (n= 125; CON: n=33, FOLL: n=42 and MAP5F/FOLL: n=50) were transferred to crossbreed recipients previously synchronized for timed embryo transfer (TET). Pregnancy diagnosis was performed 60 days after TET. Variables were analyzed by the GLIMMIX procedure of SAS. The groups FOLL (16.3±1.3) and MAP/FOLL (16.7±1.3) presented higher number of oocytes recovered than CON group (10.9±1.0; P=0.0001). Similarly, higher number of viable oocytes was found in FOLL (11.3±0.8) or MAP5/FOLL (12.9±1.0) than the CON group (7.8±0.5; P<0.0001). However, no differences were found on the blastocyst rate (CON: 35.7±3.2; FOLL: 31.8±3.9; MAP5/FOLL: 28.7±3.3; P=0.44), number of embryo produced per OPU session (CON: 2.8±0.3; FOLL: 3.5±0.4; MAP5/FOLL: 4.2±0.6; P=0.14) and number of pregnancy achieved per OPU session (CON: 1.3±0.6; FOLL: 1.6±0.4; MAP5/FOLL: 1.9±0.2; P = 0.48). In conclusion, FSH treatment prior to the OPU, with or without MAP5, increased the number of oocytes recovered; however, similar number of viable embryos was produced per OPU session in Gir cows.

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A139 OPU-IVP and ET

Spermatozoa pre-incubation increases the IVF embryo development from poor quality porcine oocytes

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Keywords: IVP, polispermy, sperm capacitation.

An important cause of low efficiency in porcine *in vitro* embryo production is the high polispermy rate, which is exacerbated in poor quality oocytes. It was already shown that the reduction in the insemination dose during IVF decreases the rate of polispermy. It is possible that the sperm pre-capacitation can result in greater polispermy reduction, mainly in poor quality oocytes. The objective of this study was to evaluate different sperm pre-capacitation periods in porcine IVF of low quality oocytes. Follicles of 3 to 6 mm of diameter were aspirated from ovaries obtained in abattoirs. Low quality oocytes (without compact cumulus cells and heterogeneous cytoplasm; with vesicles and granules) were selected to the study. The IVM was performed in TCM-199 supplemented with 26.19 mM de Sodium Bicarbonate, 25% de Follicular Fluid (FF), 0.1 mg/mL L-Cysteine, 10 ng/mL of Epidermal Growth Factor, 100 UI/mL Penicillin G, 0.1 mg/mL Streptomicine Sulfate, 0.5 mg/mL LH, 0.01 UI/mL FSH and 1 mM dbcAMP. After 22h the oocytes were transferred to an IVM media without LH, FSH and dbcAMP, for additional 18 to 20h. The oocytes were then transferred to dishes with mTBM added with 0.4 mg/mL caffeine and 2 mg/mL BSA. The semen was obtained from fresh ejaculate of an IVF pre-tested boar, and maintained at 15 to 17°C. For fertilization, semen was heated at 30°C for 10 min and sperm selection performed by mini Percoll gradient (45 and 90%). Then the semen was pre-incubated in IVF medium for different periods of times, according the experimental group: 0h (Control), 0.5h, 1h, 1.5h, with 03 replicates. At the end of pre-incubation time, the oocytes were introduced in the medium containing 62,500 spermatozoa/mL and incubated for additional 3h. After this, the zygotes were cultured in PZM-3, being added 10% of FBS in day 4 of culture. IVM, IVF and IVC were performed in an incubator at 38.8°C, at 5% CO_2 atmosphere. The cleavage and blastocyst rates were evaluated at days 2 and 7 of IVC, respectively. The results were analyzed by qui-square test ($P < 0.05$). For cleavage, the pre-incubation groups of 1h (78.8%-190/241) and 1.5h (79.9%-207/259) were similar, and both were higher than 0h (38.1%-45/118) and 0.5h (68.7%-145/211) groups. The higher blastocyst production was observed with 1.5h of pre-incubation (13.5%-35/259). The blastocyst production of the groups 0h (5.9%-7/118), 0.5h (7.6%-16/211) and 1.0h (6.6%-16/241) do not show any differences ($P > 0.05$). The results show that 1.5h of sperm pre-incubation prior to IVF increases the cleavage and blastocyst rates of low quality porcine oocytes. In conclusion, adjusts in IVP system can result in a satisfactory embryo production with low quality porcine oocytes.

A140 OPU-IVP and ET

In vitro production of bovine embryos after meiosis delay

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Keywords: forskolin, *in vitro* maturation, meiosis.

This study aimed to show if the use of forskolin is able to inhibit maturation in bovine oocytes, producing a higher rate of embryos *in vitro*. Nellore oocytes were matured in TCM-199 and to delay meiosis, the oocytes were maintained for 6 h in medium in presence of 0.1mM forskolin. Then the oocytes were cultured for 18 h in agent-free medium to resume meiosis, completing 24 h of maturation. After resume of meiosis, the oocytes were stained with Hoechst 33342 and classified in: germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), metaphase II (MII), degenerated or unidentified (D/U). Then (day 0), oocytes were fertilized in human tubal fluid (HTF) and the semen was selected by Percoll gradient and the concentration adjusted to 2 x 10^6 sperm/mL. The presumptive zygotes were culture in SOFaa in 5% CO_2, 5% O_2, 90% N_2 atmosphere until day 7, when blastocyst rate was evaluated. Apoptosis in blastocysts was accessed by TUNEL (Terminal deoxinucleotil transferase Uracil Nick End Labeling) reaction. Data were analyzed by ANOVA, followed by Tukey test using the general linear model (PROC GLM) of SAS. The level of significance was 5%. There were differences in MI phase between the control group: 8.3 ± 6.2^a (N=166) and forskolin 0.1mM group 34.1 ± 6.7^b (N=144); P<0.05. On the other hand, there were no differences in other phases between the control group (GV: 0, GVBD: 0.8 ± 0.9, MII: 67.6 ± 9.6, D/U: 7.3 ± 3.8) and forskolin 0.1mM group (GV: 0, GVBD: 1.0 ± 0.9, MII: 50.2 ± 10.4, D/U: 14.1 ± 4.1) P>0.05. No differences were observed in blastocyst production rate between control (36.7% ± 3.7) and forskolin 0.1mM (25.1% ± 3.7) (P>0.05). When we analyzed the apoptosis rate, we found differences between the control (6.0% ± $6,3^a$) and forskolin groups 0.1mM (33.4% ± 6.3^b) (P<0.05). Although forskolin was able to produce embryos with the same rates of the control group, the embryos treated with this drug presented a higher rate of cellular apoptosis. This suggests that further reduction of the concentration of forskolin used in the maturation medium is needed.

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A141 OPU-IVP and ET

Superovulatory protocol using low dose of equine pituitary extract (EPE): comparison between Crioula and American Quarter horse breed

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Keywords: donor mares, embryo transfer, folicular growth.

Equine superovulatory protocols frequently lead to a low embryo recovery rate per ovulation (around 50%). The reduction of EPE doses can be an efficient option to increase embryo recovery rates, and also to reduce the costs involved in equine ET procedures. Recently the use of biotechnologies was allowed in Crioula pony mares. This fact has generated a great quest from breeders by biotechniques, especially superovulation and embryo collection. However, there is not a suitable protocol and there is little information about using of these biotechnologies in Crioula breed. This study aimed to evaluate the superovulatory response of embryo donor mares of Crioula and American Quarter Horse (AQH) breeds, using an EPE low doses protocol. Six Crioula and eight AQH mares, with excellent sanitary and reproductive conditions, were submitted to a dairy follicular control by ultrasonography. The animals were distributed into two groups. In the control group the monitoring of follicular growth was performed until the time of natural ovulation. In the EPE group, when one or more follicles reached 20mm in diameter, was initiated the IM injection of 7mg of EPE, twice a day, with 12h interval, until the time of ovulation induction. When follicles reached 35mm of diameter, ovulation was induced by injection of 2500IU of hCG. Embryo donors were inseminated every 48h until ovulation and the embryo recovery were performed at the eighth day post ovulation. The mares were monitored during three consecutive estrous cycles, undergoing both treatments. Data were analyzed with GLM procedure of the SAS statistical package. The variables were analyzed by Tukey's test and the least squares means were adjusted for Tukey-Kramer multiple comparisons test. Crioula and AQH breed's mares showed different follicular growth rate. We observed that in Crioula mares the follicular growth was slower, leading to longer duration of superovulation therapy (6.6 days) compared to the AQH mares (4.7 Days - $P < 0.01$). The number of recovered embryos was influenced by the protocol with low doses of EPE in the Crioula breed ($P < 0.01$), but showed no effect on AQH breed mares ($P > 0.05$). The amount of recovered embryos was higher in Crioula mares treated with EPE (3.0 ± 2.0 embryos) when compared with not treated counterparts (natural ovulation, 0.83 ± 0.4 embryos), and also compared with the AQH mares treated with EPE (1.12 ± 0.8 embryos) or not treated (Control 0.5 ± 0.5 embryos) ($P < 0.05$). The superovulatory protocol using low doses of equine pituitary extract increased the number of embryos collected for the Crioula breed mares compared with AQH mares, being an interesting alternative to increase the number of embryos recovered per donor mare.

A142 OPU-IVP and ET

Effect of the use of successive hormonal stimulation and laparoscopic oocyte recovery on quanti-qualitative oocyte production in goats raised in the tropics

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Keywords: goat, laparoscopy, oocyte.

The laparoscopic oocyte recovery (LOR) can be an excellent tool for the multiplication of genetically superior goats when associated with *in vitro* embryo production (IVEP). However, for a more efficient technique it is important that it can be performed several times in the same donor. This study aimed to verify the effect of the successive use of hormonal treatment followed by LOR on the quantitative and qualitative production of oocytes in goats raised in the tropics. For this purpose, 12 adult crossbred Anglo Nubian goats, cyclical, were submitted to five successive hormonal treatments for ovarian stimulation. Intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (Progespon, Syntex, Buenos Aires, Argentina) were inserted at the beginning of the treatment (D0). On D7 75 µg of cloprostenol (Prolise, ARSA, Buenos Aires, Argentina) was given i.m. The stimulation was obtained by the injection of 180 mg pFSH (Folltropin-V, Bioniche, Belleville, Canada), divided into 5 decreasing doses with an interval of 12 h from D7 to D9 of the progestagen treatment. Thirty-six h before LOR, goats were fasted and LOR was performed using volatile anesthesia, starting with i.v. injection of 20 mg / kg thiopental (Tiopentax 2.5% Cristália, Sao Paulo, Brazil) and maintained with isoflurano (Isoforine, Cristália, São Paulo, Brazil). Under laparoscopic control, the follicles were visualized and classified as small (<3 mm), medium (3-4 mm) and large (> 4 mm). The cumulus-oocyte complexes (COCs) were aspirated with the help of a vacuum system (WTA, Cloves, Brazil) and in classified in Grades I to IV. The results were expressed as mean ± SEM and analyzed by ANOVA (repeated measures) followed by Tukey test (5%). A total of 1149 follicles (19.1 ± 2.6 follicles / donor) was punctured and 822 oocytes were collected (13.7 ± 1.6 oocytes / donor) which resulted in a total harvest rate of 71.5%. There were no statistical differences in the number of punctured follicles and oocytes collected during the five sessions LOR, respectively (21.3 ± 2.1 and 12.9 ± 1.5 *vs* 21.8 ± 3.4 and 15.6 ± 1.7 *vs* 20.0 ± 1.7 and 15.2 ± 1.5 *vs* 16.5 ± 2.3 and 12.9 ± 2.0 *vs* 16.3 ± 1.4 and 11.9 ± 1.1; P > 0.05). However, the number of large follicles observed in session 1 was different when compared to sessions 2 and 4 (5.8 ± 0.7 *vs* 2.8 ± 0.6 and 2.0 ± 0.4, P <0.05). Regarding to harvest rate, there was no statistical difference between the sessions, averaging 61.4, 72.6, 75.5, 71.7 and 73.2% (P> 0.05) for sessions one to five, respectively. Only 17% of oocytes collected were classified as degenerated (Grade IV) and 83% viable (GI-III). In conclusion, five successive hormone treatments followed by LOR did not affect the quantitative and qualitative production of oocytes in goats rose in the tropics, and may be a useful tool for genetic improvement in goats.

A143 OPU-IVP and ET

Use of forskolin as lipolytic drug production and cryopreservation of bovine embryos produced *in vitro* (PIVE)

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Keywords: bovine embryos, cryopreservation, forskolin.

Bovine embryos produced in vitro have less tolerance to cryopreservation compared to embryos produced in vivo. One of the factors related to this low tolerance is the highest concentration of intracellular lipid in vitro embryos. The forskolin is a substance derived from an Indian plant (Coleus forskohlii) has been described in the literature as a metabolic regulator in inhibiting the formation of intracellular lipid droplets (Ye, J., et al. 2,010, Regul. Pept., 161, 58-66). The aim of this study was to evaluate the effect of forskolin on the rate of blastocyst and their respective cryopreservation. Oocytes were recovered from abattoir ovaries, matured for 22 h (TCM 199 with 10% FCS, 5.0 mg / mL LH 0.5 mg / mL of FSH, 0.2 mM pyruvate and 50 g / mL gentamicin) for 18 h fertilized (TALP with heparin and PHE - 2 x 106 spermatozoa / mL) and cultured for 7 days in SOFaa (10% FCS) supplemented with 0 (control), 10, 25, and 50 mM forskolin 38.5°C and 5% CO2. On the seventh day of culture was determined blastocyst rate compared to the total number of oocytes and embryos in the blastocyst stage expanded (grade I and II) were cryopreserved by slow freezing technique, using ethylene glycol as a cryoprotectant. After freezing and thawing, embryos were co-cultured in 100mL of droplets with SOFaa cell monolayer incubated at 38.5°C, 5% CO2 and determined the percentage of re-expansion (24 h), and hatch (48 h). Data were analyzed by ANOVA followed by Tukey test ($P < 0.05$) ± standard deviation. The embryonic development of 240 oocytes were evaluated in four replications. The percentage of embryonic development on the seventh day of culture in medium supplemented with 0, 10, 25 and 50 mM forskolin was (40.08 ± 6.0, 48.26 ± 21:26, 44.42 ± 6.0 and 47.42 ± 8.34% respectively), showing no statistical difference ($P > 0.05$). The evaluation of the rate of re-expansion of the embryos treated with forskolin did not differ ($P > 0.05$) between the control group (86.0 ± 8.28%), 10 (17:13 ± 81.0%), 25 (83.0 ± 5.3%) and 50 mM (17.2 ± 76.0%). The percentage of embryos hatched after thawing was 56.0 ± 14.70, 74.0 ± 25.21, 55.0 ± 17:57 and 25:35 ± 28.0% for the groups treated with 0, 10, 25 and 50 mM forskolin, respectively, with no significant difference ($P > 0.05$). In conclusion, bovine embryos cultured in the presence of different concentrations of forskolin did not increase the rate of embryo cryopreservation and tolerance in relation to the control group.

A144 OPU-IVP and ET

Pregnancy rates following FTET of *Bos taurus* x *Bos indicus* with high, intermediate and low numbers of antral follicles

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Keywords: antral follicle count, embryo recipient, pregnancy.

The objective of this study was to evaluate the influence of the antral follicles population on pregnancy rate of embryo recipient heifers. *Bos taurus x Bos indicus* heifers (Bos indicus; n=281) with BCS of 3.0 ± 0.5 were submitted to a protocol for synchronization of ovulation. The animals received an intravaginal device (CIDR, Zoetis, Brazil) and 2mg of estradiol benzoate (EB); (Estrogin, Farmavet, Brazil). Eight days later, the animals received 0,5 mg of PGF2 (Ciosin, Intervet Schering-Plough, Brazil), 300UI of eCG (Novormon, Syntex SA, Argentina) and 0.5 mg of estradiol cipionate (EC);(ECP, Pfizer, Brazil), IM. Antral follicles ≥ 3 mm were counted (D17) using an intravaginal micro-convex array transducer and the heifers received embryos produced in vitro 17 days after the beginning of the hormone treatment. Heifers were assigned into groups with high antral follicular count (AFC; G-High ≥30 follicles, n=38) intermediate AFC (G-Intermediate, <11 follicles, n = 136) or low AFC (G-Low, ≤3 follicles, n = 75). The number of follicles was evaluated by Kruskal-Wallis and pregnancy rates were compared by Chi-square test (P ≤ 0.05). The average number of antral follicles (mean ± SD) was 12.94 ± 9.21 and the mean pregnancy rate 33.08% (93/281). The mean population of antral follicles was 25.82 ± 7.40 (G-High), 11.34 ± 2.96 (G-Intermediate) and 3.85 ± 1.35 follicles (G-Low, P<0.05). There was no difference in pregnancy rates between groups (G-High, 30.00%; G-average, 33.82%, G-Low, 34.60%, P> 0.05). It was concluded that the population of antral follicles on D17 of the TETF protocol does not affect the pregnancy rate of embryo recipient heifers.

A145 OPU-IVP and ET

Evaluation of aqueous extract of propolis as replication inhibitor of murine zygotes *in vitro* experimentally infected by bovine herpesvirus type 1 Colorado strain (BoHPV-1)

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Keywords: BoHPV-1, propolis, replication inhibitor.

Despite greater control over the transmission of pathogens by the use of biotechnologies in animal breeding, there is, paradoxically, the potential for disease transmission by bovine embryo transfer which remains a justifiable concern for regulatory agencies. The objective of this work was to evaluate whether the detrimental effects of the experimental infection of murine zygotes with bovine herpesvirus type 1 (BoHPV-1; Colorado strain, 10^8 $TCID_{50}$/mL) during 24 h could be reduced by the viral inhibitor aqueous extract (AE) of Propolis (Pp). Between six and eight weeks old female mice (Swiss lineage) were superovulated with 0.2 mL (5 UI) of eCG-hCG and mated with males from the same lineage. After 18 h, the zygotes were collected and washed with 0.5% pronase solution. The zygotes were allocated into four groups, which were exposed to: 10 µL of PBS (G1; control), 10 µL of BoHV-1 virus (G2; 10^8/mL $TCID_{50}$/mL), 10 µL of Propolis Aqueous Extract (PpAE) in 0.001% in PBS (G3), 10 µL of PpAE in 0.001% in PBS and 10 µL of BoHV-1 virus (G4). All groups were kept for 24 h at 37.5°C, 5% CO_2 in air. In this work were evaluated the embryo morphological differences, cleavage rate, and viral replication by titration (Reed and Muench test) after 72 h co-culture with Madin-Darby bovine kidney (MDBK) cells. Differences among groups in cleavage rate were compared by Fisher test and in titration by Mann-Whitney test. The non toxic Propolis Aqueous Extract concentration was 0.001%. Only the group G2 showed morphological differences, like granulations and degenerative appearance. The cleavage rates were as follows: G1, 77% (154/201); G2, 53% (138/262); G3, 82% (224/273) and 74% (185/250). There were no significance differences ($p<0.005$) in cleavage rate among the groups G1xG4 and G1xG3. There was significance difference ($p<0.05$; $P = 0.0286$) in viral replication rate between groups G2 (6.04×10^7 $TCID_{50}$/mL) and G4 (1.63×10^3 $TCID_{50}$/mL). These results support the conclusion that Propolis Aqueous Extract reduces the viral rate of replication without interfering in cellular development, and may be an alternative to sanitary control protocols.

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A146 OPU-IVP and ET

Seasonal variation of OPU-IVP results in Zebu donors (*Bos taurus indicus*)

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Keywords: climate, oocytes, zebu.

Zebu breed donors produce more oocytes and are less sensitive to seasonal variations in the results of *in vitro* production of embryos in tropical and subtropical regions (Viana, 2005. Acta Sci. Vet.,33, 213-219; Camargo *et al*, 2007, Theriogenology, 68, 626-632; Nunes. 2010, Reprod. Fertil. Dev. 22, 567-573.). This study aimed to compare the distribution of the results of IVEP over the months of the year in *Bos taurus indicus* donors, done in the south and southwest of the Minas Gerais state (Brazil) by the same veterinarian. The region has mesothermic climate of CW classification, according Koepen. Results from 1349 follicular aspirations (OPU) from Gir (594), Nelore (345) Brahman (283) e Guzerá (127) donors were used, done for a period of 12 months. In this period the total rainfall was 2.970mm, the maximum temperature was 38.7°C and the minimum was 4.6°C. The management of animals was in the pasture *(Brachiaria decumbes e Brachiaria brizanta)* with commercial mineral supplementation *ad libitum*. For OPUs was used an ultrasound device with intravaginal microconvex transducer of 7,5 MHz (Mindray DP 2200). All follicles larger than 3 mm were identified and punctured. The Cumulus oocytes complexes (COCs) recovered were counted and classified based on their morphological aspect. The viable oocytes were matured (TCM 199) for 22-24 hours after the start of OPU at a temperature of 38.5°C, 5% CO2 and saturated humidity. Sorted sexed semen of different bulls, evaluated for motility and vigor, was used for *in vitro* fertilization (IVF). After IVF the presumptive zygotes were transferred to *in vitro* culture (SOF), where they remained for seven days at a temperature of 38.5°C and controlled atmosphere (5% CO2). The average production of oocytes and embryos conversion was compared between breeds and months of the year by Dunn's test, considering a 5% significance. There was no breed effect for any variable. Donors produced an average of 20.4±16.5 total oocytes, 13.2±12.5 viable oocytes, and 4.0±3.2 embryos per OPU. No differences were observed in the production of viable and totals oocytes in the different months of the year. In April and May, the average production of embryos was lower ($P<0.05$), observed by a lower conversion of oocytes into embryos in those periods. The drop in efficiency of zebu donors during this period may be related to two situations simultaneously: possible cold stress (Hansen, 2004. Anim. Reprod. Sci, 83, 349-360) due to drop temperature during these months and indirect effect by reducing the quality of pastures (Turner 1980, J.Anim.Sci. 50, 1201 – 1205). It was concluded that zebu donors suffer seasonal variation in the intrinsic quality of the oocyte and embryo production by IVP in the region studied.

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A147 OPU-IVP and ET

Effect of HSP90 inibitor on developmental competence of bovine oocytes

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

The heat shock protein 90kda (HSP90) is a cytoprotective chaperone that influences the maturation of *Xenopus* oocytes (Nebreda and Ferby, 2000. Curr Opin Cell Biol 12:666-675). Its effect can be repressed by inhibitors as 17-(allylamino)-17-demethoxygeldanamycin (17AAG, Sigma, St. Louis, USA). This study aimed to evaluate the effect of 17AAG concentration and exposure time during *in vitro* maturation in order to identify a possible HSP90 requirement for oocyte developmental competence. 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 *in vitro* maturation. The maturation was performed in Nunc plate containing 400μL of TCM199 medium (Invitrogen, Carlsbarg, USA) supplemented with porcine FSH (pFSH - Pluset, Lab. Callier, Espanha) and 10% estrus cow serum, and incubated at 38.5ºC under 5% CO_2 and 95% humidity for 24h. Oocytes were *in vitro* fertilized (IVF) for 20h and incubated under the same maturation conditions. Semen was processed by Percoll gradient and a fertilizing dose of $2x10^6$spermatozoa/mL was used. After IVF, the presumptive zygotes were denuded in a solution of PBS plus 1% hialuronidase and then cultured in wells with 500 μL of modified CR2aa medium supplemented with 2.5% FCS (Nutricell, Campinas, Brasil) in an incubator at 38.5ºC under 5% CO_2, 5% CO_2 and saturated humidity for 8 days. Cleavage was evaluated on day three (D3) and blastocysts were evaluated on day seven (D7) and on day (D8) post-fertilization. Data from nine replicates (n=1836 oocytes) 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. Regarding to exposure time, there was no difference for cleavage and blastocysts rates in D7 and D8 between 12h and 24 hours. Blastocyst rates of 2μM 17AAG group were decreased on D7 (18.6±2.2%; $P<0.02$) and on D8 (20.4±2.2%; $P<0.01$) when compared to 0μM (29.2±2.5% and 34.0±3.3% for D7 and D8, respectively), whereas 1μM produced intermediary blastocyst rates (25.6±2.7% and 27.9±3.1% for D7 and D8, respectively). There was no interaction ($P>0.05$) between concentration and exposure time. In conclusion, inhibition of HSP90 by 17AAG decreases oocyte developmental competence and suggests that this protein is also required for maturation of bovine oocytes.

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A148 OPU-IVP and ET

Use of reverse sorted and conventional semen on "*in vitro*" embryo production

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Keywords: cleavage, embryo, sexing.

Sexed semen production using previous frozen straws (reverse sorted semen), allows produce animals of desired gender, even after male death or sterility. It is known that semen sexing is harmful to spermatozoa function (Seidel, 2002, Reproduction, 124, 733–743); however, the possibility to have more embryos from desired sex made the reverse sorted semen available to commercial laboratories of embryo IVP. However, literature data comparing embryo IVP using conventional or reverse sorted semen are rare. The aim of our work was to compare the embryo *in vitro* development rates using conventional or reverse sorted semen from same animals (6 bulls). To achieve our goal we used data from an embryo IVP commercial laboratory during 2011 and 2012. Results were analyzed by ANOVA with 5% significance level (SAEG). A total of 178 ovum pick-ups were performed in conventional semen group and 85 in sex sorted semen group, whereas 2692 e 1536 viable oocytes were obtained, respectively. No differences were observed between cleavage rate: 67% ± 2 and 70% ± 3, or embryonic production rate seven days after fertilization: 36% ± 2 and 34% ± 3, in conventional and sexed sort semen, respectively. Once that a larger number of oocytes were used and the same bulls were distributed in two treatments, it is possible to conclude that injuries caused to bulls gametes during reverse sorting process was not able to decrease in vitro embryo development rates.

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A149 OPU-IVP and ET

Influence of the inhibition of phosphodiesterases-3 and -8 on meiosis resumption of bovine oocytes

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Keywords: cAMP**,** maturation, phosphodiesterases.

Oocyte in vivo maturation is a highly orchestrated process in which meiosis is resumed by the gonadotropin surge that precedes ovulation and induces the decrease in cAMP levels in the oocyte (Gilchrist, R. B et al., 2009 Reprod. Fertil. Dev. v.22, p. 293-293). cAMP is synthesized by adenylyl cyclase (*ADCY*) and degraded by phosphodiesterases (PDE), of which some are cAMP-specific and other to cGMP (Richard *et al*., 2007, J Anim Sci, 85, 4-6). The activity of cAMP-specific PDE3 is prevalent in oocytes, while PDE8 is prevalent in cumulus cells (Sasseville *et al*., 2009, Biol Reprod, 81, 415-425). The aim of the present study was to determine the contribution of different isoforms of phosphodiesterases in resumption of meiosis when high levels of cAMP are maintained by the addition of different cAMP-specific PDE inhibitors to the maturation medium. Cumulus-oocyte complexes (COCs) were matured in vitro in the presence, absence or combination of inhibitors of cAMP-specific PDEs. COCs were aspirated from abattoir ovaries, selected (grade 1 and 2), transferred in groups of 10 to 100 μL droplets of TCM 199 + 0.1% PVA, under mineral oil, containing the treatments: 1) control (no inhibitors), 2) PDE3 inhibitor (cilostamide, 20μM) 3) PDE8 inhibitor (dipyridamole, 50 mM) and 4) combination of cilostamide and dipyridamole. Cultures were carried out for 9 h at 38.5°C and 5% CO_2 in air. For evaluation of nuclear maturation rates, the oocytes were denuded, fixed between slide and cover slip for 24 h (methanol: acetic acid, 3:1), stained with 1% acetic orcein and observed under phase contrast microscope. Oocytes in germinal vesicle (GV) were considered immature and in metaphase I (MI) as having already resumed maturation (GV breakdown). Six replicates were performed. Data analyzed for the effects of the treatments were tested by ANOVA and means compared by Tukey test (SAS, 2002), considering a level of significance of 5%. Cilostamide alone or in combination with dipyridamole reduced the proportion of oocytes reaching MI (20.9 and 28.7%, respectively) compared to control (77.7%, $P<0.05$) and dipyridamole (74.8%, $P<0.05$). Cilostamide combined with dipyridamole was similar to cilostamide alone ($P>0.05$). In conclusion, the inhibition of PDE3 (most active isoform in oocytes) with cilostamide was sufficient to reduce the proportion of oocytes resuming meiosis. Inhibition of PDE8 (most active isoform in cumulus cells) with dipyridamole did not inhibit meiosis resumption and its association to cilostamide did not contribute further for the meiosis inhibition induced by cilostamide alone. Therefore, it seems that there is a major relative contribution of cAMP levels produced by oocytes to block meiosis than of cumulus cells. However, more studies are necessary to confirm this possibility.

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A150 OPU-IVP and ET

Effect of recombinant human FSH during *in vitro* maturation on apoptose of bovine blastocysts

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Keywords: apoptotic index, maturation, recombinant hormone.

Previous study reported that recombinant human FSH (rhFSH) during in vitro maturation (IVM) allows blastocyst production at the same rate of FSH from porcine pituitary (Souza et al., 2012. Anim Reprod, 9:550). The present study aimed to evaluate the total cell number and cells in apoptosis in blastocysts generated from oocytes matured in vitro with different rhFSH concentrations. rhFSH was gently donated by Galactous Biotech Ltda (Concepción, Chile). Immature COCs obtained from slaughtered animals were randomly allocated in six treatments of *in vitro* maturation as follow: T1 - control with porcine FSH (pFSH - Pluset, Callier, Spain), T2 – without FSH; T3 – 0.0105 UI rhFSH; T4 – 0.021 UI rhFSH; T5 - 0.042 UI rhFSH and T6 – 0.084 UI rhFSH. IVM was performed in TCM199 medium (Invitrogen, Carlsbarg, USA) supplemented with 10% estrus cow serum for 24h at 38.5 ºC under 5% de CO_2 and 95% humidity. After IVM, oocytes were *in vitro* fertilized and cultured in modified CR2aa medium supplemented with 10% fetal calf serum (Nutricell, Campinas, Brasil) at 38.5 ºC under 5% de CO_2 and 95% humidity. Two hundred two blastocysts, from the different treatments, were fixed in 4% paraformaldehyde at the eighth day post-fertilization and evaluated by TUNEL technique for quantification of cells number and apoptotic index. Evaluation of total cell number and apoptotic index of inner cell mass (ICM) and trophoblast (TE) were also performed in some blastocysts (n=45). Localization of ICM and TE nuclei was based on their position in the captured images using ImageJ software. Data was submitted to analysis of variance and means compared by Student Newman Keul's test. Values are shown as mean ± SEM. There was no difference ($P>0.05$) on total cell number, total apoptotic cell number and apoptotic index in blastocysts from different treatments. When only TE was evaluated, both the total number of cells and the apoptotic index also were not different between treatments. However, in ICM, no difference in total number of cells was observed, but the blastocysts from T1 and T2 showed lower ($P<0.05$) apoptotic index (28.8±5.8 and 29.4±3.9%, respectively) than blastocysts from T3 and T4 (57.8±8.3 and 58.6±7.3%, respectively) but similar to T5 and T6 (44.8±4.4 and 46.3±3.8%, respectively). In all treatments the index of apoptotic cells in ICM (44.6±2.6) was higher than in TE (10.6±0.9). Overall, rhFSH during IVM does not influence the cells number or the index apoptotic in bovine blastocysts, nevertheless, can increase the apoptotic rate of ICM.

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A151 OPU-IVP and ET

Effect of ethylene glycol and glycerol in slow freezing of bovine embryos produced *in vitro*

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Keywords: ethylen glycol, glycerol, slow freezing.

For embryo freezing the use of intracellular cryoprotectants is recommended to protect the embryos from temperature decrease and ice crystal formation during the process. The intracellular water is replaced for the cryoprotectant while the temperature decreases. (Rodrigues, 2011; Nieman and Sommerfeld, 1999). The objective of the present work was to evaluate the viability of embryos produced *in vitro* after slow freezing with ethylene glycol or glycerol. Embryos were produced in accordance with the standardized protocol of Vitrogen. Expanded blastocysts of seven days in culture were selected and randomly divided into two groups (ethylene glycol and glycerol): Group 1 (n = 56) - exposed to ethylene glycol 1.5M with 0.4% BSA and 0.1M sucrose; Group 2 (n = 137) - exposed to glycerol in three steps: 1) 0.4% BSA (BSA) in Embriolife medium; 2) 5% Glycerol, with 0.1M sucrose in Embriolife medium, and 3) 10% Glycerol, with 0.1M sucrose in Embriolife medium, with exposure to glycerol of 5 minutes. Slow freezing was performed with the TKR (PK1000 model machine), in which each straw (0.25 mL) had seven embryos. All groups were submitted to cooling rate of 1 to 3°C/min until the temperature of -6oC, when crystallization was induced. Cooling continued to 0.3°C/min up to 35°C negative, when the straws were immersed in liquid nitrogen (N_2), and stored in liquid nitrogen containers. Thawing of Group1 and Group 2 were done in two steps: five seconds in the air and 15 seconds in water bath between 19-20° c. After this period, embryos received two five-minute baths, in two separate drops containing culture medium for removal of cryoprotectant, and returned to incubator at 38.5°C in a humidified atmosphere of 5% CO_2 in air for evaluation of reexpansion and hatching rates at 24, 48 and 72 hours post-thawing. Statistical analysis was performed using the Student t-test with a significance level of 5%. Reexpansion rates in the first 24 hours post-thawing were not different; however, at 48 and 72 hours after thawing, Group 1 (ethylene glycol) was better when compared with Group 2 (glycerol). In Group 1, the best result concerning reexpansion was obtained with 72 hours after thawing (35.7%). In Group 2, the best rate was obtained with 24 hours after thawing (19%). The bovine embryos produced in vitro and frozen slowly in medium containing ethylene glycol had higher reexpansion rate when compared to embryos frozen in the medium containing glycerol.

A152 OPU-IVP and ET

Effect of ovarian stimulation using FSH on oocyte recovery rate and *in vitro* embryos production in Sindhi breed - Preliminary results

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Keywords: in vitro fertilization, OPU, ovarian superestimulation.

The oocyte competence acquisition can be stimulated by FSH administration before follicular aspiration (FA) (Nivet et al., 2012, Reproduction 143, 165-171; Chaubal et al., 2007, Theriogenology 67, 719 - 728, Blondin et al. 2002, Biol Reprod 66, 38-43). The time between ovarian stimulation by the application of exogenous gonadotropins and FA (coasting), as well as the stage of follicular wave during ovarian overstimulation are crucial to the success of the technique (Chaubal et al., 2007, Theriogenology, 67, 719-728). The objective of this study was to compare the effect of ovarian stimulation using FSH before follicular aspiration in Sindhi cows on oocyte recovery rate and *in vitro* embryo production. Thirteen cyclic females were randomly distributed in three treatments. In all animals was performed ablation of follicles larger than five millimeters in diameter at the beginning of the treatment (D0). The animals were kept with intravaginal implant containing 1.0 g of natural progesterone (Cronipres ®, Biogenesis-Bagó SA, and Buenos Aires, Argentina) during all over the experiment, being removed only at the time of FA. In all animals a new progesterone implant was introduced in vagina just after FA. The treatment groups were: T1- Ovarian stimulation with FSH 80 mg (Folltropin-V ®, Bioniche, Bellevile, Ontario, Canada) divided in three intramuscular decreasing doses and with 24 h interval between applications (D1, D2, D3), being the last dose 54h before FA (D5,5); T2-Ovarian stimulation with FSH 80 mg, with a single dose (half dose administered subcutaneously and other half intramuscularly) 102h (D1) before FA and simultaneous with the first application of the T1 group; T3-Control group without ovarian stimulation. The hormonal administration began in the day after follicular ablation (D1). Three FA sessions for each treatment were performed within 1-week intervals between sessions. Data were analyzed by ANOVA and Tukey test. The number of follicles aspirated was 10.77±5.91 in T1, 10.22±6.41 in T2 and 9.87±1.88 for the control group. The mean of oocytes retrieved was 9.00±7.21 in T1, 8.11±2.39 in T2 and 6.00±2.39 in control group. The oocytes recovery rates were 83.50% (81/97) 79.34% (73/92) and 60.75% (48/79) respectively for the groups T1, T2 and control. Cleavage rates were 59.25%, 67.12%, 72.91% and blastocyst rates were 31.2%, 35.61% and 27.08%, respectively for T1, T2 and control group. No differences were found between groups for the characteristics evaluated. The FSH doses were not sufficient to increase the oocyte recovery rate and embryos production, being to necessary new studies for validation the stimulation protocols in this breed.

A153 OPU-IVP and ET

Effects of fatty acid supplementation in Holstein cows during the pre and postpartum period on oocyte quantity and *in vitro* production of embryos

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Keywords: *bos taurus taurus*, OPU-IVPE, polyunsaturated fatty acids.

The supplementation of dairy cattle with sources of polyunsaturated fatty acids (PUFA) can be use to increase the energy level of the diet (Van Knegsel *et al*., 2005, Reproduction Nutrition Development, 45, 665-688). The PUFA provides positive effects on reproductive functions of important tissues, including the hypothalamus, pituitary, ovaries and uterus (Funston 2004, Journal of Animal Science, 82, 154 - 161). The aims of this study were to evaluate the reproductive condition of postpartum, number of follicles, aspirates oocytes, amount of viable oocytes and the *in vitro* production of embryos (IVPE) of the Holstein multiparous donors supplemented with rich diet in protected PUFA (especially linoleic acid - n-6) and non-protected (especially linolenic acid - n-3) during pre and postpartum. The donors were allocated into three groups, a control group (C) with 6 donors (management of the farm, with no fat source supplementation), a Megalac-E group (M) with 5 donors (supplemented with 100g/donor/day in pre-partum and 300g/donor/day in postpartum) and a linseed group (L) with 5 donors (supplemented with 1kg linseed pie/donor/day pre-partum and 1.5 kg/donor/day in postpartum). The Megalac-E is a protected fat source and linseed pie is fat source not protected. The diets had been given for antepartum during thirty days and postpartum sixty days. The animals were submitted to OPU on days 30, 45 and 60 postpartum. The recovered oocytes were selected and the viable ones were submitted to procedures of the IVPE. The data from both experiments were analyzed by the method of least squares using variance analysis of proc GLM. The differences between means were compared by Tukey test with 5% significance. There was no detectable effect of treatment and aspirations of postpartum days on variables: amount of viable oocytes and viable oocytes rate (C=3.38±1.22 and 59%; M=3.20±1.34 and 70%; L=8.86±1.34 and 72%; P>0.05); IVPE and embryos production rate (C=1.00±0.24 and 29%; M=0.20±0.27 and 6%; L=1.33±0.27 and 15%; P>0.05). However, was observed in the group supplemented with linseed pie more follicles and total oocytes than group Megalac-E (C=11.27±1.49 and 5.72±1.24; M=8.46±1.63 and 4.53±1.36; L=18.33±1.63 and 12.26±1.36; P<0.05). The aspirations performed in postpartum donors supplemented with PUFA didn't increase the number of viable oocytes and IVPE. Thus, more studies are needed with higher numbers of donors and different fat sources to test the real influence of PUFA in dairy females.

A154 OPU-IVP and ET

Effect of norgestomet on *in vitro* embryo production in *Bos indicus* and *Bos taurus* cows

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Keywords: bovine, *in vitro* embryo production, Norgestomet.

The aim of this study was to determine the effect of previously used norgestomet auricular implant or of two new norgestomet implant on *in vitro* embryo production from Gir (*Bos indicus*) and Holstein (*Bos taurus*) breeds. A total of twelve pluriparous, non-lactating cows were selected in this study, 6 Gir and 6 Holstein. The animals were subjected to six follicular aspiration consecutive with fourteen days of interval between OPU. The study was performed in cross-over design, in which cows were subjected alternately to one of the hormonal treatment in each OPU session: control – animals that did not receive auricular implant, low norgestomet – animals receiving used auricular implant of norgestomet (Crestar®, Intervet, Brazil), high norgestomet – cow treated with two new norgestomet implant (6 mg of norgestomet combined). For synchronize the emergence of a new follicular wave, all cows received 3 mg of estradiol benzoate (RIC-BE®,Tecnopec, Brazil) plus 150 µg D-cloprostenol (Prolise®,Tecnopec, Brazil) to eliminated the presence of corpus luteum and endogenous progesterone influence in the treatment. The follicular aspiration was performed 7 days after the beginning of hormonal treatment. The ear devices were removed 24 hours after OPU. All follicles ≥ 3 mm were aspirated and the oocytes recovered were morphologically evaluated, selected and those considered as viable were matured for 22-24 hours in TCM 199 medium (Tecgene, Brazil). Then, CCOs were fertilized with semen from bull of known fertility, processed by Percoll discontinuous gradient and gametes were co-incubated during 18-20 hours. After this period, presumptive zygotes were cultured for 7 days in SOFaa medium supplemented with 5% of FCS. Data were analyzed by ANOVA, considering the effects of treatment and genetic group and when a significant effect was obtained ($P < 0.05$), means were compared by Tukey test. A difference was detected in the number of embryos on day 7 for the Gir cows of the low norgestomet treatment than that from control and high norgestomet (5.1 ± 1.2 vs 3.0 ± 0.7 and 2.7 ± 0.6, respectively). For performance on *in vitro* embryo production an advantage was observed in the Gir as compared to Holstein cows on number of follicles visualized (21.1±0.7 vs 15.7±0.6), number of recovered oocytes (12.7±1.1 vs 8.2±0.7), number of oocytes grade II and III (3.4±0.4 vs 1.8±0.3 and 3.1±0.4 vs 1.6±0.3), number of viable oocytes (9.0±1.0 vs 5.5±0.7), cleavage rates (81.8±3.5 vs 68.7±4.5) and number of embryos on day 7 (3.6±0.5 vs 2.1±0.4). In conclusion, the present study demonstrated that using an auricular implant of norgestomet previously used in Gir cows, there was an increase in the number of embryos on day 7. Also, Gir breed showed better performance in OPU-IVP program than Holstein cows.

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A155 OPU-IVP and ET

Seroprevalence of bovine Herpesvirus-1 in embryo recipient cows in the state of Acre

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Keywords: embryos, IBR, serological tests.

Infections that directly or indirectly affect the reproductive tract of females and males and compromise the development of embryos and fetuses, promote a great impact on the reproductive efficiency of cattle. Sero-epidemiological studies conducted in several states in Brazil have shown high percentages of seroconversion for herpesvirus type-1 (BoHV-1) in cows (Fino et al., 2012, Rev. Bras. Reprod. Anim., v36, 122-127). However, until now, there are no studies confirming the presence of BoHV-1 in Acre. Were used 235 crossbred cows (*Bos taurus* x *Bos indicus*) as embryo recipients. All animals received the same protocol for synchronization of ovulation in a program of Embryo Transfer in Fixed Time (FTET). On the 16th day after the beginning of the protocol, an in vitro produced embryo was transferred to each recipient and at the same time a sample of blood was collected by venous puncture of the coccygeal vein in vacuum tubes without anticoagulant. The samples were subjected to centrifugation for obtaining serum. The serological diagnosis of BoHV-1, a virus neutralization test was performed, and animals with title 2 or lesser were considered nonreactive. The diagnoses of pregnancy were performed on the 25th and recipients pregnant were revalued at 55th days after FTET, both by ultrasonography (Aloka SSD 550, Aloka, Japan). The serological diagnosis was performed by the R & D Center of Animal Biological Institute of São Paulo. From the total of samples 43.82% (103/235) were diagnosed as sero-reactive to BoHV-1. The utilization rate of recipients undergoing FTET was 67.23% (158/235) which resulted in 34.17% (54/158) of pregnancy rate, and 37.04% (20/54) of the pregnant recipients had titers against the virus. The revaluation of pregnant recipients on the 55th day, shows that 20.75% (11/54) had aborted and 72.73% (8/11) of these losses occurred in serum-reactive animals, for which the titles of virus neutralization were 1024 (25%), 512 (25%), 256 (37.5%) and 128 (12.5%). The abortion rate, was evaluated by the qui-square test being observed relation between recipients serum positivity and the abortion rate ($p < 0.05$). The high viral concentrations in these recipients show the IBR virus circulation, being responsible for the increased rate of abortion.

A156 OPU-IVP and ET

Slaughterhouse and laparoscopic ovum pick up (LOPU) derived goat oocytes have different IVM kinetics and requirements for embryo development

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Keywords: blastocyst, IVF, oocyte maturation.

Oocytes used for goat IVP can be collected either from slaughterhouse (SLAUG) ovaries or by LOPU from live animals. Most studies aiming at setting up and improving goat IVP conditions were performed using SLAUG ovaries and then these results were inferred to LOPU oocytes, whereas oocytes from both sources may have different requirements. A total of 2581 goat oocytes were used. In Experiment 1, the aim was to evaluate the effect of oocyte source (SLAUG, *n* = 545 and LOPU, *n* = 423) on the kinetics of IVM (18 *vs.* 22 *vs.* 26 h) when submitted to semi defined or defined maturation media. The maturation medium consisted in TCM199 supplemented either with: 1) EGF (10 ng/mL) and cysteamine (100 µM) (defined); or 2) EGF (10 ng/mL), 5 IU/mL hCG, 10 IU/mL eCG, 19 ng/mL IGF-1, 2.2 ng/mL FGF, 5 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL selenium, 90 µg/mL L-cystein, 0.1 mM β-mercaptoethanol, 75 µg/mL vitamin C, 720 µg/mL glycine, 0.1 mg/mL glutamine and 110 µg/mL pyruvate (semi defined). In Experiment 2, we determined the differences on embryo development between both oocyte sources (SLAUG, *n* = 1043 and LOPU, *n* = 570) when submitted to both media and to either, IVF (Souza *et al.*, 2013, Anim Reprod Sci 138, 82-89) or parthenogenetic activation (PA). Embryos from all groups were vitrified and their viability evaluated after thawing. In Experiment 1, in defined medium, more SLAUG oocytes reached metaphase (M II) stage than LOPU ones at 18 and 22 h ($P<0.05$). Furthermore, for SLAUG oocytes, M II rate did not change among 18 (87%), 22 (90%) and 26 h (79%), whereas for LOPU oocytes, M II increased significantly ($P<0.05$) from 53% (18 h) to 72% (22 h), being similar to 26 h (65%). In semi defined medium, no difference in number of oocytes that reached M II stage was observed when oocytes from both origins were matured. These results suggest that the kinetics of IVM is different between oocyte sources and depending on the medium used. In Experiment 2, cleavage rate was significantly higher ($P<0.001$) after PA than after IVF for all groups. Interestingly, cleavage rate after PA was similar for SLAUG oocytes matured in both media (~90%) whereas it was improved when LOPU oocytes were matured in semi defined (93%) as compared to defined (83%) medium ($P<0.05$). After IVF, SLAUG oocytes had higher cleavage rate (~67%) as compared to LOPU ones (~39%) ($P<0.05$), whereas the percentage of blastocysts from cleaved embryos was not different (68 and 67%, respectively). Therefore, SLAUG oocytes developed to blastocyst stage in a greater number than LOPU ones. Vitrified-thawed blastocysts showed similar results in survival (~67%) and hatching (~55%) rates between oocyte sources, maturation media or activation method. In conclusion, SLAUG and LOPU derived oocytes may have different IVM kinetics. Although IVM in goats still need improvement in order to enhance embryo yield, it was possible to generate good quality embryos from LOPU and SLAUG derived oocytes.

A157 OPU-IVP and ET

Embryonic development of bovine oocyte matured *in vitro* in the presence of GDF-9 and BMP-15

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Keywords: BMP-15,GDF-9, IVM.

The GDF-9 (Growth Differentiation Factor 9) and 15-BMP (Bone Morphogenetic Protein 15) are growth factors of TGF-β family. They are produced by the oocyte, and are involved in regulation of important ovarian functions. Studies have suggested that addition of these factors during maturation improves embryonic development in vitro. The aim of this study was to evaluate the effect of GDF-9 and BMP-15 during *in vitro* maturation of bovine oocytes on *in vitro* embryo production. Cumulus Oocyte Complexes (COCs) were aspirated from slaughterhouse ovaries and, after selection COCs classified as I and II were distributed into 4 treatment, performed in 5 replicates: Cont: control oocytes matured in TCM 199 supplemented with FSH (0.01 IU / ml), l-glutamine (0.1 mg / ml), amikacin (0.075 mg / ml) and 0.4% BSA; GDF: oocytes matured in control medium supplemented with GDF-9 (100 ng / ml; R&D systems); BMP: oocytes matured in control medium supplemented with BMP-15 (10 ng/ml; R&D systems), and BMP + GDF: oocytes matured in control medium supplemented with GDF-9 (100 ng / ml) and BMP-15 (10 ng / ml). After maturation, COCs were fertilized and embryos cultured in SOF medium and evaluated on D2, D7 and D8. For statistical analysis, Chi-square test ($P < .05$) was used to compare embryonic development among different treatments. None of the treatments, Cont (n = 134), GDF (n = 128), BMP (n = 130) and BMP + GDF (n = 147) showed differences during embryonic development, when evaluated at D2 (79.1%, 81.3%, 83.8% and 79.6%), D7 (33.6%, 43%, 44.6% and 38.8%) and D8 (36.6%, 43%, 44.6% and 38.8%) respectively. We conclude that the addition of growth factors GDF-9 and BMP-15, alone or in combination, did not influence embryonic *in vitro* development.

A158 OPU-IVP and ET

Is it possible to increase the efficiency of in vitro embryo production in Holstein preselecting donors with more oocyte production?

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Keywords: donor, embryos, Holstein.

The commercial availability of sex-sorted semen resulted in a significant Increase of *in vitro* embryo production (IVEP) in dairy breeds. Despite the lower oocyte production (Palhão et al., 2011), cleavage rates, and embryo production (Grázia et al., 2012) than in zebu dairy breeds, there is a great interest in the use of IVEP in Holstein. The present study aimed to evaluate whether the selection of donors based on the production of cumulus-oocyte complexes (COC) can compensate the lower IVEP efficiency in this breed. Data from follicular aspiration and *in vitro* cultures performed in Gir (N = 266) and Holstein (N = 270) donors from 2011 to 2013 in the same IVPE laboratory were used. Data were analyzed by ANOVA and differences between groups were compared by Tukey's test. Percentage differences were compared by Chi-square test. The results are shown as mean ± SEM. As expected, the average of total COCs production and number of viable COCs were higher in Gir than in Holstein donors (19.1±0.9 and 11.6±0.6 vs. 13.3±0.6 and 6.8±0.3, respectively; P <0.0001) and the total embryo production rate was also higher in Gir breed (55.1%±0.01% vs. 36.3%±0.02%). The percentage of IVEP batches with 40 to 100% of embryo production was also higher in Gir (68.7% vs. 39.2%) than in Holstein donors (P<0.05). In Holstein, correlations between total oocytes recovered or total viable oocyte and embryo production rate per IVEP batch were negative (R = -0.02 and R = -0.05, respectively; P>0.05). The retrospective analysis in Holstein breed showed that the IVEP batches with results of 0 to 20% and 80 to 100% were associated with aspiration sessions that produced the same number of total and viable COC (10.3±0.8 and 5.2±0.5 vs. 12.0±1.4 and 5.6±0.7, respectively; P<0.0001). Coherently, the hypothetical selection of Holstein donors ranked in the first and second quartiles of total oocyte production would still result in a total number of embryos produced and embryo production rate lower than in Gir donors (4.0±0.3 and 41.0% vs. 6,5±0,3 and 55,1%, respectively; P<0.001). These results demonstrate that the selection of donors based on COCs production is not the best strategy to optimize IVEP results in Holstein, and highlight the need to investigate other potential predictive parameters for the system efficiency.

A159 OPU-IVP and ET

Investigation on the presence of glucocorticoid receptors in bovine cumulus-oocyte complex, oocyte and embryos produced in vitro

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Keywords: Glucocorticoid receptor, cumulus-oocyte complex, embryo.

Glucocorticoid Receptors (GR) are transcription factors that reside in the cytoplasm while inactive, and are translocated to the nucleus upon activation by binding to their corresponding hormone (Bosscher & Haegeman, 2009, Molecular Endocrinology, 23, 281–291). During research performed in our laboratory, it was observed that the addition of cortisol to the culture medium influenced embryo quality during *in vitro* production, which suggests GRs play an active role during early embryogenesis. Therefore, the objective of this study was to verify the presence of GRs in bovine cumulus-oocyte complexes (COCs), immature and mature oocytes, and embryos produced *in vitro*. For immunocytochemistry analysis, COCs, immature and 22-hour matured oocytes, and embryos at stages 2-4 cells (48h), 8-16 cells (72h), morula (120h) and hatched blastocyst (168 h) were fixed in 4% paraformaldehyde (PFA) for 15 minutes. Fixed samples were permeabilized with 0.25% Triton X-100 for 10 minutes and blocked with 1% BSA and 0.3 M glycine for 30 minutes. They were then incubated overnight in the primary antibody solution (1:50) and then for 1 hour with the secondary antibody (1:250) conjugated with FITC. Nuclei were counterstained with propidium iodide. 15 samples per stage were analyzed. All samples were found to be positive for GR at all of the studied stages (100%), and the corresponding negative controls (without antibody 1º) were found to be negative. GRs were observed to show a more peripheral localization in immature COCs. In other stages studied (matured COCs, immature and matured oocytes, and embryos), GRs were observed to be more diffusely distributed throughout the cytoplasm. Therefore, we conclude GRs are present, display stage specific localization during early embryogenesis, and that this may allow these early stages to respond to cortisol therapy.

A160 OPU-IVP and ET

Different sperm selection methods used for ovine in vitro embryo production

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Keywords: IVP ovine, sperm gradients, swimn up.

The sperm selection method is one step of in vitro embryo production systems and influences the embryo development rates. The aim of this study was to compare the efficiency of different sperm selection methods used in ovine IVP systems in terms of embryo development rates. Methods as Swim up (SW), Mini Optiprep® (MO), Mini Percol® (MP) and Mini Isolate® (MI) were compared in terms of cleavage rates at day 2 and development rates at day 8 (blastocysts/oocytes inseminated). Cumulus oocyte complexes (COCs) were aspirated from ovine ovaries obtained at a local slaughterhouse, selected and matured for 22-24h. The same batch of ovine fresh semen was used in the different sperm selection methods in the experiment. The SW method was performed by layering an aliquot of semen under tris glucose citric acid medium. After 30 min of sperm migration in controlled conditions (39^{o}C), the upper portion was removed and centrifuged at 200G for 5 min. The small volume gradients (MP and MI) were both prepared at 90 and 45%. Semen aliquots were layered over MP and MI and centrifuged at 700G for 5 min. The small volume gradient MO was prepared at 30, 28 and 26% and after semen aliquots were layered it was centrifuged at 900G for 15 min. The sperm pellet was isolated and centrifuged at 700G for 5 min in fertilization media, in all treatments. The *in vitro* matured COCs were randomly distributed in four treatments: SW (n=130), MO (n=152), MP (n=120) and MI (n=110) and inseminated with 1x 10^6 sptz/mL spermatozoa maintained in fertilization media during 18h. Embryo culture was performed during 8 days in SOFaa media with 0.8% BSA in bag system in an atmosphere arrangement of 5% CO_2, 5% O_2, and 90% N_2. Embryo development rates were compared using variance analysis and Duncan test at 5% of significance level (SAS). No differences were observed in the cleavage rates (71%, 76%, 75% and 81%) and development rates at D8 (21%, 24%, 16% and 17%) (P>0,05) for SW, MO, MP and MI respectively. The experiment results showed no influence of the different sperm preparation methods over the embryo development rates found in the ovine *in vitro* embryo production systems.

A161 OPU-IVP and ET

Effect of semen incubation prior to insemination on in vitro fertilization rate of bovine oocyte

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Keywords: bovine, embryo ivp optimization, spermatozoa.

In several situations, the same mating is used for oocyte insemination of different donors that are submitted to ovum pick up in the same day. The use of the same semen straw for IVF in distinct periods of time could decrease the technique cost, especially when using sexed semen. Therefore, the aim of this study was to evaluate the *in vitro* fertilization rate of bovine oocytes, using sperm doses incubated prior to the IVF procedure. An ejaculate of Nellore bulls (n = 4) was collected and frozen in three fractions: non-sexed (NS) – control group, sexed X (SX) and sexed Y (SY). A fourth group was formed by a pool of X and Y spermatozoa (SXY). Semen from each group/bull was used for IVF of oocytes grades I and II, obtained from slaughterhouse ovaries. One straw of each group/bull was thawed and washed in SOF 700 *g*/ 5 min. Semen from each treatment was incubated in SOF medium with a final concentration of $2x10^6$ sperm/mL for 0h, 2h and 4h until the *in vitro* insemination time. Twelve and 18 hours post-insemination (pi), the zygotes (n=2051) were denuded, fixed and stained with Lacmoid for evaluation of *in vitro* fertilization rate. Data were analyzed by ANOVA with Tukey test ($P < 0.05$). There was no difference in fertilization rates between 12 and 18 h pi for groups and incubation period. The NS group (63.5 ± 12.3, 37.2 ± 12.4, 30.8 ± 12.1) showed higher fertilization rates, compared to the SXY group (34.7 ± 12.2, 19.5 ± 10.2, 8.9 ± 14.5) at 0, 2 and 4 h pre-incubation periods, respectively. However, both groups showed lower fertilization rate at 2 h and 4 h pre-incubation, compared to 0h. For the groups SX and SY, no significant difference was observed for fertilization rates at 0 h (41.5 ± 12.6 and 34.9 ± 12.2), 2 h (22.7 ± 10.7 and 32.8 ± 12 5) and 4 h (11.5 ± 8.1 and 15.8 ± 9.5). Nevertheless, a decrease in fertilization rate occurred at 2 h pre-incubation in group SX, while in group SY this decrease only was observed when sperm pre-incubated for 4 h were used. Since bovine sperm pre-incubation decreased *in vitro* fertilization rates for both sexed and non-sexed sperm cells, we can conclude that it is not an alternative for IVP optimization. However, sperm containing Y chromosome kept the same fertilization capacity after 2 h of incubation, being probably more resistant in culture conditions than X sperm.

A162 OPU-IVP and ET

Ovarian follicle stimulation prior to opu and in vitro embryo production in holstein cows

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Keywords: bovine, follicle aspiration, FSH.

Due to the growing demand for *in vitro* embryo production (IVEP), researchers have focused in developing strategies to enhance the efficiency of this biotechnique. The goal of this study was to evaluate IVEP in Holstein cows exposed to ovarian follicle stimulation prior ovum pick-up (OPU), as proposed by Nivet et al. (2012, Reproduction, v.143, p.165-171). Non-lactating Holstein cows were randomly assigned in a crossover design to the following experimental groups: Control: OPU at a random day of the estrous cycle (n = 35); FSH group: OPU after follicle wave synchronization and superstimulation with FSH (n = 35). At Day 0 of the protocol (which is the random day of the estrous cycle), a norgestomet ear implant (Crestar, Intervet, Brazil) was added and follicles ≥ 7 mm in diameter were aspirated for wave synchronization. Treatment with FSH (Folltropin-V, Bioniche Animal Health, Ontario, Canada) started 36 h later (six injections of 40 mg 12 h apart). The OPU was performed 44 h after the last FSH injection. In both groups, all of the follicles > 2.5 mm were aspirated and the oocytes were classified for subsequent IVEP. The rate of viable oocytes was higher in the FSH compared to control group (60.0 ± 3.5 vs. 57.1 ± 3.7%; P = 0.01). The blastocyst rate was also higher in the FSH group (18.8 ± 2.4 vs. 13.2 ± 2.3%; P = 0.09). However, there was no difference in the average total number of oocytes retrieved (16.5 ± 2.0 vs. 20.5 ± 2.4; P = 0.16), viable oocytes (11.1 ± 1.5 vs. 12 3 ± 1.7; P = 0.13), and embryos (3.1 ± 0.6 vs. 2.7 ± 0.5; P = 0.22) per cow per OPU between the FSH and control groups, respectively. Therefore, utilizing the protocol for ovarian follicle stimulation did not improve IVEP results in Holstein cows. These results are indicative that there are no economic advantages of using a protocol of ovarian follicle stimulation prior to OPU for IVEP.

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A163 OPU-IVP and ET

Influence of BSA and/or FBS suplementation during IVM on oocyte lipid droplets and mitochondria behavior, and embryo lipid accumulation in bovines

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Keywords: IVM, lipids, mitochondria.

Lipid oxidation in oocyte is responsible for energy intake and it is related to maturation and embryo development. This study aimed to evaluate the influence of BSA and/or FBS on oocyte Lipid Droplets (LD) and Mitochondria (M) behavior and embryonic LD. Three experiments were designed, and the oocytes underwent IVM with: 8mg/mL BSA (BSA group, GBSA), 10%FBS (GFBS) or 6mg/mL BSA + 5%FBS (GB+F). In the first experiment we evaluated the LD and M migration during IVM by confocal microscopy using fluorimetric techniques (RED Mitotracker CMXRos and LipidTox Green Neutral LipidStain, Molecular Probes, USA). In the second experiment, we studied the size (<2µm, 2 to 6µm or >6µm in diameter) and the amount of LD (by the sum of the area), and the amount of mitochondria (by Fluorescence Intensity-FI, with values between 0 and 255 for each pixel in the selected area) in immature and mature oocytes. In experiments I and II the results were compared to those obtained in *in vivo* matured oocytes (GIV), which were also evaluated for progression to MII by nuclear staining with Hoechst33342 (Molecular Probes). In experiment III, rate of BL and embryonic lipid accumulation was evaluated as mentioned in experiment II. Data from experiment I and the rate of Bl were evaluated by X^2 test and the remaining data by Mann Whitney. Regarding LD and M migration from a peripheral localization to a dispersed localization, in experiment I, the groups submitted to IVM failed to achieve the migration rate obtained for GIV (30/58-51.7%). Also, GFBS (21/65-32.3%) had higher rates than GBSA (7/61-11.5%) and GB+F (10/66-15.1%). The groups FBS and B+F showed higher LD migration rates (63/65-96.9%, 63/66-95.4%, respectively) compared to GBSA (44/61-72.1%), whereas in GIV when nuclear mature and immature oocytes were separated, all oocytes in MII (38/58-65%) also showed the same LD migration pattern. In Experiment II, we observed that IVM caused an increase in oocyte lipid content that was not present in GIV (361.7µm^2; immature oocytes:440.2µm^2), with no differences in the sizes of droplets between groups. Furthermore, GFBS showed higher LD accumulation (746.9 µm^2) and a smaller number of M (FI of 11.7) than the GBSA (LD:565.1µm^2 and M:15.71) without differing from GB+F (LD:663.7µm^2 and M:12.82). GIV was the only group able to maintain a correlation between both organelles (r^2 = 0.73). In experiment III, GBSA failed to achieve BL rates seen on GFBS and GB+F (279/1410-19.79%, 336/927-36.25% and 368/1062-34.65%, respectively). There were no differences in embryonic lipid accumulation between groups (GBSA:2526µm^2, GFBS:2588µm^2 and GB+F:2163µm^2), however, for droplet size, a higher percentage of LD between 2-6µm (53.3%) was observed in GFBS in comparison to GB+F (43.9%). Therefore, FBS in IVM does not promote greater embryo lipid accumulation than BSA, but it causes differences in comparison to *in vivo* maturation in regards to distribution and quantification of oocyte LD and M.

A164 OPU-IVP and ET

Validation of an *in vitro* bovine embryos production program with oocytes and embryos transportation through long distances

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Keywords: *Bos indicus*, embryo transfer, sexed semen.

The large territory associated with the distance between bovine production sites and *in vitro* Embryos Production (IVEP) laboratories, for many times have limited the use of this technology in large scale in Brazil (Marinho *et al*., 2012, Animal Reproduction, 9, 323-328). Thus, the objective of this work was evaluate the viability of an IVEP program in which oocyte maturation and embryo culture occurred partially during transportation, and to determine the effect of the fertilization with sexed semen upon the number of cleaved oocytes, the embryo production and the pregnancy rate of recipients. For this purpose, 123 oocytes from Nelore donors raised in Bahia state were obtained by ultrasound guided follicular aspiration (OPU). These oocytes began their maturation process in cryotubes containing TCM-99 modified bicarbonate, high humidity and 5% of CO_2 in air, and they were submitted to transport inside a portable incubator under 38,5°C temperature to a commercial laboratory in the state of São Paulo. The transportion lasted around 18 and 24 hours and the maturation was finished in the laboratory, followed by *in vitro* fertilization and culture. The fertilization was done with conventional semen (group 1 – CONV) or sexed semen for female (group 2 – SEX). Six days after fertilization, embryos produced were sent to Bahia state, under similar conditions of the oocytes, except by the medium appropriate for embryo culture. Embryos were transferred to recipients in the seventh day after *in vitro* fertilization. Pregnancy diagnosis was done by transrectal ultrasonography 30 days after IVF, using a 5,0 MHz linear transducer. Data were analyzed in *Statistical Package for Social Science* (SPSS, 19th version). Mean and standard deviation from variables were obtained by descriptive analysis; differences between number of cleaved structures and embryos produced according to CONV and SEX groups were compared using Student's T test; pregnancy rates between CONV and SEX group were compared using the chi-square (X^2) test. Embryo rate was 32,85%, with an average 10,09±6,2 of embryos produced/ OPU. Pregnancy rate was 33,12% corresponding to an average of 2,71±1,2 pregnancies/ OPU. The number of cleaved oocytes did not differ (P=0,49) between conventional (CONV=61,49%) or sexed semen (SEX=63,43%). The number of embryos produced was similar (CONV=38,11% *vs.* SEX=47,76%, P=0,054), as well as pregnancy rate (CONV=33,37% *vs*. SEX=31,93%, P=0,586). The transportation of oocytes and embryos has been proved to be efficient, even when sexed semen was utilized, showing that IVEP programs are viable when donors and recipients are located far from the production laboratories.

A165 OPU-IVP and ET

Efficiency of superovulatory treatment started near the time of emergence of the first or last follicular waves of progesterone protocol in Santa Ines ewes during non-breeding season

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Keywords: embryos, follicular wave emergence, superovulation.

This study was designed to investigate if the time of onset of FSH treatment (near the emergence of first or last follicular wave of P4 protocol) influenced the superovulatory response and embryo yield in Santa Ines ewes during non-breeding season (between the months of July to November). Days of emergence of follicular waves were defined in a previous study that evaluated the follicular dynamic during estrus synchronization treatments (Oliveira et al., Acta Scientiae Veterinariae, v.40, p.361, 2011). Twenty Santa Ines ewes were submitted to one of two superovulatory protocols according to the time FSH treatments were initiated (G-first wave, n=10 and G-last wave, n=10). On Day 0 all ewes received a P4 device (CIDR®) and injection of 37.5 µg of D-cloprostenol, i.m. The FSH treatments started on Days 4 and 10 of protocol for G-first and G-last, respectively. The superovulatory regimen consisted of eight i.m. injections of pFSH administrated twice daily (40, 40, 30, 30, 20, 20, 10 and 10 mg of pFSH). The P4 device was removed on Day 6 and 12 for G-first and G-last, respectively. At CIDR removal, all ewes received another injection of 37.5 µg of D-cloprostenol and a dose of 200IU of eCG. During four days after the P4 device removal, ewes were mated by a fertile ram. The superovulatory response was evaluated through examination of the ovaries by ultrasonography (three times daily, during the mating period) and laparoscopy (concomitantly the embryo collections). Embryo collections were accomplished 7 days after CIDR withdrawal by laparotomy, and classified according to their development. A sample number of embryos of each treatment were also fixed and stained by TUNEL techniques to assess the apoptotic cells percentage. Data were analyzed by GLIMMIX using SAS. There was no effect between treatments (P>0.05) for the superovulatory response (percentage of ovulated follicles: 89.20±4.15% vs. 83.50±6.17%; number of ovulations: 12.40±0.95 vs. 12.60±1.87; number of luteinized unovulated follicles: 1.70±0.70 vs. 3.10±1.59 for G-first wave and G-last wave, respectively). Similarly, there was no effect (P>0.05) on embryos yields (recovery rate: 69.90±5.61% vs. 51.70±7.50%; mean number of structures recovered: 8.60±1.01 vs. 5.90±0.90; number of viable embryos: 3.20±0.81 vs. 1.80±0.80; and viability rate: 40.50±11.93 vs. 32.70±11.74, for G-first wave and G-last wave, respectively). Moreover, there was no effect between treatments (P>0.05) for the apoptotic cells percentage (G-first wave: 3.10±1.66% and G-Last wave: 12.76±4.34%). In conclusion, there were no differences in superovulatory response and embryo yield between FSH treatments initiated during the first or last follicular waves of progesterone treatment in Santa Ines ewes during non-breeding season.

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A166 OPU-IVP and ET

Influence of the number of oocytes obtained per Nelore donor in the *in vitro* embryo production

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Keywords: bovine, IVP, oocyte production.

The aim of the present study was to compare the embryo production among bovine females with high, intermediate and low oocyte production obtained after OPU. Nelore cattle (*Bos indicus*, n = 66, 72-96-month-old) underwent ultrasound-guided follicular aspiration using a 7.5-convex intravaginal array transducer. Briefly, COCs recovered were classified and transported to the laboratory for IVEP. IVF was performed with semen from a bull of known fertility. Cattle were assigned to groups according the oocyte production, as follows: G-High (n = 22, ≥40 oocytes), G-Intermediate (n = 25, 18-25 oocytes) and G-Low (n = 19, ≤7 oocytes). Data were analyzed using the Qui-square test (P≤0.05). The mean number of COCs recovered was 50.4±11.30 (G-High), 21.4±3.04 (G-Intermediate) and 5.3±1.50 (G-Low, P<0.05). The mean number of viable oocytes was 40.4±10.60 (G-High), 14.8±3.02 (G-Intermediate) and 3.8±1.08 (G-Low, P<0.05) and the proportion of viable oocytes was 80% (888/1109, G-High), 69% (371/534, G-Intermediate) and 71% (72/101, G-Low, P<0.05). Cleavage rates were 79% (762/965, G-High), 74% (348/472, G-Intermediate) and 71% (65/92, G-Low, P<0.05) and blastocyst rates were 42% (405/965, G-High), 32% (153/472, G-Intermediate) and 13% (12/92, G-Low, P<0.05). The mean number of viable embryos was 18.4±6.71 (G-High), 6.1±3.57 (G-Intermediate) and 0.6±0.68 (G-Low, P<0.05) and the percentage of vitrifiable embryos was 81% (329/405, G-High), 77% (118/153, G-Intermediate) and 58% (7/12, G-Low, P<0.05). It is concluded that Nelore cows with high oocyte production had greater percentage of viable oocytes, blastocyst rates and percentage of vitrifiable embryos compared to the cattle with low oocyte production following OPU/IVP. Cattle with high oocyte production produced ~30-fold more viable embryos compared to the low ones. Thus, Nelore cattle with high oocyte production had greater performance after *in vitro* embryo production.

A167 OPU-IVP and ET

Use of cilostamide in pre-maturing of bovine oocytes

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Keywords: block, competency,meiosis.

Several substances have been tested in order to block the resumption of meiosis, providing additional time for the acquisition of oocyte competence (Luciano *et al*., 2011, Bio. of Reproduction, 85, 1252-9; Thomas *et al*., 2004, Bio. of Reproduction, 71, 1142-9). However, no increase in embryo production has been observed when oocytes are subjected to meiotic arrest. High concentrations of cAMP are responsible for maintaining the oocyte in germinal vesicle. In this context, the cilostamide, which is an oocyte specific phosphodiesterase type-3, acts directly on the cAMP concentration, and can be an alternative to be used in the meiotic arrest (Vanhoutte *et al*., 2008, Mol. Reproduction and Development, 75, 1021 -30; Thomas *et al*., 2004, Bio. of Reproduction, 71, 1142-9). The present study aimed to evaluate the effect of pre-maturation, in which the meiotic arrest was performed for 8 and 24 hours in the presence of cilostamide (10 µm; Sigma®) in IVP of bovine embryos. Cumulus oocyte complexes (COCs) were obtained from slaughterhouse ovaries and, after selection, were divided into 4 groups: C18: control where COCs were matured for 18h (n = 117); C24: control where COCs were matured for 24h (n = 120); PM8: COCs pre-matured for 8 and matured for 18h (n = 112) and PM24: COCs pre-matured for 24 hours and matured for 18h (n = 118). After maturation, COCs from all groups were fertilized and cultured until day 7 (D7) of development. Cleavage and blastocyst rates were evaluated in D2 and D7, respectively. To assess embryo quality only the embryos measuring >160µm on D7 were stained with Hoechst 33342 and used to quantify the nuclei number. Data for the measurement of embryos and nuclei number were analyzed by Kruskal-Wallis test and embryonic development by chi-square test ($P < .05$). Cleavage and blastocyst rates were higher for C24 (90.8 ± 4.4% and 47.51 ± 3.5%) and PM8 (89.3 ± 6.7% and 44.64 ± 4.2%) compared to C18 groups (76.9 ± 3.6% and 40.17 ± 2.2%) and PM24 (70.3 ± 6.1% and 27.1 ± 2.8%). The percentage of D7 embryos > 160µm, and the nuclei number were lower in PM 24 (53.2% and 105.1 ± 14.3) when compared to others, that were similar (C18: 72.3% and 121.6±14.3; C24: 70.1% and 127.5±13.8; PM8: 72.0% and 121.3±12.8). Results suggest that the addition of cilostamide in pre-maturation for 8h followed by 18h IVM does not impaired embryo development, but had no beneficial effect on embryo yield and quality. Conversely, pre-maturating for 24h followed by 18h IVM had detrimental effect on embryo yield and quality.

A168 OPU-IVP and ET

In vitro production of bovine embryos in physico substrate borosilicate

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Keywords: borosilicate, IVEP, polystyrene.

Despite the advances obtained in the systems of *in vitro* Embryos Production (IVEP) in cattle, especially in relation to embryo quality, studies about the environment and culture conditions are still necessary. In this context, due the possible leaching of bioactive chemicals components from plastic material (McDonald et al., 2008, Science 322, 917) to the cell culture medium and can be harmful to the embryonary development, the aim of this study was to evaluate the influence different physical supports (polystyrene petri dish x borosilicate glass petri dish) in the IVEP. For this purpose, ovaries from abattoir were aspirated and the selected oocytes randomly distributed between two Experimental Groups: *in vitro* maturation, fertilization and embryonic development in Polystyrene Dish (PD) or Borosilicate Dish (BD). The cleavage rates, blastocyst formation, morphological analysis, kinetics of development and total number of cells were subjected to ANOVA (Bonferroni post-test, with significance level of 5%). There was no statistical difference (P>0.05) between Groups PD e BD in none of the analyzed criteria: cleavage rates (80±16 and 72±14.4%, respectively), blastocyst formation (40±8 and 37±7.4%, respectively), total number of cells (102±39.4 and 84±28.4, respectively), qualitative morphological analysis – classified as Grade 1, 2 and 3 (PD: 45.2±12.7; 29.9±18.5; 17.1±4.0 e BD: 48.9±5.6; 29.8±3.6; 21.3±5.9, respectively) and kinetics of development (PD: 15.0±0.8; 22.5±0.8; 35±0.4 e 27.5±0.4 e BD: 18.9±0.5; 29.7±0.8; 29.7±1.1 e 21.6±0.5, respectively to Early Blastocyst, Blastocyst, Expanded Blastocyst and Hatched Blastocyst). In conclusion there is no difference on the type of physical support used in IVEP, although more studies are necessary, since only the morphological evaluation can't be enough for qualitative diagnosis embryo.

A169 OPU-IVP and ET

Variables associated with pregnancy rate in embryo recipient mares

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Keywords: embryo, mare, recipient.

The present study aimed to evaluate the possible variables on pregnancy rate in a program of embryo transfer in mares, in the breeding season of the years 2010, 2011 and 2012. One hundred and seventy-seven mares between three and fifteen years of age, located in the city of Limeira, São Paulo State, were submitted to a gynecological examination on the day of embryo transfer and classified according to the number of births, asynchrony with ovulation of donor, uterine tonus, uterine echogenicity and opening degree of the cervix. The results were submitted to analysis of variance (P <.05). The pregnancy rate was similar (p> 0.05) between nulliparous, primiparous and multiparous mares (84%, 78% and 76%, respectively). The transfers done on the fifth day of the estrous cycle showed an increase in pregnancy rate (87.2%, P = 0.05) compared to the pregnancy rate on days four (76.7%), six (61.9%) and seven (66.6%). The pregnancy rate was similar (P> 0.05) between recipients with intermediary uterine tonus (75.3%) and firm and tubular tonus (75%). In the ultrasonographic evaluation, recipients with moderately heterogeneous echogenicity and presence of endometrial folds showed a numerical increase (80.5%, p = 0.06) in pregnancy rate compared to recipients with heterogeneous echogenicity, presence of endometrial folds (63%) and with homogeneous echogenicity, with no evidence of endometrial folds (72%). The opening degree of the cervix had no influence on the results (p> 0.05) (open cervix: 82.3%; partially open cervix: 73% and close cervix: 80%). Although gynecological examination is of fundamental importance, the results showed a greater relevance of asynchrony between donor and recipient.

A170 OPU-IVP and ET

Low sperm concentration reduces polyspermy and enables the birth of piglets after *in vitro* produced embryo transfer

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Keywords: *in vitro* fertilization, pig IVP, semen.

The pig IVP until now is inefficient, with failures in many steps of production. The high rate of polyspermy after IVF is one of the problems. This study aimed to adjust the sperm concentration in IVF of porcine oocytes, allowing obtaining embryos that result in newborns piglets. Follicles 3 to 6 mm in diameter were aspirated from ovaries of prepubertal gilts, obtained at slaughterhouse, and IVM in TCM-199 medium, supplemented with 26,19mM Sodium Bicarbonate, 25% de Follicular Fluid, 0.1mg/mL L-Cysteine, 10ng/mL EGF, 100UI/mL G Penicillin, 0,1mg/mL Streptomycin, 0.5mg/mL LH, 0.01UI/mL de FSH and 1mM de dbcAMP. After 22 h of maturation, oocytes were transferred to a maturation medium free of LH, FSH and dbcAMP for additional 18 to 20 h. After maturation and mechanically denudation, oocytes were selected for the presence of first polar body. Only maturated oocytes (n=179) in three replications were submitted to IVF in mTBM fertilization medium supplemented with 0.4mg/mL Caffeine and 2mg/mL BSA. Semen was obtained from fresh ejaculate, and selection of viable spermatozoa was performed by mini-Percoll method. Selected sperm were incubated with oocytes in one of the following concentrations: 62,500, 125,000 or 250,000 sperm/mL. After a 3-h co-incubation period, the excess of spermatozoa adhered to the zone pellucid was mechanically removed, and the presumptive zygotes were fixed to verify the penetration and polyspermy rates. Data was evaluated by Qui Square test with 5% significance level. Higher penetration rates (100.0% 56/56) was observed with 250,000 spermatozoa/mL compared with 62,500 (92.4% 61/66) and 125,000 (89.5% 51/57) which did not differ between them. In the monospermy evaluation, 62,500 spermatozoa concentration showed the higher rate (42.6% 26/61) that was not different from 125,000 spermatozoa concentration (27.5% 14/51), being this one similar to that observed with 250.000 spermatozoa concentration (17.9% 10/56). With basis on the obtained results oocytes (n=319) were IVM and IVF with 62,500 sperm/mL, and cultured in PZM-3 medium supplemented with 3mM/mL BSA in an incubator at 38.8 °C under 5% CO_2 atmosphere. After cleavage evaluation (72.3% 230/319), cleaved zygotes were surgically transferred into one of the oviducts of two previously synchronized gilts (100 and 101 zygotes). Remaining zygotes (n=29) were cultured resulting in 34.5% blastocyst rate. Both the recipients were confirmed pregnant by ultrasound at day 30 of pregnancy. However, only one remained pregnant to term. During parturition there was need for obstetric aid. Six piglets were delivered (5 females and 1 male, with 1,183KG average weight. All six piglets were healthy and had a developmental performance similar to their counterparts. We concluded that *in vitro* fertilization with a reduced sperm concentration (62,500 / mL) reduces the rate of polyspermy in pig oocytes, allowing to obtain embryos that result in the birth of healthy piglets.

A171 OPU-IVP and ET

Luteal vascularization of bovine embryo transfer recipients with color Doppler ultrassonography

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Keywords: *corpus luteum*, Doppler, progesterone.

Luteal angiogenesis is an active and variable process according to the estrous cycle. Luteal blood vessels are important to an efficient functioning of the corpus luteum (CL) as an endocrine gland (SHIRASUNA et al., J. Reprod. Dev. 56: 124-130, 2010). The CL irrigated area increases aside with a volume and a progesterone plasmatic concentration (ACOSTA et al., Reproduction, 125: 759-767, 2003). The aim of this trial was to evaluate the CL vascularization score of bovine embryo transfer recipients in the transfer day and compare this result with the pregnancy rate. Were used 43 Nelore breed cows who received a vaginal device with 1.0g of progesterone (Sincrogest, Ouro Fino, Brazil) and 2mg estradiol benzoate (Sincrodiol, Ouro Fino, Brazil) intramuscular. Eight days after that the progesterone devices were removed and they received 250µg cloprostenol (Sincrocio, Ouro Fino, Brazil) and 0.3mg estradiol cypionate (E.C.P®, Pfizer, Brazil) intramuscular. Seventeen days after the hormonal treatment the cows received *in vitro* produced embryos. The ovaries with CL were evaluated before the embryo transfer by transretal ultrassonography at color Doppler mode (My Lab®30 Vet Gold, Esaote, Italy) using a 7.5 MHz scanner probe, recording the images for 30 seconds with the scanner probe positioned in the area which provided the best clearance in the observation of the CL and blood flow. The corpus CL vascularization was scored by a trained technician upon the study of the recorded images with the ultrasound device own program (My LabDesk, Esaote, Italy) and expressed by the percentage of vascularization area by total CL area. The pregnancy diagnosis was made 23 days after the embryo transfer with the same ultrasound device at B mode. The vascularization scores and the pregnancy rates were analyzed by Fisher test and the CL total area by Tukey test ($P<0.05\%$). Among the studied animals was found a minimal value of 40% and a maximum of 70% of vascularization, then four groups were provided between this interval by the vascularization score of 40, 50, 60, 70% and correlated with the pregnancy rate after transfer. The CL total area showed no difference ($P>0.05$) between the groups 40%, 50%, 60% and 70%, which resulted in 17.51mm, 16.69mm, 16.84mm and 17.56mm, respectively. The group 40% showed 0% (0/12) pregnancy which differed significantly ($P<0.05$) with the other groups 50, 60, 70% that resulted in the pregnancy rates of 40% (4/10), 45.5% (5/11) and 60% (6/10), similar ($P>0.05$). The result suggests that the evaluation of the CL functional activity can be more accountancy than the volumetrical evaluation to indicate the recipients suitable for embryo transfer.

A172 OPU-IVP and ET

Effect of the use of a chemical regulator of PI3K/AKT pathway in *in vitro* production of bovine embryos

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Keywords: embryo, PI3K/AKT, regulator.

The PI3K/AKT pathway is involved in several cell functions as protein synthesis, cell proliferation and differentiation and microtubule dynamics. This pathway acts by phosphorylation of initiation factors, components of the cell division cycle, transcription factors and proteins involved in microtubule formation and cell adhesion. Recently, it has been reported that this pathway can regulate the meiosis of oocytes in particular the metaphase I/II transition, being part of the MAPK3/1 and MAPK14 cascade in oocyte and cumulus cells in cattle. (Uzbekova et al., 2009, Reproduction, 138, 235-246). Thus, the aim of this study was to evaluate the influence of the PI3K/AKT pathway in the early embryo development, when a chemical regulator of this pathway – Wortmannin - is added to the maturation medium in an only concentration (which value is in interval of 5 to 100nM). Thus, we used 210 oocytes from slaughterhouse ovaries and classified as grade I and II. After selection, IVM was performed in drops of 100 µL (medium 199 + FBS + antibiotics) in the presence or absence (control) of the regulator, in an incubator at 5% of CO_2, 38,5ºC for 22h. For IVF, sperm were selected by the technique of mini-percoll. The matured COC_s were transferred to 100µL IVF drops using the fertilizing dose of $2x10^6$ spermatozoa/ mL for 18h. After IVF, the presumptive zygotes (10/drop) were cultured for 8 days in 100µL drops (medium 199+ FBS + antibiotics). Evaluation of cleavage and blastocyst rates was done 72h and 8 days after IVF, respectively, and compared using the T Test (LSD) at a 5% significance level. There was no difference in cleavage and blastocyst rate, respectively, between the control group (75.5% and 30.2%) and treated group (76.5% and 38.1%). In conclusion, the use of the PI3K/AKT pathway in regulating the maturation medium in the concentration used in this study did not improve the cleavage and blastocyst rates of bovine embryos produced *in vitro*.

A173 OPU-IVP and ET

Association of FSH and LH prior to follicular aspiration as a way to increase *in vitro* embryo production in the Gyr breed

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Keywords: Gyr ,OPU, PIVE.

The knowledge in physiology associated with hormonal therapies available can improve the results of follicular aspiration and PIVE. The use of FSH and LH can reduce variations quantitative and / or qualitative oocyte recovery by follicular aspiration. The objective was to compare the results of PIVE in zebu donors associating FSH and LH prior to follicular aspiration. In the study, 24 Gyr donors underwent three treatments (three groups, G1, G2 and G3) with an interval of 21 days between them, in a crossover design. In the control group (G1) cows were aspirated without any hormonal intervention in follicular wave, whereas the G2 and G3 were administered FSH (Folltropin, Bioniche) 20mg + LH (Lutropin, Bioniche) 1.25 mg by intramuscularly depth, 48 and 24 h prior to follicular aspiration, respectively. The PIVE was performed in the laboratory of the EMDGA (Station Breeding and Dissemination of Animal Genetics), with semen of the bull same for all groups. The mean oocyte retrieval and embryo production were compared by Tukey test at 5% probability. The number of oocytes (mean ± standard deviation) obtained by aspiration was 19.7 ± 8.3, 22.2 ± 9.5 and 21.4 ± 9, and embryos (mean ± SD) 4.1 ± 2, 4, 6.6 ± 2.5 and 6.5 ± 2.7 for G1, G2 and G3, respectively, not registering statistical differences between the results obtained for the number of oocytes. In the conditions proposed by the study, the treatments were not efficient to increase the number of oocytes, however G2 and G3 association with FSH and LH before follicular aspiration promoted an improvement in the number of embryos per aspiration. The G2 and G3 showed no significant statistical differences among themselves, indicating little difference between the time intervals practiced.

A174 OPU-IVP and ET

Effect of meiotic arrest with roscovitine, butyrolactone and this association on *in vitro* production embryos bovine

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Keywords: bovine, meiosis inhibition, oocyte.

One alternative to improve the quality of oocyte and consequently the quality of embryo is the use of drugs that induce inhibition of meiotic maturation (nuclear), causing at the same time a better cytoplasmic maturation. This study, evaluated the effect of the addition of a meiotic arrest roscovitine (ROS) and butyrolactone-I on in vitro production of bovine embryos. Nelore oocytes were matured in TCM-199 with Earle's salt + 10% FCS, FSH and LH, in 5% CO_2 atmosphere. To delay meiosis, the oocytes were maintained for 6 h in medium in presence of Roscovitine (12.5µM), Butyrolactone I (50µM) and Roscovitine (6.25µM + Butyrolactone I (25µM). Then the oocytes were cultured for 18 h in agent-free medium to meiosis resume, completing 24 h of maturation. After 24 h of maturation (day 0), oocytes were fertilized in human tubal fluid (HTF – Irvine, New Zeland) under the same condition above. Semen was selected through Percoll gradient and the concentration adjusted to 2 x 10^6 sperm/mL. The presumably zygotes were culture in 90µL droplets of SOFaa + 0.6% BSA + 2.5% FCS in 5% CO_2, 5% O_2, 90% N_2 atmosphere until day 7, when blastocyst rate was evaluated. There were made 5 replicates (200 oocytes/replicate). Data were analyzed with ANOVA, followed by Tukey test using the general linear model (PROC GLM) of SAS 9.2. The level of significance adopted was 5%. No statistical differences were observed in blastocyst production rate: Control: 42.3 ± 2.7%; Roscovitine 12.5µM: 39.6 ± 3.0%; Butyrolactone I 50 µM: 42.2 ± 2.3% and Roscovitine (6.25 µM) + Butyrolactone I (50 µM): 38.0 ± 4.5%. Thus, the addition of inhibitors of meiosis has not compromised embryonic development.

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A175 OPU-IVP and ET

Evaluation of melatonin antioxidant properties upon *in vitro* bovine embryo production system

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Keywords: bovine, reactive oxygen species, tbars.

This study aimed to evaluate melatonin effect over bovine *in vitro* embryo embryo production (IVEP) system. Embryo IVP rates were assessed and *in vitro* embryo culture medium thiobarbituric acid assays (TBARS) were performed. Melatonin is an effective free radical scavenger (Tan et al, 2002, Current Topics in Medical Chemistry 2, 181-197) and beneficial effects of melatonin have been demonstrated in IVP for some species (Tamura et al., 2008, J. Pineal Res. 2008, 44, 280-287). Follicles (2 to 7 mm) were aspirated from slaughterhouse-derived ovaries. Cumulus–oocyte complexes (COCs) who had a compact cumulus and oocyte with homogeneous cytoplasm were selected and randomly allocated (35 to 55 per group) on either control Group (CG) or Melatonin Treatment Group (MG). COC's were in vitro matured (IVM) for 22 h in 400µl of TCM199 (Day -1). For *in vitro* fertilization (IVF) COC's were moved to fertilization medium 400µl and were inseminated with frozen–thawed semen previously submitted to discontinuous Percoll gradient and incubated for 18 h (Day 0). After IVF presumptive zygotes were striped from remaining cumulus cells by incubation with hyaluronidase and gentile pipetting, then moved to 400µl of KSOM- BSA (Day 1). On Day 3 embryos were inspected under a stereomicroscope to evaluate cleavage rate, and 400µl KSOM-20% FCS was added. On Day 5 200µl of medium were removed, and kept for TBARS and 400µl KSOM-10% FCS was added. On Day 7 embryos were evaluated regarding their developmental stage, percentages were calculated over the total of presumptive zygotes. On Day 8 200µl of medium were removed, and kept for TBARS. All procedures were performed on a Nunc Multidishes 4 well dish without mineral oil overlay. During IVM and IVC groups received either 0ng/mL or 50 ng/mL of melatonin, CG and MG respectively. TBARS are expressed in nanograms of thiobarbituric reactive substances per mL. Statistical analysis was performed using SAS system for Windows 9.2 parametric data was submitted to student T-test and non parametric to Wilcoxon, presented mean $\pm$ Standard error and median (1^{st}; 3^{rd} quartile) respectively. Differences were considered meaningful when $p<0.05$. Melatonin treatment neither influenced cleavage (MG 73.94$\pm$2.23; CG 73.11$\pm$2.46) nor blastocyst rates on Day 7: % initial blastocysts MG 2.27 (0; 2.94) CG 2.47 (1.67; 4.55); % of Blastocysts MG 8.51 (6.15; 12.24) CG 10 (5.88; 9.62); % Expanded blastocysts MG 6.25 (3.03; 10.53) CG 5.96 (3.17; 9.62); % Total Blastocysts MG 19.72 (12.82; 26.53) CG 20.92 (14.67; 28.57). Unexpectedly melatonin treatment increased TBARS levels on medium both at D5, CG (141.7$\pm$6.87); MG (240.4$\pm$9.04), and at D8, CG (204.8$\pm$14.53); MG (268.3$\pm$16.68). Melatonin did not affect bovine in vitro embryo production rates as well as antioxidant properties were not demonstrated.

A176 OPU-IVP and ET

Effect of quercetin on glutathione levels in bovine oocyte maturation *in vitro*

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Keywords: glutatione, oocytes, quercetin.

Reactive oxygens species (ROS) are produced by physiological metabolism of oocytes and excess can cause deleterious effects on cell function, inducing apoptosis (Yang et al., 1998, Hum Reprod., 13, 998-1002). Glutathione (GSH) is the main molecule of defense against ROS in oocytes (De Matos et al., 1995, Mol Reprod Dev., 42, 432-436). When cumulus-oocyte complexes (COCs) are removed from their natural environment is increased production of ROS in this complex, justifying the use of antioxidant in the technique of in vitro production of embryos. Quercetin is a flavonoid and has excelled in scientific circles for their antioxidant (Liu et al., 2010, Toxicol In vitro., 24, 516-522). The aim of this study was to evaluate the effect of quercetin on the levels of intracellular GSH in bovine oocytes matured in vitro, compared with oocytes matured in the presence of cysteamine, which is a precursor of GSH. The COCs were aspirated from slaughterhouse ovaries and selected (grade I and II). A group of immature oocytes was used to determine the levels of GSH and the other three groups were subjected to maturation in 100 mL drops (TCM 199 with 10% FCS, 5.0 mg / mL LH 0.5 mg / FSH mL, 0.2 mM pyruvate and 50 g / ml gentamicin) supplemented with 2 mM quercetin, 100 mM cysteamine and in the absence of antioxidants (control), for 22 hours at 38.8 ° C 5% CO2. Each drop contained about 20 oocytes. To assess the levels of GSH, oocytes were mechanically separated from cumulus cells in PBS and transferred to a 10 mL droplet of PBS-PVA containing 50 mM CMF2HC fluorescent probe for 30 minutes at 38.8 ° C. To control technique, a group of matured oocytes were treated with 0.009% H2O2 for 30 minutes and determined the levels of GSH. The fluorescence emission was recorded by a camera Infinity1-1 attached to the microscope Nikon Eclipse Ti with UV-2A filter. The captured images were analyzed using the Infinity V6.2.0 software and fluorescence intensity was converted to arbitrary values in all groups. Statistical analyzes were performed using ANOVA followed by Bonferroni's test ($p < 0.05$). In the experiment we used 210 oocytes, randomly divided into five treatments with three replicates. The intracellular GSH level in immature oocytes was determined as a value (1.00), and was lower ($P < 0.05$) than oocytes maturated MIV/H2O2 (1,31) and control (1.67). GSH levels did not differ significantly ($P > 0.05$) between control and quercetin (1.64), but both differed ($P < 0.05$) in the group with cysteamine (1,76). In conclusion, matured bovine oocytes have higher GSH concentration compared to immature oocytes. Additionally, cysteamine increase GSH levels, while quercetin maintained the same level found in the control treatment.

A177 OPU-IVP and ET

Effect of mefenamic acid on pregnancy rates in recipient mares on day 10 pos ovulation (d10)

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Keywords: mefenamic acid, embryo transfer, receptor mares.

An embryo exposed to an asynchronous uterus may be subject to developmental factors and hormone levels that do not correspond to its developmental stage, what may result in changes in the rates of development or embryo death (Barnes, 2000, Theriogenology, 53, 649-658). Mares that ovulate after the embryo donor are usually better recipient candidates than those ovulating earlier, particularly if ovulation occurred 2 days before (McKinnon and Voss, 1992, Equine Reproduction, 19, 179-185). The pregnancy rate of embryo recipients under natural conditions reduces after day 9 of ovulation, limiting their use thereafter (Whitewashed et al., 2007, Brazilian Journal of Animal Science, 36, 360-368). The aim of this study was to investigate the anti-inflammatory effect of mefenamic acid on pregnancy rates after embryo transfer in recipients on day 10 of the estrous cycle (D10). For the production of embryos, donor mares had their cycle monitored by rectal palpation and ultrasonography. When follicles reached approximately 35 mm and endometrial edema was detected, ovulation was induced with 1.0 mg of deslorelin acetate IM and 24 h later the animals were inseminated with 500x106 viable spermatozoa previously diluted in the skim milk based dilutor Botu-cum (Biotech- Botucatu SP). Eight days after confirmation of ovulation uterine flush was performed to recover the embryos. A total of 33 animals were used as recipients. They were examined daily by rectal palpation and ultrasonography until the day of ovulation and randomly distributed into two groups: group 1 (n = 18) Control: embryo transfer ten days after ovulation without any treatment; and group 2 (n = 15) embryo transfer ten days after ovulation, treatmentd with 1 g of mefenamic acid orally manipulated in paste form (Powervet handling veterinary, SP-Brazil) on the day of transfer and for two more days. The transcervical transfer technique was used and only grade 1 and 2 embryos (McKinnon and Squires, 1988, J. Am Vet Med. Ass, 192, 401-406) were transferred. Pregnancy diagnosis was performed six days after ET. We used the Fisher exact test for analysis of the percentages of gestation between groups, which did not differ ($P > 0.05$). The pregnancy rate in the group treated with mefenamic acid was 33.3% (05/15) and in the control group 33.3% (06/18). Thus, it can be inferred in this experiment, that there was no effect of the treatment of mefenamic acid on pregnancy rates in recipients D10.

A178 OPU-IVP and ET

Characterization of individual differences in the efficiency of superovulation in the Gyr breed

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Keywords: follicular growth, FSH, zebu.

Most of the previous studies aiming to improve superovulation evaluated only differences in the number of corpora lutea (CL) observed and of embryos recovered. Therefore, differences in total follicular population before hormonal stimulation and in response parameters associated to follicle growth were frequently neglected, causing bias in the conclusion. The aim of the present study was to characterize individual variation in both relative and absolute efficiency of superovulation, based on ovarian follicular population. Non-lactating Gyr breed cows (n=2) and heifers (n=15), kept under the same management, were used. Follicle growth was synchronized (D0) using an intravaginal progesterone device and an injection of estradiol benzoate. Superovulations started on D5 with the injection of 200 UI FSHp, following a conventional protocol. The number and diameter of the follicles present before (D4) and during superovulation (D5 to D8), as well as the number of CL at flushing (D16), were evaluated by ultrasonography. Follicular population was ranked according to size (≤4 mm, 5-7 mm, ≥8 mm), and changes in the percentage of follicles in each size class were used to calculate relative efficiency. The absolute efficiency was determined by the ratio number of embryos recovered: number of follicles ≤4 mm on D5. Results are shown as mean±SEM. As expected, there was a great individual variation in the superovulation outcomes, both considering the number of CL (0 to 28, mean 12.6±2.1; CV=68.2%) and embryos collected (0 to 15, mean 5.1±1.1; CV=92.4%). There was no increase (P>0.05) in total follicular population during treatment, and the correlation between the number of follicles during superovulation and the further number of CL or embryos remained relatively constant between D5 and D8 (r=0.56 to 0.65 and r=0.70 to 0.79, respectively; P<0.01). FSH treatment induced a progressive (P<0.05) but partial mobilization of small follicles to larger size classes. The relative efficiency of the follicle growth stimulation was 41.9±5.5% (0 to 75.6%), and this was the endpoint with the largest correlation (R=0.80; P<0.0001) with the absolute efficiency of the process (12.2±2.1%, ranging from 0.0 to 25.0%). Retrospective analysis demonstrated that donors with relative efficiency >50% had a number of follicles ≤4 mm on D1 similar to those with efficiency <50% (41.6±6.8 vs 42.1±3.1; P<0.001), but produced more CL and embryos (17.8±2.5 and 7.6±1.7 vs 6.9±2.1 and 2.4±0.9; respectively, P<0.001). In conclusion, individual differences in follicular population and in the follicle response to FSH are important sources of variation in superovulation results, and shall be taken into account for experimental design.

Acknowledgments: FAPEMIG, CAPES, Biotran LTDA.

A179 OPU-IVP and ET

Bovine oocyte transportation in environment with or without control gas atmosphere

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Keywords: gas atmosphere, IVP, maturation.

Maturation stage includes all events that enable maximum expression of oocyte potential for development after fertilization. Indeed, it is one of the most important phases of IVP because in this period the oocyte reaches the ability to complete the next events (IVF and IVC). Several factors such as nutrients, atmosphere, temperature and pH are important and should be controlled during in vitro production targeting to simulate the characteristics of in vivo follicular environment. The aim of this study was to evaluate the effect of time (1, 8 and 24h) and of oocytes transportation device (with and without automatic control of gaseous atmosphere) on *in vitro* embryo production. Cumulus-oocyte complexes (COCs) were obtained from slaughterhouse ovaries, selected (only grades 1, 2 and 3), and grouped in 15 - 20 structures in cryotubes with TCM 199 supplemented with bicarbonate plus LH, FSH, estradiol, sodium pyruvate and fetal calf serum (FCS). Then, cryotubes were filled with a gas mixture containing 5% CO_2, 5% O_2 established in N_2, and placed in two different transportation devices: with controlled temperature and gaseous atmosphere (L1, Carrier Lab Mix Touch, WTA Watanabe Applied Technology Ltda EPP, Cravinhos, Brazil) and only with control of temperature (L2, Carrier oocytes MOD toi-16i, WTA Watanabe Applied Technology Ltda EPP, Cravinhos, Brazil). Cryotubes were kept on transportation devices for 1, 8 and 24 hours (T1, T2 e T3, respectively). A total of 679 viable oocytes were assigned into 6 treatments in 8 replicates. After the exposure period, COCs were transferred to IVM medium and cultured at 38 °C and 5% CO_2 until they completed the 24-hour period of maturation. The embryo production was assessed on the seventh (D7) and tenth (D10) days after in vitro fertilization. The experiment was arranged in 3 x 2 factorial design (time x carrier). Data were analyzed by GLM (general linear models), with means compared by Student Newman-Keuls (SAS), considering the effects of time, transportation device and interaction. There was no effect (P> 0.05) of transportation device (L1 and L2) on the cleavage rate (68.3 ± 3.1 and 71.7 ± 2.5%, respectively), blastocysts production on D7 (26, 9 ± 2.6 and 29.4 ± 2.4%, respectively) and on D10 (31.1 ± 2.5 and 31.0 ± 2.5%, respectively). Similarly, there was no effect of time (1, 8 and 24 hours) on the cleavage rate (74.1 ± 3.4, 67.2 ± 3.3 and 68.7 ± 3.7%, respectively), blastocysts on D7 (26.8 ± 2.9, 31.4 ± 3.0 and 26.2 ± 3.4%, respectively) or on D10 (29.6 ± 3.1, 35.2 ± 2.6 and 28.2 ± 3.4%, respectively), or interaction between time and transportation device (P> 0.05). In conclusion, bovine oocytes can be transported in incubators with or without automatic control of the gaseous environment by extended period of time without affecting the viability of the oocytes or embryonic development potential.

Acknowledgments: Fapemig.

A180 OPU-IVP and ET

Comparison of recovery rates and pregnancy rates of embryos from quarter horses mares of different ages

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Keywords: age, embryo transfer, mare.

The objective of this study was to evaluate the rate of embryo recovery, embryo quality, and pregnancy rate in mares of different ages. The study was made in two farms located in Limeira/SP, in breeding seasons 2011/2012 and 2012/2013. Were analyzed 342 uterine flushings of Quarter Horse mares (n=162). The procedures divided into three groups according to age, mares from 2 to 7 years (G1, n = 191), 8-17 years (G2, n = 99) and > 18 years (G3, n = 52). Donors with follicles of 35 mm and significant uterine edema were treated with 1 mg of deslorelin or 1250 IU of hCG. The mares were inseminated with cooled semen 24h after ovulation induction and examined 24 h later to verify the occurrence of ovulation. Was used the semen of 25 stallions at a dose of 500 million to 2 billion sperm with progressive motility. Uterine flushing were collected seven to nine days after ovulation and embryo transfer was performed in healthy mares, between the fifth and ninth days of the estrous cycle, with a closed cervix, uterus with homogeneous echogenic, and content without edema on ultrasound evaluation. Pregnancy diagnosis was performed at 15 days of gestation by transrectal ultrasonography. Results were compared by analysis of variance (P <0.05). The results were grouped because there was no effect of location on the variables analyzed. The recovery rate of embryos was similar (P <0.05) between groups G1: 71.2% (136/191); G2: 66.6% (66/99); G3: 61.5% (32/52). The stage of embryonic development was similar between groups resulting in 87.5% (119/136), 77.3% (51/66) and 93.8% (30/32) of expanded blastocysts; 2.9% (4/136), 7.6% (5/66) and zero blastocysts; and 9.6% (13/136), 15.2% (10/66) and 6.3% (2/32) of morulaes, respectively for G1, G2 and G3. There was a predominance of embryos of excellent quality among the groups (G1: 91.9%; G2: 97.0% e G3: 84.4%). Pregnancy rates were similar between groups G1: 80.9% (110/136); G2: 87.9% (58/66); G3: 68.8% (22/32). Pregnancy per embryo collection was higher (P = 0.05) between donors of G1: 57.6% (110/191) and G2: 58.6% (58/99) relative to G3: 42.3% (22/52). From these results, we observed an effect of age on the reproductive efficiency in large scale embryo transfer.

A181 OPU-IVP and ET

Effect of metabolic state in oocytes competence from nulliparous and multiparous crossbred dairy cows

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Keywords:follicular aspiration, heifers, reproductive efficiency.

Infertility in dairy cows is a multifactorial problem, since it depends on the climate, the management, the herd characteristics, and the physiological status of the animals (Westwood et al. 2002, Journal of Dairy Science, 64, 1153-69). The aim of this study was to analyze the effect of metabolic profile in the nuclear maturation rate of crossbred dairy cows (Holstein/Gyr), with 19.0 kg/day milk production. The study consisted of 3 groups: Group 1: Nul - (n = 30) nulliparous cows; Group 2: Mult < 45 (n = 30), multiparous cows during 30 - 45 days postpartum and Group 3: Mult > 45 (n = 30) - multiparous cows during 60 - 90 days postpartum. Oocytes were recovered by ultrasound-guided follicle aspiration and were classified as viable (grade I, II and III) and degenerate (without cumulus, expanded, atresic and degenerate). The viable oocytes were matured in vitro and after 22 h, and were analyzed for the emission of polar body in confocal microscopy. Blood samples of the coccygeal artery/vein and follicular fluid (FF) of the dominant follicle were collected. The following biochemical analyzes were performed: glucose (GLU), β-hydroxybutyrate (BHBA), serum gamaglutamiltransferase (GGT), sodium (Na), potassium (K) and calcium (Ca). Data were analyzed by BioStat 5.0 software. The average number of oocytes recovered was 7.8 (Group 1), 5.8 (Group 2) and 7.4 (Group 3). In groups 1, 2 and 3, an average of 5.0, 2.9 and 3.6 of viable oocytes were recovered, respectively, whereas in these same groups an average of 2.8, 2.8 and 3.7 were classified as degenerated. No significant difference was found ($P > 0.05$) in quality of oocytes between groups, while the nuclear maturation rate was higher in group 1 when compared to groups 2 and 3 ($P < 0.05$). When serum metabolites were analyzed, the rate of GLI was higher ($P < 0.05$) in group 1 compared to the concentrations of the animals in groups 2 and 3, whereas the concentrations of BHBA and GGT were lower in Group 1 when compared to groups 2 and 3 ($P < 0.05$). The serum levels of Na, K and Ca did not differ significantly between groups. The concentrations of GLI, Na and K of the FF were higher in group 1 when compared with group 2.3 ($P < 0.05$), whereas no significant difference in concentrations of BHBA and Ca of the FF between groups was observed. So, it can be concluded that crossbred nulliparous dairy cows had higher maturation rate, and together with the multiparous cows above 45 days postpartum had better physiological state after analyzing the data.

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Different Pluset superestimation protocols in Holstein lactating cows submitted to OPU

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Keywords: bovine, FSH, oocytes.

In vitro embryo production (IVEP) can accelerate bovine genetic improvement of superior animals. However, the efficiency of this technique can be limited due to variation in the number and quality of oocytes recovered through follicular aspiration (OPU). Hormonal protocols designed to control follicular emergence and development can minimize these variation (Blondin et al., 2002. Biol. Reprod., 66, 38–43). The objective was to evaluate different synchronization and follicular-stimulation protocols during OPU in Holstein donors. Fifteen lactating Holstein donors were used with average milk production of 28.8 liters/day. All cows were submitted to four treatments before OPU (Crossover design). The OPU was performed with an interval of 21 days by the same veterinarian. At OPU, variables evaluated and compared between treatments were the diameter of follicles and the number of total and viable recovered oocytes. The four treatments were as follows: T1: at day 0 (D0) - Intravaginal P4 device (Ciclovar®-Hertape-Calier, Juatuba, Minas Gerais, Brasil[1]) + 0.15mg of D-Cloprostenol (Veteglan®-Hertape-Calier[1]), day 1 (D1) - 3mg of Estradiol Benzoate EB (Benzoato-HC® Hertape-Calier[1]), day 4 (D4) - single dose of 150UI FSH (Pluset®-Hertape-Calier[1]) and day 7 (D7) – OPU; T2: D0 - P4 device + 0.15mg of D-Cloprostenol, D1 - 3mg of EB, D4 to D6 - 50UI FSH twice a day at 12h intervals, D7 - OPU; T3: D0 - P4 device + 0.15mg of D-Cloprostenol, D1 - 3mg of BE, D4 - 100 IU of FSH twice, D5-150UI FSH twice and D6 - 150UI FSH twice, D7 – OPU; T4: D0 - P4 device + 0.15mg of D-Cloprostenol, D1 - 3mg of EB. Follicles largest than 3 mm were aspirated. The average milk production, follicle size and production of viable and total oocytes were compared among groups by Tukey test, at 5% significance level. No differences were found in milk production between treatments ($P>0.05$). Animals of T3 had the greatest average of follicular development than other treatments (T1: 0.41 ± 0.84, T2: 1.38 ± 1.27, T3: 3.63 ± 1.77 and T4: 3.58 ± 1.80; $P< 0.05$). Concerning total oocytes, T1 and T3 had greater number of structures than T4 (9.06 ± 0.33, 11.86 ± 2.83 and 6.86 ± 2.16, respectively). T3 was the group in which best results of viable oocytes were obtained (T1: 2.06 ± 2.61, T2: 5.20 ± 0.51, T3: 9.40 ± 4.71 and T4: 2.06 ± 2.61, respectively). T4 showed greater number of non-viable oocytes. In conclusion, stimulation with 400 IU FSH in six applications can improve the results OPU in Holstein lactating cows.

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Antioxidant effect of quercetin in reducing ROS in bovine oocytes

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Reactive oxygen species (ROS) are produced during cellular metabolism like hydrogen peroxide (H_2O_2). In the in vitro production system (IVP) of embryos the concentration of ROS can be increased due to factors such as O_2 voltage, light exposure and oocytes/embryos manipulation, causing cellular damage. Thus, the use of antioxidants in IVP embryos system with high O_2 voltages (~20%) is necessary. The quercetin antioxidant action has high capacity for free radicals eliminating, preventing cellular damage. The objective of this study was to evaluate the quercetin ability to remove hydrogen peroxide bovine oocytes treated with 0.009% H_2O_2. The oocytes were obtained from slaughterhouse ovaries and grade I and II were selected. To determine the H_2O_2 concentration to be used in oocytes a pre-experiment was made with different concentrations (data not shown). To assess the ability of quercetin to remove H_2O_2, oocytes (without cumulus cells) were treated with several concentrations of quercetin (0,4, 2, 10 and 50 µM), H_2O_2 (0.009%) and 10 µM of 2',7'-Dichlorofluorescin diacetate (H2DCFDA - used as an indicator for ROS) for 30 minutes at 38.5 °C. Two control groups were also evaluated: Positive and Negative Control (with and without addition of 0.009% H_2O_2, respectively) with 10 µM of H2DCFDA for 30 minutes at 38.5 °C. The fluorescence emission of oocytes was captured by camera Infinity1-1 attached to the microscope Nikon Eclipse Ti with filter B_2E/C 495 absorption/emission 519, and the images analyzed by Infinity software V6.2.0. Arbitrary fluorescence values were analyzed by ANOVA (BioEstat 5.0) and the Bonferroni test ($p < 0.05$). 240 oocytes were evaluated in three replicates and levels of ROS in negative control group were lower (0.56 ± 0.04) than positive control group (1.69 ± 0.16). However, when oocytes were treated with quercetin (0.4, 2, 10 and 50 µM) a decrease in ROS levels (1.51±0.21, 1.32 ± 0.16, 1.34 ± 0.16, 0.89 ± 0.13, respectively) was observed in comparison to Positive control (1.69 ± 0,16), but remained above levels from Negative control (0.56 ± 0.04). According to results obtained, quercetin was effective in reducing hydrogen peroxide concentrations in immature oocytes due its antioxidant effect, showing that can be used on *in vitro* embryo production.