A107 OPU-IVP and ET

Evaluation of the effects of anti-inflammatory drugs on ovarian scar tissue formation in heifers under OPU

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Keywords: anti-inflammatory drugs, bovine, OPU.

The IVP associated with the OPU improved the reproduction of cows. However, it may cause morphological and functional changes in the ovaries. This study aims to evaluate the effects of anti-inflammatory drugs on the formation of scar tissue in the ovary of heifers submitted to follicular aspiration. Ten OPU sessions were performed with an interval of 15 days between them, and 18 heifers were divided into three groups: Group I (GI): control animals treated with 5ml of saline; Group II (GII): animals treated with 1.1 mg / kg flunixin meglumine (GII - FM: Banamine®) and Group III: animals treated with 0.5 mg / kg meloxican (GIII - M: Maxicam®), immediately after each OPU session. At the end of the OPU's all the animals were slaughtered, their ovaries removed, of which the fragments were subjected to histological technique by Gomori Trichrome. For histological analysis, in each ovary, right (RO) and left (LO), and group, quantitatively, the average number of viable follicles, atretic follicles and corpora lutea, and qualitatively, as mild, moderate or severe, the presence of collagen in ovarian tissue were considered. From quantitative analysis by the Dunnett Test, it was observed that the average number of viable and atretic follicles, present in the ovaries was similar between groups I, II and III ($p < 0.05$), 15.50 + 6.66, 15.17 and 12.17 + 6.05 + 5.00, and 1.83 + 0.41 1.83 + 2.00 + 0.41 and 0.00, respectively. The average number of CL present in the ovaries of the FM-treated group (GII) was greater than observed in the ovaries of the control group (GI) and treated with M (GIII), 1.33 + 0.52, 0.50 + 0.55 e 0.50 + 0.55 ($p<0,05$), respectively. From qualitative analysis by the Kruskall-Wallis Test, all ovaries evaluated the presence of collagen was observed, but the most severe grade was observed in the ovaries of the Control Group attaining 41.7% (05/12) of the ovaries with severe lesions and only 16.7% (02/12) with mild injury ($p<0,05$), whereas the ovaries of Group II showed 33.3% (04/12) of minor injuries and Group III 25% (03/12) of minor injuries and 33.3% (04/12) of severe lesions. The results indicate that treatment with anti-inflammatories (M and FM) possibly decreased accumulation of collagen in ovarian tissue after OPU sessions.

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A108 OPU-IVP and ET

Use of artificial lighting and intravaginal progesterone devices in anticipating reproductive cyclicity in anestrous mares

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Keywords: artificial lighting, intravaginal progesterone device, mare.

The use of artificial lighting programs in equine species aims to hasten the breeding season by increasing exposition to luminosity. For the same purpose, synthetic progestins are used in the transitional period, simulating the corpus luteum effect by inhibiting progesterone (P4) action on hypothalamic hormones. The aim of this study was to evaluate the efficiency of artificial lighting and intravaginal progesterone devices, associated or not, to hasten the equine breeding season. Forty mares were distributed into 2 anestrous mares groups: Light and Light+P4; 1 transitional mares group: P4; and 1 cycling mares group: Control (n=10 mares/group). Animals from Light and Light+P4 groups were kept for 60 days, from winter solstice, in paddocks with incandescent lamps (100lux/lamp), totaling 16 hours of daily light. Once in transitional phase (follicles between 20 to 25 mm in diameter), a progesterone intravaginal device was placed (Primer®, 1g of P4 without estradiol capsule) and kept during 12 days in mares from P4 and Light+P4 groups. Daily evaluations were performed through rectal palpation and ultrassonography to monitor follicular development until first spontaneous ovulation. In order to determinate plasmatic P4, blood samples were collected daily by jugular puncture in heparinized tubes, during the 12 days of P4 device permanence in treated groups and in the corresponding days in Control group. Hormones concentrations were assessed by radioimmunoassay. The duration of two intervals was analyzed, from the beginning of artificial light exposure until the largest follicle reached 35 mm in diameter (solstice-35) and until ovulation (solstice-ov). The plasma concentration of P4 and P4 groups light + P4 increased abruptly to the insertion device, reaching maximum concentrations after 1 day (4.9085 ng / ml), with a gradual decrease until its removal, ranging from 0.2154 ng / ml and 1.0851 ng / ml (D0-D11, whereas the placing on D0 of the device). While in the control group was maintained throughout the basal time (0.0924 and 0.1113 ng / ml). For the characteristics evaluated, the experimental design was completely randomized, whereas for P4 plasmatic concentration a factorial scheme 3X12 (groups X days) was used. The Tukey test was used at 5% significance. At Light and Light+P4 groups, a decrease in solstice-35 and solstice-ov intervals was observed in relation to Control group ($P<0,05$). There was no difference in these intervals duration between Control and P4 groups and among P4, Light and Light+P4 groups ($P<0,05$). Progesterone plasmatic concentration at P4 and Light+P4 groups increased sharply with insertion of the device, reaching maximum concentrations 1 to 2 days after insertion, showing a gradual decrease until its removal, while in Control group it remained basal during all the time. The present study suggests that artificial lighting programs associated or not with exogenous progestins devices anticipates mares breeding season.

A109 OPU-IVP and ET

Use of sperm preincubation as a strategy to improve bovine embryos *in vitro* production

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Keywords: blastocysts, capacitation, *in vitro* fertilization.

Increasing rates of *in vitro* production (IVP) bovine embryos has been the goal of several research teams in recent years. Despite advances, it is estimated that only 40% of slaughterhouses oocytes achieve development to the blastocyst (BL) stage (VARAGO et al. Rev.. Bras. Reprod. Anim. v.32 (2008) 100-109). At this way, it is important to investigate the IVP protocols and develop simple strategies to solve this problem. In experiment 1, 277 bovine oocytes from cattle ovaries from slaughterhouses of Campos dos Goytacazes, RJ, Brazil, were matured (22 hs) and *in vitro* fertilized with bovine frozen-thawed semen and selected by Percoll discontinuous gradient. Prior to fertilization, sperm was preincubated in the IVF medium (FEC-TALP supplemented with heparin 20 mg/mL) for 1 hour (G1h) or 4 hours (G4h) in IVF droplet (100 µL) adjusted to a final concentration of $1x10^6$ espermatozóides/mL, in a humidified atmosphere of 5% CO_2 in air at 38 °C. At the control group (G0h), there was no sperm pre-incubation. The IVC was carried out in TCM199 medium supplemented with 10% FCS. The cleavage and blastocyst rates were analyzed on days 3 and 8 of development, respectively, by ANOVA test (SAS, 2002). In experiment 2, samples of 100µL of medium with spermatozoa under the same conditions of experimental groups were stained by chlortetracycline (CTC, 20µM) and analysed by light microscopic. A number of 100 cell/slides was counted and classified into three different patterns: not capacited (NC) with uniform fluorescence sperm head; capacited with intact acrosome (C), capacited with reacted acrosome (RA) (adapted from WANG et al., 1995). The experiment 1 and 2 were repeated 5 and 4 times, respectively. All reagents used were obtained from Sigma (St. Louis, USA). The cleavage rates were similar between the experimental groups G1h, G4h and G0h (59.40 ± 18.82, 43.60 ± 17.76, 63.80 ± 10.03, respectively). The group G1h resulted in a higher rate of blastocyst production (37.20 ± 19.43) compared with G0h (28.20 ± 15.75) and G4h (11.00 ± 5.83). In relation to CTC assessment, there was significant difference between the groups (Test T). At G0h, only 15.75 ± 2.87 sperm analyzed were classified as capacited (NC: 70 ± 6.73 and AR: 13.5 ± 4.5). After pre-incubation for one hour, most of the sperm (62.25 ± 1.70) were capacited (CN: 21.75 ± 3.4, and RA: 16 ± 2.16). At G4h, 82.5±1.73 had already suffered acrosome reaction and only 10 ± 2.58 were classified as capacited (NC: 7 ± 1.4). It is possible that pre-incubation for 1 hour is the time required for *in vitro* capacitation to occur appropriately, because it leaded to higher rates of IVP blastocyst on D8 and a greater number of capacitated sperm by CTC assessment. This technique is easy and inexpensive, that's way can be introduced in IVP routine laboratories in order to increase the number of *in vitro*-produced bovine embryos.

A110 OPU-IVP and ET

Effect of presence of insulin, transferrin, selenium (ITS), L-ascorbic acid (AA) and cilostamide during prematuration of bovine oocytes on *in vitro* embryo development

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Keywords: antioxidans, cilostamide, prematuration.

Oocytes used for IVP are obtained from a heterogeneous population of ovarian follicles that when recovered from their follicles resume meiosis, regardless of their competence level. One possible strategy to improve the quality of oocytes submitted to IVM would be to provide prematuration culture conditions, which would allow additional time to complete the acquisition of competence without resuming meiosis. The aim of this study was to evaluate the effect of adding ITS and AA, associated with a phosphodiesterase type 3 inhibitor, cilostamide, during prematuration of bovine oocytes on subsequent embryonic development. The COCs (n=647) obtained from slaughterhouse ovaries were selected and distributed into three groups: 1) control group (CG), immediately placed in IVM; 2) prematuration for 8 hours, followed by IVM for 22 hours (PM8+IVM22) and 3) prematuration for 24 hours, followed by IVM for 22 hours. The prematuration medium used was TCM-199 supplemented with FCS 10%, 10μM of cilostamide, 0.5 mg/ml of ITS and 100μg/ml of AA. The IVM medium consisted of TCM-199 supplemented with FCS 10% and FSH (0.01 UI/ml) and antibiotics. After IVM, the COCs of the three groups were submitted to IVF (D=0) and IVC, and were evaluated for cleavage rate on D2 and blastocyst rate on D7. The D7 blastocysts were measured with the assistance of a camera Motic Image Plus 2.0 and were divided into three categories (120-140 μm; 140-160 μm and >160 μm). Only those embryos with size >160 μm were evaluated for number of cells using Hoechst stain. Data of embryo development and size categories were analyzed by chi-square test ($P<0.05$), and cell numbers and size of embryos by the Mann-Whitney ($P<0.05$). Cleavage rate was similar ($P>0.05$) for CG (82.4) and PM8+IVM22 (81.7), but lower ($P<0.05$) for PM24+IVM22 group (72.6%). The blastocyst rate was highest ($P<0.05$) in CG (54.8%), intermediate in PM8+IVM22 (29.3) and lowest in PM24+IVM 22 (15.0%). The CG also showed higher percentage of embryos with size >160 μm (75.2%) than the PM8+IVM22 group (56.7%), being the percentage of both groups higher ($P>0.05$) than the PM24+IVM22 group (0%). Although the cell number of embryos >160 μm did not differ between the groups CG (122±5.7) and PM8+IVM22 (111±6.0) their mean size was highest in GC (192.4 μm vs. 177.1±25.0 μm). The results suggested that, regardless the period of prematuration, the presence of ITS, AA and cilostamide in culture, had a detrimental effect on yield and quality of embryos.

A111 OPU-IVP and ET

Effect of donor breed on the *in vitro* production of bovine embryos

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Keywords: breed, oocytes, ovum pick up.

The *in vitro* production of bovine embryos (IVP) is growing quickly in the dairy market due to growing efficiency of the use of sex-sorted semen in the IVP system, which makes possible the production of great number of females for the industry of dairy products. However, besides the fertility between different cattle breeds, there are reproductive peculiarities between animals of different genetic groups that must be taken into account when reproductive techniques are used as, for instance, the estrus length, the dominant follicle diameter and the number of recruited follicles (Baruselli et al; Revised Brazilian Animal Reproduction, v.31, n.2, p.205-211, 2007). So, the aim of this study was to compare the effect of oocyte donor breed (*Bos taurus taurus* versus *Bos taurus indicus*) on the IVP bovine embryos comparing the rates of viability oocyte, cleavage and blastocysts rates. The data was provided by In Vitro Rio, located in Barra do Piraí-RJ, Brazil. None of the oocyte donors were submitted to any hormonal treatment previously to OPU. For this study, data was related to 500 OPU sessions in Holstein donors (*Bos taurus*, n= 250) and in Gir (*Bos indicus*, n=250). The embryos were obtained with the use of female sexed semen from Holstein bulls for both oocyte donors breed. The viable oocytes, cleavage and blastocyst rates were analyzed by chi-square test ($p \leq 0.05$). The percentage of viable oocytes (viable oocytes / recovered oocytes) was 58.4% (2093/3583) for Holstein females and 58.5% for Gir (2341/4000) ($p = 0.941$) and cleavage rate (cleaved / oocytes viable) was 65.7% (1377/2093) for Holstein and for Gir 59.6% (1397/2341) ($p = 0.0001$). The blastocyst rate (blastocyst / viable oocytes) was 28.1% (590/2093) for Holstein and for Gir 26.8% (628/2341) ($p = 0.3264$). Therefore, this study showed no statistical difference between breeds concerning viable oocytes and blastocysts rates, but, there was statistical difference between the breeds in cleavage rate. Thus, we conclude that the donor oocyte breed does not affect the viable oocytes and blastocyst rates.

A112 OPU-IVP and ET

In vitro embryo production of Holstein cows presents distinct performance according to the season of the year and the type of sperm utilized

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Keywords: embryo, oocyte, OPU.

The environmental factors, such as food intake and heat stress could influence the fertility, especially in high production Holstein dairy cows. Likewise, it is known that a spermatozoid subjected to the sex sort process by flow cytometry could have its viability altered. Thus, the objective of this study was to investigate the influence of season of the year and the use of sexed semen in *in vitro* embryo production of oocytes harvested by ovum pick-up (OPU). A total of 1869 OPU procedures were performed in non-lactating and lactating Holstein cows, previously selected by high ovarian follicular population (>20 follicles per donor). The females were from three farms in southeastern Brazil and the OPU were performed during the summer (n = 1217 OPU) and winter (n = 652 OPU) of the 2010 and 2011. The oocytes were subjected to the *in vitro* embryo production and were fertilized with sex sorted sperm (n = 1566 OPU) or non-sex sorted sperm (n = 303 OPU). The variables were analyzed using the GLIMMIX procedure of SAS. The overall efficiency was: 20.8 ± 0.3 total oocytes harvested, 15.3 ± 0.3 viable oocytes, 3.5 ± 0.1 embryos per OPU session. The proportion of oocytes suitable for *in vitro* production was 72.2 ± 0.5% and the embryo production by the viable oocytes was 25.6 ± 0.6%. The number of viable embryos produced by OPU session (3.3 ± 0.1 summer, winter 3.9 ± 0.2, P = 0.004) and embryo production per viable oocyte (summer 23.8 ± 0.4%; winter 28.9±1.0%, P <0.0001) were reduced when the OPU were performed during the summer than those performed during the winter. Regarding the type of semen used, there was a reduction in the number of embryos produced by OPU session (P <0.0001) and the embryo production per viable oocyte (P <0.0001) when sex sorted sperm was used (3.3 ± 0.1 and 23.7 ± 0.6%) when compared with the use of non sex-sorted sperm (4.5 ± 0.2 and 35.1 ± 1.5%). Therefore, the current study indicates that *in vitro* embryo production of the Holstein cows is decreased when OPU is performed during the summer. In addition, lower embryo production is achieved when sex sorted sperm is applied to *in vitro* fertilization.

A113 OPU-IVP and ET

Effects of oxygen tension and oocyte density by volume of medium on nuclear and cytoplasmic maturation of bovine oocytes

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Keywords: cytoplasmic maturation, nuclear maturation, oocytes.

The *in vitro* maturation (IVM) comprises the events of nuclear and cytoplasmic maturation, which can be influenced by several factors, including oxygen tension and density of oocytes per volume of medium used for IVM. The objective of this study was to evaluate the influence of association between O_2 tension (5% or 20%) and different density of oocytes per volume of medium (1:10 or 1:20 µl) on the nuclear and cytoplasmic maturation. Oocytes were obtained from abattoir ovaries by aspiration of the follicles 2-8 mm. Following selection, they were homogeneously divided into 15 oocytes by treatment T1: 1:10 in 5% O_2, T2: 1:10 in 20% O_2, T3: 1:20 in 5% O_2, T4: 1:20 to 20% O_2. The oocytes were matured in TCM 199 plus 10% of estrous mare serum, EGF, LH, FSH and pyruvate for 22 to 24 hours at a temperature of 39 °C and saturated humidity. After IVM the cumulus cells were removed and denuded oocytes were stained in drops of 5µl of IVM medium supplemented with 10ug/ml of bisbenzimide (Hoechst33342) and 220nM of Mitotracker Green FM and analyzed under an epifluorescence microscope. Extrusion of the first polar body was considered for assessment of nuclear maturation (NM), and mitochondrial reorganization was considered for assessment of cytoplasmic maturation (CM). The oocytes that had dispersed mitochondria, with homogeneous distribution in the cytoplasm, were considered oocytes with CM. Means of each group were compared by ANOVA and Tukey's test, with significance of 5%. For nuclear maturation, 219 oocytes were evaluated (4 replications), divided into four treatments, with rates of NM similar among treatments (67.9%, 74.5%, 66% and 67.8% for T1, T2, T3 and T4, respectively). For the cytoplasmic maturation, 264 oocytes (5 replications) were evaluated, and the CM rate also similar between treatments (69.7%, 76.6%, 71.2% and 72.1% for T1, T2, T3 and T4, respectively). These data show that the association of an atmosphere of 5% or 20% O_2 with a density of 1:10 or 1:20 oocytes per microliter of IVM medium did not influence the rate of nuclear and cytoplasmic maturation of oocytes matured *in vitro*. These results do not exclude possible influences on future embryo quality and other studies should be conducted to evaluate the production of reactive oxygen species (ROS) in the IVM and IVP systems and their influence on quality of embryos produced *in vitro*.

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A114 OPU-IVP and ET

Increment of embryo recovery rate in mares submitted to a novel uterus washing method

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Keywords: uterus washing, embryo transfer, mare

In bovine species, an addition of 30% in the total number of embryos can be obtained after filling up the uterus and taking the donator for a walk (Castro Neto et al., Theriog. 63, 1249-55, 2005). The purpose of this study was to evaluate the embryo recuperation rate of mares submitted to a walk with full uterus after third wash. American Quarter mares (n = 34) were submitted to daily ultrasonography examination for detection of 1 or more follicles ≥ to 35 mm and the presence of uterine edema level 3 (scale 1 to 3), when ovulation was induced with hCG (1667UI IV). On the following day, IA was conducted with fresh or refrigerated semen. After 36 hours, ovulation was detected. On D8, they were submitted to collection of embryos through unique system with 3 uterine washes. Each wash was composed of 1 to 1.5 liters of ringer with lactate and posterior recuperation of fluid in filters. In test group (n = 25) after washing the uterus twice, the uterus was filled up with the collection medium, with a catheter closed by forceps haemostatic so the uterus would be kept filled for a period of 10 to 15 minutes, during which the donator was conducted through a harness for a walk. In Control Group, the mares (n = 9) were submitted to conventional procedure of embryo collection, which consisted of 3 consecutive uterine washes, without filling the uterus and without the walk. The content of each uterine wash was divided in individual Petri plates and a search for embryos was conducted. In Test Group, from the 87 procedures of collection, 47 embryos (54%) were collected: 21 (44.6%) of the recovered ones in the first wash, 16 (34%) in the second and 10 (21.2%) in after the third wash. In Control Group, from the 28 procedures, 19 embryos (67%) were recovered: 10 (35.7%) in the first wash, 7 (25%) in the second and 2 (7%) in the third one. A analysis of variance was conducted, and the significance was found in the groups and washes ($p<0,05$). The majority of recovered embryos in equine species came up right after the first uterine wash (35 and 44%) and a few embryos in the following washes, as described (Fleury et al. Braz. J. vet. Res. anim. Sci.,38, n. 1, 29-33, 2001). In this experiment, significant differences were found (21 vs. 7%) in embryo recovery rate of mares if a 10-to-15-minute walk was conducted while uterus was filled up with fluid. This may have occurred due to some embryos being among endometrial folds or maybe in the oviduct-uterus junction. Keeping the uterus distended with fluid and combining the movement of the walk could contribute to unfolding the endometrial folds and/or favoring the release of embryos adhered in the limit of the uterus and the tube. Therefore, being this a non-additional cost practice, which requires a very little time, it is suggested the use of it when embryos are not recovered in the first two washes.

A115 OPU-IVP and ET

Developmental capacity of bovine oocytes matured *in vitro* with recombinat human FSH

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Keywords: *in vitro* culture, *in vitro* maturation, *in vitro* production.

Follicle Stimulating Hormone (FSH) has beneficial effects on *in vitro* maturation (IVM) by favoring the cumulus cells expansion, fertilization and cleavage. Most of IVM protocols use FSH extracted from porcine pituitary, despite the risks of biological contamination and the batch-to-batch inconsistency. These problems might be avoided by using recombinant FSH. This study aimed to evaluate the effects of different concentrations of recombinant human FSH (rhFSH) on *in vitro* production of bovine embryos. The rhFSH was gently donated by Galactous Biotech Ltda. (Concepción, Chile). Immature COCs (n=793 in at least five replicates) obtained from slaughtered animals were randomly allocated in six groups of *in vitro* maturation as follow: G1 - control with porcine FSH (pFSH - Pluset, Callier, Spain); G2 – without FSH; G3 – 0.0105 UI rhFSH; G4 – 0.021 UI rhFSH; G5 - 0.042 UI rhFSH and G6 – 0.084 UI rhFSH. The maturation was performed in TCM199 medium (Invitrogen, Carlsbarg, USA) supplemented with 10% estrus cow serum for 24h at 38.5 ºC under 5% CO_2 and 95% humidity. After IVM, the oocytes were *in vitro* fertilized with 2 x 10^6 spermatozoa/mL for 18-20h. The presumptive zygotes were partially denuded and then cultured in modified CR2aa medium supplemented with 10% fetal calf serum (Nutricell, Campinas, Brasil) at 38.5 ºC under 5% CO_2 and 95% humidity. Cleavage and blastocyst rates at day seven (D7) and day eight (D8) post-fertilization were evaluated by analysis of variance and means compared by Duncan's test. Values are shown as mean±SEM. Expansion of cumulus cells were observed in all groups, except in the group without FSH (G2). There was no difference (P>0.05) on cleavage rate among pFSH (88.6±3.9%) and groups with different rhFSH concentrations (89.6±2.7%; 85.2±3.5%; 92.1±2.7% e 90.3±5.0% for G3, G4, G5 and G6, respectively). Nevertheless, the group without FSH (G2) had the lowest (P<0.05) cleavage rate (72.2±3,8%). There was no difference (P>0.05) among groups regarding blastocyst rate at D7. However, the blastocysts rate at D8 for the group with 0.0105 UI rhFSH (G3; 53.0±3.6%) was higher (P<0.05) than for groups without FSH (G2; 33.5±4.5%) and 0.084 UI rhFSH (G6; 40.2±4.8%), but similar (P>0.05) to pFSH (G1; 45.3±3.8%), 0.021 UI rhFSH (G4; 44.8±2.6%) and 0.042 UI rhFSH (G5; 47.0±2.5%) groups. In conclusion, rhFSH may be used for *in vitro* maturation of bovine oocytes, with results similar to pFSH for embryos and with concentrations ranging from 0.0105 to 0.042 UI.

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A116 OPU-IVP and ET

Application of placental gonadotropins on the *in vitro* production of bovine embryos

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Keywords: embryos, *in vitro* maturation, placental hormones.

The *in vitro* maturation (IVM) is one of the most challenging stages in bovine IVP. The IVM medium should provide satisfactory conditions for the nuclear and cytoplasmic maturation to occur as close as possible of the physiological. The pituitary gonadotropins are essential components for these changes to occur synchronically in order to produce competent oocytes. However, there is controversy whether these hormones (pituitary and/or placental) are really necessary and also about the concentrations to be used. Given this disagreement, this study aimed to compare the effect of different combination of gonadotropins (on the IVM medium) on production of bovine blastocysts. Cumulus-oocyte complexes (COC, n=399, grades I and II), obtained from bovine ovaries (abattoir Fribom, Assis, SP), were distributed into three groups: G1) FSH (0.1 µg/mL) and LH (50 µg/mL, n=114 COC in 4 replicates); G2) hCG (2 UI/mL) and FSH (0.1 µg/mL, n=139 COC in 5 replicates); G3) eCG (4 UI/mL) and LH (50 µg/mL, n=146 COC in 5 replicates). The selected COC were matured in TCM 199 supplemented with 10% fetal calf serum. After 24 h matured COC were subjected to *in vitro* fertilization (IVF, D0) in TALP-FIV for 22-24 h. The semen was selected for viability by Percoll gradient method and then evaluated for motility and sperm concentration, to calculate the insemination dose ($1x10^6$ sperm/mL). Presumptive zygotes (PZ) were cultured in SOF (Synthetic Oviduct Fluid) supplemented with 2,5% fetal calf serum and BSA (5mg/ml), in an incubator (38.3°C, 5% CO_2 and maximum humidity). Embryo development was evaluated by the cleavage, blastocyst and hatching rates (D3, D7 and D10, respectively) compared to the PZ. The average rates of replicates were analyzed by ANOVA and Tukey-Kramer and significance was considered when $P<0.05$. There was no statistical difference between the mean values (±SD) of cleavage (76±13%, 84±11% and 73±13%), blastocyst (34±24%, 38±21% and 40±12%) and hatching (20±16%, 24±15% and 22±14%, respectively, for G1, G2 and G3). The partial replacement of the pituitary gonadotropins LH (for hCG in G2) and FSH (for eCG in G3) resulted in development rates similar to the G1 (control group with FSH and LH). In this experimental condition, we conclude that the placental hormones are a viable and effective alternative for replacement of FSH or LH on the IVM of bovine oocytes, to be used in the commercial IVP of bovine embryos.

A117 OPU-IVP and ET

Utilization of different cell culture media to produce bovine embryos *in vitro* in abscence of fetal calf serum

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Keywords: bovine, cell culture, embryo.

Introduction: The media used for *in vitro* production of bovine embryos affects development after fertilization, interfering with metabolism, gene expression and pregnancy rate. Bovine embryos are routinely cultured in medium supplemented with fetal calf serum (FCS). However, the use of this compound has been associated with metabolic distress and lipid accumulation, reducing embryo viability after cryopreservation. Objective: The present study aimed to produce bovine embryos in media used for stem cells culture, such as DMEM, alpha-MEM and F12, in the absence of FBS. A comparison was made to the SOFaa, usually used for embryo culture. Material and Methods: Abattoir ovaries were used to obtain the oocytes from follicles with 2-8 mm in diameter. Grade I and II oocytes were selected and submitted to *in vitro* maturation (IVM) in a 5% CO_2 in air and 100% humidity atmosphere at a temperature of 38.5 ^{o}C, for 22-24 hours. Subsequently, IVF was performed with semen from bulls with known fertility. The sperm were selected by Percoll gradient and the concentration was adjusted to $2x10^6$ sperm/ml. After 18 hours of IVF, presumptive zygotes were stripped and transferred to 90µl drops of culture medium. Embryo culture was conducted on 4 groups according to the medium composition: Group 1 (n = 245) composed of DMEM/F12 supplemented with 10 mg/ml BSA. Group 2 (n = 285) composed by alfa-MEM/F12 supplemented with 10 mg/ml BSA. Group 3 (n = 234) composed by SOFaa supplemented with 10 mg/ml BSA. Group 4 (control: n = 234) = SOFaa supplemented with 5mg/ml BSA and 10% FBS. The embryos were cultivated in a 5% CO_2 and 5% O_2 in air atmosphere, saturated with humidity, at 38.5 °C until 7 days when blastocyst production was estimated. Eleven replicates were performed with all groups being repeated in all replicates. Data were analyzed by ANOVA, followed by Tukey test using the general linear model (PROC GLM) of SAS 9.2. Results: The overall blastocyst production was 24.80% for the control group, 20.51% for G3 (SOF + BSA), 12.65% for G2 (alpha-MEM + BSA) and 13.90% for G1 (DMEM + BSA). Conclusions: The media α-MEM and DMEM in association with nutrient mixture F-12 HAM are widely used for the cultivation of various cell types, including embryonic stem cells. However, the results obtained so far still indicate the superiority of SOFaa for *in vitro* production of bovine embryos. Nevertheless, there are no published reports of the use of these media for maintenance of embryo development *in vitro*, and this study indicates the possibility of embryo production in cell culture media, making this an important new research in the area.

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A118 OPU-IVP and ET

In vitro production of bovine embryos from oocytes matured in the presence of forskolin

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

The oocyte *in vitro* maturation (IVM) is an important reproductive technology that generates mature oocytes that are capable of supporting embryo pre-implantation development and full development to term (Gilchrist and Thompson, 2007; Theriogenology, v.67, p.6-15). However, one of the most widely used techniques in maturation is inhibition or delay in the nuclear meiotic of oocytes, which would allow a more regulated and appropriate transition from prophase I to metaphase II. In this matter, the forskolin (Sigma-Aldrich, St. Louis) is an efficient inhibitor of nuclear maturation because of its ability in raising the levels of intracellular cAMP. This study aimed to show if the use of forskolin alone is able to inhibit meiosis in bovine oocytes and produce a higher rate of *in vitro* produced embryos. Nelore oocytes were matured in TCM-199 with Earle's salt + 10% FCS, FSH and LH, in 5% CO_2 in air atmosphere. To delay meiosis, the oocytes were maintained for 6 hours in medium in presence of 3 different concentrations of forskolin: 1mM, 0.05mM, 0.025mM. Then the oocytes were cultured for 18 hours in agent-free medium to resume meiosis, completing 24 hours of maturation. After 24 hours 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. Eight replicates were made. Data were analyzed by 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: 36.7% ± 3.7; Forskolin 1mM: 25.1% ± 3.7; Forskolin 0.05mM: 29.2% ± 3.7; Forskolin 0.025mM: 32.6% ± 3.7 (P=0.168). The forskolin was able to produce embryos without degeneration and with similar qualities to the agent-free group. However, to really prove if its action does not compromise embryonic development, techniques such as TUNEL (Terminal deoxinucleotil transferase Uracil Nick End Labeling) are being performed for the *in situ* detection of apoptotic cells.

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A119 OPU-IVP and ET

Induced interruption of pregnancy did not change subsequent fertility of embryo recipient heifers

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Keywords: bovine, embryo, recipient

In dairy farms, the birth of the male calf sometimes is not desired and the culling price of these animals is not significant for the dairy production system. Thus, with the available biotechnologies for animal reproduction, this problem is even bigger because of the costs to produce one pregnancy. The aim of this study was to evaluate the further fertility of the 60 cross-breed (dairy-beef) recipients, after the current pregnancy was interrupted. The embryos were *in vitro* produced and the oocytes fertilized with a commercial sex sorted semen to produce female. Between 50 and 65 days after the first embryo transfer, the animals were checked by transrectal ultrasonography (5 MHz linear array, Mindray DP2200) to access the fetus gender. The recipients diagnosed with a male fetus were immediately treated with an intramuscular prostaglandin injection (0.150 mg of the d-cloprostenol, Veteglan® – Hertape Calier). Using the available commercial sex sorted semen for female, the likelihood of the male pregnancy was 12.3% (60/486). Between 12 and 15 days after first prostaglandin injection, the animals were checked again and a second shot was given when an ovarian corpus luteum was detected. Twenty days after the second prostaglandin injection, the animals were assembled in a new protocol for estrous synchronization and timed embryo transfer (TET). The fertility was compared between two different periods: at the first time the heifer was used as embryo recipient; and at the second time after pregnancy interruption. The average number of embryos to produce one pregnancy was compared between these two periods with the Student t test, and the probability of 5% was used for statistical significance. The treatment was 91.7% (55/60) efficient to produce abortion. The intervals from treatment to first embryo transference and to conception were 40.0+8.0 e 67.1+12.8 days, respectively. The number of embryos transferred to produce one pregnancy did not differ (P>0,05) between the periods (2.3+0.4 vs 2.5+0.8; P>0.05). The induction of the pregnancy outbreak around day 60 of the gestation did not change the fertility of the cross-breed heifers after IVF embryo transfer.

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A120 OPU-IVP and ET

The *in vitro* production of porcine embryos is increased with PZM-3s culture medium

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Keywords: *in vitro* culture, IVF, NCSU-37.

The porcine embryo *in vitro* culture systems do not provide the same developmental conditions as observed in vivo, thus negatively affecting the quality and viability of the *in vitro* produced embryos. The search for appropriate culture conditions is then necessary. This study compared the porcine embryo *in vitro* developing rates after culture in three different culture media routinely used for the specie. Gilt ovaries obtained from slaughterhouse were transported in saline solution at 30 °C. Follicles between 3 and 6 mm diameter were aspirated with the aid of a vacuum pump, and selected under stereomicroscopic. Only cumullus oocytes complexes with compact layers of cumulus cells, and smooth cytoplasm were used. Maturation was initially performed in TCM 199 medium supplemented with 0.5mg LH, 0.01 IU/ml FSH, 10ng/mL EGF, 25% follicular fluid, 1mMdbcAMP, 2.19 mM sodium pyruvate, 0.1 mg/mL cysteine and3,05mM glucose for 22 hours period. At this point LH, FSH and dbcAMP were removed. Ooocytes were cultured for additional 19 hours. Fertilization was performed with 250 x 10^3 fresh sperm/mL, selected by Percoll gradients (45 to 90%). Sperm and oocytes were incubated for 4.5 hours. Presumptive zygotes were randomly assigned to one of three experimental groups (five replications). Group 1: NCSU-37. Group 2 Sequential NCSU-37 (0.56 mg/mL pyruvate, 100mg/mL BSA, 54.66 mg Sorbitol, and 5.92µL/mL of sodium lactate from day 0 to day 2. After day 2 the lactate and pyruvate were removed and 25 mg/mL of glucose was added). Group 3 Sequential PZM-3 (3mM BSA between days 0 and 4 and 10% of fetal bovine serum after day 4 of culture). Embryo developing rates (morula and blastocyst) were evaluated on day 7 of culture, and data analyzed statistically by Chi-square test ($P < 0.05$). The highest embryo rate (101/154 - 65.6%) was obtained with sequential PZM-3 media ($P < 0.05$). The embryo rate of sequential NCSU-37 medium (26/197 - 13.2%) and NCSU-37 medium (10/147 - 6.8%) do not differ ($P > 0.05$). Results showed that under our conditions the sequential PZM-3 media is the most appropriate, increasing the production of porcine embryos.

A121 OPU-IVP and ET

Short estrus synchronization protocol for ovine embryo recipients with new, second and third use of a CIDR device

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Keywords: CIDR, ovine, recipients.

The aim of this study was to evaluate the new, second and third use of the vaginal progesterone releasing device Eazi-breed CIDR® (CIDR, Pfizer, Brazil) on a six day estrus synchronization protocol for ovine embryo recipients. Three hundred and ninety recipients of Santa Inês and Dorper crossbred were synchronized, of which 207 with a new device (CIDR1), 110 with a second use device (CIDR2) and 73 with a third use device (CIDR3). Of these animals, 181 were yearling lambs and 209 ewes. Only four animals lost the device (1%) reducing groups CIDR1, 2 and 3 to 205, 109 e 72 animals respectively. At device withdrawal every animal received 10mg of Dinoprost (Lutalyse, Pfizer, Brazil) and 400UI of eCG (Folligon, Intervet, Brazil). Teasers were used for sexual stimulation and estrus detection that was identified twice a day. Estrus behavior was observed in 350 females among all treatments and 272 were used as recipients, 154 ewes and 118 yearling lambs. Data were analyzed by logistic regression (PROC LOGISTIC from SAS software, 2010), with a significance level of 5%. Statistical model included the effect of treatment (CIDR1 vs. CIDR2 vs. CIDR3) and animal category (yearling lamb vs. ewe) on estrus behavior and pregnancy rate. All treatments resulted in adequate estrus behavior rates, however it was lower ($p<0.02$) on group CIDR2 (84.4%) compared to CIDR1 (92.3%) and CIDR3 (93.1%). Group CIDR1 had a better response in yearling lambs (97.6%; $p<0.03$) than ewes (90.1%), as 81 of the 83 yearling lambs synchronized with a new CIDR displayed estrus. No effect of treatment (CIDR1: 60.4%; CIDR2: 72.1%; CIDR3: 57.8%) or category (ewes: 59.5%; yearling lambs: 67.8%) was verified over pregnancy rates. Even though plasma progesterone levels are lower in animals synchronized with second and third use CIDR in short protocols (Vilariño et al., ActaScie Vet, s479, 2008), both CIDR2 and CIDR3 treatments resulted on adequate estrus behavior and pregnancy rates in ewes, but specially in yearling lambs, comparatively to new device, that allows recommend them, indiscriminately, on estrus synchronization protocol for ovine embryo recipients.

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A122 OPU-IVP and ET

Effects of fatty acid supplementation in primiparous Nelore cows during the pre and postpartum periods on oocyte quality and *in vitro* production of embryos

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Keywords: IVP, linseed, megalac-e.

The objective of this study was to evaluate the resumption of cyclicity in postpartum period, oocyte quality and IVP embryos of primiparous Nelore cows supplemented with high fat diet. For this, the donors were divided into three groups of seven animals, a control group (C or 1) with routine feeding of the Institute of Animal Science of Sertãozinho, group 2 (M) supplemented with 100g of megalac-E per day per animal and group 3 (L) supplemented with 1kg of linseed per day per animal. The animals in groups 2 and 3 were treated for 30 days pre-partum and 60 days postpartum period. Cows were first aspirated between 25° to 32° days postpartum and other aspirations at intervals of 14 days between them, totaling four aspirations. After aspiration, oocytes were evaluated regarding the number and quality, and then taken to the laboratory of animal reproduction of FCAV-UNESP where they were subjected to *in vitro* production of embryos. After the final aspiration all animals entered the natural breeding season (BS) for 90 days. Thirty days after the departure of BS was carried out pregnancy diagnosis by ultrasonography. Statistical analysis was performed in PROC GLM of using the command PDIFF. The group L showed a higher number of follicles per cow and corpus luteum (CL) by ultrasonography than to group C and M (L = 20.4 and 8, C = 14.4 and 2 and M = 14.2 and 1, $p < 0.05$). For total and viable oocytes there was no significant difference between the three study groups (C = 15.96 ± 2.43 and 11.88 ± 1.85, M = 17.5 ± 2.29 and 13.10 ± 1, 75, L = 18.64 ± 2.43 and 12.2 ± 1.85, $P > 0.05$). There was also no significant difference between the supplemented groups in embryonic development assessed by the cleavage rate and embryo production (C = 7.24 ± 1.19 and 3.84 ± 0.83, M = 6.71 ± 1.13 and 2.67 ± 0.78 and L = 5.36 ± 1.19 and 2.24 ± 0.83, $P > 0.05$). Thirty days after BS was over, diagnosis of pregnancy was performed and the groups supplemented with linseed and megalac-E showed higher pregnancy rates than the control (L = 75%, M = 71.4% and C = 42.8%). We conclude that supplementation with linseed and megalac-E did not influence the quality of oocyte and embryo production. However cows supplemented with linseed soon returned to estrus more quickly in comparison to the control and megalac-E. And the groups linseed and megalac-E showed higher pregnancy rates than the control group.

A123 OPU-IVP and ET

Transcervical embryo recovery on Crioula Lanada sheep: preliminary results

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Keywords: crioula lanada breed, ovine, transcervical embryo recovery.

Unscientific breeding practices diluted the genetic resources of various Brazilian sheep breeds limiting their genetic variability and resulting in the loss of important genes with potential for future use. The Crioula Lanada is one of the few sheep breeds brought to southern Brazil by European settlers that can be used as a basis for genetic material preservation, since it remains without genetic contamination, Along with sperm cryopreservation, embryo cryopreservation is fundamental to maintain racial purity. However, the surgical embryo collection method traditionally used in ewes often results in internal adherences in the donors' reproductive tract that compromise their subsequent reproductive life. Thus, the development of an embryo recovery method that does not damage the reproductive system of the donors is of interest. Considering the scarce information available about the Crioula Lanada breed and the great variation in technical efficiency among breeds, this study evaluated a method of transcervical embryo recovery in Crioula Lanadas ewes. The estrus cycle of ten adult ewes with body condition of 3.5 was synchronized using Eazi-Breed CIDR® (Pfizer, New Zealand) associated with superovulatory treatment with 200 mg of FSHp (Folltropin®, Vetrepharm Inc., Canada). Ewes were inseminated by laparoscopy and embryos were recovered after six days. Four ewes were treated with misoprostol (Prostokos®, Infan, Brazil) and the embryos were recovered by the transcervical method (Gusmão, Arq. Bras. Med. Vet. Zootec. 61, 313, 2009). In one female, the cervix could not be exposed due to adherences caused by previous interventions and embryo recovery was not done. In the remaining ewes, the uterus was washed with ten infusions of 20 ml PBS in each uterus horn. However, only two of them produced structures. The remaining ewes were submitted to conventional surgical embryo recovery, but collections were not done in three of them: one due to the presence of adherences; and two that did not respond to the supervoulation protocol. Seven embryos were recovered by the transcervical method and eighteen were recovered by the surgical method. Considering the average of viable embryos (grade 1-3, IETS) recovered by the transcervical (2.3) and the surgical (2.8) methods, we conclude that Crioula Lanada ewes may be submitted to both embryo recovery techniques with acceptable efficiency. Further studies should be conducted to validate the results of this pilot study and to produce embryos for storage in the National Bank of Animal Germoplasm.

A124 OPU-IVP and ET

Effect of norgestomet on *in vitro* embryo production in Gyr and Holstein donors – preliminary results

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Keywords: blastocysts rate, norgestomet, OPU.

The effect of norgestomet on *in vitro* production of embryos was evaluated in oocytes obtained from donors Gyr (*Bos indicus*) (n=6) and Holstein (*Bos taurus*) (n=6) breeds. Cows were submitted to four ovum pick-up (OPU), with 14 days of interval, and in each ovum pick-up the cows were subjected alternately to one of the hormonal treatments, consisting in used ear device of norgestomet (Crestar®, Intervet, Brazil) (Low norgestomet group), two new devices (High norgestomet group) and absence ear device (Control group). At the beginning of hormonal protocol, all cows received 150 µg D-cloprostenol (Prolise®,Tecnopec, Brazil) to eliminate the presence of corpus luteum and endogenous progesterone influence in the treatment, and 3 mg of estradiol benzoate (RIC-BE®,Tecnopec, Brazil), to synchronize the emergence of a new follicular wave. The OPU was performed 7 days after the beginning of hormonal treatment and ear devices were removed 24 hours after OPU. Recovered oocytes were classified morphologically and the COC's considered viable were matured for 22-24 hours in TCM 199 medium (Tecgene, Brazil) and then, fertilized with semen from bull of known fertility, processed by Percoll discontinuos gradient and gametes were co-incubated for 18-20 hours. After this period, presumptive zygotes were cultured 7 days in SOFaa medium supplemented with 5% of FBS. The difference in number of viable oocytes was analyzed by ANOVA, considering the effects of breed, treatment and their interaction, and means were compared using *t* - test. Rates of viable oocytes and blastocysts were analyzed by Chi-square test ($P<0,05$). The number of viable oocytes was not affected by treatment with norgestomet ($P>0,05$) obtained values were 7,31 ± 4,22, 7,69 ± 8,09, 6,75 ± 4,31 for the control, low norgestomet and high norgestomet groups, respectively. Gyr cows produced higher number ($P<0,05$) of viable oocytes 9,38 ± 6,65, compared to Holstein breed 5,13 ± 3,61. This difference also remained for blastocysts rate, which was 37,50% (84/224) for Gyr cows, higher ($P<0,05$) than the 26,50% (31/117) observed in cows from Holstein breed. Blastocysts rate was lower ($P<0,05$) in high norgestomet group (28,97%, 31/107) than in control group (38,60%, 44/114) and low norgestomet group (33,33%, 40/120). The preliminary results suggest that norgestomet did not affect groups regarding mean viable oocytes. However, between breeds, number of viable oocytes obtained from Gyr cows was higher compared to the Holstein breed. Also, administration of two norgestomet devices had detrimental effect on blastocysts rate.

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A125 OPU-IVP and ET

Effect of heat shock on the expression of bovine oocyte apoptosis and competence related genes

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Keywords: *Bos indicus*, gene expression, heat shock.

Heat stress promotes, among several physiological and cellular alterations, changes in the reproductive tract microenvironment, reducing oocyte nuclear maturation, fertilization and embryo development to the blastocyst stage. However, the cellular mechanisms triggered by elevated temperature in bovine oocytes are poorly understood, as well as the molecular events that cause oocyte death or survival in response to stress. Therefore, the objective of this study was to evaluate the effect of heat shock (*in vitro* elevated temperature) during *in vitro* maturation (IVM) on the expression of oocyte competence and apoptosis related genes (BAX, CASPASE 3, BMP15 and Histone H2A). Cumulus-oocyte complexes (COCs) collected from ovaries of crossbred cows *Bos indicus* derived from slaughterhouse were subjected to heat shock model (control: 38.5°C for 22 hours and heat shock: 41°C for 12 hours followed by 38.5°C for 10 hours). Oocytes were denuded in 10.000 IU/ml hyaluronidase after aspiration (immature) and after IVM treatments. Groups of 30 oocytes were collected from each experimental group per replicate (immature, control and heat shock, n=3 replicates) and subjected to total RNA extraction (RNeasy Mini kit, Qiagen). The expression of target genes was determined by real time RT-PCR. Ribosomal Protein 30 (RPL30) expression was used as housekeeping gene. The relative gene expression values were obtained by ΔΔCt method corrected for amplification efficiency for each gene (Pfaffl equation). Data were subjected to least squares analysis of variance using JMP statistical program of SAS. Target gene mRNA levels were compared by Tukey-Kramer´s HSD test. Histone H2A mRNA levels were higher for immature as compared to mature oocytes (control and heat shock). Relative BAX, CASPASE 3 and BMP15 gene expression did not differ among groups. In conclusion, heat shock did not affect expression of apoptosis and oocyte competence related genes. However, *in vitro* oocyte maturation reduced Histone H2A mRNA level, suggesting that this transcript was degraded during maturation.

A126 OPU-IVP and ET

Effects of reduction or replacement of fetal calf serum by other protein sources during *in vitro* maturation of bovine oocytes

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Keywords: fetal calf serum (FCS), *in vitro* maturation (IVM), oocytes.

Recently, research efforts aiming the improvement of *in vitro* production of bovine embryos (IVPE) are mainly related to replacement of fetal calf serum (FCS) for alternative protein sources in culture media. In this study, bovine serum albumin (BSA) and embryonic fluid (EF) (Sigma-Aldrich, St. Louis, MO, USA) were used separately or together in different concentrations in order to replace or reduce the concentration of FCS during *in vitro* maturation (IVM). For this purpose, bovine oocytes were *in vitro* matured in TCM199 medium containing 1.0µg/mL FSH, 50µg/mL hCG, 1.0 µg/mL estradiol, 0.20mM pyruvate and 83.4µg/mL amikacin. According to protein supplementation, the following groups (G) were studied G1 (control) - 10% FCS, G2 - 8mg/mL BSA, G3 - 10% EF G4 - 6mg/mL BSA + 5% FCS, G5 - 6mg/mL BSA + 3.5% FCS + 1.5% EF, G6 - 6mg/mL BSA + 1.5% FCS + 3.5% EF, G7 - 6mg/mL BSA + 5% EF, and G8 - 5% FCS + 5% EF). After 24 hours of IVM, oocytes were classified according to the meiotic progression and migration of cortical granules (CHERR et al., J. Exp. Zoo., 246:81-93,1988, modified). Maturation rates were evaluated by chi-square test ($\chi 2$) or, when appropriate, by Fisher's exact test using GraphPad Prism 4.0 software (GraphPad Prism Inc., San Diego, USA). Despite the lack of statistical difference among groups in terms of nuclear maturation (G1: 104/117 – 88,89%, G2: 69/92 – 75%, G3: 87/109 – 79,8%, G4: 95/106 – 89,62%, G5: 87/100 – 87%, G6: 92/111 – 82,88%, G7: 97/117 – 82,91%, G8: 105/121 – 86,78%), we observed significant differences related to cytoplasmic maturation. In G2, G7, G6 and G3 groups, cytoplasmic maturation rates were reduced: 78/125 - 43,9%, 54/125 - 43,2%, 59/ 137 – 43,07% e 42/115 - 36,52%, respectively, compared to the control medium (G1), which corresponded to average values of 78/125 - 62.4%. A decrease of 3,5% in FCS concentration in IVM was possible without affecting cytoplasmic maturation rates (group G5: 65/128 - 50.78% oocytes presenting CG arranged peripherally), and those groups containing 5% FCS + 5% EF (G8) and BSA + 5% FCS (G4) reached rates of 70/118 - 59.32% and 58/113 - 51.33% respectively, values close to those obtained by the control group. Therefore, we concluded that it is possible to reduce the concentration of FCS in IVM medium up to 3.5% without affecting nuclear and cytoplasmic maturation rates.

A127 OPU-IVP and ET

Effect of the supplementation with injectable tonic, organic phosphorus based associated with vitamin B12 (B12 Catosal ®) in the *in vitro* embryo production of Gyr donors

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Keywords: IVF, mineral, nutrition.

The effect of the supplementation with injectable tonic, organic phosphorus based associated with vitamin B12 (B12 Catosal®) was evaluated in the *in vitro* production of bovine embryo of Gyr. A total of 16 lactating Gyr cows were used. Before the study started, all the follicles present in ovaries of the donors were aspirated in order to synchronize the wave emergence. After the follicular aspiration (OPU) related to the synchronization, cows were assigned according to oocytes production into one of two experimental groups: Control and Catosal group. No treatment was administered to the donors from the control group. In the Catosal group, cows received a subcutaneous injection of 25ml of tonic organic phosphorus based and vitamin B12 (B12 Catosal®, Bayer SA, Brazil). The treatment consisted in the administration of two doses of Catosal® B12, the first dose was performed five days before and the second at the moment of the OPU. All donors were submitted to five consecutive follicular aspiration 14 days apart, the first two before and three post-treatment. The oocytes recovered from OPU were fertilized with the same bull (Gyr) and the produced embryos were transferred into recipients. Statistical analysis was performed using SAS software (PROC GLIMMIX). In evaluations related to quality and quantity of oocyte and *in vitro* embryo production, there was no interaction between treatment and follicular aspirations (P> 0.05). Although the blastocyst rate is similar among treatments [42.0% (68/165) for control group and 40.3% (108/260) for Catosal group, P = 0.79], it was found that donors of the Catosal group had higher number of recovered structures (6.45 ± 0.49 for Control group and 8.10 ± 0.54 for Catosal group, P = 0.03), viable oocytes (4.13 ± 0, 39 for Control group and 6.50 ± 0.47 for Catosal group, P = 0.001), produced embryos (1.70 ± 0.26 for Control group and 2.7 ± 0.28 for Catosal group; P = 0.001) and viable oocytes rate [65.7% (165/258) for Control group and 79.8% (260/324) for Catosal group, P = 0.009]. The conception rate of the *in vitro* produced embryos (n = 176) was similar between the experimental groups [42.6% (29/68) for Control group and 44.4% (48/108) for Catosal group, P = 0.72]. In conclusion, the subcutaneous administration of Catosal® B12 increased quantity and improved quality of recovered oocytes by OPU and *in vitro* produced embryos, however, no effect was observed in the blastocyst and pregnancy rates of the Gyr donors' embryos.

A128 OPU-IVP and ET

Effect of follicular growth wave synchronization and treatment with bST or eCG in the OPU-IVP of Nelore, Brangus and Holstein donors

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Keywords: breed, OPU, synchronization.

The effect of the follicular wave synchronization, bST and eCG were evaluated in the OPU-IVP of Nelore and Brangus cows and Holstein heifers. In each study, 12 oocyte donors were allocated into one of four treatments (control, P4+E2, bST and eCG) arranged in a cross-over design. In the control group, the OPU was performed on a random day of the estrous cycle. In P4E2 group, donors were synchronized with 2 mg of estradiol benzoate (EB-RIC, Agener), 150µg of PGF (Prolise, Agener) and insertion of an intravaginal progesterone device (Primer, Agener) on D0. The bST and eCG groups, the donors were synchronized as the P4E2 group and received 500mg bST (Boostin, MSD) on D0 or 400IU of eCG (Novormon, MSD) on D3, respectively. On D5 (P4+E2, bST and eCG groups) the device of progesterone was removed and the donors aspirated together with the donors of Control Group. Statistical analysis was performed using SAS software (PROC GLIMMIX). In Nelore, the donors of the eCG group had higher number of recovered structures compared to the other treatments (Control, 16.1±2.8^{b}; P4+E2, 19.7±2.7^{b}; bST, 14.7±1.9^{b}; eCG, 20.8±3.5^{a}; P=0.002). The number of viable oocytes was higher in eCG group compared to the bST group and similar to control and P4+E2 groups (Control, 13.6±2.6^{ab}; P4+E2, 14.8±2.2^{ab}; bST, 11.8±1.4^{b}; eCG, 16.5±3.1^{a}; P=0.03). There was no difference between experimental groups in viable oocytes rate, embryo number and embryo rate. Among the Brangus breed, there were differences between experimental groups in the number of recovered structures (Control, 8,9±1.2^{c}; P4+E2, 12.5±0.6^{b}; bST, 12.2±1.8^{b} and eCG, 19.1±1.7^{a}; P = 0.001) and viable oocytes (Control, 6.4±1.1^{c}, P4+E2, 7.8±0.8^{bc}; bST, 8.8±1.3^{b} and eCG, 13.3±1.1^{a}; P=0.001). There was no difference between groups in the viable oocytes rate, number of embryos and embryo rate. In Holstein heifers, difference was observed between the experimental groups in the number of viable oocytes (Control, 2.8±0.5^{b}; P4+E2, 4.4±0.8^{a}; bST, 5.0±0.7^{a}; eCG, 5.2±0.8^{a}; P = 0.04) and number of embryos (Control, 0.8±0.2^{b}; P4+E2, 1.1±0.3^{b}; bST, 2.1±0.5^{a}; eCG, 2.1±0.4^{a}, P=0.04). There were no differences in the number of structures recovered, viable oocytes and embryo rates. In conclusion, eCG administration increased the number (Nelore and Brangus) and quality of recovered oocytes (Brangus and Holstein) and number of embryos (Holstein). Furthermore, the bST administration increased (Brangus) and quality of recovered oocytes (Brangus and Holstein) and number of embryos (Holstein) in oocyte donors undergoing *in vitro* embryos production.

A129 OPU-IVP and ET

Association of plasma IGF1 concentration and oocyte yield and embryo production in Gyr (*Bos indicus*) donors

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Keywords: embryo, Gyr, IGF.

The insulin-like growth factor type I (IGF1) affects follicular recruitment and growth during antral phase and therefore can influence the number of follicles emerging in each follicular wave in cattle. The relationship between IGF1 plasma concentration and oocyte yield for *in vitro* embryo production, however, remains unclear. The aim of the present study was to evaluate a possible association of plasma IGF1concentration and oocyte recovery by OPU in Gyr breed donors. Data from IVEP of Gyr donors (N=60) kept in the same facilities but originally coming from 6 different herds with independent breeding programs were used. All aspirations (N=593) were performed by the same team and sent to a single IVEP laboratory in the period 2009 to 2011. Plasma samples were collected and kept under refrigeration until centrifugation at 600 X g. To avoid possible effects of synchronization protocols used to prepare the donors, all collections were performed at least 30 days after the previous aspiration. Plasma samples were identified and frozen at -20oC. IGF 1 analyzes were performed by ELISA using an specific kit for cattle (Enzyme-linked Immunosorbent Assay Kit for IGF1 receptor, E90050BO, USCN, Life Sci. Inc., Missouri, USA) in the Cell Biology Laboratory of the Biology Department, UFJF. Incubation and reading (450nm) of plates were performed in a Varioskan Multimode Reader (Thermo Scientific, Wyman Street, Waltham, USA). The data for the effects of the donor, herd and order of collection were submitted to variance analysis and the correlations by the Pearson method. The results are presented as mean ± EPM. The average numbers of recovered oocytes, viable oocytes and embryos produced in the period were 23.2±0.6; 13.3±0.4 and 4.1±0.1; respectively. There was a high variability in IGF1 concentration among individuals (mean 74.7±10.1 ng/mL, VC=93.3%). There was a significant donor effect ($P<0.05$), and differences among herds were determined by individual differences within herd, but there was no ($P>0.05$) OPU order effect. There was no correlation ($P>0.05$) of IGF1 concentration and the total number of oocytes recovered or viable, but there was an unexpected negative (although low) correlation of IGF1 and embryo production ($R=-0.16$; $P<0.001$). There was no difference in IGF1 concentration among herds ($P>0.05$). These results suggest that the direct association between IGF1 concentration and potential for oocyte yield and embryo production in Gyr breed is weak and therefore has limited use as criteria for donor selection.

A130 OPU-IVP and ET

Efficiency of *in vitro* production of F1 crossbred embryos using Gyr (*Bos taurus indicus*) or Holstein (*Bos taurus taurus*) donors

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Keywords: embryos, Gyr, Holstein.

There is an increasing demand for the use of *in vitro* embryo production (IVEP) technology as a tool for multiplication of high genetic value animals and also to produce the animal replacement for the commercial herds, such as gyr x holstein crossbred dairy females. The higher potential of gyr female as oocyte donor compared to holstein one was previously demonstrated (Palhão et al., XXV Annual Meeting of the Brazilian Society of Embryo Technology, 2011). The aim of the present study was to evaluate differences of the *in vitro* developmental potential of cumulus-oocyte complexes (COCs) obtained from donors of different breeds. Data from follicular aspiration and IVEP obtained by the same laboratory during 2011 were analyzed. The following crossings were compared (dam x sire): G1 - holstein x holstein (n=109), G2 - gyr x gyr (n=68), G3 – holstein x gyr (n=65), G4 – gyr x holstein (n=86). Data were evaluated by anova and differences among groups compared by tukey's test. Differences in percentages were compared by chi-square. Results are shown as means ± sem. Gyr donors produced more total and viable COCs (16.3±1.0 and 10.6±0.7) compared to holstein (12.7±0.8 and 6.4±0.4, $p<0.0001$). The proportion of viable and Grade I COCs in regard to the total was also greater in Gyr (65.1% and 5.6% vs. 50.0% and 3.1%, respectively; $P<0.01$) than in Holstein. There was no difference in IVEP among groups with donors of the same breed. Females of G2 and G4 groups presented the greater number of cleaved embryos (10.2±1.1 and 8.5±0.7 vs. 4.5±0.4 and 5.3±0.6; $p<0.0001$), embryos on D7 (6.1±0.8 and 5.0±0.5 vs. 1.8±0.3 and 2.2±0.3; $p<0.0001$), and total embryos (7.2±0.9 and 6.0±0.5 vs. 2.1±0.3 and 2.6±0.4; $p<0.0001$) compared to G1 and G3 ones, respectively. Cleavage rates in G2 and G4 (86.6% and 87.2%) were higher than that in G1 (82.8%; $p<0.05$), which was higher than that in G3 (61.3%; $p<0.05$). Embryo rate (total embryos / total viable COCs) was also higher ($p<0.01$) in G2 and G4 (61.3 and 61.6%) compared to G1 and G3 (38.2 and 32.8%). There was no difference among groups ($p>0.05$) regarding the percentage of embryos in D7 or D8. We conclude that the greatest embryos yield in gyr females is associated with a greater quality and *in vitro* developmental potential, regardless of sire breed. Furthermore, gyr dam vs. holstein sire presented the best efficiency to produce f1 crossbred embryos.

A131 OPU-IVP and ET

Effect of the syncronyzation protocol and the recombinant bovine somatotropin (rbST) on the superovulatory response, recovery rate and *in vivo* maturation of goat oocytes

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Keywords: Cumulus-oocyte complex, goat, rbST.

The aim of this study was to evaluate the effects of standard (PC; Veiga-Lopez et al., Theriogenology 2005; 63:1973-83) and short (PD0; Menchaca et al., Theriogenology 2007; 68:1111-7) synchronization protocols, and the use of recombinant bovine somatotropin (0, 125, 250 mg of rbST) on superovulatory response, oocyte recovery rate and in vivo maturation of goat oocytes. Intravaginal P4 devices inserted in 92 does on D0 were maintained for six (PD0, n=46) or 12 days (PC, n=46). In each protocol (PC or PD0), 15 animals received a 125-mg dose of rbST (Boostin®, Intervet/Schering-Ploug, São Paulo, Brazil; via SC) on D1 and another on D7; another group of 15 females received a single 125-mg rbST injection on D7; and 16 animals did not receive rbST (controls). For the PD0, 200 IU eCG (IM) and 50 µg of cloprostenol (IM) were given at the time of intravaginal P4 device removal (D6), and 25 µg of lecirelin (IM) was given on D8. On D10, 50 µg cloprostenol (IM) were given to animals in PC, with the onset of the FSH treatment (180 mg), in six doses, 12 h apart (36, 36, 36, 36, 18, 18 mg; IM). In the last two FSH doses (D12), 50 µg cloprostenol (IM) was given to the donors in PD0, and P4 insert removal in PC, followed by 25 mg of lecirelin (IM) on D13 in both protocols. Follicular aspiration (OPU) was performed on D14, with recovered COCs evaluated for morphological viability and nuclear maturation. After nine replications, the number of follicles (FOLs), corpora hemorrhagica (CH), total COCs, viable COCs/female, and recovery (COCs/follicles) and maturation (MII) rates were compared by ANOVA or the $\chi 2$ test ($P<0.05$). The PD0 and PC protocols resulted in similar mean numbers of FOLs (14.0 ± 1.1 vs. 13.0 ± 1.1), CH (3.1 ± 0.6 vs. 3.8 ± 0.6) and COCs/female (13.9 ± 1.1 vs. 11.5 ± 1.0), irrespective of the rbST. However, a lower recovery of expanded (5.7 ± 0.9 vs. 11.1 ± 1.0), total COCs (88.5% vs. 99.1%), expanded COCs (49.6% vs. 79.7%), MII/total COCs (29.1% vs. 57.7%), and MII/expanded COCs (58.6% vs. 72.4%) were seen in the PD0 than in the PC protocol, respectively. When comparing the rbST doses, no significant differences were observed in the mean numbers of FOLs, CH and total COCs on a per female basis. However, the recovery rates of expanded COCs (72.4%) and MII/COCs (52.7%) were higher in the 125 mg rbST treatment group than the 0 mg (65.7% and 45.2%) and 250 mg (60.3% and 46.3%) groups. When considering the interaction between the protocol and the rbST dose, the use of the PC protocol and a 125-mg rbST dose resulted in a higher recovery of expanded COCs (75.0%), and higher proportion of MII/total COCs (75.0%) and MII/expanded COCs (100.0%) than the other groups. The conventional synchronization protocol was more effective to obtain mature COCs, and females synchronized with the standard protocol and treated with 125 mg rbST resulted in a greater proportion of expanded and matured COCs than the other treatment groups.

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A132 OPU-IVP and ET

Cytoplasmic maturation and ultrastructural changes in sheep COCs prematured *in vitro* with roscovitine and cycloheximide

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Keywords: meiosis inhibitors, oocytes, ultrastructure,

Meiosis inhibitors have been studied in several animal species with the aim of increasing the efficiency of *in vitro* embryo production. However, most of these studies are based only on the assessment of oocyte chromosomal configuration to predict the *in vitro* development potential of oocyte and embryo. Thus, the objective of this research was to evaluate the progression of cytoplasmic maturation and ultrastrucutral changes in cumulus-oocyte complexes (COCs) of sheep *in vitro* premature with roscovitine or cycloheximide. For this, COCs grade 1and 2 from slaughterhouse ovaries were cultured in maturation medium consisting of TCM199, fetal bovine serum, cysteamine, pyruvate, penicillin, LH and FSH (control group) containing 100 μM roscotivine or 1ug/mL cycloheximide (treatment group) for 24 hours at 38.5ºC in a 5% CO2 saturated humidity air atmosphere. The concentrations of meiosis inhibitors were based on previous studies and literature information. After culture, COCs were fixed in 2.5% glutaraldehyde and prepared according to the protocol established by the Center of Electron Microscopy of IBB-UNESP-Botucatu-São Paulo. In each experimental group, a sample of 10 COCs was randomly selected for analysis in a transmission electron microscope. The experimental design was completely randomized with 3 experimental groups, five repetitions and 100 oocytes for each group, a total of 300 oocytes. COCs were evaluated according to the maturity parameters described by Hyttel et al. (1989, J. Reprod. Fertil., 38, 35-47). As expected, COCs of control group showed maturity signs characterized by cumulus cells expansion, absence of junctional complexes, pleomorphic mitochondria spread throughout the ooplasm, some of them in association with lipid droplets and smooth endoplasmic reticulum forming metabolic units. Large amount of cortical granules were aligned with the cytoplasmic membrane. In contrast, COCs treated with 100μM roscovitine had partial cumulus expansion, substantial reduction of junctional complexes, the mitochondria were swollen, less electrodense and some of them with blackened spots inside. Furthermore, there were few cortical granules and degenerations signs in cumulus cells. In the treatment with 1μg/mL cycloheximide, there were the same maturity characteristics of control group without signs of degeneration. Our results indicate the roscovitine, in the concentration and inhibition time proposed in the present study, resulted in significant structural changes different form that observed in the treatment with cycloheximide at 1μg/mL. However, further studies are essential to verify the feasibility of using these drugs in the *in vitro* production of ovine embryos.

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A133 OPU-IVP and ET

Synchronization of estrus and ultrasound monitoring in cyclic Toggenburg goats destined to superovulatory treatments

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Keywords: follicular dynamics, goat, superovulation, syncronization of estrus.

The efficacy of the superovulation depends on the profile of ovarian follicular growth in the earlier administration of FSH. Treatments started more closely to the emergence of the follicular wave growth have shown better results (Gonzalez-Bulnes et al. 1999 Small Rum Res 34, 65-69; Fonseca et al., 2006, Acta Vet Sci 32, 65-70; Menchaca et al., 2006, Acta Vet Sci 34, 51-58). The aim of this study was to determine the number and diameters of ovarian follicles, and the day of the follicular emergency in cyclic Toggenburg goats and use these parameters as the basis for the beginning of superovulatory treatment. Twelve cyclic goats were assigned according to body condition score and lactation status in two treatments, T1 and T2. T1 goats had their estrus synchronized with two doses of 37.5µg prostaglandin lateralvulvar with intervals of seven days (d-cloprostenol; Prolise®, Tecnopec LTDA, São Paulo-SP, Brazil) and T2 through the insertion of intravaginal device containing progesterone for 10 days (CIDR®; Pfizer Animal Health, São Paulo, Brazil). Ultrasound scanning, initiated in D0, was performed twice daily for 10 days to measure the number and diameter of ovarian follicles and days of the follicular wave emergency. Follicles were grouped according to their diameter in Class 1 (2.0 to 3.9 mm), 2 (4.0 to 4.9 mm), 3 (5.0 to 5.9 mm) and 4 (≥6mm). In 48 hours after the second prostaglandin administration (T1) and 36 hours after CIDR insertion (T2),66.6% (4/6 and 4/6) of animals from both treatments had follicles of class 1 (15.4±6.7) and 2 (4.3±5.3) mainly in relation to Class 3 follicles (1.83 ± 0.98) and 4 (1.0±0.0). The profile of classes 1 and 2 suggests that the follicular emergency is happening around this time for T1 and T2 animals. Furthermore, the profile of classes 3 and 4 suggests that the deleterious effects of dominant follicles may be minimal in the same period. These parameters can be considered to start the superovulation in cyclic Toggenburg goats.

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A134 OPU-IVP and ET

Influence of sperm processing on IVP of bovine embryos

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Keywords: bovine, IVP, sperm preparation method.

The IVP rates of bovine embryos are within acceptable levels, although several factors contribute to reduce the percentage of transferable embryos. The effect of the bull used as sperm donor is one of such factors, but information about the processing of the sperm used in IVF is still scarce. The present study aimed to determine the influence of cryoprotectants and sperm selection gradient on sperm recovery and integrity, and the IVP rate of bovine embryos. Initially, sperm of a bull with known fertility for IVF was split in two fractions for cryopreservation: 5% glycerol (GLY); and 3% ethylene glycol (EG). At sperm selection, each group was centrifuged at 5000 g for 5 min in discontinuous gradients of Percoll® (90 and 45%; Per) or OptiPrep® (40 and 26%; OP) with reduced volume (600µl), forming four treatments: Per + GLY, Per + EG, OP + GLY, and OP + EG. Grade I and II cumulus-oocyte complexes (COCs) obtained from ovaries collected in slaughterhouse were randomly divided in 4 groups for IVM, in TCM 199 supplemented with gonadotropins and 10% estrus mare serum (EMS). Each IVM routine (n = 7) used one semen straw from each treatment (GLY and EG). Sperm concentration, vigor, motility and membrane integrity were evaluated after thawing and after selection. Each COCs group was inseminated (D0) with 1x106 spermatozoa / ml. After 18 hours of incubation in TALP-Fert, presumptive zygotes were denuded and transferred (n = 725) to SOFaaci medium supplemented with 5% of EMS and cultured at 39 °C under humid atmosphere with 5% O2 + 5% CO2 + 90% N2 in a bag-system. The results were evaluated by Statistix9®. Cryoprotectant did not influence (P> 0.05) recovery and sperm membrane integrity (GLY = 26.0% and 62.7%, EG = 24.9% and 67.5%, respectively). However, the selection gradient affected (P <0.001) recovery rates and sperm membrane integrity (Per = 37% and 79.6%, OP = 13% and 50.6%, respectively). Embryo development (D8 blastocysts) was similar (P> 0.05) for Per + GLY (19.3%) and Per + EG (25.4%), but both rates were higher than those observed for OP + GLY (11.1%) and OP + EG (11.0%) (P <0.001). The tested cryoprotectants did not affect the evaluated sperm parameters and the rate of embryo development. However, the Percoll gradient was more efficient than the OptiPrep for sperm selection, providing greater recovery rate and sperm integrity and higher efficiency in IVP of bovine embryos.

A135 OPU-IVP and ET

Effect of heat stress in the maturation rate, fecundation and development of bovine embryos fertilized *in vitro*

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Keywords: embryo, heat stress, oocyte.

There are indications that heat stress affects the physiological responses, damaging ovarian dynamics, and the quality and competency of oocytes in females. The aim of this study was to evaluate the effect of 2°C increase in temperature during maturation, fecundation and development of "*in vitro*"- derived embryos. Bovine oocytes were obtained from ovaries of slaughterhouse's animals and were divided into six groups totaling two experiments, being, control 1 (GC1) and exposed 1 (GE1) for the experiment I, and control 2 (GC2), exposed 2A (GE2A) exposed 2B (GE2B) and exposed 2C (GE2C) for experiment II. The oocytes of the group GC1 (n=316), GC2 (n=447) and GE2C (n=297) were cultured at 38°C and the oocytes of group GE1 (n=365), GE2A (n=337) and GE2B (n=321) cultured at 40°C during the maturation period (24 hours) with 5% CO2 and 95% humidity. After the maturation period, oocytes of group GC1 and GE1 were evaluated for their morphology in optical microscope and, after removal of cumulus cells evaluated the maturation rate by the presence of polar body. In experiment II, after the maturation period, oocytes of group GC2, GE2A, GE2B e GE2C were fecundated with semen treated by Percoll discontinuous gradient. The oocytes of group GC2, GE2B and GE2C were cultured at 38°C, and the group GE2A was cultured at 40°C throughout the period of fecundation (IVF; 18-20h). On the second day, after the IVF, the control group 2 (GC2) and group GE2B remained at 38°C, and the group GE2A at 40°C, but the group GE2C was cultured at 40°C during embryonic development. The embryos were evaluated for cleavage rate, morula and blastocyst by means of optical microscopy. In control group 1 (GC1), the oocytes showed uniform expansion of cumulus cells, classified as moderate to high, with brown color and uniform appearance of the ooplasm, with a maturation rate of 69.62% (220/316). In the oocytes exposed to 40°C (GE1), we observed a decrease in the expansion of cumulus cells, and the same showed rounded appearance and retraction of the ooplasm with dark coloration, verifying a maturation rate of 49.04% (179/365). In the control group 2 (GC2), after the "*in vitro*" fecundation period, the rates of cleavage, morula and blastocyst were 68.23% (305/447), 50.16% (153/305) and 43.28 % (132/305), respectively. For the group GE2A, it was not observed the formation of zygotes (0/337). The group GE2B showed 31.46% (101/321) and 35.64% (36/101) of cleavage rate and morula respectively, without formation of blastocyst (0.0%), and in group GE2C, the cleavage rate was 3.7% (11/297), however, it was not observed the formation of morula and blastocysts (0.0%). For statistical analysis, the x2 test ($p>0.05$) was used to determine significance. These data suggest that, in all stages of exposure, embryo and the gamete cells are susceptible to heat stress. Furthermore, exposure decreases embryonic development.

A136 OPU-IVP and ET

Manual incision of zona pellucida increases the hatching rate of *in vitro*-produced porcine embryos

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Keywords: *in vitro* culture, pronase,PZM-3s.

Porcine embryo *in vitro* production (IVP) experienced important advances in recent years, but there are still many specific points requiring solutions. The quality of porcine IVP embryos is still lower in comparison to other species, such as the bovine. As a result, pig embryos often fail to hatch out of the zona pellucida (ZP). Failure on zona lysis (i.e. formation of the nick through where hatching starts) leads to infertility due to hatching failure embryonic loss. In order to increase hatching rates of porcine IVP embryos, this study evaluated alternatives to weaken or smooth the ZP. Embryos were allocated into four experimental groups as follows: Pronase group (n = 31): submission to partial digestion of ZP with 0.2% pronase; Pronase + incision group (n = 35): pronase partial digestion associated to a small incision on ZP; Incision group (n = 40): only the incision on ZP, and Control group (n = 31): untreated embryos. For embryo production, gilt ovaries were obtained at an abattoir. Follicles of 3 - 6 mm of diameter were aspirated and oocytes were recovered, and selected in follicular fluid. Oocytes were IVM in TCM 199 supplemented with 0.5mg LH, 0.01 IU/mL FSH, 10ng/mL EGF, 25% follicular fluid, 1mMdbcAMP, 2.19 mM sodium pyruvate, 0.1 mg/mL cysteine and 3.05 mM glucose for 22 hours. At this point the LH, FSH and dbcAMP were removed, and the oocytes were cultured for additional 19 hours. After IVM, oocytes and sperm were co-incubated in m-TBM medium for 3 hours at a concentration of 62,500 sperm / mL. Sperm were selected through Percoll gradients (45 and 90%). Presumptive zygotes were cultured in sequential PZM-3, where 5% of FCS was added at day 5. On day 4, structures from Pronase and Pronase + Incision groups were exposed to a 0.2% pronase solution for 30 seconds. On day 6, embryos from Pronase + Incision and Incision groups were placed on TCM-Hepes + 20% FBS droplets covered with mineral oil, where a small incision was made in their ZP, with the aid of a biopsy blade (Bioniche Animal Health Canada Inc). Hatching rates were evaluated between days 7 and 8 of culture, and data were analyzed by Chi-square test ($P < 0.05$). There was a significant increase in hatching rates of Incision group (15/40 - 37.5%) when compared to Pronase (2/31 - 6.5%) and Control (3/31 - 9.7%) groups. No significant difference was showed between hatching rates of Pronase + Incision (7/35 - 20%) and Incision groups. We conclude that an incision on porcine embryos ZP increases the hatching rates, regardless of a previous exposure to pronase.

A137 OPU-IVP and ET

Conception rate of bovine embryo recipients in response to ato hormonal protocol or natural estrus expression

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Keywords: embryo transfer, pregnancy, synchronization.

It has been increasingly frequent the use of reproductive technology in order to increase the number of offspring born in a herd. Among these reproductive biotechnologies we can highlight the *in vitro* produced embryos (IVP), which has as main advantage the optimization of embryo production. However, this increased embryo production associated with its low freezability, makes the embryo recipients a critical point in the production system. The preparation of these animals as recipients can be performed through two methods: observing the natural estrus expression or using hormonal protocols with the aim of cycle synchronization. The first method requires a large number of animals and efficient estrus detection. In the second method the number of animals can be reduced and there is no need of estrus detection, on the other hand, there is hormonal cost. Thus, the aim of this study was to compare the conception rate of bovine embryo recipients using different methods of preparation of these animals. To this end, the data used in this study were collected from Minerembryo Company, located in Alfenas, Minas Gerais, referring to 1802 recipient heifers. The recipients were divided into two groups (control; n=401 and FTET; n=1401). In control group, the animals received no hormonal protocol which natural estrus was observed. In FTET group, the recipients were synchronized by hormonal protocol started on a random day of the estrous cycle, with the insertion of an intravaginal implant with 1 g of progestogen and administration of 2 mg of estradiol benzoate, and this was considered day 0 (D0). On D8, the intravaginal implant was removed and 0.15 mg of PGF2α analogue (D-cloprostenol), 0.5 mg of estradiol cipionate and 300 IU of eCG was administered. The embryo transfer was performed 7 days after estrus detection in the control group and on D17 in FTET group. The pregnancy diagnosis was performed with the aid of ultrasound 30 days after transfer. The data were evaluated by Chi-square test with a significance level of 5%. There was no significant difference ($P=0.3295$; $P<0.05$) between groups (control group: 32.7%; FTET group: 30.1%). By this work, we conclude that using of recipients that expressed estrus naturally presented the same efficiency in relation to the use of hormonal protocols related to conception rate.

Acknowledgments: Minerembryo.

A138 OPU-IVP and ET

Influence of follicular fluid supplementation to the maturation media on the quality of *in vitro* produced bovine embryos

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Keywords: follicular fluid, *in vitro* maturation, oocyte.

The components of follicular fluid participate actively in the process of nuclear and cytoplasmatic maturation on in vivo environment. This study aimed to evaluate the effects of follicular fluid (FF) added to maturation medium on the quality of *in vitro* produced bovine embryos. In a first experiment, oocytes were matured in five different media containing several proportions of FF (0%, 25%, 50%, 75% or 100% FF) during four maturation times (22, 24, 26 or 28 h) and classified according to the meiotic progression and migration of cortical granules (CHERR et al., J. Exp. Zoo., 246:81-93,1988, modified). In the second experiment, oocytes were matured in the same media used in the first experiment, fertilized at different times (24, 26 or 28 h) and subsequently it was evaluated the rates of cleavage and blastocyst and embryo quality. Embryo quality was evaluated by the ratio of the cells number in the inner cell mass (ICM) and trophoblast cells (TF) compared to the total cells number (T), stained by differential staining modified by fluorochrome (IWASAKI et al., J. Rep. Fert. 90:279-85,1990) and were considered superior those embryos that exhibited a proportion greater than 1:2. The FF in the proportion ≥75% slowed the progression of meiotic regardless time of maturation ($P<0.05$), reducing the nuclear maturation rate from 77.61% to 55.35%. The FF in the proportion ≥ 25% negatively affected the cytoplasmatic maturation rate in the time of 24 and 26 h of maturation ($P <0.05$). The FF in the proportion ≥ 75% reduced the cleavage rates from 78.74% to 67.01% and in proportion ≥50% also reduced the blastocyst rates from 27.38% to 9.63% regardless of time of maturation ($P <0.05$). The ratio ICM:TF in all proportions of FF were around 1:2, and the percentage of higher embryos were 62.50%; 67.50%; 55.00% in the same order. In conclusion, the supplementation with follicular fluid to the *in vitro* maturation medium retards the meiotic progression and migration of cortical granules, promotes progressive reduction of the embryo development rates, and provides increased total cell number of produced embryos.

A139 OPU-IVP and ET

Effects of the use of embryonic fluid during *in vitro* maturation of bovine oocytes on embryonic development and survival to vitrification

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Keywords: cryopreservation, embryonic fluid, *in vitro* maturation.

The excessive accumulation of lipid droplets in IVP embryos can influence embryonic cryosensitivity, which is directly related to the usage of FCS in IVP medium (ABE et al., Mol. Reprod. Dev., 61: 57, 2002). This study aimed to evaluate the effects of total or partial FCS replacement for bovine serum albumin (BSA) and for a commercial product called embryonic fluid (EF) (Sigma-Aldrich, St. Louis, MO, USA), and their associations during *in vitro* maturation (IVM) of bovine oocytes regarding blastocyst development and viability after vitrification. For that, IVM of oocytes was performed in TCM 199 with 25mM sodium bicarbonate; 1,0µg/mL FSH; 50UI/mL hCG; 1,0µg/mL estradiol; 0,2mM sodium pyruvate; 83,4µg/mL amikacin; 2µg/mL ITS and 1µg/mL antioxidant. According to the protein source used, the following treatments were delineated: control group with FCS (10%); BSA (8mg/mL); EF (10%); BSA (6 mg/mL) + FBS (2.5%); BSA (6mg/mL) + EF (2.5%); FBS (5.0%) + EF (5.0%) e BSA (6mg/mL) + FBS (2.5%) + EF (2.5%). After 24h of IVM, the oocytes were co-incubated with sperm in TALP-FIV containing 6 mg/ml BSA for 20 h approximately. The *in vitro* culture (IVC) was performed in SOFaa supplemented with 6 mg/mL BSA and 2.5% FBS. All cultures during IVP process were performed in an incubator at 38.5 ° C and in 5% CO_2 in air. After seven days of IVC, blastocyst rates were evaluated, and the embryos produced were vitrified. Embryonic survival after vitrification was assessed by embryonic hatching rate post-thawing; embryos were cultivated for 24h under the same conditions. Three replicates were performed, and results were evaluated by chi-square test (χ^2) in SAS v.8.2 software ($p = 0.05$). Initially, when embryo rate was evaluated, it was observed that EF group was similar to the control group (41.88% and 44.59% respectively) and BSA, when used alone, was inferior to the other protein sources, with 34.47% of blastocysts produced. When embryo survival post thawing was evaluated, BSA group displayed hatching rates similar to control group (21.40% and 22.74%, respectively). However, the EF group was higher (28.46%) than BSA and FBS groups. When we used association of protein sources, it was observed that embryo production in BSA + FBS, FBS + EF, EF+ BSA + FBS groups did not differ (46.69%, 43.53% and 46%, respectively), and that BSA + EF combination was lower than other groups - with 33.23% blastocysts formed. Besides the numerically superior rates of hatched blastocysts in the group containing the three protein sources (BSA + EF + FBS) (31.56% vs. control: 22.74%; BSA: 21.40%; EF 28.46%; BSA+FBS: 23.20%; BSA+EF: 26.52% e FBS+EF: 26.53%), this association enables the use of a lower FBS concentration, and possibly will reflect in a decreased lipid accumulation in these embryos. Therefore, we conclude that EF supplemented alone or in association with other protein sources during oocyte maturation, improves embryonic survival after cryopreservation.

A140 OPU-IVP and ET

Effect of addition of LH in different moments of the maturation on the *in vitro* production of bovine embryos

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Keywords: bovine embryos, gonadotropins, *in vitro* maturation.

The gonadotropins FSH and LH are the most used hormones added to the *in vitro* maturation system. Reports indicate a direct action of the FSH receptor in the communication between cumulus cells and the oocyte, which results in the acquisition of competence for oocyte development. Other studies demonstrate that the amount of mRNA of LH receptors increases after 6 hours of maturation. The present study aimed to compare the effect on the blastocyst production of the recombinant human FSH (hFSH) followed or not by the addition of luteinizing hormone (LH) in two different moments of the IVM. Oocytes from abattoir were divided into three different groups of hormone supplementation: G1 (total=346) control group with LH and hFSH; G2 (total=308) with hFSH; and G3 (total=308) with hFSH in the first 6 h of culture followed by LH addition. Looking for a closer relationship with the commercial IVP model the maturation medium used was the TCM-199 bicarbonate supplemented with 10% of FCS, LH and hFSH, both 0.5 µg/mL (according to each group). The FSH for all groups was the hFSH (0.5 µg/mL) in order to avoid any possible LH contamination. After 24 h of IVM, oocytes were fertilized and cultured in a 5 % CO2 at 39 °C. Rates of cleavage and of viable blastocysts after seven days of culture were assessed to evaluate the effectiveness of each treatment in the IVP. The total variable of cleaved oocytes was analyzed in an approach of Poisson regression model, considering the total number of oocytes as the exposure variable and the type of treatment as a covariate. For the total variable of embryos, was also considered the total number of oocytes as an exposure variable, however, the negative binomial model was adopted. The rates of cleavage of treatments G1, G2 and G3 were respectively 70 %, 76 % and 69 % and the blastocyst rates were 43 %, 50 % and 42 %. The approach indicates that the cleavage rate is not influenced by treatment (P=0.5394). The same conclusion stands for the rate of embryos (P=0.7958). In this experimental condition, we can conclude that the IVM after adding LH integrally (24 h), partially (6 h) or in the absence of LH does not interfere with the rate of cleavage and embryo development to the blastocyst stage. We can infer that the addition of LH during IVM does not play a crucial role in the acquisition of oocyte competence.

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hCG is more effective in the treatment of follicular cyst in Nelore donors compared to GnRH

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Keywords: bovine, follicle, pathology.

Two inductors of ovulation (GnRH and hCG) were evaluated on the cyclicity return of Nelore donors with follicular cyst. A total of 98 oocytes Nelore donors were used in the study with body weight average of 628.8 ± 152.9 kg and with follicular cyst(s). The diagnose of a cyst was considered by the presence of one or more follicular structures with a diameter≥ 20mm and absence of corpus luteum (CL) in the ovary. An ultrasonography exam (US) was conducted before the treatments to diagnose the cystic cows. The cystic animals were allocated into two experimental groups: GnRH group and hCG group. On D0, all cows received an intravaginal progesterone device (DIB®, MSD, Brazil). In addition, 250µg of gonadorelin (Fertagyl®, MSD, Brazil) was administered in the cows of the GnRH group and 2,500UI of hCG (Chorulon®, MSD, Brazil) in the ones of the hCG group. After day seven (D7), the progesterone device was removed and the animals received 0.530mg of D-Cloprostenol, IM (Preloban®, MSD, Brazil). On D9, the donors of the GnRH and hCG group received one more dose of 250 µg of gonadorelin (Fertagyl®, MSD, Brasil) and 2,500UI of hCG (Chorulon®, MSD, Brasil), respectively. Ultrasonography's examinations were performed between D15 and D25 and between D36 and D46 to evaluate the ovarian structures (presence of CL or follicular cyst). Treated cows were considered when a CL was visualized after two ultrasonographic evaluations. Evaluations were also performed in cows that had CL or absence of cystic structures (follicles less than 20 mm). Statistical analysis was performed using SAS software (PROC GLIMMIX). There were statistical differences between the experimental group in the presence of corpus luteum between days 36 and 46 [GnRH group – 18.4% (9/49) and hCG group – 53.1% (26/49); p=0.0008], cows with CL or absence of cystic structures rate between days 15 and 25 [GnRH group – 46.9% (23/49) and hCG group – 69.4% (34/49); p=0.03] and between days 36 and 46 [GnRH group – 30.6% (15/49) and hCG group – 63.3% (31/49); p=0.002]. In conclusion, the use of hCG in the follicular cysts treatment of oocyte Nelore donors is more efficient than GnRH.

A142 OPU-IVP and ET

Effect of fibroblast growth factor 10 (FGF10) on the *in vitro* oocyte maturation and on the *in vitro* production of bovine embryos

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Keywords: bovine, FGF10, *in vitro* production.

The fibroblast growth factor 10 (FGF10) is involved in paracrine signaling in cumulus-oocyte complex, which increases the expression of genes related to oocyte maturation and embryo competence. The aim of this study was to evaluate the effect of FGF10 on oocyte nuclear maturation, on the apoptosis in oocytes, on the rate of *in vitro* production of embryos and on the expression of genes related to embryo quality and competence. Oocytes of predominantly Nelore cows from abattoir were matured for 22 hours in TCM199 supplemented with different doses of FGF10 (0, 2.5, 10 and 50 ng/mL). In experiment 1, after maturation, the oocytes were fixed and stained with Hoechst-33342 to identify the different stages of meiosis: metaphase 1 and metaphase 2 intermediate phases. In experiment 2, after maturation, the oocytes were stained by TUNEL to determine the percentage of oocytes undergoing apoptosis. In experiment 3, oocytes were fertilized with Nelore semen and cultured to the blastocyst stage. In Experiment 4, we evaluated by RT-qPCR in real time the expression of genes PLAC8, COX2 and CDX2 in blastocysts. The stages of maturation, cleavage, morula and blastocyst were analyzed by ANOVA using PROC GLM of SAS (SAS for Windows). The percentage data were transformed into arccosine before analysis. The percentage of TUNEL-positive oocytes was analyzed by logistic regression. To evaluate the effect of FGF10 on target gene expression in bovine blastocysts, ANOVA was used and means values were compared by orthogonal contrast. Differences were considered significant when $P<0.05$ and a trend was considered when $0,05<P<0,10$. The FGF10 increased the percentage of oocytes in metaphase 2 at a dose 2.5 ng/mL when compared to other groups ($P<0.05$). The addition of FGF10 at any dose decreased the percentage of apoptosis in oocytes, with lower values in groups treated with the doses of 10 ng/mL and 50 ng/mL compared to the dose 2.5 ng/mL ($P<0, 05$). *In vitro* production of embryos did not differ among groups. The addition of FGF10 tended to increase the expression of COX2 and PLAC8 under 50 ng/mL ($P=0.09$ and $P=0.07$, respectively) and also the expression of mRNA of CDX2 under 10 ng/mL ($P=0.08$). The results indicate that the addition of FGF10 on the *in vitro* maturation medium seems to increase oocyte competence through the improvement of nuclear maturation and decrease of apoptosis rate. In addition, although the *in vitro* production of blastocysts was not increased, FGF10 tends to increase the expression of genes involved in embryo competence (PLAC8, CDX2 COX2).

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A143 OPU-IVP and ET

Ultrasonographyc evaluation and number of embryonic structures collected by non-surgical method in dairy goats

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Keywords: corpus luteum, goats, ultrasound.

The aim of this study was to compare the number of corpora lutea (CL) measured by ultrasound and the number of embryonic structures recovered in superovulated dairy goats. The study was performed in the city of Piau, Zona da Mata region of Minas Gerais. Fifteen (n=15) pluriparous Toggenburg goats were selected by ultrasonografic evaluation, weighing 56.4±0.8kg and 3.0±0.5 body condition score. For synchronization of estrus and superovulation device CIDR® (Pfizer - Animal Health, São Paulo, Brazil) was used for six days. A total dose of 300 IU of FSH (Pluset® - Hertape Calier-Animal Health, Juatuba - Brazil) i.m. was administered twice daily for three days at decreasing doses (25-25-15-15-10-10%) from D4. In D5 CIDR® was removed along with the administration of 5 mg of PGF2 (Lutalyse ® - Pfizer - Animal Health, São Paulo - Brazil) laterovulvar. In the morning of D8 250 IU hCG (Vetecor®, Hertape Calier-Animal Health, Juatuba - Brazil) i.m. were administered. The natural breeding was conducted for three days with four bucks, twice a day after device removal. Six to seven days after first mating, embryos were collected by transcervical method described by Fonseca et al. 2011. One day before embryo collection, transrectal ultrassound was performed to evaluate SOV protocol and the presence of CL, to relate to the number of recovered structures. Of 15 superovulated goats, three did not respond the protocol and one had a one follicular cyst prior to embryo collection. From 11 goats remaining, 6 had the number of CL consistent with the number of recovered structures (9/9, 8/8, 5/5, 9/9, 5/5 and 7/7), three goats show more CL than recovered structures (14/10, 9/8 and 5/3), and two goats had more structures than the CL observed (19/21 and 20/23). From 110 structures visualized by ultrasound, 108 were recovered: 38 blastocyst, 35 morulae, 22 unfertilized and 13 degenerated embryos. The ultrasonografic evaluation prior to embryo collection can be used to estimate the superovulatory response and the efficiency of nonsurgical flushing in goats. In addition, the ultrasonografic evaluation can reduce the cost of uterine flushing in non-responsive goats.

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A144 OPU-IVP and ET

Effect of quercetin on *in vitro* development of bovine oocytes

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Keywords: embryos, oocytes, quercetin.

The conditions for IVM of oocytes are essentials for *in vitro* production (IVP) of embryos and can be influenced by different factors, such as gaseous atmosphere, the culture medium, temperature, protein supplementation and growth factors. In the IVP system for embryos, reactive oxygen species (ROS) are produced from the oxygen tension, light exposure and excessive handling. Therefore, the use of antioxidants is an important factor in the IVP process; they are substances that can decrease the harmful effects caused by ROS. Quercetin is a flavonoid found widely throughout nature and it has been characterized by their antioxidant activity. The objective of this study was to evaluate the effect of quercetin during IVM of bovine oocytes on blastocyst rates. Oocytes recovered from slaughterhouse ovaries were matured (TCM199 with 10% FCS, 5.0 µg/ml of LH, 0.5 µg/ml of FSH, 0.2 mM pyruvate and 50 µg/ml gentamicin) for 22h. IVM was done separately in the presence of 0,4; 2; 10 or 50 µM quercetin or 100 µM cysteamine or absence of antioxidants (Control). After maturation, oocytes were fertilized in TALP medium for 18h and cultured in SOFaa (10% FCS) for seven days at 38.5°C and 5% CO2. A total of 1,684 oocytes were used in the six experimental groups with seven repetitions for each group. Statistical analysis were performed using ANOVA followed by Tukey test ($p<0.05$). Embryonic development rates varied among treatment groups, the highest rates were obtained in the presence of antioxidants when compared with control group. The mean (± standard deviation) of blastocysts (D7) obtained for treatments using 0.4, 2, 10 and 50 µM of quercetin were 56.9 (± 3.3), 59.6 (± 1.9), 53.7 (± 4.9) and 49.7 (± 3.1)%, respectively. For the treatment using 100 µM cysteamine and the control group, the development rates were 50.4 (± 3.8)% and 42.3 (± 4.2)%, respectively. Comparison between antioxidants groups showed that use of 0.4 and 2 µM quercetin were higher in the production of embryos regarding the use of cysteamine, while concentrations of 10 and 50 µM quercetin showed similar rates to those observed with the use of cysteamine. The analysis of the development rates for the treatment with quercetin showed that treatments using 0.4 and 2 µM were similar ($p>0.05$), but differed in the rates found for concentrations of 10 and 50 µM and these last were similar. The supplementation of IVM with quercetin or cysteamine increased blastocyst rates compared to control. The use of quercetin showed the highest rate of embryonic development than cysteamine and suggests a potential to reduce the gap between the embryonic development *in vivo* and *in vitro*.

A145 OPU-IVP and ET

Effect of a dietary supplementation rich in fatty acids to Nelore cows on oocyte quality and *in vitro* production of embryos

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Keywords: fatty acids, embryo, oocyte.

Cattle industry has grown rapidly in Brazil, and it requires continuous improvement of animal reproduction biotechnologies. *In vitro* embryo production following ovum pick-up (OPU) maximize the reproductive process through multiplication of high genetic merit animals. Moreover, diets rich in fatty acids have been evaluated to improve oocyte quality and oocyte *in vitro* development capacity. The aim of this study was to evaluate the quality of oocytes recovered and the embryo production of non lactating Nelore cows receiving fat supplementation. The donors were divided according to the number of ovarian follicles population into one of two experimental groups (n=6 per group): Group 1 (control) and Group 2 (supplemented with Megalac-E; Arm & Hammer Animal Nutrition, QGN, Feira de Santana-BA, Brazil). Animals from Group 1 were supplemented with 1.39Kg of corn bran, 50g of urea and 60g of mineral salt. Animals from Group 2 were fed with 100g of Megalac-E, 1.29Kg of corn bran, 50g of urea and 60g of mineral salt, totalizing 1.5Kg of diet/day for each animal of both groups, in a period of 40 days. Both groups were kept in separated pickets with ad libitum hay and water. OPU and animals fed supplementation started at the same time. The second OPU was performed 21 days after the first OPU session, and the third OPU was performed on day 40 of supplementation, totalizing three aspirations. After each OPU, oocytes were conducted to reproduction laboratory of FCAV – UNESP and their visual quality evaluated. Then, the oocytes were subjected to *in vitro* embryo production. Data were analyzed using ANOVA and the means compared by Tukey test, considering 5% level to significance. There was no statistical difference between groups regarding number of follicles (means±SD), despite the decrease on the third aspiration (P=0.02): 18.00±12.60, 18.83±10.68 and 14.50±7.58 for Group 1 and 19.00±7.48, 20.16±6.67 e 14.00±4.24 for Group 2. However, the number of oocytes aspirated in Group 1 was significantly greater (13.00±10.65, 15.66±9.04 e 10.33±6.88) than in Group 2 (8.16±1.72, 13.16±6.11 e 7.00±5.93; P=0.03). Thereby, oocyte recovery rates on 1st, 2nd and 3rd aspiration of group 1 were 72%, 83% and 71%, and of group 2 were 42%, 65% and 50%, respectively. No statistical difference was observed for number of viable oocytes or in the embryos produced between animals from Group 1 (3.50±3.27, 3.33±1.86, 4.50±2.88) or Group 2 (1.83±1.72, 0±0, 3.66±3.77). In conclusion, Megalac-E supplementation in non-lactating Nelore cows did not increase the number of follicles, number or quality of oocytes or their *in vitro* embryo development capacity.

A146 OPU-IVP and ET

Effects of time of AI on the sex of foals and pregnancy rate in anestrous and cyclic recipients in equine embryo transfer programs

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Keywords: equine embryo transfer, pregnancy rate, sex.

Objectives: 1) To determine the effect of time of AI on the variation in the sex ratio of the foal, 2) to determine the effect of hormonal P4 treatment in recipient on the pregnancy rate in ET program in horses. MM: Mangalarga Marchador mares (n = 84) aged 3-23 years were used as embryo donors. The mares had their ovaries monitored by rectal palpation and ultrasonography (US) every two days until the detection of a follicle≥ 35mm in diameter. At this time, monitoring was performed daily and the mares were inseminated with fresh diluted semen (500 x106 mobile spermatozoa) of fertile stallions every 48 hours until the detection of ovulation (OV; Day 0). Embryos were collected (D7-D9) and transferred to 2 recipients groups between D2-D9: 1) G1: recipients with natural OV and 2) G2: recipients treated with P4 (three consecutive daily applications of decreasing doses of E2 5, 3 and 2 mg of Estradiol Benzoate (EB) followed by 1500 mg P4 LA (D0)). Recipients treated with P4 received one more dose of P4 LA (1500 mg) at the time of ET, and the dose was repeated every 7 days prior to diagnosis (DG) of 15 days or 120 days of gestation. Recipients that received embryos in G1 received a dose of P4 (1500mg) at the time of ET. All recipients received a dose of Flunixin meglumine (1.1mg/kg IV) at the time of ET. To test the effect of time of AI on the variation of sex ratio of the foal, the cycles were divided into: AI performed 0-24 hours (C1) or 24-48 hours (C2) prior to detection of OV. The sex of the foal was confirmed after birth. Data for pregnancy rate, embryonic loss and sex ratio after different times of AI were analyzed by chi-square test. Differences with $p<0.05$ were considered significant. Results: A total of 300 embryos transferred were recorded. Of these, 231 and 69 embryos were transferred into recipients of G1 and G2, respectively. There was no difference ($P> 0.05$) between groups in pregnancy rate (81.0% vs 81.2%), embryonic loss (4.8% vs 7.0%) or pregnancy rate between the different days of synchrony (D2 -D9;$P>0.05$) for G1 and G2, respectively. A retrospective analysis of 230 pregnancies resulted in 113 cycles inseminated for C1 and 117 cycles inseminated for C2. There was no significant difference ($P>0.05$) in the proportion of females or males for two moments of AI (65/113 and 67/117 or 48/113 and 50/117) for mares inseminated from 0-24h or 24-48h, respectively. In conclusion, pregnancy rate of transferred embryos to recipients induced with P4 is similar to recipients with natural OV supplemented or not with P4. The time of AI had no effect on the variation in sex ratio of the foal as assessed in this study.

A147 OPU-IVP and ET

In vitro embryo production of high producing Holstein cows at different moments postpartum

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Keywords: Holstein, OPU/IVP, postpartum.

This study evaluated the *in vitro* embryo production of high producing Holstein cows at different moments postpartum. The experiment was conducted in Santa Rita farm (Agrindus S/A), Descalvado - SP. After birth, 22 high producing Holstein cows were selected to be aspirated every 14 days at five different moments postpartum (30.2±3; 44.2±3; 58.2±3; 72.3±3 and 86.3±3 days postpartum). At the time of ovum pick up (OPU), the number of follicles and body condition score (BCS) were evaluated. The procedures for *in vitro* embryo production (IVP; maturation, fertilization and culture) were carried in the laboratory of Bioembryo, Bauru - SP. Statistical analysis was performed by the GLIMMIX procedure of SAS. No differences at different moments postpartum were observed (30.2±3; 44.2±3; 58.2±3; 72.3±3 and 86.3±3 days in milk) in relation to the total number of visualized follicles (18.1±3.9; 15.5±2.1; 14.7±1.6; 16.3±2.2; 17.9±2.2; P=0.14), the total number of oocytes recovered (12.4±2.6; 10.8±1.3; 11.1±1.9; 13.5±2.4; 13.9±2.3, P=0.69), the number of viable oocytes (9.3±2.3; 8.0±1.1; 7.7±1.6; 10.3±2.1; 10.7±2.0; P=0.54), the cleavage rate [56.9% (116/204) 57.4% (101/176) 63.3% (107/169), 63.1% (137/217), 61.7% (145/235); P=0.68] and blastocyst rate [5.4% (11/204), 9.7% (17/176) 11.8% (20 / 169), 10.6% (23/217), 13.2% (31/235); P=0.11], respectively. Multiparous donors showed higher cleavage rate [65.7% (461/702) vs 50.8% (202/398); P=0.001] and blastocyst rate [12.5% (88/702) vs 5.8 % (23/398); P=0.01] that primiparous donors. The cows of low BCS ($\leq$ 2.75) showed higher cleavage rate [64.9% (395/609) vs 54.6% (268/491); P=0.001] and blastocyst rate [12.3% (75/609) vs 7.3% (36/491); P=0.01] for the donors that had higher BCS ($\geq$ 3.00). It was concluded that the *in vitro* embryo production of high producing Holstein cows had no difference until 90 days postpartum. The body condition and animal category interfere on *in vitro* embryo production of high producing Holstein cows subjected to OPU/IVP until 90 days postpartum.

Acknowledgment: Santa Rita farm (Agrindus S / A), Bioembryo - Biotechnology of Animal Reproduction, Veterinary Clinic – SAMVET and Alta Genetics Brazil.

A148 OPU-IVP and ET

Effect of seasonal variation in the bovine oocytes quality selected by brilliant Cresil Blue method

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Keywords: BCB, bovine, oocyte.

The brilliant cresil blue (BCB) is a vital dye that has been used in several species with the aim of selecting the most competent oocytes when associated with morphological selection method. Thus, this study aimed to use BCB to evaluate the season effect on meiotic competence of bovine oocytes. Oocytes were obtained by aspiration from slaughterhouse ovaries during the summer and winter of 2010 and 2011. Only oocytes classified as grade I and II were used. After selecting the oocytes were placed in incubation solution (medium 199 supplemented with antibiotics and fetal calf serum) during 90 minutes in the absence (control) or presence of 26 μM of BCB (treatment). Then the oocytes incubated with BCB were separated according to their color into two sub-groups: oocytes with some degree of blue staining in the cytoplasm (BCB+, more competent), and oocytes with no blue color (BCB-, less competent). After that, all oocytes were *in vitro* matured. The oocytes were maintained in drops of 100 μL of maturation media (20-25 oocytes per drop), in mineral oil for a period of 22 hours at 38.5°C and 5% CO_2. After IVM, oocytes were stained by acetic orcein method determining the nuclear maturation. The comparison between the oocytes rate that completed nuclear maturation (which reached the M II stage) in different treatments and the comparison of BCB + and BCB- oocytes proportion in summer and winter was performed by Chi-square test with 95% confidence. The oocytes percentage from the control groups, BCB+ and BCB- that reached the MII stage during summer 2010 from a total of 305 (69.9%a, 78.0%a and 47.5%b, respectively) were similar to the same period of 2011 from a total of 208 oocytes (80.6%a, 79.4%a and 46.6%b, respectively). This was also observed during winter 2010 in the control groups, BCB+ and BCB- in a total of 293 oocytes (66.3%a, 73.8%a and 47.8%b, respectively) and in 2011 in a total of 288 (70.4%a, 79.8%a and 46.8%b, respectively). In both years studied and both seasons, the BCB- group showed a significantly lower amount of MII oocytes when compared to control group and BCB+. There was also a significant effect ($p<0.05\%$) of the year in the proportion of BCB+ and BCB- oocytes collected in the summer and winter of 2010 from a total of 490 (BCB+ 56.8%a and BCB- 43.2%a; BCB+ 45,4%b and BCB- 54.6%b, respectively) and 2011 from a total of 426 oocytes (BCB+ 58.9%a and BCB- 41,1%a; BCB+ 51,1%a and BCB- 48,9%a , respectively). Therefore, it can be concluded that regardless of the season variation and the years analyzed in this work, BCB- group presented a lower oocyte number that reached M II. On the other hand, the year influenced the relationship BCB+/BCB-, being the winter of 2011 the period that presented a higher number of low competence oocytes (BCB-).

A149 OPU-IVP and ET

Effect of meiotic arrest with roscovitine on the *in vitro* production of bovine embryos

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Keywords: meiotic arrest, oocyte maturation, roscovitine.

In mammalian oocytes, the nuclear and cytoplasmic maturation occur at different times and this fact seriously undertakes the viability of *in vitro* matured oocytes. Thus, 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. Thus, this study, evaluated the effect of the addition of a meiotic arrest roscovitine (ROS; Sigma-Aldrich, St. Louis) 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 hours in medium in presence of 2 different concentrations of Roscovitine: 12,5μM and 25μM. Then the oocytes were cultured for 18 hours in agent-free medium to meiosis resume, completing 24 hours of maturation. After 24 hours of maturation (day 0), oocytes were fertilized in human tubal fluid (HTF – Irvine, New Zealand) under the same condition above. Semen was selected through Percoll gradient and the concentration adjusted to 2 x 106 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 routines (200 oocytes/routine). 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%; Roscovitine 25μM: 49,2 ± 3,9% (P=0,205). The roscovitine was able to produce embryos without degeneration and with similar qualities to the agent-free group. However, to really prove if its action does not compromise embryonic development, techniques such as TUNEL (Terminal deoxinucleotil transferase Uracil Nick End Labeling) are being performed for the *in situ* detection of apoptotic cells.

A150 OPU-IVP and ET

Melatonin improves bovine blastocyst quality but not embryo rates in IVP

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Keywords: antioxidant, bovine, embryo.

This study aimed to evaluate melatonin effect over bovine embryo quality and rates on IVP. Melatonin is an effective free radical scavenger (Tan et al, 2002, Current Topics in Medical Chemistry 2, 181-197) and has beneficial effects in IVP for some species (Tamura et al., 2008, J. Pineal Res. 2008, 44, 280-287). Embryo production sessions (n=12) were performed; 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 underwent IVM for 22 hours in 400µl of TCM199 (Day -1). For IVF, COC's were moved to fertilization medium (400µl), inseminated with frozen–thawed semen, previously submitted to discontinuous Percolltm gradient,, and incubated for 18 hours (Day 0). After IVF, presumptive zygotes were striped from remaining cumulus cells by incubation with hyaluronidase and pipetting and 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 400µl KSOM-10% FCS was added. On Day 7 embryos were evaluated regarding their developmental stage, percentages (%) were calculated relative to the total of presumptive zygotes. All procedures were performed on a Nunc Multidishestm 4 well dish without mineral oil overlay. During IVM and IVC groups received either 0ng/ml or 50ng/ml of melatonin, CG and MG, respectively. On Day 7, out of 4 sessions, all expanded blastocysts (n=66) were dyed with Propidium Iodide and Hoechst 33342. Statistical analysis was performed using SAS system for Windows 9.2TM parametric data was submitted to student T-test and non parametric to Wilcoxon, presented mean + standard error and median (1st; 3rd quartile) respectively. Differences were considered meaningful when $p<0,05$. Expanded blastocysts from MG presented more cells (44.87+2.36) than the ones from CG (30.82+2.02). Also MG presented less cells with membrane rupture 8.89(3.33; 21.88) than CG ones 25 (11.11; 37.50). Melatonin treatment neither influenced cleavage (MG 73.94+2.23; CG 73.11+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). The results demonstrate that melatonin improves bovine embryo quality however it had no effect over embryo rates on IVP.

A151 OPU-IVP and ET

Effect of the presence of insulin-transferrin-selenium (ITS) and L-ascorbic acid (AA) during maturation on bovine *in vitro* embryo production

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Keywords: antioxidants, maturation, oocyte.

Aiming to improve IVP rates, various substances, such as gonadotropins, steroids, growth factors and antioxidants have been added into maturation and *in vitro* culture medium. Among those, ITS and AA have been associated with a reduction of the reactive oxygen species production in cell culture media. Therefore, the objective of this study was to test the effect of the presence of AA and ITS during IVM, on the quality and production of IVP embryos. Oocytes obtained from slaughterhouse ovaries were distributed into four groups: 1) C (N = 220), IVM media without ITS and AA, 2) ITS + AA-1st 12hM (N = 224), IVM media supplemented with 0.5 mg/ml of ITS and 100μg/ml of AA in the first 12 hours of maturation, 3) ITS + AA 2nd 12hM (N = 213), IVM media supplemented with 0.5 mg / ml of ITS and 100μg/ml of AA in the last 12 hours of maturation and 4) ITS + AA-24hM (N = 209), IVM media supplemented with 0.5 mg/ml of ITS and 100μg/ml of AA during 24 hours of maturation. After IVM, oocytes were fertilized and cultured *in vitro*, being the cleavage and blastocyst rates evaluated on D2 (D0=day of IVF) and D7 day of culture, respectively. The D7 embryos were measured, with a camera Motic Images Plus 2.0 and were divided into three categories: 1) 120-140mm, 2) 140-160mm, and 3) ≥ 160mm. Only those with size ≥ 160 mm were evaluated for cell number by Hoechst staining. Data of embryo development and size category were analyzed by chi-square test ($P < 0.05$) and the cell number by Kruskal-Wallis test ($P<0.05$). The cleavage rate was similar ($P>0.05$) among all treatments, however the production of blastocysts on D7 was higher ($P<0.05$) in ITS + AA-2nd 12hM (51.6%) than in C (39.5%), ITS + AA-1st 12hM (40.2%) and ITS + AA-24hM group (41.1%), which did not differ ($P>0.05$) among them. The percentage of embryos ≥ 160mm was higher ($P<0.05$) in ITS + AA -2nd 12hM (83.7%) group when compared to ITS + AA-1st 12hM (68.9%) and ITS + AA-24hM groups (71.0%) and similar ($P>0.05$) to C group (74.7%). The blastocyst from group ITS +AA-2nd 12hM had higher ($P<0.05$) cell number (143.2 ± 49.9) than those from ITS+AA-1st 12hM group (122.3 ± 46.1), but did not differ ($P> 0.05$) from the embryos of the groups, C (131.9±44.7) and ITS + AA-24hM (124.3±35.3). It can be concluded that addition of ITS and AA in the last 12 hours of maturation improved not only embryo production, but also the quality of the embryos.

A152 OPU-IVP and ET

Effect of the number of laparoscopic oocyte recovery sessions in Canindé goats on the efficiency of oocyte production in an IVEP system

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Keywords: goat, laparoscopy, oocyte.

In vitro embryo production (IVEP) can become an excellent tool for genetic improvement and preservation of goat breeds. However, for this, it is necessary the use of gametes (spermatozoa and oocytes) derived from genetically superior and pure specimen. Concerning the female, laparoscopic oocyte recovery (LOR) can be the appropriate method. However, in Canindé goats there is no study on the effect of repeated LOR in the same oocyte donor. The objective of this study was to observe the response of donors after successive sessions for the oocyte production in an IVEP system. For this purpose, it was used 16 adult and cyclic Canindé goats, which were submitted to two or three treatments for hormonal ovarian stimulation followed by LOR. All oocyte donors received intravaginal sponges containing 60 mg MAP (Progespon, Syntex, Buenos Aires, Argentina) for 11 days, combined with an intramuscular (im) injection of 50 µg d-cloprostenol (Ciosin, Coopers, São Paulo, Brazil) on day 8 of progestagen treatment. For ovarian stimulation, goats received a single im injection of 70 mg NIH-FSH-P1 (Folltropin-V, Vetrepharm, Belleville, Canada) plus 200 IU eCG (Novormon, Syntex, Buenos Aires, Argentina) 36 h before sponge removal. The interval between each hormonal treatment/LOR was 14 days. LOR was performed under volatile anesthesia and according the procedure cited by Avelar et al. (2012, Anim Reprod, 9, 27-32). The pressure was set at -30 mmHg and all follicles larger than 2 mm were aspirated. The collection medium used was TCM199 supplemented with HEPES, heparin and gentamicin. Once the LOR was completed, each ovary was gently flushed with a heparinized saline. The effect of repeated LOR was analyzed using repeated-measures ANOVA and Tukey's test. There were no statistical differences between the three LOR sessions in the number of visualized/punctured follicles (15.3 ± 5.1/12.7 ± 4.5, 15.5 ± 4.2/12.8 ± 3,9 and 14.7 ± 6.4/11.9 ± 4.9, $P > 0.05$). Concerning the recovery rate, there was also no statistical difference between the different sessions, with an average of 71.2, 74.8 and 74.4% ($P > 0.05$) for the first, second and third session, respectively. In conclusion, three LOR sessions did not affect the oocyte production in Canindé goats submitted to hormonal ovarian stimulation aimed at subsequent IVEP.

A153 OPU-IVP and ET

Quercetin effect on *in vitro* embryo production

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Keywords: embryo, oocyte, quercetin.

During *in vitro* culture system, the embryos are exposed to higher oxygen tension than those produced *in vivo* and this leads to constant production of free radicals that can cause extensive cellular damage. Thus, to optimize the *in vitro* production of bovine embryos is necessary a culture system that minimizes the oxidative stress, resulting from the oxygen tension, light exposure or by manipulation. The use of antioxidants on *in vitro* culture systems with high oxygen tension, approximately 20% O_2, is considered an important factor to embryo production due to their protective actions against the effects of free radicals. Quercetin is a flavonoid of the polyphenolic compounds group widely found throughout nature (apple, grape, etc.) that stood out in the scientific environment by its antioxidant action. The objective of the present study was to assess the effect of quercetin on *in vitro* embryo development. Bovine oocytes recovered from slaughterhouse ovaries were matured for 22 h (TCM199 with 10% FCS, 5.0 µg/ml of LH, 0.5 µg/ml of FSH, 0.2 mM pyruvate and 50 µg/ml gentamicin), fertilized by 18 h (TALP with PHE and heparin – 2 x 10^6 spermatozoa/ml) and cultivated for 7 days in medium SOFaa (10% FCS) supplemented with 0, 0.4, 2, 10 and 50 µM quercetin at 38.5°C in a 5% CO_2 humidified atmosphere. Data were analyzed using the ANOVA followed by Tukey test ($p<0.05$) ± standard deviation. The embryonic development of 895 oocytes was evaluated in four replications and it differed among treatment group of quercetin. It was found a higher percentage of blastocyst ($p<0.05$) at concentration of 10 µM of quercetin (61.1±3.7%) when compared to other concentrations of 0 (49.2±5.1% - control), 0.4 (49.3±1.1%), 2 (50.8±3.8%) and 50 (41.4±2.0%) µM quercetin. Blastocysts production was not different ($p>0.05$) among concentrations of 0, 0.4, 2 and 50 µM quercetin (49.2±5.1, 49.3±1.1, 50.8±3.8 e 41.4±2.0%, respectively). The supplementation of 10 µM quercetin on *in vitro* culture provides an increase in blastocyst rates compared to the other treatments. The addition of quercetin on *in vitro* culture of bovine embryos has the potential to reduce disparities between the embryonic development *in vivo* and *in vitro*.

A154 OPU-IVP and ET

Successfully obtaining equine pregnancy using embryos produced by intracytoplasmic sperm injection (ICSI)

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Keywords: equine, ICSI, oocyte.

An alternative for *in vitro* equine oocyte fertilization is through intracytoplasmic sperm injection (ICSI) followed by transfer to recipient mares. The purpose of this study was to evaluate the efficiency of ICSI at *in vitro* production of equine embryos. Transvaginal follicle aspiration was conducted in 102 mares between May and August 2011. Oocytes recovered were placed in TCM maturation medium and were transported to the lab in gasified environment at 38.5°C. Afterwards, they were cultured for 24-32 hours in DMEM/F12 medium supplemented with LH, FSH, estradiol, FCS, IGF (insulin-like growth factor), EGF (epidermal growth factor), ITS (insulin-transferrin-selenium), and gentamicin, under mineral oil in incubator containing 5% of CO_2 in air kept at 38.5°C. Those oocytes in metaphase II of the first meiotic division were considered maturated. For the ICSI, 200 ul of fresh or unfrozen semen were placed in 15-ml falcon tubes containing 1 ml of sp-TALP and were incubated in atmosphere of 5% of CO_2 in air for swim up. After 30-40 minutes, a volume of 0.6 ml of medium was collected from the supernatant and then centrifuged at 1000 rpm during 5 minutes. The pellet was suspended once more and washed with the same medium and, finally, the supernatant was removed and the pellet was used for ICSI. Immediately before injection, 10 μl of the suspension were placed in 10 μl of sp-TALP containing 10% of W/v and PVP (Sigma). The injection was managed in one separate drop of 50 μl of BO. The oocytes were injected in culture medium at the incubator for 7 to 8 days for embryo production. Then, they were transferred via transcervical to previously synchronized recipients. From a total of 204 aspirated ovaries, 414 oocytes were obtained, i.e., an average of 4.1 oocytes per mare. From the 63 oocytes injected through intracytoplasmic injection, 21 (33.3%) were cleaved in 24-72 hours of *in vitro* culture, from which 5 (23.8%) developed to blastocyst stage with 7-8 days of *in vitro* culture. From the blastocysts transferred (n = 5), two recipients got pregnant (40%). The average of recovered oocytes by OPU in the current study (4.1) was greater than the one reported by Galli, An Repr Sci,98, 39-45, (3.2), while the production of blastocyst is similar to the one described in literature (23-44%; Hinrichs,Theriog.,64,535-41; Galli, An Repr Sci,98,39-45). Despite that *in vitro* production of embryos requires improvements, it is possible to verify the feasibility of embryo production through ICSI, a technique highly recommended in case of low quality semen and small number of oocytes.

A155 OPU-IVP and ET

Comparison of embryo production after OPU/IVP and MOET/ET among bovine females with high and low antral follicular counts: preliminary results

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Keywords: antral follicular population, bovine, embryo production.

The aim of the present study was to compare the embryo production among bovine females with high and low follicular counts obtained after OPU/IVP and MOET/ET. Braford females (5/8 Nelore x 3/8 Hereford, n=137, 9±1-month-old) were submitted to six ovarian ultrasonographic scans (Áquila PRO, Pie medical, Maastricht, The Netherlands) using a 7,5-convex intravaginal array transducer, with intervals of 60 days, and were assigned to two groups, according the number of antral follicles ≥3 mm: G-High (n=20, mean ≥40 follicles) and G-Low (n=20, mean ≤10 follicles). When the females reached 24 mo of age, they (n=40) were used for OPU/IVP. Briefly, COCs recovered were classified and transported to the laboratory for IVP. The number of COCs recovered, viable oocytes, cleavage rates, blastocyst rates and transferable embryos were analyzed. One week later, the same females were submitted to superovulation treatment. Each female received an auricular device (Crestar®, Intervet-Schering Plough, Brazil) randomly during their estrus cycle (D0) and 2.5 mg EB (Estrogin®, Farmavet, Brazil), IM. Five days later, FSH treatment (200 mg, IM, Foltropin-V®, Bioniche, Canada) was given twice for four consecutive days. On D7 in the morning, females were injected with 2.5 ml PGF2α (Ciosin®, IM, Intervet-Schering Plough, Brazil) and, on D8, with 200 IU eCG (Novormon®, IM, Syntex SA, Argentina) in the morning and in the afternoon. The device was removed on D8, after the second dose of eCG. On D9 in the morning, females received 12.5 mg LH (Lutropin-V®, IM, Bioniche, Canada) and insemination was performed 12 to 24 h later. Embryos were recovered eight days after LH injection. The number of recovered structures and transferable embryos were analyzed. After follicular aspiration, the mean number of COCs recovered was 36.9±14 (G-High) and 5.8±3 (G-Low). The mean number of viable oocytes was 22±10 (G-High) and 3±2 (G-Low). Cleavage rates were 61.25% (452/738, G-High) and 68.10% (79/116, G-Low). Blastocyst rates were 16.53% (122/738, G-High) and 11.21% (13/116, G-Low) and the mean number of transferable embryos was 6.1±4.5 (G-High) and 0.55±0.82 (G-Low). After embryo collection, the mean number of recovered structures was 8.8±6.8 (G-High) and 2.3±2.6 (G-Low) and the mean number of viable embryos was 6.4±10.0 (G-High) and 2.0±2.4 (G-Low). Animals with high population of antral follicles had the greatest embryo production in both OPU/IVP and MOET/ET procedures.