A080 OPU-IVP and ET

Inbreeding and its relationship with oocyte production in the Gir (*Bos indicus*) breed: preliminary results

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The Gir breed is native from India. This breed is characterized by low weight (and thus low nutritional requirements), tolerance to heat and parasites, as well as a potential for milk production under tropical conditions. Live animals were imported to Brazil mainly between 1870 and 1962, but less than 700 cattle were actually brought. Due to the small number of imported animals that were used as founders of Brazilian Gir cattle herd, it is important to study the population structure and to control the inbreeding levels. One of the main consequences of high inbreeding levels in cattle herds is the reduction of the genetic variability, decreasing heterozygosis and causing inbreeding depression. Inbreeding can negatively affect animal breeding programs and cause low genetic gains. The aim of this study was to estimate the coefficient of inbreeding means by the analysis of pedigree of a Gir population, and its possible effects on oocyte yield in Gir donors. The pedigrees of 6,108 Gir animals from a commercial farm of Minas Gerais State, Brazil, were used. The database editing and structuring, as well as the calculation of inbreeding coefficients, were performed using the SAS and CFC software, respectively. The estimated coefficient of inbreeding was 4.49%, with a maximum F of 32.03% based on genealogical information from 1,384 females. From the population studied, 22.65% was considered as endogamic, while 69.21% of the individuals showed F <5%. The association between inbreeding coefficient and *in vitro* embryo production (IVEP) outcomes is currently under evaluation in this population. Preliminary analyzes have shown that F> 0.06 is associated with a reduction in the average number of total and viable oocytes retrieved per donor. In summary, high coefficient of inbreeding can be found within Gir herds, and the resulting inbreeding depression may also affect oocyte yield in donors, potentially reducing IVEP outcomes.

A081 OPU-IVP and ET

Influence of season and genetic group on commercial production of bovine embryos *in vitro*

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The beginning of the commercial work of OPU-PIVE in Brazil occurred with the implantation of the first laboratory, in the year of 2000. The Nelore breed adapted to this technique extraordinarily and its use quickly spread among the ranchers. Brazil currently accounts for about 70% of the total number of embryos produced *in vitro* in the world. Despite the evolution and potential of PIVE, there is great variability in the rates of blastocyst (10 to 40%) and pregnancy (30 to 50%), factors that directly influence the commercial success of the technique. The objective of this study was to evaluate the influence of the seasons of the year (spring/summer and autumn/winter) and the genetic group of the donor *Bos indicus* (Nelore), *Bos indicus* x *Bos taurus* (Brangus and Girolando) and *Bos taurus* (Dutch and Senepol) in the production of viable oocytes, cleavage and production of blastocysts. Data from a commercial laboratory, from 2014 to 2016, were analyzed for 776 ultrasound-guided follicular aspiration (OPU) sessions in 225 cows of Nelore (n=83), Girolando (n=73), Brangus (n=49), Dutch (n=10) and Senepol (n=10), which were grouped according to the genetic group (*Bos indicus,* n=83; *Bos indicus* x *Bos taurus*, n=122; *Bos taurus* n=20). The influence of the donor's seasonality and genetic group on the production of viable oocytes, cleavage and blastocyst production were analyzed. Statistical analysis was performed using the Chi-square test using the SAS statistical program. Regarding the season, we observed a higher percentage of viable oocytes in spring/summer when compared to autumn/winter (70.8 *vs* 67.2% respectively, P=0.0001). There was no difference in the cleavage rate (75.3 and 74.7% respectively, P=0.506) and blastocyst production (27.1 and 27.8% respectively, P= 0.0403). As for the genetic group, there was a higher oocyte production in *Bos indicus* females, followed by *Bos indicus* x *Bos taurus*, and *Bos taurus* (74.1%, 69.2% and 63.9% respectively, P<0.0001). The cleavage rate in *Bos indicus* was not different than *Bos indicus* x *Bos Taurus*, 76.8% in both groups. However, the *Bos indicus* and *Bos indicus* x *Bos taurus* groups had a higher cleavage rate than *Bos taurus* (76.8%, 53.4%, P<0.0001). The blastocyst rate differed between the groups, it was higher in the *Bos indicus* group followed by *Bos indicus* x *Bos Taurus* and *Bos Taurus* (31.1%, 26.8% and 12.4% respectively, P<0.0001). According to the data presented, it is concluded that, regardless of the genetic group, the seasons of the year influence the production of viable oocytes, but do not exert effects on the production of embryos and; in relation to the genetic group, this in turn influences the production of embryos, where the females of the *Bos indicus* species present better performance among all studied variables (production of viable oocytes, cleavage rate and formation of the blastocyst), when compared to females *Bos indicus* x *Bos taurus* and *Bos taurus*.
Aknowledgement: FAPESP, UNOESTE.

A082 OPU-IVP and ET

Study on superovulation with FSH coasting in bovine female

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The Superovulation Technique (SOV) is one of the fundamental stages in embryo transfer programs in bovines, therefore, to stimulate the development of a larger number of follicles capable of ovulating, several protocols are studied, among them the association of superovulation followed by FSH coasting. Coasting is a period of absence of FSH, which simulates the natural physiological changes that occur before ovulation. Although many studies have shown that this association (SOV + FSH coasting) results in improved oocyte competence, more research should be done to prove the effect of this protocol. Objective: The objective of this work was to evaluate the efficiency of follicular superovulation associated with FSH coasting in bovine donor females. Methodology: Sixteen adult Nelore bovine females, from a homogeneous genetic group, aged 3 to 7 years, with an average weight of 400 kg, were distributed in two treatments. In group 1, control (CON) (n = 8) the animals were submitted to OPU without follicular wave synchronization; group 2, treatment (TRAT) (n = 8) at day zero (D0) the animals underwent a follicular wave synchronization process, where the intravaginal slow release progesterone implant (Sincrogest, OF) + 2.0 mg of estradiol benzoate (BE) IM (Synchrodiol, OF) + 0.15 mg D-cloprostenol IM (Croniben, BB). From D3 to D6 the animals received 200UI of FSH and 200 IU of LH IM (Pluset, Ceva) and in D8 the implant was removed and performed at OPU.The recovered cumulus oocyte (CCOS) complexes were washed, transferred to petri dish, counted and graded. The parameters considered were total number of viable CCOS and CCOS, blastocyst rate and hatch rate. Statistical analysis was performed using a T-test with 5% significance. Results: The number of total and viable CCOS in the CON group (n = 15 and 11.9) didn't differ from the TRAT group (n = 16.1 and 14.4), respectively. Blastocyst and hatching rates also didn't differ (P> 0.05) between the CON (38.5 and 64.8%) and TRAT (44.5 and 59.6%) groups, respectively. Conclusions: Ovarian stimulation associated with FSH coasting didn't increase the number and quality of total and viable CCOS, blastocyst and hatching rates.

A083 OPU-IVP and ET

Crossbreed effect (*Bos taurus* and *Bos indicus)* on blastocyst and pregnancy rates in a comercial *in vitro* embryo production program

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The *in vitro* embryo production (IVEP) showed significant growth in dairy sector in last decades. However, breed aspects can cause variations in reproductive rates of a IVEP commercial program. The aim of this work was to evaluate the blastocyst and pregnancy rates from crosses of Gir and Holstein sires with Gir, Holstein and ½ Holstein-Gir donnors. A total of 363 Gir, 109 Holstein and 145 ½ Holstein-Gir donors were used to OPU. The oocytes were classified according to IETS manual, and viable oocytes (Grades 1, 2 and 3) were matured, fertilized (D0) with Gir (n=18) or Holstein (n=51) sexed sêmen, and cultured *in vitro* for 7 days (D7). For *in vitro* fertilization, bulls with previously known fertility were used. All embryos produced were transferred in D7 to synchronized recipients. The pregnancy diagnosis was performed by transrectal ultrasonography 60 days of pregnancy. Mann-Whitney U test ($P \leq 0.05$) was used to compare groups. No difference was found among all crosses. For Gir donors, crossed with Gir and Holstein sires, blastocyst and pregnancy rates were 24.2% *vs* 27.4% and 42.1% *vs* 37.4%, respectively. Holstein donors showed blastocyst and pregnancy rates of 22.5% *vs* 14.8% and 35.0% *vs* 35.8% crossed with Gir and Holstein sires, respectively. In addition, crosses of ½ Holstein-Gir donors with Gir and Holstein sires results on blastocyst and pregnancy rates of 35.9% *vs* 23.0% and 31.2% *vs* 36.4%, respectively. In conclusion, the Gir and Holstein bulls crosses with Gir, Holstein and ½ Gir / Holstein donors do not affect the blastocyst and pregnancy rates in a commercial IVEP program.

A084 OPU-IVP and ET

Seasonality and stage of embryo development influence the pregnancy rate in commercial *in vitro* production of bovine embryos

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Despite the great evolution and potential of follicular aspiration followed by *in vitro* embryo production (OPU-IVP) in recent years, such biotechnology presents variability in the success of blastocyst production and pregnancy rate, factors that compromise the success of the technique. In commercial scale, the use of recipients of different breeds and origins, with different management, as well as different protocols and technical, evidences the need of researches that reported the reality of the commercial production of embryos and that can contribute to the improvement of such limiting indices. The aim of this study was to verify the effect of seasonality and the stage of embryonic development on the embryo production rate of embryos produced *in vitro* (IVP) in a commercial scale in the Western region of São Paulo. We used retrospective data on 20,297 IVP embryos transferred in the seasons: autumn/winter (n=6804 embryo transfers) and spring/summer (n=13493 embryo transfers) from 2009 to 2016 from 1,400 oocyte donors of breeds for the production of meat and milk created, in the West Paulista, Mato Grosso do Sul and North of Paraná. The IVP followed the protocols established by the laboratory, which is site in the city of Regente Feijó-SP, being the work done by a team composed of a laboratory and two field technicians. In order to evaluate the influence of the embryo stage, embryos in D7 with quality 1 and 2, classified in morula (n=41, stage 4), initial blastocyst (n=109, stage 5), blastocyst (n=203, stage 6), expanded blastocyst (n=257, stage 7) and hatched blastocyst (n=13, stage 8) were used. In the statistical analysis, the chi-square test was used at the 5% level of significance ($p < 0.05$). Pregnancy rate in spring/summer (42.7%) was higher when compared to autumn/winter (37.7%, $p < 0.0001$), regardless of breed. Regarding the stage of development, the pregnancy rate did not differ in stages 6, 7 and 8 (44.3%, 48.2% and 46.1%, respectively, $P > 0.05$), however, such embryos resulted in higher pregnancy ($P=0.0046$) when compared to the embryo transfers in the early stages of embryo development, that is, 4 (24.4%) and 5 (31.2%). It is concluded that the IVP embryo pregnancy rate obtained by the analyzed laboratory, regardless of breed, is higher in spring/summer; embryos innovated in more advanced stages of development (blastocyst, expanded blastocyst and hatch) result in better pregnancy rates.

A085 OPU-IVP and ET

Physiological and oogenesis characteristics of breeds adapted to the tropics under different environmental stress conditions

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In order to evaluate the influence of thermal stress on physiological parameters (level of the proteins HSP 70 and HSP 90) and the oogenesis of *Bos taurus* x *Bos indicus* 3/4 (Girolando; n = 12) and *Bos taurus* (Pantaneira; n = 12) cows, twelve sessions of ultrasound guided follicular aspiration were performed, among January and November 2014. The recovered oocyte-cumulus complexes (COCs) were selected and classified immediately after OPU. Part of the viable COCs was submitted to immunofluorescence analysis under confocal microscopy for identification of HSP 70 and HSP 90 proteins. Before each OPU, the thermal stress was estimated by the black globe humidity index (BGHI), which was calculated according to the formula described by BUFFINGTON et al. (Black Globe-Humidity Index (BGHI) as Comfort Equation for Dairy Cows. Transactions of the ASAE. 1981; 24:711) and classified according to the National Weather Service. The BGHI was calculated on the day of the OPUs and 90 days before each of them. Data were submitted to analysis of variance by program R (version 3.3.1). When differences were found, the means were compared by the Tukey test, with a 5% probability. In twelve OPU routines, 272 oocytes from the Girolando cows and 306 oocytes from the Pantaneira cows were collected. And signal intensity of HSP70 and 90 were affected by BGHI ($P<0.05$). The BGHI 90 days before OPU influenced the viability of oocytes of both breeds. HSP in the oocytes was influenced by the BGHIs for both breeds, but with opposite behaviors. Pantaneira breed had lower oocyte quality when BGHIs <78 demonstrating and BGHIs >78 have a deleterious effect on the quality of oocytes Girolando.

A086 OPU-IVP and ET

Obtaining embryos produced *in vitro* from oocytes retrieved by ultrasound-guided follicular aspiration in Holstein heifers

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There is great interest in the use of calves as oocyte donors for IVEP to increase genetic improvement by reducing the interval between generations. However, the results obtained so far in this animal category are unsatisfactory due to lower oocyte competence, low fecundation rate and low *in vitro* embryo development. This study aimed to compare the rate of production of embryos with sex-sorted or conventional semen in Holstein females up to 12 months of age. Holstein donors (*Bos taurus taurus*, N=101) were used, allocated into groups by age category: 6 to 9 months (n = 39 fertilized with conventional semen, n = 18 fertilized with sex-sorted semen) and 10 to 12 months (n = 15 for conventional semen, n = 29 for sex-sorted semen). No protocol for follicle growth synchronization was used, and oocytes were collected by ultrasound-guided transvaginal follicular aspiration. The ultrasound used was the ExaPad MiniTM coupled to an OPU guide with a radius of curvature measuring 10mm. Oocytes were *in vitro* matured for 24 hours, fertilized with conventional semen (1x106 sperm / mL) or sexed (1x105 sperm / mL) and cultured *in vitro*. The blastocyst rates between the groups were compared by the chi-square test adopting $P \leq 0.05$. The mean number of oocytes recovered per group was 19 and 16 for heifers from 6 to 9 months with the use of conventional and sexed semen, respectively. For females from 10 to 12 months, the mean number of oocytes recovered was 10 for the group fertilized with conventional semen and 12 for the group fertilized with sexed sêmen. The percentages of embryos produced with conventional and sexed semen were, respectively, 22.8% and 7.4% for calves of 6 - 9 months; and 37.1% and 18.8% for females of 10-12 months. Blastocyst rates differed between age groups, both for conventional and for sex-sorted semen ($P<0.0001$). It was observed a lower rate of embryo production for calves of 6 - 9 months, however, we highlight the time gain between generations with the possibility of OPU in very young animals. The use of sexed semen resulted in lower rates of embryos produced, but the best genetic targeting of the herd should be considered when obtaining desired sex animals.

A087 OPU-IVP and ET

Factors affecting pregnancy outcome in a commercial bovine embryo technology center in Minas Gerais Brazil

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The objective was to evaluate the effect of factors associated with the gestation outcome of *in vitro* produced bovine embryos transferred non-surgically. The factors analyzed were: parity (nulliparous, primiparous and multiparous), semen type (sex-sorted or conventional), sire (n = 44), embryo stage at transfer (morula (Mo), initial blastocyst (Bi), blastocyst (BL), expanded blastocyst (BX) and hatching blastocyst (BN)), the use or not of GnRH at the time of transfer and technician (n = 4). Gestation was diagnosed by ultrasound at 30 days post ovulation. A total of 3991 embryo transfers were analyzed from a four years period. Not all transfer could be traced to the independent variables, thus the number of response events for each parameter is variable. For the analysis of the bull effect, only bulls with more than 30 diagnoses were considered. All data were submitted to chi-square under a generalized linear model procedure, after tests of normality under the Univariate procedure of SAS®, considering the binomial distribution and the parameter estimation adjustments of the Overdispertion by Pearson Chisq / DF, Bias and link logit function of the JMP-12 Pro® package (SAS, Cary, NC, USA). The differences between the independent variables of the model were compared by orthogonal contrasts. The effects of sire (P = 0.005), semen type (P = 0.05), parity (P< 0.0001), GnRH (P = 0.02), embryo stage (P = 0.009) and technician (P = 0.004) was significant. Pregnancy rate was higher in nulliparous (53.4±2.2%) compared to multiparous (37.3±2.1%) recipients. The highest pregnancy rate sire achieved 53.9±0.8%, while the lowest 25.0±0.07%. The pregnancy rate was higher for the expanded blastocyst (45.0±1.1%) compared to the blastocyst (35.8±1.4%) stage. The pregnancy rate was higher (p = 0.05) for sex-sorted semen (41.51±3.39%; n= 212) compared to conventional (38.99±0.08%; n = 3775) semen. The pregnancy rate was higher (P= 0.02) for GnRH-injected-recipients (42.90±1.21; n = 2313) compared to the non-injected controls (36.92±1.00; n = 1656). It is surprising that sex-sorted semen achieved better pregnancy rates in this study, as it goes against the literature, however, since the effect of sire was significant, but was not considered in the semen type analysis it may be speculated that the positive result for sex-sorted semen is confounded by for this latter effect. All the other effects were influenced the gestation outcome and should therefore be carefully accounted for embryo centers in the pursuit of result improvements.

A088 OPU-IVP and ET

Effect of *in vitro* maturation (IVM) method and gas tension during culture upon *in vitro* production of F1 embryos (Nelore x Angus) using Y-sorted semen

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The availability of X-sorted sperm was a turning point for the use of *in vitro* embryo production (IVEP) in dairy breeds in Brazil. Due to a repressed demand for crossbred dairy heifers, IVEP using female sex-sorted semen has boosted up in the past 10 years. On the other hand, less attention was given to the use of Y-sorted semen for IVEP, despite the great potential for large-scale production of F1 male beef calves. Optimization of IVEP in beef, however, must take into consideration some particularities of extensive beef cattle operations, including the usual long distances between the site of oocyte collection and the IVEP laboratory. In this regard, *in vitro* maturation (IVM) during oocyte transportation may represent a critical step for the entire process. The aim of the present study was to evaluate the effects of IVM in tubes versus Petri dishes followed by *in vitro* culture (IVC) under two distinct O_2 tensions, i.e., a randomized complete design with a 4x4 arrangement of treatments: IVM in tube-IVC in high O_2 (n=1043); IVM in tube-IVC in low O_2 (n=1131); IVM in Petri dish-IVC in high O_2 (n=866), and IVM in Petri dish-IVC in low O_2 (n=846). Cumulus-oocyte complexes (COCs) were collected from ovaries of Nelore cows at a slaughterhouse and randomly assigned for IVM in either 5mL tubes (25 COCs per tube in 400mL of medium TCM199) or microdrops on a Petri dish (25 COCs per 90mL drops of TCM199), at 38.5º C, for 22-24h in 5% CO_2. Matured COCs were *in vitro* fertilized (IVF) with commercial Y-sorted semen from a single Angus sire. Sperm preparation included centrifugation in a mini-Percoll gradient followed by co-incubation of gametes for 18-22h. After IVF, putative zygotes were cultured in SOF medium at 38.8°C with two distinct gas tensions: 5.5% CO_2 and 20% O_2 (high O_2) or 5.5% CO_2 and 5% O_2 (low O_2). The endpoints cleavage rate, blastocyst rates at days 7 and 8 (late embryo development), and proportion of grade I embryos were analyzed using the SAS GLM procedure and differences among means compared by the Tukey´s post hoc test. We did not observe an interaction between type of IVM and gas tension during IVC. Nonetheless, IVM in tubes increased cleavage and day 7 blastocyst rates compared to IVM in petri dish (54.2±1.9% *vs* 41.0±2.4% and 19.5±1.5% *vs* 12.7±1.1%, respectively, $P<0.05$), but had no effect ($P>0.05$) on the proportion of grade I embryos nor day 8 blastocysts. O_2 tension did not affect ($P>0.05$) blastocyst rate and proportion of grade I embryos, but low O_2 reduced day 8 blastocyst rate compared to high O_2 (10.5±2.6% *vs* 20.5±3.0%, respectively, $P<0.05$). These results demonstrate that IVM in tubes is a feasible alternative for COC maturation during transport to the laboratory. In addition, IVC performed at high O_2 delayed embryo development, with consequences for the logistics of embryo transfers into recipients. Thus, we conclude that both IVM and IVC still need optimization for large-scale IVEP programs using Y-sorted beef cattle semen.

A089 OPU-IVP and ET

Superovulation and transcervical embryo recovery in Lacaune ewes raised under tropical conditions

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This study assessed two superovulatory treatments and the feasibility of transcervical embryo recovery in Lacaune ewes. Ewes (n=23) received medroxyprogesterone acetate sponges (60mg, Progespon®, Syntex, Buenos Aires, Argentina) for nine (G9, n=23) or six (G6, n=23) days, 37.5 µg d-cloprostenol i.m. (Prolise®, Tecnopec, São Paulo, Brazil) 24 h before sponge removal and were superovulated with 133 mg of porcine FSH i.m. (Folltropin®-V; Bioniche Animal Health, Belleville, Canada) in six decreasing doses (twice daily) at 60 h before sponge removal, under a crossover design. Ewes were checked for estrus twice daily and were naturally mated by fertile rams (4:1 ratio) while in estrus. Transretal ovarian ultrasonography (Mindray M5VET®, Shenzen, China - 8.0 MHz) was performed at first FSH injection to count and measure the follicles, and at the 5th day after estrus to count the number of CL with Doppler mode activated. All ewes received 37.5 µg d-cloprostenol and 1 mg estradiol benzoate (Sincrodiol®, OuroFino, Cravinhos, Brazil) i.m. 16 h and 50 IU oxytocin (Ocitocina forte UCB®, São Paulo, Brasil) i.v. 20 min before uterine flushing. Embryo recovery was performed at the 6th day after estrus by transcervical method. Qualitative data were analyzed by generalized linear models (GLM), with binomial distribution and logit link function. Quantitative data were analysed by GLM with normal or Poisson distribution (log transformation) when variables not assumed presumptions of ANOVA. Association of variables was evaluated by Pearson correlation, using software SPSS Statistics (IBM®Inc., Chicago, USA). The percentage of donors in estrus was 78% in both treatments, and the percentage of responding donors (>2CL) was 67.8±17.6% (G9) and 73.0±17.8% (G6, P>0.05). The numbers of follicles at first FSH with <3mm or >5mm were 11.7±3.9 and 0.8±0.2 for G9 and 12.5±4.3 and 1.2±02 for G6, respectively (P>0.05). The numbers of CL were 6.5±0.5 (G9) and 6.7±0.5 (G6), and did not differ (P>0.05). The number of follicles >5mm (r=0.38) were positively correlated (P<0.05) with ovulatory response. Overall, cervical transposition and uterine flushing were possible in 91% (31/34) of mated ewes, and did not differ between treatments (87 *vs* 94%). The time of cervical surpass and the total time of procedure were 4.7±0.6 and 24.1±1.7 minutes in G9 and 5.7±0.6 and 24.0±1.5 min in G6 (P>0.05). The number of recovered structures (5.6±0.6 *vs* 4.0±0.5) and viable embryos (2.8±0.5 *vs* 1.4±0.3) per ewe collected, did not differ (P>0.05) between G9 and G6. In conclusion, both treatments showed high variability in ovulatory response which might reduce the embryo yield average from donors. The protocol for cervical relaxation allowed the transcervical embryo recovery in high percentage of Lacaune ewes.
Financial support: EMBRAPA (Project 02.08.02.005.00.04) and Fapemig (Project CVZ-PPM 00201-17).

A090 OPU-IVP and ET

Follicular population and oocyte quality from Holstein x Gyr cows under heat stress in climatic chamber and supplemented with organic chromium

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Changes in energy metabolism were related as an important cause of losses change in productive and reproductive performance. It is observed that cows under heat stress have higher peripheral glucose metabolism, reducing the blood flow to the reproductive tract, impacting ovarian physiology. Chromium enhances the function of insulin, increasing its efficiency and optimizing the absorption of glucose by cells. This study evaluated the follicular population and the quality of oocytes from Holstein x Gyr dairy cows submitted to heat stress and supplemented with organic chromium in the diet. Were used thirty-six ¾ Holstein x Gyr cows, average 113 days in milk, in a 2 x 3 factorial design, two diets (control and diet with 0.08 mg Cr/kg metabolic weight) and three environmental conditions: heat stress in climatic chamber - temperature and humidity index (THI) 85 for eight hours daily, thermoneutral environment in free stall - THI 68 feeding ad libitum and pair-fed group in same thermoneutral environment, totaling 6 contemporaneous groups with 6 animals each. The cows were submitted to ultrasound evaluation and OPU while all the animals were in thermoneutral environment (first moment) and after being distributed under the three environmental conditions, in two days of collection, after three and six days (second moment). Fisher's exact test was used to evaluate the categorical variables and the significance level was $P<0.05$. No significant difference was observed in the follicular population in any of the effects studied (diet, environmental conditions and moment of collection), presenting a mean of 14.6 follicles in the assessments ($P>0.05$). The cows that underwent heat stress in the climatic chamber showed a significant difference in oocyte quality between the two moments of collection, with a higher proportion of grade 1 oocytes (60.68% *vs* 39.32%), lower proportion of grade 2 oocytes (49.38% *vs*. 50.62%) and degenerated oocytes (37.25% *vs*. 62.75%) in the first and second moment of collection, respective ($P<0.05$). The cows that received the control diet and suffered heat stress in the climatic chamber showed a significant difference in the oocyte quality between collections, higher proportion of grade 1 oocytes (72.73% *vs*. 27.27%) and a lower proportion of degenerated oocytes (35.71% *vs*. 64.29%) in the first and second moment of collection, respective ($P<0.05$). However, the animals treated with the diet with organic chromium that suffered heat stress, did not present differences in oocyte quality between these collections. The oocyte quality is closely related to the environment in which it is found. Increases in body temperature have direct and adverse effect on cellular function compromising their quality (Hansen, J Anim Sci, 80: 33-44, 2002). In this study, animals treated with chromium did not present reduced oocyte quality when submitted to heat stress. Thus, chromium was effective on maintaining a healthy follicular environment under heat stress.

A091 OPU-IVP and ET

Comparison of stress and animal welfare caused by the procedures of embryo collection by either surgical or non-surgical via in sheep

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In ovine species, embryo collection is commonly done by laparotomy (LP). However, this technique promotes adhesions in the reproductive organs leading to fertility impairment and affecting animal welfare. The non-surgical method, done by transcervical route (TC), although less invasive and expensive, can also affect animal welfare due to the cervix mechanical manipulation. In this perspective, biochemical markers of inflammation, as acute phase proteins, are considered reliable parameters of the systemic response to inflammatory processes. In addition, the measurement serum glucose can indicate high stress levels. Thus, this study aimed to determine the levels of acute phase proteins (total protein and albumin) and of stress (glycemia) in ewes submitted to either LP or TC embryo collection. Santa Inês ewes (n=27) were superovulated using the Day Zero protocol (Menchaca et al., Theriogenology, 68: 1111-17, 2007), followed by natural mating. Cervical dilation was induced in all ewes with estradiol benzoate i.v. (20 μg/mL; RIC-BE; Agener União, São Paulo, Brazil) and cloprostenol i.m. (0.12 mg; Estron; Agener União) 12 h prior to collection and oxytocin (100 IU, Ocitocina Forte UCB, Centrovet, Goiânia, Brazil), 15 min prior to embryo collection. A cervical transposition test was performed to define which method should be conducted. LP collection (n=11) was performed under general anesthesia (Lima et al., Animal Production Science, 56:1463-8, 2015). TC collection (n=16) was performed as proposed by Fonseca et al., Small Ruminant Research, 111: 96-9, 2013, with a circuit closed system. Blood samples were collected before fasting (M1) and of the sedation (M2), during collection (M3) and at various moments after collection: immediately (M4), and 1 h (M5), 3 h (M6), 6 h (M7), 12 h (M8), 24 h (M9), 48 h (M10). Data were evaluated by Kruskal-Wallis test at 5% of significance. LP and TC collection differed in serum glucose concentrations in M4 (LP: 72.7 ± 17.4 *vs*. TC: 102.2 ± 22.9 mg/dL), in M9 (LP: 64.0 ± 12.3 *vs*. TC: 55.1 ± 4.3 mg/dL) and in M10 (LP: 61.3 ± 11.8 *vs*. TC: 52.6 ± 4.1 mg/dL) ($P<0.05$). In M10, there were differences in albumin concentrations (LP: 1.9 ± 0.4 *vs*. TC: 2.1 ± 0.3 g/dL) and serum proteins (LP: 5.3 ± 0.5 *vs*. TC: 5.7 ± 0.4 g/dL) ($P<0.05$). In both methods, there was no effect ($P>0.05$) of the evaluation moment on serum proteins and albumin concentrations. In TC method, an increase in glucose in M3, M4, M5 and M6 ($P<0.05$) was observed compared to other moments. A similar pattern was observed in the LP method where the increase was concentrated in M3, M5 and M6 ($P<0.05$). In conclusion, the LP method induced a greater inflammatory response due to serum albumin drop 48 h after collection, and higher level of stress due to elevated serum glucose.

Acknowledgements: Embrapa (Project 02.13.06.026.00.02) and CNPq (400785/2016-1).

A092 OPU-IVP and ET

Peripheral resistance to insulin in Holstein repeat breeders cows (*Bos Taurus*) submitted to drying

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The aim of this study was to evaluate the peripheral insulin resistance (PIR) in repeat breeder (RB) Holstein cows (*Bos taurus*) submitted to drying on IVEP and pregnancy rate after FTET. Twenty-six cows were distributed into three groups: Initial lactation [IL; DIM<90 days; n=9; 27.9 ± 2.01 kg/day/milk; BCS = 2.86 ± 0.11]; End of lactation and repeat breeders [EL- RB; DIM> 300 days; n = 8; 19.4 ± 1.66 kg / day / milk; BCS = 3.22 ± 0.06] and dry repeat breeder cows [DC -RB; Dry period ≥60 days; n = 9; BCS = 3.24 ± 0.07]. For the glucose tolerance test (GTT), blood samples were collected after 4 hours of fasting at -20, -10 and 0 min, and an infusion of 0.3 g / kg of sterile glucose solution [50% of glucose (iv)] was performed at the 0 minutes mark during 5 min. At 5, 10, 20, 30, 40, 60, 80, 100 and 120 subsequent minutes, blood samples were collected to calculate the metabolism rate (k), and half-life (T½) of the plasmatic glucose. The insulin response (INS) after GTT was evaluated by the circulating insulin concentration and by the insulin increase ([INS] peak - [INS] basal; Δmax). To evaluate the quality of the oocyte, an OPU were performed in all follicles ≥ 3 mm in diameter at random phase of the estrus cycle. Statistical analyzes were performed by orthogonal contrasts [Contrast 1 (C1) = Lactation Phase (IL *vs* EL-RB + EL-P) and Contrast 2 (C2) = Reproductive Status (EL-RB *vs* EL-DC)] using logistics regression by SAS GLIMMIX PROC. No difference was found between the groups and the variables INS , k and T½ (min ; P> 0.05) . When the physiological status was compared the INS peak and Δ max (μUI / ml) were higher for dry cows than lactating cows [IL=70.3±10.9b + EL-RB =72.7±17.7b *vs* DC-RB=241±58.1a; P=0.0004] and [IL = 51 ± 9.3b + RB = 55.1 ± 16.6b *vs*. DC-RB = 219.6 ± 56.5a; P = 0.0003], respectively. Considering the physiological status, differences were observed between the groups for the variables insulin concentration in the interval between 5-120 minutes [IL = 4719.8 ± 595.1b + EL-RB = 4277.2 ± 631.8b *vs*. DC-RB = 8871.0 ± 1594.7a; P = 0.002]. The antral follicle count (AFC), recovered oocytes, viable oocytes and cleavage rate did not differ between groups (P>0.05). However, blastocysts rate and embryonic development were higher for dry when compared to lactating cows [C1; IL = 35.2%b + EL-RB = 34.4%b *vs*. DC-RB = 53.1%a; P = 0.003) and [IL = 42 %b + EL-RB = 45.6%b *vs*. DC-RB = 60.3%a; P = 0.02), respectively. No differences were observed in the pregnancy rate [IL = 37.5% (3/8); EL-RB = 21.4% (3/14); DC-RB = 33.3% (12/42); C1, P = 0.76 and C2, P = 0.44]. It was concluded that repeat breeder dry cows presented greater amount of insulin released in response to TTG, which contributed to the establishment of the PIR, although it has not been proven if there is differences in oocyte quality between dry and lactating cows.

Acknowledgements: APATA, WTA IVF, FAPESP 2015/19563-0.

A093 OPU-IVP and ET

In vitro produced embryos with 12 and 24 month old Nellore (*Bos indicus*) heifers treated or not with FSH

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This present study aims to evaluate the *in vitro* production of 12 (prepubertal) and 24 (pubertal) month old Nellore heifers and response to treatment with FSH with the objective of reducing the age of reproduction and the interval between generations to accelerate genetic gains of the herds. For this, 7 heifers aging 12 months and 10 heifers aging 24 months were submitted to two OPU (with an interval of 40 days; cross-over) in a 2 x 2 factor design [heifers aging 12 months without FSH (G12C); heifers aging 12 months with FSH (G12FSH); heifers aging 24 months without FSH (G24C); heifers aging 24 months with FSH (G24FSH)]. All heifers were synchronized receiving 2mg of estradiol benzoate (RIC-BE®, Agener União – Saúde Animal, São Paulo, SP), 0.530mg of cloprostenol sodium (Estron®, Agener União – Saúde Animal, São Paulo, SP) and one intravaginal progesterone device with 360mg progesterone on day 0 (Primer PR®, Agener União – Saúde Animal, São Paulo, SP). The heifers of the FSH-treated group received two doses of 30mg of FSH (Folltropin®, Agener União – Saúde Animal, São Paulo, SP) on day 4 (morning and afternoon) and two doses of 20mg FSH on day 5 (morning and afternoon). On day 7 (after a coasting period of 44 hours) the device was removed and each heifer was submitted to OPU (guide EC9-5 Novilha, WTA, Cravinhos, SP; ultrassom S8®, SonoScape, China). The oocytes were selected and send to IVF. For the fertilization of the oocytes, semen of the same bull and the same batch was used. Statistical analyzes were performed using the SAS® GLIMMIX procedure. No category and treatment interaction was observed for the studied variables (P>0.05), except for oocyte recovery rate. The 24 month old heifers presented higher follicular population (G24C: 33.5±4.0^{a} *vs*. G12C: 20.9±4.2^{b}; P=0.013) and more recovered oocytes (G24C: 26.1±3.5^{a} *vs*. G12C: 18.4±4.0^{b}; P=0.018) than 12-month-old heifers. The treatment decreased the oocyte recovery rate in both categories (G12C: 87%^{b}, G12FSH: 52%^{a}; G24C: 79%^{b}, G24FSH: 55%^{a}; P=0.04). The rate of viable oocytes was higher for 24-month-old animals (G12C: 64%^{b}, G24C: 74%^{a}; P=0.039). The 24-month-old heifers produced more embryos per OPU (G12C: 2.9^{b}, G24C: 8.5^{a}; P=0.002) than 12-month-old heifers. The treatment with FSH increased the rate of blastocyst production on total oocytes of prepubertal heifers (G12C: 18%^{b}, GT: 28%^{a}; P=0.035). At first, treatment with FSH improves the rate of blastocyst production in prepubertal Nellore heifers of 12 months, but it is necessary to increase the number of animal in this study to confirm this results.

Acknowledgements: IZ de Sertãozinho, Bovigênese, WTA, NeoGen, Cnpq.

A094 OPU-IVP and ET

Comparison of the *in vitro* embryos production of bovine (*Bos indicus*) treated with different concentrations of natriuretic peptide C (NPPC) in maturation or *in vitro* culture

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The aim of this study was to compare the effect of different concentrations of natriuretic peptide C (NPPC) supplementation in the medium *in vitro* maturation (IVM) or *in vitro* culture (IVC), comparing the rate of development of the bovine embryos produced *in vitro*. Ovaries (n = 524) from Nelore cows (*Bos indicus;* n = 262) were obtained from the slaughterhouse in 7 replicates and transported in saline solution at 35 ° C to the laboratory to obtain oocytes. After follicular aspiration, the cumulus-oocyte complexes (COCs) were selected (level I and II; n=3,125) and randomly divided into 7 experimental groups according to NPPC supplementation: a) Control (without supplementation; n = 438); b) 50 IVM (50 nM supplementation in IVM; n = 484); c) 100 IVM (100 nM supplementation in IVM; n= 508); d) 150 IVM (150 nM supplementation in IVM; n= 498); e) 50 IVC (50 nM supplementation in IVC; n= 351); f) 100 nM IVC (100 nM supplementation in IVC; n=430); f) 150 nM CIV (150 nM supplementation in IVC; n=416). After the selection the COCs were placed for *in vitro* maturation, but for the 50, 100 and 150 nM MIV groups the maturation medium was supplemented with NPPC throughout the IVM stage (24 hours). On day 0 (D0) all COCs were fertilized with sperm from a single bull previously tested. After the period of fertilization (D1) presumptive zygotes were denuded and cultured *in vitro*. On day 5 (D5) of the culture the probable zygotes of the 50, 100 and 150 nM IVC groups were treated with NPPC and remained with the supplementation until day 7 (D7). In D7 the embryo production rates were evaluated for comparison of the effect of NPPC supplementation at different times and concentrations. The results were compared by the logistic regression test in the Minitab® statistical program, version 18.1. Effect was considered significant when p value was ≤ 0.05. When comparing the blastocyst rate of the Control group (31.05%) with the 50 IVM groups (33.47%, P= 0.427), 100 IVM (35.24%, P= 0.165), 150 IVM (32.53%; P= 0.625); 50 IVC (28.49%, P= 0.439); 100 IVC (27.67%, P= 0.282); 150 IVC (26.92%, P= 0.192), there was no difference. Although blastocyst rates did not differ between groups, our data provide references to other research that seeks to improve the efficiency of *in vitro* production of bovine embryos.

A095 OPU-IVP and ET

Ethanolic extracts of cerrado plants on the oxidative stress of *in vitro*-produced bovine embryos

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The *in vitro* embryo culture induces an excessive production of reactive oxygen species (ROS) and affects blastocysts production. Medium supplementation with antioxidants agents in culture is an interesting alternative to minimize those effects. The present study evaluated the effect of ethanolic extracts obtained from cerrado plants on the oxidative stress of IVP embryos. Bovine ovaries from slaughterhouse were used to obtain grade I and II oocytes, which were submitted to maturation, fertilization (D0) and *in vitro* culture. Four groups were used in the culture: a control group subject to high oxygen tension (G20%), a group subject to low oxygen tension (G5%), besides two groups cultivated under high oxygen tension, supplemented with 0.01mg/mL of cagaite extract (GCag) and another group with 0.01mg/mL of murici extract (GMuri). Cleavage (D2) and blastocyst rates (D6 and D7) were evaluated. D7 expanded blastocysts were used to evaluate ROS, glutathione (GSH) and gene expression. ROS levels were analyzed by confocal microscopy using H2DCFDA (6-carboxy-20,70-dichlorodihydrofluorescein diacetate), and GSH on epifluorescence microscopy with Cell Tracker Blue (4-chloromethyl- 6,8-difluoro-7-hydroxyocoumarin). The transcripts level of genes involved in apoptosis (BAX, BCL21L, CASP3 and CASP8) and in the ROS metabolic pathways (SOD2, CAT, GPx4 and PRDX3) was determined by qPCR, with GAPDH used as a housekeeping gene. Data were analyzed by ANOVA and the means compared by Tukey test, at 5%. A total of 2135 oocytes were used and 893 embryos were produced (41.8%). Embryo production was similar (P>0.05) among G20% (cleavage: 87.6 ± 8.1%, D6: 26.5 ± 12.2% and D7: 42.7 ± 6.2%), GCag (89 ± 7.3%, 23.9 ± 10.3%, 43 ± 6.2%), GMuri (89 ± 9.5%, 26.7 ± 16% and 40.1 ± 8.4%) and G5% (88 ± 9.5, 26.7 ± 16, 40.1 ± 8.4). ROS and GSH emitted fluorescence were also similar (P> 0.05) among G20% (105.24 ± 26.04 and 156.36 ± 11.39), GCag (125, 92 ± 31.82 and 159.98 ± 10.89), GMuri (135.25 ± 29.05 and 155.36 ± 14.07) and G5% (116.05 ± 27.51 and 151.37 ± 17.45). The results showed that transcripts levels of apoptosis-related genes were similar (P>0.05) among the groups. However, genes involved in ROS metabolism were differentially expressed in the treatments. Abundance of GPX4 transcripts was higher (p <0.05) in the groups cultured with cagaite and murici than the G5% group, and PRDX3 transcripts was higher (p <0.05) in the GMuri group than the G5% group. The other transcripts analyzed (SOD2 and CAT) were similar among treatments (P>0.05). Supplementation of culture medium with extracts (0.01mg / mL) of cagaita and murici increased transcripts levels for genes related to antioxidant function (GPx4 and PRDX3), although it did not increased embryo production. Therefore, those extracts can be an alternative to reduce oxidative stress caused by IVP adverse conditions.

A096 OPU-IVP and ET

Peripheral insulin resistance in Holstein (*Bos taurus*) repeat breeder cows

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The aim of this study was to evaluate the establishment of peripheral insulin resistance (PIR) in Holstein (*Bos taurus*) repeat breeder (RB) cows and the effect on the *in vitro* embryo production (IVEP). Twenty-eight cows were distributed in three experimental groups: Initial Lactation [IL; DIM <50 days; n=9; 24.9±1.7kg/d of milk; BCS=2.9±0.07]; End Lactation and Repeat Breeders (EL-RB) [DIM>300 days; n=10; 17.2±0.9kg/d of milk; BCS=3.4±0.12] and End Lactation and Pregnant [EL-P; DIM>300 days; n=9; 19.6±1.3kg/d of milk; BCS=3.4±0.06]. For the glucose tolerance test (GTT), blood samples were collected after 4 hours of fasting at -20, -10 and 0 min, and an infusion of 0.3 g / kg of sterile glucose solution [50% of glucose (iv)] was performed at the 0 minutes moment during 5 min. At 5, 10, 20, 30, 40, 60, 80, 100 and 120 subsequent minutes, blood samples were collected to calculate the metabolism rate (k), and half-life (T½) of the plasmatic glucose. The insulin response (INS) after GTT was evaluated by the circulating insulin concentration and by the insulin increase ([INS] peak - [INS] basal; Δmax). An ovarium pick-up (OPU) were performed in a random day of estrus cycle to determinate oocytes quality and a US (Mindray® DP2200 Vet) evaluation was performed for antral follicle count (CFA). Statistical analyzes were performed by orthogonal contrasts [Contrast 1 (C1) = Lactation Phase (IL *vs* FL-RS + FL-P) and Contrast 2 (C2) = Reproductive Status (FL-RB *vs* FL-P)] using logistics regression by GLIMMIX PROC of SAS. Statistical differences were found considering lactation phase, for the variables area under the curve (AUC) between 5 and 120 minutes after infusion [IL=4367.8±927.1 *vs* RB=9524.5±1746.3 + EL-P=8047.6±1113.8; P=0.02]. Antral follicle count (CFA) was higher in cows at the beginning of lactation when compared to cows at the end of lactation [IL=21.3±6.0 *vs* EL-RS=13.6±4.3 + EL-P=10.8±2.2; P=0.03 (C1)] but the number of recovered oocytes, viable oocytes, oocyte recovery rate, and cleavage rate did not differ between groups (P> 0.05). A difference was found for blastocyst rate [IL = 10.0% *vs* EL-RB = 18.9% + EL-P = 22.8%; P = 0.01] and for embryo development rate [IL = 14.0% *vs* EL-RB = 41% + EL-P = 38.1%; P = 0.03] considering the moment of lactation. In conclusion, repeat breeder cows and pregnant cows at the end of lactation have higher insulin in response to TTG, evidencing the establishment of the PIR. However, poor oocyte quality was not demonstrated when compared to cows at the beginning of lactation.

Acknowledgments: FAPESP (2014/19460-4), Instituto de Zootecnia - Nova Odessa, SP, WTA FIV.

A097 OPU-IVP and ET

Effect of treatment with GnRH at the moment of embryos transfer on the conception rate of water buffalo recipients (preliminary results)

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Fixed Time Embryo Transfer (FTET) is an important technique for increasing reproductive efficiency of embryo recipients. The objective of the present study was to evaluate the effect of GnRH treatment at the time of FTET on the conception rate of buffalo embryo recipients. The hypothesis of the present study was that treatment with GnRH on the FTET day induces the formation of an accessory corpus luteum CL, increases the plasma concentrations of progesterone P4, generating embryos that have greater capacity for synthesis and release of Interferon-Tau (bIFN-τ; Mann et al., J Reprod Fertile Suppl., 54: 317-328, 1999), thus reducing embryonic mortality and, consequently, increasing conception rate (Vecchio et al., *Reprod Dom Ani* 45,614-618, 2010). This study involved 291 Murrah buffaloes, aged from 2 to 15 years, without fertility problems and with a good body condition score (BCS> 2.5). The experiment was carried out in 2 different farms: Estância Santa Olga (Road 81, Formosa, Argentina), and in the Ribeira Valley Regional Pole (Unit of Research and Development, Registro, São Paulo, Brazil). The recipients were synchronized with the following protocol: on day 0 the buffaloes received the insertion of an intravaginal device containing 0.5 g of P4 (DIB® 0.5; Syntex, Argentina), associated with i.m. application of 0.5 mg of estradiol benzoate (Syntex®, Argentina) and 0.53 mg of sodium cloprostenol (PGF2α; Cyclase DL, Syntex, Argentina). On day 9, the P4 device was removed and 0.53 mg PGF, 400 IU equine chorionic gonadotrophin (eCG; Novormon®, Syntex, Argentina) and 1.0 mg estradiol cypionate (Cipiosyn®, Syntex, Argentina) were applied (I.M.). On day 18 of the protocol (sixth day of the estrous cycle), an ultrasound evaluation (DP 2200®, Mindray; China) was performed. Buffaloes that had a CL> 14 cm (n = 168) received a vitrified embryo produced *in vitro* (IVEP). The rate of responsive animals to synchronization protocol was 64.8% for Estancia Santa Olaga (70/108) and 53.5% for the Regional Pole (98 / 183). At FTET, recipients were divided into two groups: GnRH Group (G-GnRH) which received 10 μg of acetate of gonadorelin i.m. (GnRH, Gonasyn GDR, Syntex, Argentina); and the Control Group (G-CONT), without treatment. After 30 days of FTET, a new ultrasound examination was performed to evaluate the conception rate of the recipients. Statistical analyzes were performed using the SAS® GLIMMIX procedure. No significant difference was observed between the conception rate of the two groups (G-GnRH: 20.9%, G-CONT: 14.8%, P = 0.31). In addition, there was no interaction of the quality of transferred embryos: blastocyst (BL), expanded blastocyst (EXBL), hatched blastocyst (HBL) (P = 0.78); farm (P = 0.73) and treatment / farm (P = 0.71). Treatment with GnRH at the time of FTET did not increase conception rate in buffaloes after FTET.

A098 OPU-IVP and ET

Lactation induction in dry repeat breeder hostein cows (*Bos taurus*) reduce peripheral insulin resistance

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The aim of this study was to evaluate the peripheral insulin resistance (PIR) in repeat-breeder (RB) Holstein cows *(Bos Taurus)* submitted to drying and subsequent induction of lactation (IL) on *in vitro* embryo production (IVEP) and pregnancy rate after FTET. A total of 34 cows were used, homogeneously distributed into four experimental groups: Early - physiological lactation [EPL; DIM<90d; n=9; 28.7± 1.4 kg/d of milk; BCS= 2.7±0.04]; Early lactation – IL [EIL; DIM<90d; n=9; 17.7±1.56 kg/d of milk; BCS= 3.0±0.04]; end of lactation - RB [EL-RB; DIM>300d; n=7; 16.7±1.26 kg/d of milk; BCS=3.0±0.08] and dry cows - RB [DC-RB; dry period >300d; n=9; BCS=3.7±0.09]. RB cows were characterized as not becoming pregnant after four or more AIs. For the glucose tolerance test (GTT), blood samples were collected after 4 hours of fasting at -20, -10 and 0 min, and an infusion of 0.3 g / kg of sterile glucose solution [50% of glucose (iv)] was performed at the 0 minutes mark during 5 min. At 5, 10, 20, 30, 40, 60, 80, 100 and 120 subsequent minutes, blood samples were collected to calculate the metabolism rate (k), and half-life (T) of the plasmatic glucose. The insulin response (INS) after GTT was evaluated by the circulating insulin concentration and by the insulin increase ([INS] peak - [INS] basal; Δmax). In order to evaluate antral follicle count (AFC) and oocyte quality, three OPUs were performed with a 30 day interval, at a random day of the cycle. Statistical analyses were performed using the GLIMMIX of SAS. No difference was found between the groups at the level of INS, k and T½ (P>0.05). The peak of insulin (P=0.0007) and Δ max (μUI/mL; P=0.005) was higher in DC-RB cows when compared to EPL and EIL but similar to EL-RB [DC-RB (318.5±44,6^{a} and 268.5±37,9^{a}); EPL (173.3±27,7^{b} and 132.2±28,8^{b}); ILI (181.1±26,0^{b} and 142.1±21,3^{b}) and EL-RB (238.3±42,0^{ab} and 197.7±41,9^{ab})]. Differences were observed between the categories for the INS concentration in the range of 5-60min [EPL=7486.3±1168.9^{ab}; EIL=6880.4±1066.8^{b} EL-RB=9416.9±1396.9^{ab}; DC-RB=11907.6±1954.4^{a}; P=0.05]. The AFC was higher in EL-RB and DC-RB cows when compared to EIL but similar to EPL (respectively 16.7±2.2^{a}, 18.1±2.6^{a}, 9.3±1.1^{b}, 14.2±1.6^{ab}, P=0.04). Statistical differences in the number of viable oocytes between the groups was found (EPL=5.0±0.7^{bc}, EIL=3.2±0.5^{c}, EL-RB=7.7±1.0^{ab}, DC-RB=8.0±1.4^{a}, P=0.02). The viable oocytes rate was higher in EL-RB and DC-RB cows when compared to EPL but similar to EIL (57.7%^{a}, 52.0%^{a}, 42.5%^{b}, 44.0%^{ab}, P=0.05). There are differences on blastocysts rates (EPL=15.0%^{ab}; EIL=8.4%^{b}; EL-RB=20.4%^{a}; DC-RB=17.8%^{ab}; P =0.03). No differences were observed in the pregnancy rate [EPL=45.0% (9/20); EIL=40.0% (2/5) EL-RB=41.4% (17/41); DC-RB=38.7% (12/31); P=0.81]. It was concluded that the lactation induction protocol decreased the RPI status, however it was not efficient in improvement in IVEP and pregnancy rate after FTET.

Acknowledgments: FAPESP (2014/19460-4), IZ - Nova Odessa, SP.

A099 OPU-IVP and ET

Effect of rbst treatment on fertility and milk production in lactation water buffalo (*Bubalus bubalis*)

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The objective of this trial was to evaluate the fertility and milk production during the entire lactation after treatment with bovine recombinant somatotropin (rbST - Boostin® - MSD Animal Health) in buffalo. This study was conducted at Paineiras da Ingaí farm in Sarapuí-SP, Brazil, in 95 lactating Murrah buffalo during the period of 12 months (from March, 2017 to April, 2018). The buffalo were homogeneously divided in two experimental groups: control, CON (n=48) and treated, rbST (n=47), according the lactation number [4,2±0,3 (EPM) lactations], entire production of previous lactation (3.395,1±91,9 L), occurrence of twin births (one in each group), weight at calve (620,89±76,90 kg) and days in milk to begin the treatments (60,8±3,7 dias). The rbST applications were subcutaneous each 14 days, in a total of 18.9±0,4 applications/lactation. The buffalo were submited to natural servisse at the proportion of one bull for 60 buffalo. An ultrassonografic ovum pick-up (OPU) was performed to an *in vitro* embryo production (IVEP) 80,7±4,6 days postpartum to evaluate the treatment effect on oocyte quality in 24 buffalo (CON=12 and rbST=12), and were used two bulls. The statistical analysis were performed using the PROC GLIMMIX of SAS. There was no effect of treatment with rbST in the days-conception period (CON=86,2±11,0 and rbST=88,1±11,5 days P=0,96) and on pregnancy rate at the end of lactation period [CON=97,9%(48/49) and rbST=95,9%(47/49), P=0,54]. Also, there was no treatment effect on the recovered quantity of complex cumulus-oocyte at OPU (CCO - CON=8,0±0,8 and rbST=8,3±0,9 oocyte/buffalo, P=0,51) and at IVEP (CON=1,7±0,4 and rbST=1,6±0,4 embryos/buffalo, P=0,25). The rbST treatment increased the daily milk average (CON=10.3±0.1 and rbST=12.2±0.2 L/buffalo/day, P<0.0001). To milk production curve, there was effect of time (P<0.0001) and interaction time-treatment (P=0.03). The rbST group increased the milk production in all the periods of lactation (P=0.03). Besides, there was effect in the total milk production on lactation (CON=3.119±111.3 and rbST=3.576±119.3 L/buffalo/lactation, P=0.02). The use of rbST did not presente effect on fertility, however, increased daily milk average and total milk production during the entire lactation of Murrah buffalos.

A100 OPU-IVP and ET

Effect of FSH and/ or rBST treatment on IVEP of Holstein calves (4 to 9 months of age)

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The aim of the present study was to evaluate the effect of FSH and/or rBST treatment on IVEP of Holstein calves (*Bos Taurus*). A total of 27 Holstein females were used, in which 22 were prepubertal and 5 pubertal. The prepubertal animals were distributed into four groups: Control group: (CTLG, n=6); Treated only with rBST group (BSTG, n=5); Treated only with FSH group (FSHG, n=6); and Treated with rBST and FSH (rBST+FSHG, n=5). The pubertal heifers (PHG) were included as a positive control for the laboratory. All the animals received a norgestomet auricular device (Crestar®, MSD Saúde Animal, São Paulo, SP, Brazil) and 1 mg IM of estradiol benzoate (Fertilcare Sincronização®, MSD Saúde Animal) at the onset of the protocol (D0). The animals of CTLG group received no additional treatment. The animals of BSTG received 500 mg IM of rBST (Boostin®, MSD Saúde Animal) on day -2 (D-2). The animals of FSHG received 140 mg IM of FSH (Folltropin®, Agener União – Saúde Animal, São Paulo, SP, Brazil), peformed in four injection twice a day on decreasing doses (40 mg [day 4 PM], 40 mg [day 5, AM], 30 mg [day 5, PM], and 30 mg [day 6, AM]; coasting period of 24 hours). The animals of BST+FSHG received 500 mg IM of rBST on D-2 and the same FSH treatment protocol mentioned above. The Heifers of PHG were not treated with any additional treatment. On day 7 the auricular devices were removed and the animals of all groups were submitted to an epidural anesthesia (2% lidocaíne) followed by ovum pick-up guided by transvaginal ultrasound (guide EC9-5 Heifer, WTA, Cravinhos, SP; ultrasound S8®, SonoScape, China). The recovered oocytes were sent to an IVEP commercial lab, and the embryonic development (cleavage and blastocyst rates) evaluation were performed. The oocytes were fertilized with sexed semen from two Holstein bulls (*Bos Taurus*). The obtained data were analyzed by the GLIMIX procedure of SAS®. No statistical differences were found between groups on number of blastocysts (P=0.7), number of cleaved oocytes (P=0.57), total recovered oocytes (P=0.11), blastocyst rates (on total oocytes, P=0.36) and cleavage rate (on total oocytes, P=0.3). On day 7 the FSHG group had more (P=0.006) medium follicles (14.2±3.09a) than BSTG, CTLG and PHG (1.1±1.20b; 1.5±1.18b e 1.3±1.16b, respectively) and the BST+FSHG had more (P=0.002) large follicles (2.5±0.67a) than the CTLG (0.1±0.31b). The BSTG oocytes recovery rate was lower (P=0.01) than CTLG (55.0%a *vs*. 70.2%b, respectively). The number of viable oocytes was greater (P=0.03) on FSHG (14.6±0.28a) than BSTG group (3.2±0.25b). Apparently, treatment with FSH, rBST or the association of both treatments had no influence on IVEP in this animal category; however, further studies with greater number of animals should be done in order to conclude these results.

Acknowledgements: CAPES; Bovigenese; WTA; Vitrogen; Laboratório de Pesquisa em Bovinos de Leite (USP).

A101 OPU-IVP and ET

Effect of different reproductive biotechnologies (AI, ET and IVF) on reproductive and productive performance of Holstein females

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The objective of this study was to evaluate the effect of different reproductive biotechnologies (AI, ET and IVF) on reproductive performance of lactating Holstein cows, and to evaluate the reproductive and productive performance of the female Holstein calves. The study was conducted at Santa Rita farm/Agrindus S.A. in Descalvado, São Paulo, Brazil. The reproductive data of the lactating cows that received contemporaneously these three biotechnologies during the years of 2013 to 2015 (AI=8382, ET=6381 and IVF=1503) were analyzed, and cows were submitted to the same conditions. In cows, the variables gestation length (GL) and retained placenta (RP) were analyzed, and in calves the mortality from birth to weaning (MBW; AI=659, ET=572 and IVF=90) and genetic merit (GM; AI=619, ET=545 and IVF=84) were analyzed through the Gestor Leite – CRV Lagoa program. Additionally, weight at birth and at weaning (WB and WW; AI=574, ET=504 and IVF=73) were analyzed in the calves and age at first conception (AFC; AI=552, ET=456 and IVF=61) and milk production on the first lactation (MP1L; AI=216, ET=151 and IVF=16) were partially analyzed. The data were collected during periods of heat (spring and summer) and of cold (autumn and winter). The data were analyzed by the PROC GENMOD and GLIMMIX of SAS. In the cows, GL differed according to the time of year in which it occurred (Heat=275.5±0.3^{A} and Cold=274.3±0.2^{B} days, P=0.045), and there was no difference according to the biotechnology (AI=275.0±0.3^{AB}; ET=274.4±0.2^{B} and IVF=276.4±0.6^{A} days, P=0.068). RP did not differ according to the biotechnology [AI=22.0% (145/659), ET=19.9% (114/572) and IVF=23.3% (21/90), P=0,546]. In the calves, MBW differed according to the biotechnology [AI=6.8% (45/659)^{AB}; ET=4.4% (25/572)^{B} and IVF=10.0% (9/90)^{A}, P=0.047], as well as the WB (AI=39.9±0.1^{B}, ET=39.8±0.2^{B} and IVF=41.7±0.5^{A} kg, P<0.0001), the AFC (AI=474.0±3,2^{AB}, ET=467.8±3.3^{B} and IVF=491.9±10.5^{A} days, P=0.060), and the GM (AI=9241.4±21.94^{B}, ET=9301.6±22.6^{B} and IVF=9497.7±57.3^{A} kg, P=0.0001). However, WW did not differ according to the biotechnology (AI=98.2±0.4, ET=98.8±0.4 and IVF=96.2±1.3 kg, P=0.565), as well as the MP1L (AI=10172.1±150.8, ET=10230.8±156.3 and IVF=11118.7±353.8 kg, P=0.163). As a conclusion, there was difference on the time of the year for GL and there was difference according to the biotechnology for GL, MBW, WB, AFC and GM. The RP, WW and the MP1L did not differ according to the biotechnology. At first, the biotechnologies might have influence on the reproductive performance. However, more data is needed to conclude the influence on the productive performance of Holstein females.

A102 OPU-IVP and ET

Influence of the animal category, follicular wave control and administration of exogenous FSH on oocyte recovery and *in vitro* embryo production in Holstein females

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Holstein females created in the subtropical climate regions present low efficiency in the number, quality and competence of the oocytes to produce *in vitro* embryos. The control of follicular dynamics associated with the administration of exogenous FSH can increase the number and improve oocytes quality to embryo production. Twenty-one Holstein female were used, 07 heifers (H), 07 dry cows (DC) and 07 cows in lactation (LC) in a randomized "crossover" of four treatments performed at 21 days interval: T1 – Follicular aspiration at random in any time of the estrous cycle; T2 – Follicular aspiration three days after dominant follicle removal; T3 – Follicular aspiration three days after dominant follicle removal and twenty-four hours after administration and of a single dose FSH (100 IU for heifers and 200 IU for cows); T4 – Follicular aspiration after administration of FSH at 200 IU for heifers and 400 IU for cows, subdivided into 8 decreasing doses, luteolysis induction with the sixth dose of FSH and LH peak induction with GnRH 36 hours after administration of PGF2a and follicular aspiration 26 hours after that the application of GnRH. The recovered oocytes were classified as viable immature (compact cumulus and homogeneous cytoplasm), atresic (compacted or expanded cumulus and irregular cytoplasm) or matured *in vivo* (cumulus expanded with the first polar body extrusion). The results were evaluated by analysis of variance by the F test. When the F value was significant at the 5% the Duncan test was applied to compare the means. Considering the three categories of animals, the highest average of oocytes retrieved (H=10.21, DC=8.39 and LC=6.71) and viable oocytes (H = 8.28, DC = 6.78 and LC = 5.61) were obtained with heifers. The dry cows had the highest average of embryo production (H = 1.28, DC = 2.86 and LC = 0.39), being $p < 0.05$. Analyzing the treatments, there was only significant differences ($p < 0.05$) in the variable viable oocytes, with T4 being higher average treatment (T1=4.52; T2=6.48; T3=7.38 e T4=9.19). The results showed that the dry cow category is more efficient to produce *in vitro* embryos, and that treatments with follicular wave control and FSH stimulation were insufficient to increase *in vitro* embryo production IN Holstein cows and heifers.

A103 OPU-IVP and ET

Metabolic profile and *in vitro* embryo production of primiparous canchim cows kept in areas of intensive grazing or silvipastoral

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In regions with a tropical climate, shading may determine greater animal comfort (Garcia et al, 2011), impacting on productivity. Thus, the aim was to study the influence of the silvopastoral system on the metabolic profile and the *in vitro* embryo production of primiparous Canchim cows. We used 18 donors, with 477.0 ± 12.0 kg of body weight and 26.2 ± 2.2 days postpartum, at the beginning of the experiment. The females were managed in two grazing systems: 1) with trees (n = 10; Silvipastoral-SP, eucalyptus trees at 15x2m spacing); 2) without trees (n = 8; Intensive Rotation-IR). The experiment was carried out at Embrapa Livestock Southeast, in São Carlos, SP, Brazil, from January to May 2017. The following procedures were performed monthly: black globe humidity index (BGHI) and heat load index (HLI) (P4), β-hydroxy butyrate (BHBA), glucose (GLU), non-esterified fatty acids (NEFA) and follicular aspiration (OPU). After counting the follicles observed in OPU (OF), the recovery rate (RR) was calculated. Cumulus oocyte (COC) complexes evaluated with degrees I to III were matured and used in PIVE, performed with semen of the same bull, of known fertility. The total number of oocytes (TO), number of viable oocytes (VO), D3 cleavage rate (CA), blastocyst rate in D7 (number of embryos / number of viable oocytes, BR). The discrete variables were analyzed by PROC GENMOD, while the continuous variables were PROC MIXED. The results were presented as mean ± SEM. BGHI and HLI were higher (P <0.01) in RI (83.2 ± 0.7 and 694.2 ± 11.3) than in SP (81.2 ± 0.7 and 610 ± 8, 9). Cows grazing in IR had a higher concentration of P4 than those in SP 1.4 ± 0.4 and 1.1 ± 0.2, respectively; P = 0.02), but the system did not influence the concentrations of NEFA (P = 0.65), GLU (P = 0.31) and BHBA (P = 0.09) during the experimental period. The OF (P = 0.72), RR (P = 0.73), TO (SP: n=1043, RI: n=788, P = 0.27), and VO (P = 0.97) were not different between grazing areas. CR (85.9 ± 2.6574/643] *vs* 82.8 ± 1.4[400/470], respectively, P = 0.04) and BR (43.0 ± 4.2[245/574] *vs* 36.6 ± 4.3[159/400], respectively; P = 0.04) was higher in cows in SP than in those in IR. It was evident in this study, the difference of the microclimate provided by the RI and SP areas. In this sense, the highest concentrations of P4 observed in cows in the IR area indicate a possible stress condition in this group of animals (Cooke et al, 2009). It was not observed, by the BHBA, NEFA and GLU dosages, a severe energy balance after calving, however considering the possible condition of thermal stress of the cows in IR, the embryo production was higher in the cows in SP.

Acknowledgments: Embrapa (Biotec Network, Pecus Network, Adapt +), Byoembryo and Vitrogen Laboratories, GS Animal Reproduction, FAPESP (#2015/26627-5), CAPES.

A104 OPU-IVP and ET

Associations between dinoprost tromethamine and deslorelin acetate for ovulation induction in Mangalarga mares

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The objective was to determine the efficiency of dinoprost tromethamine (DT; Lutalyse®, Zoetis, EUA) and its association with the synthetic analogue of GnRH, deslorelin acetate (DA;Sincrorrelin®, Ouro Fino, Brasil) in inducing ovulation of cyclic mares. *Mangalarga* mares (n=30), aged between 4 and 15 years and body condition score 5 and 6 (1 skinny – 9 overweight) were examined daily via transrectal ultrasonography. Endometrial edema 2 (0 no edema – 3 maximum edema), open cervix and a follicular diameter of at least 35 mm were minimum conditions of mare inclusion in this study. Estrous cycles (n=52) were randomly allocated to one of five treatments: 7.5 mg of DT (T1; n= 17); 1.0 mg of DA (positive control; T2; n= 10); 0.5 mg of DA (T3; n= 4); 1.0 mg of DA + 7.5 mg of DT (T4; n= 7) and 0.5 mg of DA + 7.5 mg DT (T5; n = 14). The animals were examined every six hours – Dominant follicle diameter and endometrial edema were recorded until ovulation and pregnancy diagnosis performed at 12 days post ovulation. Number of treatments performed (52) and number of gestational diagnoses were recorded (52). Data were analyzed by chi-square analysis, GENMOD procedure with the binomial option, SAS® (Cary - NC, USA). Ovulation rates (%) did not differ ($P>0.05$) among treatments 1-5 (65, 90, 75, 100 and 100), respectively. Effects of treatment, transitional period, pre-ovulation edema and edema at the time of induction were considered significant if $P<0.05$. The mean time-interval between induction and ovulation was 54.52 ± 5.19 h. Mean pre-ovulatory follicle diameter (39.71±0.23 mm) did not differ among treatments. There was no effect of treatment ($P= 0.67$) on gestation rate. Gestation rates were not affected by edema at the time of induction ($P= 0.66$) and pre-ovulatory edema ($P= 0.45$). The similar ovulation rates obtained in this trial, support the hypothesis that the associations between prostaglandin and GnRH analogues may sustain the ovulatory mechanism compared to the individual use of the latter. This complimentary effect may reduce the cost of synchronization protocols currently used which rely solely in the more expensive GnRH analogues. The results corroborate the active role of prostaglandins in the ovulation mechanism.

Development institution: National Council for Scientific and Technological Development (CNPq).

A105 OPU-IVP and ET

Use of the Center of Gravity methodology to analyze the dynamics of Brazilian embryo industry: preliminary results

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The Center of Gravity (CG) methodology has been used to evaluate the geographic mobility of crop production (DE MORI et al., Boletim de Pesquisa e Desenvolvimento, 37:6, 2007). In the current study, this tool was used to evaluate the geographic changes of cattle embryo production in Brazil. We hypothesized that the CG moves according to changes in embryo production in dairy and beef breeds. As a case study, we used data from the Brazilian Zebu Cattle Breeders Association (ABCZ), stratified by year, breed, and technology used (*in vitro* production [IVP] or *in vivo* derived [IVD]). The local of production was arbitrarily defined as the geographic coordinates (latitude and longitude) of the ABCZ offices were embryo transfer was reported. These coordinates were then used to calculate weighted averages for the spatial distribution of variables, and to define the CG. The coordinates obtained were compared within technology and period to obtain the distance and direction of changes in CG. Embryo production data from the years 2004, 2011, and 2016, which correspond to the embryo production growth cycles in beef and dairy, respectively (VIANA et al., Anim Reprod 14:476-481, 2017), were used. In 2004, the IVD CG was in the Triângulo Mineiro region, MG State. In the period 2004-2011, this CG moved 370.4 Km N to Goiás State. This change was mainly associated to the trends of the CG in the Nelore breed, which moved 450.8 Km N between 2004 and 2011. In the following cycle, IVD CG moved back a similar distance (380.5 Km) towards MG State, ending up in 2016 only 15.5 Km from its 2004 original position, however, during this period IVD embryo production in Zebu breeds decreased 91.9%. In the Gir breed, on the other hand, the CG moved NE and then E, and in 2016 was at 473.1Km of its original 2004 position, while still in MG State. Differently from IVD, IVP CG moved only 76.9 km S and 63.1Km NW in the two periods, respectively, oscillating in the Triângulo Mineiro – South of Goiás axis. In both Gir and Nelore breeds, changes in IVP CG were restricted within a 50 Km radius, centered in theUberaba City region and in border of MG and GO States, respectively. The lesserinfluence of embryo production cycles on IVP CG is possibly associated to the higherdependency of laboratory facilities, which is still concentrated in the Southwest region. On the other hand, the use of IVP as the technique of choice for embryo production inZebu breeds displaced IVD embryo Market to marginal areas. Our preliminary results show that the CG methodology is useful in the analysis of cattle embryo market trendsand in the construction of scenario.

Acknowledgements: Fapemig, FAPDF, Embrapa Projeto 01.13.06.01.002, PIBIC-CNPq.

A106 OPU-IVP and ET

DNA fragmentation in oocytes of Holstein x Gyr cows supplemented with organic chromium under heat stress in climatic chamber

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Heat stress may cause reversible or irreversible cellular damage, causing an adaptive response or cell death. Considering that apoptosis is important for the reduction of number of oocytes in mammals, it is possible that it plays an essential role in oocytes exposed to heat stress conditions (Paula-Lopes et al., Animal Reproduction,9:395-403, 2012). It is observed that cows under heat stress have higher peripheral glucose metabolism, a strategy for greater heat dissipation. Chromium enhances the function of insulin, acting as a cofactor and increasing its efficiency, aiding in the absorption of glucose by the cells, including the reproductive tract. The present study aimed to evaluate the DNA fragmentation of oocytes from Holstein x Gyr dairy cows submitted to heat stress in climatic chamber supplemented with organic chromium in the diet. It was used thirty-six ¾ Holstein x Gyr cows, average 113 days in milk, in a 2 x 3 factorial design, two diets (control and diet with 0.08 mg Cr/kg metabolic weight) and three environmental conditions: heat stress in climatic chamber - temperature and humidity index (THI) 85 for eight hours daily, thermoneutral environment in free stall - THI 68 feeding *ad libitum* and pair-fed group in same thermoneutral environment, totaling 6 contemporaneous groups with 6 animals each. The cows were submitted to OPU while all the animals were in thermoneutral environment (first collection) and after three days of being distributed under the three environmental conditions (second collection). The viable oocytes were matured *in vitro* and the "terminal deoxynucleotidyl transferase-mediated dUTP nick and labeling" (TUNEL) test was performed to determine DNA fragmentation, in which, TUNEL-positive: cells with DNA fragmentation; TUNEL-negative: cells without DNA fragmentation. Fisher's exact test was used to evaluate the categorical variable and the significance level was $P<0.05$. In the pre-heat stress collection, 40.51% (n=32) of oocytes were TUNEL-positive while 59.49% (n=47) were TUNEL-positive in the second collection ($P<0.05$). Evaluating only the second time of collection, which was caused the heat stress in a group in the climatic chamber, there was no difference between the different environments or between diets offered ($P>0.05$). However, comparing the diets offered in the two moments of collection, the cows that were fed with diet supplemented with organic chromium presented no difference in the percentage of DNA fragmentation: 43.18% (n=19) in first collection and 56.82% (n=25) in second collection ($P>0.05$). While those fed with control diet presented 37.14% (n=13) of TUNEL-positive oocytes in first collection and 62.38% (n=22) of TUNEL-positive oocytes in the second collection ($P<0.05$). Although there was no difference between the environments in the second collection, the dietary supplementation of organic chromium is a promising management in promoting provide protection against genetic material damage in dairy cows.

A107 OPU-IVP and ET

The collection technique and the stage of estrous cycle affect the recovery of good quality oocytes in domestic cats

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The use of biotechnologies such as in IVF and cloning are dependent of the adequate number recovery of good quality oocytes. It has already been shown that different collection methods can influence these parameters. Therefore, this study aimed to evaluate the effect of both: different techniques of oocyte collection and the stage of the estrous cycle on the quantity and quality of oocytes recovered in domestic cats. The study was performed at the Veterinary Hospital - UNIGRANRIO, in Duque de Caxias/RJ. After elective ovariohisterectomy, 43 pairs of cat ovaries were used, which were maintained in PBS for a maximum of 4 h and evaluated according to the estrous phase in: follicular, luteal or inactive (Uchikura, et al. J. Vet. Med. Sci., 73:561-566, 2011). The groups formed were PUN: (oocytes were recovered by puncturing follicles with 21 g needle), PUN + SLI (oocytes were recovered by slicing (SLI) the ovary that was previously PUN) or SLI (oocytes were recovered only by slicing). The oocytes were quantified and classified in Grade I, II, III and IV (Wood and Wildt, J. Reprod. Fertil., 110:355-360, 1997). The parametric variables were analyzed by ANOVA, followed by the Tukey test, while the non-parametric variables were analyzed by the chi-square test ($P<0.05$). A total of 974 oocytes (~ 23 oocytes/animal) was obtained. In the follicular phase, 476 oocytes were recovered, the technique of PUN + SLI (177/476) and SLI (244/476) recovered similar number ($P>0.05$) of oocytes and greater number than PUN (55/476), and there was no difference ($P>0.05$) in their quality. Regarding the stage of estrous cycle, 46.5% of animals/ovaries were in the follicular, 21.0% in luteal phase and 32.5% were inactive. There was no difference in the number of oocytes recovered. However, the inactive stage resulted in higher ($P<0.05$) number of good quality (Grade I and II) oocytes than luteal phase and the others (inactive *vs* follicular and luteal *vs* follicular) were similar ($P>0.05$). The results found in the present study indicate that the SLI technique recovers more oocytes, not affecting their quality and the inactive and follicular phases recover oocytes of better quality in cats. Therefore, to optimize the use of biotechnologies the stage of the estrous cycle and the collection technique used must be taken into consideration.

A108 OPU-IVP and ET

The effect of COCs morphology on bovine *in vitro* embryo production

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The potential of embryonic production can be estimated by the cumulus-oocyte complex (COC) morphology in the bovine *in vitro* embryo production (IVP). This paper presents the evaluation of the effect of the quality of COCs (through the evaluation of the COC morphology) on the efficiency in the IVP. The data of this study were obtained from the commercial laboratory of IVP - MÚLTIPLA EMBRIÕES LTDA (Ji-Paraná - Rondônia - Brazil), between March 2015 and October 2016. The COCs, obtained through the aspiration of ovaries from slaughterhouse, were classified according to their morphology in grade I: more than three layers of cumulus compact cells, oocyte with homogeneous ooplasm, filling the entire inner part of the zona pellucida; and grade II: when they had three or fewer layers of cumulus cells, and / or cumulus cells with small expansion, and / or ooplasm with slight granulation. Grade III COCs (oocytes without cumulus cells, and / or cumulus cells with expansion, and / or ooplasm with granulation, and / or ooplasm regressed with space between the cell membrane and zona pellucida) were not used in the laboratory tests. After classification, a total of 1,219 COCs were submitted to *in vitro* maturation (IVM), followed by *in vitro* fertilization (IVF) and after *in vitro* culture (IVC), at 38.8ºC, 5% CO2 and high humidity. The rate of cleavage of the fertilized oocytes was evaluated on the third day of embryonic development. Only those embryos with a minimum of four cells that did not have degeneracy characteristics were considered cleaved. On the seventh day of culture, the number of embryos produced (blastocysts), classified according to the International Embryo Technology Society (IETS), was evaluated and the blastocyst rate was evaluated in relation to the number of COCs submitted to IVP. Statistical analysis was performed through the statistical program SAS (1998). The rates of cleavage and blastocyst development were analyzed by the Chi-square test, considering a level of significance $P < 0.05$. Data from 13 procedures were used with 1,219 COCs subjected to IVP. Of these, 554 COCs were considered as grade I, and 665 COCs were considered as grade II. The grade I COCs obtained 58.12% (322/554) of cleavage rate while grade II COCs had 62.86% (418/665) ($P = 0.09$). The rate of blastocysts for grade I COCs was 38.81% (215/554) and for grade II was 43.16% (287/665) ($P = 0.12$). The grade II COCs tended to have a higher cleavage rate than the grade I COCs. There was no difference in the rate of blastocysts between the two classes studied, however, more detailed researches, on the aspects of COCs that interfere with the success of IVP, should be investigated.
Acknowledgment: PIBIC/CNPq.

A109 OPU-IVP and ET

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

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In the present study, the effect of forskolin (FSK) exposure time to induce lipolysis in bovine oocytes may improve the cryotolerance of *in vitro* produced embryos. Oocytes were aspirated from the ovaries of zebu cows obtained from a commercial slaughterhouse. Selected oocytes (N = 674) , were transferred in groups (20-25), to 90 µL IVM medium (TCM 199 with 1 mg/mL FSH, 5 mg/mL LH and 11 mg/mL pyruvate) containing or not 10% FBS, in the presence or absence of 50 µM forskolin (FSK) in the following groups, cultured for 24 h: +FBS (control, N = 167), -FBS (serum free IVM, N = 163) , +FBS/FSK (IVM with serum and FSK for 6h and then the next 18h without, N = 169), -FBS/FSK (IVM without serum, but with FSK for 6h and the next 18h without, N = 175). Five replicates were performed. IVM was carried out in an incubator with 5% CO_2 in air, at 38.5 °C and high humidity. These oocytes, from the different groups, were fertilized *in vitro* with frozen sperm from the single Nelore bull (*Bos Taurus indicus*) separated by Percoll gradient (Nutricell) and final concentration at $2x10^6$ sperm/mL. Presumptive zygotes were cultured in SOFaa medium plus 5 mg/mL BSA, 2.5% FBS and 0.11 mg/mL sodium pyruvate. The other part of the oocytes (N=210) was stained with 1 µg/mL Nile red to assess lipid content. The pictures obtained from the stained oocytes were taken on an epifluorescence microscope with a magnification of 20X and the fluorescence intensity was measured with the software Image J. software. The lipid content is presented as mean fluorescence intensity per μm^2 ($FI/\mu m^2$). For statistical analysis, the dependent variables were submitted to ANOVA by the least squares method using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC, USA). If the ANOVA was significant, the data were analyzed using the SEM and the level of significance was 5% ($P<0.05$). -FBS group (0.37 ± 0.03^b $FI/\mu m^2$) had higher lipid contents than ($P <0.05$) FBS (0.14 ± 0.01^a $FI/\mu m^2$), FBS/FSK ($0.14 \pm 0, 01^a$ $FI/\mu m^2$) and -FBS/FSK (0.15 ± 0.02^a $FI/\mu m^2$). Cleavage rates [FBS (83.8 ± 4.4 - 140/167), -FBS (81.6 ± 6.1 - 131/163), FBS/FSK (77.3 ± 5.0 - 130/169) and -FBS/FSK (73.6 ± 4.4 - 131/175)] and blastocyst development [FBS (46.4 ± 3.9 - 77/167), -FBS (45.6 ± 3,7 - 73/163), FBS/FSK (33.0 ± 6.6 - 54/169) and -FBS/FSK (31.1 ± 4.1 ± 54/175), were similar for all treatments ($P>0.05$). In conclusion, FSK only reduced lipid content when oocytes were matured in the absence of FBS. On the other hand, in the presence of serum, FSK had no lipolytic effect. Therefore, 6h of exposure of oocytes to FSK, but only without serum, is sufficient time to reduce lipids without harmful effects on subsequent embryo development.
Acknowledgments: Fapesp 2014/21289-1.

A110 OPU-IVP and ET

Cervical transposition test at estrus as a tool to select ewes for transcervical embryo collection

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The use of multiple ovulation and embryo transfer (MOET) program in sheep is limited by anatomical particularities of ovine cervix, making non-surgical embryo collection method (transcervical) more difficult or even impracticable. Therefore, the cervical morphology screening prior MOET becomes an interesting strategy. Thus, this study aimed to evaluate a cervical transposition method as a tool to select ewes able of being submitted to non-surgical embryo collection. Adult Santa Inês ewes (n=50) were superovulated using Day zero protocol (Pinto et al., Theriogenology, 113:146-52, 2018) followed by natural mating. The cervix transposition test was performed with Hegar dilator at estrus and at the embryo collection time. The latter test determined whether the ewe would be submitted to non-surgical or surgical embryo collection method. Prior the test, in both moments, ewes were sedated with acepromazine maleate i.v. (0.1 $mg.kg^{-1}$, Acepran, Vetnil, Louveira, Brazil) and diazepam i.v. (0.4 $mg.kg^{-1}$ Diazepam, Teuto, Anápolis, Brazil). The test at the embryo collection time was preceded by a hormonal protocol of cervical dilation based on estradiol benzoate i.v. (20 μg/mL; RIC-BE; Agener União, São Paulo, Brazil) and cloprostenol i.m. (0.12 mg; Estron; Agener União, São Paulo, Brazil) both administered 12 h prior to the moment of embryo collection, oxytocin i.v. (100 IU; Ocitocina Forte UCB, Centrovet, Goiânia, Brazil) administered 15 min prior to embryo collection and epidural anesthetic with ketamine (2.0 $mg.kg^{-1}$; Cetamin; Syntec, São Paulo, Brazil). A maximum of three attempts were performed to insert the Hegar dilator through the cervix. The test outcome was considered positive when Hegar dilator was successfully inserted through the cervix in any attempt, otherwise it was considered negative. Results of the first test were related to results obtained in the second test and classified as follows: true positive (TP, animals with positive results in both tests), true negative (TN, animals with negative results in both tests), false positive (FP, animals with positive result in the first but negative in the second test) or false negative (FN, animals with negative result in the first but positive in the second test). The cervix transposition test was evaluated calculating sensitivity (SENS), specificity (SPEC), positive (PPV) and negative predictive value (NPV), accuracy (Acc), and Kappa index (κ). The SENS, SPEC, PPV, NPV, and Acc were 85.7, 66.6, 85.7, 66.6, and 80.0%, respectively. Agreement between both test was considered moderate (κ = 0.52). The high SENS and Acc verified in the study demonstrated that cervical transposition test at the estrus using a Hegar dilator has the potential to be included in MOET programs as a screening strategy to direct ewes for a surgical or non-surgical embryo collection.

Acknowledgements: Embrapa (Project 02.13.06.026.00.02) and CNPq (400785/2016-1).

A111 OPU-IVP and ET

Docosahexaenoic acid (DHA) in the *in vitro* maturation medium reduces the lipid content of swine oocytes and embryos

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Previous results of our research group revealed that addition of 50.0 μM DHA to the *in vitro* maturation (IVM) medium improved the cleavage and blastocysts rates (Hoyos-Marulanda, et al., 2017. Anais XVII Congresso da Abraves 2017). Considering that DHA is able to reduce lipid content in other cell types, this experiment was designed to evaluate the effect of this polyunsaturated fatty acid on the lipid content of swine oocytes and embryos. Ovaries were collected from prepubertal gilts at a local slaughterhouse and follicles of 3-6 mm diameter were aspirated and, after being morphological selected, cumulus-oocyte complexes (COCs) were matured in medium supplemented with 50 μM DHA previously diluted in ethanol, and a control group with 10% of follicular fluid of pubertal sows. IVM was performed at 38.5°C with 5% CO_2 in air, for 44 h; with gonadotrophin supplementation only in the first 22 h. COCs were collected and denuded after 22 and 44 h of IVM. The remaining structures were parthenogenetically activated and cultivated until blastocyst stage. The lipid content of the structures (MIV 22 (n=195) and 44h (n=188) and D7 blastocysts (n=166)) was determined by the level of fluorescence emission obtained with the excitation of the Nile Red dye. The images were captured using G2A filter with 5.44 ms exposure. The images were analyzed by Image J® software, transforming the fluorescence intensity on a logarithmic scale. The response was compared among the treatments, within each category of structures, through analysis of variance with means comparison using the Fisher - LSD test (Statistix®, 2013). Swine oocytes matured in the IVM medium supplemented with 50 μM DHA presented lower concentration of lipid droplets after 22 h and 44 h ($P < 0.05$) of maturation. This reduction in lipid content was also observed in embryos at D7 ($P < 0.05$). Supplementation of IVM medium with 50 μM DHA reduced the lipid content of oocytes and blastocysts, which may be responsible for the improved rates of cleavage and blastocyst observed in previous experiments. Further studies should be carried out to investigate the mechanisms involved in the DHA action during IVM of swine oocytes, especially regarding to lipid metabolism and droplet formation.

A112 OPU-IVP and ET

Does the addition of the selective agonist of the peroxisome proliferator-activated receptor delta (PPARδ) L-165041 in bovine embryos produced *in vitro* improve cryotolerance?

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Aims: decrease the apoptoctic index and increase cryotolerance of bovine embryos produced *in vitro* with the addition of 1 μM of L-165041. Materials and methods: on day 1 (D1), presumed zygotes were cultivated with 1 μM of L-165041 (PPARδ selective agonist, Sigma, St. Louis, USA**)** n = 441, and without the agonist (control group) n = 450. The cleavage rate (%) was evaluated on day 2 (D2) and the development of blastocysts on day 7 (D7). Embryos before and after vitrification were fixed for the TUNEL trial. After vitrification, the embryos were heated and re-cultivated to evaluate the hatching rate at 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h and were frozen at 12 h of re-cultivated embryos for mass spectrometry (MALDI-MS). Statistical analyses of deviance were carried out considering generalized mixed linear models, and the effect of the collection day (block) was considered as random. For the count variables, the Poisson distribution and the log link function were considered. In the cases of variables represent by rates, binomial distribution and the logit link function were used. In the study of cryotolerance, analysis of variance of the hatching rate for each one of the times evaluated was carried out. In cases of significance of the effect of treatments, the Dunnett test was applied to compare treatments. Multivariate and univariate statistical models were used for analysis of MALDI-MS. All analyses were made using the GLIMMIX procedure of the SAS software (SAS Institute, Cary, NC, USA). Results: The cleavage rate and blastocyst development were not different among the groups. The number of cells per embryo was not affected ($P>0.05$) by the addition of 1 μM of L-165041 in fresh blastocysts. The total apoptosis rate decreased ($P<0.05$) in embryos before and after vitrification (5.92% and 10.10%, respectively) by the addition of 1 μM of L-165041 compared to the control group (9.62% and 18.56%, respectively). The apoptosis rate of the inner cell mass (ICM) in the embryos before and after vitrification (7.42 % and 51.35%, respectively) decreased compared to the control group (11.03% and 79.79%, respectively). The ICM rate of the fresh embryos in the L-165041 group (61.95%) increased compared to the control group (57.00%) ($P<0.05$). The number of cells per embryo in devitrified blastocysts increased ($P<0.05$), and the apoptosis rate decreased ($P<0.05$) from the addition by 1 μM of L-165041. The hatching rates at 48 h, 60 h, and 72 h after devitrification were greater (P^+ and [oxidized PC (36:1) + $H]^+$ were more abundant ($P<0.05$) in embryos cultivated with L-165041, and are considered positive biomarkers of cryotolerance. Conclusions: the addition of 1 μM of L-165041 in the culture medium decreased the apoptotic index and increased cryotolerance.
Acknowledgments: CAPES, FAPEMIG, PPGCV/UFLA, CENATTE EMBRIÕES.

A113 OPU-IVP and ET

Pregnancy rate of embryo senepol produced *in vitro*

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The Senepol breed stands out in Brazilian beef cattle breeding by the capacity of development, carcass quality, rusticity and adaptation to adverse conditions mainly of pasture and climate. Associated with these characteristics, the use of reproductive biotechniques, like IVP, which contributes to the expressiveness of the breed in the current scenario. The objective of this study was to evaluatete the embryo pregnancy rate Senepol IVP. For this, the pregnancy rate and the effects on the same of the location of the farm were evaluated (A: Amazonas State and B: Rondônia State), of the year (2016 and 2017), of the time of year (waters: december to march and dry season: april to november), the technician who performed the ET (B and C), the stage of development of the embryo and the number of ET already performed in the recipient (one, two or three-four). Percentage values were compared by Chi square whereas 5% of significance and processed for SAS. The pregnancy rate was of 43.3% (469/1083), being similar in the different locations of farms (43.09% - 374/868 and 44.1% - 95/215, in Amazonas and Rondônia States, respectively), years of the ET (43.2% - 269/622 and 43.20% - 200/461, in 2016 and 2017, respectively), in the season of the ET (42.7% - 313/733 and 44.6% - 156/350 for the seasons of the waters and dry, respectively) and the technician who performed the ET (41.1% - 166/404 and 44.6% - 303/679 for technicians B and C respectively) all considering $P>0.05$. However, a similar pregnancy rate was observed after ET in blastocyst and blastocyst expanded ($P<0.05$), being of 49.5% - 284/574, 43% - 71/165 and 28% - 29/103, respectively. The ET in morula and blastocyst initial have the pregnancy rate similar ($P>0.05$). The ET in blastocyst initial and blastocyst have the pregnancy rate similar (28% - 29/103, 35.3% - 85/241 and 43% - 71/165, respectively, $P>0.05$). The pregnancy rate was higher in receipts already used three to four times for this purpose ($P<0.05$), than in recipients used once or twice, which presented similar pregnancy rate ($P>0.05$), been 62.64% (57/91), 41.5% (297/715) and 41.5% (115/277), respectively. Under the conditions of this study, was conclude that embryo pregnancy rate Senepol IVP not influenced by the location of the farm, of the year ET, of the water or dry season or the technician. However, the pregnancy rate was higher when the ET was performed with more advanced stages of embryonic development, for recipients already used for three to four times.

A114 OPU-IVP and ET

Antioxidant effect of essential oil of *Syzygium aromaticum* on *in vitro* nuclear maturation of bovine oocytes

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In vitro production (IVP) of bovine embryos presents a variable efficiency and the oxidative stress is cited as one of the reasons in this variation. In this sense, the use of natural antioxidants during culture can be an alternative to minimize the effects of this phenomenon. Therefore, the aim was to evaluate the antioxidant effect of the essential oil of *Syzygium aromaticum* (EOSA) on the *in vitro* nuclear maturation (IVM) rates of bovine oocytes. The main constituent of EOSA is eugenol. Then, cow's ovaries were collected from slaughterhouses and transported to the laboratory. The *cumulus*-oocyte complexes (COCs) were aspirated and oocytes classified with more than one layer of *cumulus* cells and homogeneous cytoplasm were matured *in vitro* in TCM199 medium containing 20 µg/mL FSH/LH, 10% fetal bovine serum, 1% antibiotics and with antioxidants, according to the five groups: EOSA0 (absence of EOSA), EOSA 10 (10 µg/mL of EOSA), EOSA15 (15 µg/mL of EOSA), EOSA20 (20 µg/mL of EOSA) and CYS (100 µM of cysteamine). The dilution of EOSA was performed with 0.25% DMSO in IVM medium. After 24 h of IVM (38.5°C and 5% CO_2), denuded oocytes were evaluated for maturation according to the presence of the first polar body and for the antioxidant effect of oil and cysteamine by the labeling with H_2DCFDA (5 µM) and MitoTracker Red (500 nM) to quantify the levels in units of arbitrary fluorescence of reactive oxygen species (ROS) and mitochondrial membrane potential (ΔΨm), respectively. Then, matured oocytes were visualized under a fluorescence microscope and images were analyzed with ImageJ software. All data were expressed as mean±standard error with nuclear maturation rates and ROS and ΔΨm levels being analyzed by the chi-square test and ANOVA followed by Tukey's test, respectively ($P<0.05$). After twelve replicates, no difference was observed in the IVM rate [EOSA0: 69.1%±3.4 (114/165), EOSA10: 74.7%±4.7 (124/166), EOSA15: 66.2%±3.8 (102/154), EOSA20: 66.9%±5.4 (105/157) and CYS: 66.1%±4.3 (109/165)]. Moreover, although a numerical reduction can be observed in all groups containing the oil or cysteamine, no difference was observed for ROS levels [OESA0: 1.00±0.14, OESA10: 0.89±0.08, EOSA15: 0.74±0.07, EOSA20: 0.86±0.09 and CYS: 0.76±0.04]. Nevertheless, when evaluating ΔΨm, oocytes derived from the EOSA15 (0.73±0.01; P=0.004), EOSA20 (0.72±0.03; P=0.001) and CYS (0.80±0.06; P=0.04) groups showed significantly lower ΔΨm when compared to EOSA0 group (1.00±0.04). Additionally, EOSA20 oocytes showed significantly lower ΔΨm when compared to EOSA10 (0.94±0.00; P=0.02). Thus, it is suggested that reduction of ΔΨm in oocytes matured in the presence of EOSA15 and EOSA20 can reduce the levels of ROS. Therefore, it can be indicated that EOSA at concentrations of 15 and 20 µg/mL promoted a reduction of ΔΨm similar to the effect promoted by cysteamine in matured oocytes, and EOSA could be used during IVP in cattle.

A115 OPU-IVP and ET

Embryos production from bovine oocytes matured *in vivo* using intrafollicular transfer of immature oocytes (TIFOI)

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Intrafollicular transfer of immature oocytes (TIFOI) provides an entirely *in vivo* environmental condition and may be a good alternative to produce embryos in cattle. However, several aspects of the technique have yet to be established. The aim of this study was to evaluate if the time in which the oocytes remain in the follicle after the TIFOI is enough to allow the oocytes a complete maturation, fertilization and embryo development. Ovulators cows were submitted to a standard estrus synchronization protocol, using a vaginal progesterone device, estradiol benzoate and prostaglandin. On D8, the progesterone device was removed and 52 hours later animals with had a dominant follicles greater than 10 mm was subjected to TIFOI A total of 1297 imature oocytes were obtained from slaughterhouse ovaries, in which 609 were used for TIFOI and the remainder was used as control. In the control group, the oocytes were placed in IVM and IVF was performed at 12, 16 and 22h of culture. For TIFOI 30 to 50 oocytes were transferred to ovulator cow, which at 12 h post-injection were recovered by ovum pick up (OPU). The recovered oocytes were divided into three groups: one was submitted to IVF immediately after aspiration (12h after TIFOI), and the remaining oocytes were placed in IVM for another 4 and 10 hours prior to IVF (16 and 22h after TIFOI). Oocytes and sperm were co-incubated for 12 h and the possible zygotes were transferred to culture drops, where they remained until day 7 (D7). Treatments and number of oocytes per treatment were: control 12h (n=223); control 16h (n=229); control 22h (n=236); TIFOI 12h (n=239); TIFOI 4h (n=185); TIFOI 22h (n=185). The cleavage (D2) rate, blastocyst rates (D6 and D7) and apoptotic index in D7 embryos (Terminal deoxinucleotidyl transferase dUTP nick end labeling [TUNEL]) were evaluated. The cleavage and blastocyst rate data were analyzed by Chi-square test ($P<0.05$). The total number of cells, apoptotic index and ratio between the two were analyzed by ANOVA and Tukey's test ($P<0.05$). The blastocysts rate on D7 was similar ($P>0.05$) among the control groups (control 12h=35.4%, control 16h=36.7% and control 22h=40.7%), as well as among TIFOI groups (TIFOI 12h=18.8%, TIFOI 16h=17.3% and TIFOI 22h=21.6%). The blastocysts rate on D7 was higher ($P<0.05$) in the control groups than in the TIFOI groups. Regarding the total cells number, there was no difference ($P>0.05$) among all groups. The percentage of apoptotic cells in the TIFOI 22h group (4.1%) differed ($P<0.05$) only from the control 12h (7.2%) and control 16h (7.1%) and did not differ from the others ($P>0.05$) (control 22h=6.3%, TIFOI 12h=5.1%, TIFOI 16h=6.3%). Despite the lower embryonic development, observed in the TIFOI group, it can be concluded that the time of 12 hours is sufficient for the oocytes to be ready to be fertilized and to develop to blastocyst stage. It seems that the time after the injection to fertilization is not the main obstacle for the embryonic development.

A116 OPU-IVP and ET

Characterization of expression pattern of genes involved on the activity of PGE2 and PGF2α in bovine COCs with different levels of competence during *in vitro* maturation

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During the preparation of the follicle for ovulation and during maturation of the cumulus-oocyte complexes (COC) an increase of PGE2 and PGF2α levels occurs in the follicular fluid of several species, suggesting that these substances play an important role in those processes. Studies have reported that oocytes from large follicles have greater developmental capacity than oocytes derived from small follicles, resulting in high rates of *in vitro* embryo production. The aim of this study was to determine the level of transcripts for genes involved in the activity of PGE2 and PGF2α and to evaluate whether their expression profile varies during maturation according to the competence level of the COCs. The ovaries were collected in local slaughterhouses and the COCs were obtained from follicles of 1.0-2.9 mm (incompetent oocytes; INC) and 6.0-8.0 mm of diameter (competent oocytes; COM) by dissection, and from aspirated 3-8 mm diameter follicles as the control (CTL). The expression pattern of the PTGS2 (PGE2 and PGF2α synthesis), PTGES1 (PGE2 specific synthase) and AKR1B1 (PGF2α specific synthase) were evaluated in cumulus cells (CCs) and PTGER2 (PGE2 specific receptor) and PTGFR (PGF2α specific receptor) in oocyte from COCs of different categories. For each group four pools of CCs obtained from 17 COCs and three pools of 6 oocyte were used for gene analysis by RT-qPCR.The expression values were normalized using GAPDH for CCs and PPIA for oocytes as constitutive gene data of the gene expression were analyzed by analysis of variance and Tukey's test or by Kruskall-Wallis and Mann-Whitney, if they presented normal or non-normal distribution, respectively. Initially, levels of transcripts of genes before and after IVM were compared in CCs of the different groups of COCs, the results showed that after 24 hours of IVM the expression of PTGES1 increased ($P<0.05$) in the INC and COM groups, and that of PTGS2 in the INC group. Regarding the AKR1B1 gene the expression level decreased in the INC group ($P<0.05$). When the expression of genes was compared between CCs from the different groups of COCs at the same time of maturation (0 or 24 hours), the level of transcripts of all genes evaluated were similar between groups ($P>0.05$), both at 0 and 24 hours of IVM. With the exception of the PTGS2 gene, which presented lower expression in the COM group ($P<0.05$) than the INC and CTL groups at 24 hours. Subsequently, the expression of the PTGER2 and PTGFR genes was quantified in oocytes from the three groups of COCs, however they were not detected. Based on the results it can be concluded that although the specific receptors of PGE2 and PGF2α are not expressed in oocytes, genes related to the synthesis of these PGs are differentially expressed during maturation and the PTGS2 gene showed to be a good marker for competence of mature COCs.

A117 OPU-IVP and ET

Effect of embryo stage in pregnancy rate of nelore and wagyu *in vitro* produced embryos

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Among commercial reproductive biotechnologies, *in vitro* production technique (IVP) is an important tool for multiplying superior merit genetic material. Instability in the success of IVP embryo transfer is still a point to be adjusted. Thus, the objective was to evaluate the effect of the embryo development stage on the conception rate (CT) of embryos *in vitro* in Nelore and Wagyu breeds. A retrospective study of IVP data (June/17 to April/18) of pure embryos of Nelore (*Bos indicus*) and Wagyu (*Bos Taurus*) breeds was carried out, and the recipients were crossbred. The results of 1632 embryo transfers (677 Nelore and 952 Wagyu). The IVP was performed in the laboratory by viable oocyte maturation (22-24h) in TCM 199 (10 % SFB, FSH 0.1µg / mL and LH 50µg / mL); the *in vitro* fertilization in TALP-IVF medium (22-24h) with 1x106 spermatozoa/mL of Nelore or Wagyu pure bulls, each to fertilize the oocytes of the corresponding race. Cultivation in SOF medium (10% FBS and 5mg/ml BSA) at 38.3°C, maximum humidity (5% O2, 5% CO2 and 90% N2). On day 7, viable embryos were classified in developmental stages: initial blastocyst (Bi), blastocyst (Bl), and expanded blastocyst (Bx); and all viable embryos of these stages were innovated in the recipients. After 30 days of fertilization, the gestation diagnosis was performed to determine the conception rate. The chi-square test was applied in each breed to assess the homogeneity of Bi, Bl and Bx ratios, and also between breeds. Total CT was similar in Nelore 36.63% (248/677) and in Wagyu 33.93% (323/952). In the evaluation of the embryo stage the Nelore CT was all different - Bi: 17.44% (15/86); Bl: 34.55% (114/330); Bx: 45.59% (119/261). In the Wagyu embryos CT also differed in the stages -Bi: 13.54% (26/192); B1: 34.68% (155/447) and Bx: 45.37% (142/313). The comparison between the races did not reject the hypothesis of different conception rates (p-value = 0.1991), indicating that the Wagyu breed follows the same pattern of successful design of embryos produced *in vitro* as the Nelore breed. In summary, in the statistical analyzes of the embryonic stages for both races the hypothesis of homogeneity (p-value <0.001) is rejected, indicating that there was a significant difference between the embryonic stages in both races. Thus, the chance of gestation is 1.9 times higher in the embryo at the blastocyst stage, reaching 2.5 times higher in embryos in the expanded blastocyst stage, when compared to the initial blastocysts. Therefore, to obtain regularity in the CT of an IVP program, the ideal methodology would be prioritize the embryos transfers in stages of more advanced blastocysts, Bl and Bx. This criterion reduces the initial gestational losses in recipients by increasing the efficiency and sustainability of production.

A118 OPU-IVP and ET

Blastocyst and pregnancy rates in bovine IVP in relation to breed, age and physiological state of donors

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The aim of this study was to evaluate effects of breed, physiological state and age of oocyte donors on blastocyst production (BlxR) and pregnancy (PxR) rates after embryo transfers (ET). The breeds of donors from Nelore (NE) (n= 114), Gir (GI) (n= 116), Girolando (GO) (n= 61) and Senepol (SE) (n= 82) breeds with different physiological states (heifers, non-lactating cows, lactating cows for up to 100 days, 101 - 205 days and over 205 days) and age groups (0 - 12 months, 13 - 24 months, 25 - 36 months, 3 - 8 years and more than 8 years) were used. The blastocyst rates were assessed 168 hours after insemination; the IVP procedures were carried out in Embriza laboratory (Campo Grande, Mato Grosso do Sul, Brazil) and the media were produced by Cenatte Embriões laboratory (Pedro Leopoldo, Minas Gerais, Brazil). A total of 1274 ET were carried out between March and August of 2017. The PxR was confirmed by ultrasonography 45 days after the transfer. The effects of breed, physiological state and age on BlxR and PxR were tested by analysis of variance and the means were compared by SNK test ($P<0.05$). The effects of the interactions between breeds and physiological states were similar for NE, GI and GO breeds. There were, however, differences between others breeds and the SE. Effects of breed and physiological state, on BlxR and PxR were detected ($P<0.05$). There was no effect of age on either BlxR or PxR. The BlxR and PxR results obtained from NE (36.8±26.7 and 49.8±32.2), GI (34.2±28.1 and 55.6±35.5) and GO (32.0±27.3 and 38.05±27.8) respectively, were superior to those obtained from SE (25.1±24.4 and 34.4±33.8). Regarding physiological state, the non-lactating and up-to-100-day lactating cows showed superior BlxR results (40.7±28.4 and 37.2±25.5, respectively), whereas the results for heifers, 101-to-205-day and over-205-day lactating cows were inferior (25.5±25.1, 28.2±24.5 and 23.6±27.8, respectively). The PxR means according to physiological state were superior in cows lactating for over 205 days (55.6±33.1), while heifers exhibited the lowest averages (32.9±32.7). The other categories exhibited average values and did not differ among the groups. The inferior performance of SE (*Bos Taurus taurus*) oocyte donor cows in comparison to the zebuine breeds (*Bos indicus*) (NE e GI) or *Bos taurus indicus* (GO) has been confirmed in relation to BlxR and PxR in IVP programs. Due to their better performance, non-lactating or up-to-100-day lactating cows are recommended to improve BlxR in IVP programs. More studies are needed to confirm the use of cows lactating for over 205 days as donors in order to obtain superior pregnancy rates after ET.

A119 OPU-IVP and ET

Effect of Progesterone Supplementation on the Conception and Resynchronization Rate of Nelore Breed Embryo Recipients

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The *in vitro* embryo production of bovine has been one of the main methodologies used to multiply animals with superior genetic merit. The goal of this trial was to study the effect of progesterone (P4) and the resynchronization of Nellore recipients, two experiments were performed. In experiment 1, multiparous cows of the Nelore breed (n = 396) were initially submitted to a conventional protocol for fixed-time embryo transfer (TETF), they received first-use CIDR plus 2 mg of estradiol benzoate in D-11. In D-8, all animals received 12.5 mg of dinoprostrometamine, 0.5 mg of estradiol cypionate, 300 IU equine chorionic gonadotropin, and withdraw of the implant. In D8, the recipients were evaluated by ultrasonography (U.S.) and TETF. For the control group (T1) (n = 132) the animals did not receive any type of progesterone supplementation. In contrast, the treatment group (T2) (n = 137) in the TETF received a previously used CIDR device, remaining for thirteen days, in order to evaluate the effect of progesterone supplementation of the animals. The diagnosis of gestation (DG) for both groups were performed in D31. The results showed that there was no effect (P>0.05) of the P4 supplementation in relation to the conception rate (control 37.9%, treatment 39.7%), as well as for CL diameter (control 17.5 ± 3.2mm , treatment 18.1 ± 3.4mm). Experiment 2 was performed using the animals with negative DG from the previous experiment, evaluating the hypothesis of resynchronization of the animals that received the implant for thirteen days, in an attempt to make them for a second TETF. The formation of the control group, coming from the negative result of the T1 of experiment 1 (n = 69), in the same direction, for the formation of the animals of the treatment group, came from the T2 group of experiment 1 (n = 74). Thus, only the recipients who presented LC received a new embryo. The DG for experiment 2 was performed 23 days after TETF2. There was no statistical difference between the two groups for conception rates (T1: 22.2% *vs* T2: 35.7%, P>0.05), recipient use, and LC diameter. However, there was similarity in conception rate between conventional TETF of experiment 1 (38.8%) and resynchronization protocol in experiment 2 (35.7%). This result shows that resynchronization may be an innovative strategy for the application of current TETF programs.

A120 OPU-IVP and ET

In vitro production of bovine embryos at low oxygen tension increases the rate of blastocyst hatching

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The aim of this study was to evaluate the effect of different oxygen tensions on *in vitro* production of bovine embryos. The ovaries were collected from local slaughterhouse and transported to the laboratory in 0.9% NaCl solution plus antibiotics at a temperature of 35-37°C. Follicles 3-8 mm in diameter were punctured and the cumulus-oophorus complexes (COCs) recovered were classified according to the appearance and distribution of cumulus cells and cytoplasmic uniformity. Only the COCs classified as Grade 1 and Grade 2 were used. After selection, the COCs were divided into two groups: HIGHO2 (HIGH oxygen tension - 20% O2) and LOWO2 (LOW tension of O2-5%), matured *in vitro* (IVM - MIV - TCM 199 + 10% de FCS + Sodium pyruvate + FSH + LH + amikacin. Matured oocytes were fertilized *in vitro* with semen prepared using Percoll and were fertilized (HTF + BSA + Sodium pyruvate + Caffeine, Heparin, Penicillamine, Hypotaurine, Lepinephrine, Amikacin). Presumptive zygotes were cultured *in vitro* (SOFaa +hmyo-inositol+Sodium Pyruvate+BSA+ FCS + Amikacin) according to the treatments, where they remained for 9 days. All steps of *in vitro* embryo production (PIVE) were performed at LOW or HIGH O2 tension. In this experiment 11 replicates, in a total of 562 oocytes, were performed and in each of them the two groups were tested simultaneously. The variables evaluated were D3 cleavage rate (n° cleavage/COCs), blastocyst rate in D7 (n° blastocysts/COCs) and hatch rate in D9 (n° hatched/ n° blastcysts), all expressed as percentage. The percentages were transformed using sine-arc. The data were submitted to analysis of variance (ANOVA) using the statistical program R. There was an effect ($P<0.05$) of the oxygen tension in the cleavage rate (88.4% HIGHO2 (252/282) *vs*. 78.5% LOWO2 (226/280). On the other hand, there was no effect ($P>0.05$) on the rates of blastocysts (31.2% HIGHO2 (89/282) *vs*. 27.1% LOW2 (78/280). However, the D9 hatching rate was higher ($P<0.05$) at the LOW O2 tension (31% HIGHO2 (35/113) *vs*. 43.6% LOWO2 (44/101)). It was concluded that IVP performed in incubator with LOW oxygen tension generated a lower cleavage rate but a higher hatching rate of blastocysts and therefore better embryos quality.

A121 OPU-IVP and ET

Gene expression analysis of *in vitro* produced bovine embryos cultured in uterine mesenchymal stromal cells conditioned media

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There is evidence that conditioned medium (CM) obtained through mesenchymal stromal cells (MSCs) culture contains proteins with tissue remodeling activity and also improves cell proliferation rate. Embryo culture system has a dramatic effect on the mRNA expression of several important genes for embryonic development as OCT4 and CDX2 and genes related to apoptosis as HSP70 and BAX. This study aimed to evaluate the rate of gene expression of HSP70, BAX, OCT4 and CDX2 genes in bovine embryos produced *in vitro* in different culture media. MSCs derived from bovine endometrium were obtained by enzymatic digestion. Cells were plated and cultured in medium composed of 80% DMEM/F12, 20% FBS, antibiotics and antimicotic until 70% confluence. To obtain the CM, bottle was washed five times with PBS and the culture medium replaced with medium without FBS for 96 hours. The CM obtained was centrifuged, filtered and stored at -80ºC. Cumulus-oocyte complex (COC) (n=20/drop) of grade I and II obtained from slaughterhouse ovaries were selected in Dulbecco's modified PBS containing FBS and transferred to TCM HEPES. COCs were matured *in vitro* with TCM199 with pyruvate, FSH, antibiotics and 10% of FBS. IVM was performed in petri dishes with 90 µL droplets, covered with mineral oil at 38.5ºC and 5% CO2 in humidified air for 24 hours. IVF was performed in FIV-fert in a concentration of $2x10^6$ sperm/ml. Presumptive zygotes were cultured in SOFaaci (supplemented with antibiotics and amino acid) with the respective addition of 0,5% BSA (BSA), 0,5% BSA + 2,5% FBS (FBS) and 0,5% of BSA + 20% CM (CM). The parameters analyzed were embryo development and temporal expression of genes related to embryo quality (HSP70, BAX, OCT4 and CDX2). Data were normalized using the reference gene PPIA and analyzed by analysis of variance (ANOVA). Tukey test was used to compare the means. Groups BSA (n=223) and FBS (n=198) showed a higher (P=0.0014) blastocyst production rate compared to group CM (n=265), 28, 42 and 16% respectively. The results of gene expression were obtained from 5 replicates (3 expanded blastocyst/pool) from each group. Genes evaluation showed the same (P>0.05) expression pattern in the three different groups. With the presented results it is possible to conclude it is feasible to produce bovine embryos in culture medium without FBS. Moreover, the CM produces similar quality embryos compared with FBS.

A122 OPU-IVP and ET

Effect of histone deacetylase inhibitor during pre-maturation and/or *in vitro* maturation of bovine oocytes on embryonic development

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The mRNAs stocks present in the oocyte at the time of its removal from the follicle is associated with its developmental competence. Considering that high acetylation allows gene transcription, we hypothesized that the presence of a deacetylase inhibitor prior to IVM would allow a greater accumulation of RNA improving oocyte competence. The objective of this study was to evaluate the effect of Scriptaid, a histone deacetylase inhibitor, during pre-IVM (PMIV) and/or IVM in the *in vitro* embryos development in bovine. Cumulus-oocyte complex (COC's) were obtained from slaughterhouse ovaries and were submitted to PIVM for 6 h using 100nM of C-type natriuretic peptide (NPPC), in the presence or absence of 500nM of Scriptaid. COCs were divided into 5 groups: T1-IVM for 22h; T2-PIVM for 6 h and IVM for 22 h; PIVM with Scriptaid for 6 h and IVM for 22 h; T4-PIVM for 6 h and IVM with Scriptaid for 22 h; and T5-PIVM with Scriptaid for 6 h and IVM with Scriptaid for 22 h. Nuclear maturation, cumulus cell expansion, embryo development and embryo quality (differential cell staining) were evaluated. Data from nuclear maturation and embryonic development were evaluated by Chi-Square ($P<0.05$). Differential staining and cumulus cell expansion data were evaluated by ANOVA and, when non-parametric by Kruskal-Wallis ($P<0.05$). All treatments submitted to PMIV, when evaluated at the beginning of maturation (0 hours), presented the majority of their oocytes at germinal vesicle stage (T2= 87%, 47/54, T3= 85%, 41/48) 0.05) which was similar to the control group (T1= 96%, 66/69). After 22 hours of IVM, all groups had the majority of oocytes at metaphase II (T1= 94%, 56/57, T2= 96%, 46/48, T3= 92%, 48/52, T4= 96% 52/54 ($P> 0.05$), except for T5 (88%, 47/56), which presented a lower rate than the T1 ($P<0.05$). Cumulus cell expansion was similar between groups, with the exception of T5 which had a lower ($P <0.05$) expansion than T2. Regarding to embryo development at D7, T3 (32%, 65/203) had a lower rate than T2 (37%, 71/190) but was similar to control (35%, 82/236). The groups receiving Scriptaid in IVM (T4 = 23%, 47/207 and T5= 18%, 32/177) had lower rates of blastocysts ($P <0.05$) then the other treatments (T1 = 35%, T2 = 37% and T3 = 32%). Embryos from T5 (165, n= 30) presented lower amounts of cells ($p <0.05$) in comparison to T1 (192.59, n = 39) and T3 (189.53, n = 32). In relation to the proportion of internal cell mass and total cells, the T5 group also presented lower ($p <0.05$) the number of embryos with a proportion of 20-40% of internal cell mass (T5 = 67%, 20/30) in relation to the T1 group (87%, 34/39). It can be concluded that the presence of Scriptaid in PMIV and IVM simultaneously affects nuclear maturation, cumulus cell expansion, embryo development and embryo quality.

Support: Embrapa e CAPES.

A123 OPU-IVP and ET

Analysis of the comercial production of bovine embryo surplus *in vitro*

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The production of *in vitro* bovine embryos (IVP) results in a good amount of great quality surplus embryos. This is a problem for the embryo industry because the cryopreservation techniques for *in vitro* embryos are not well establised and surplus embryos should be discarded. Given the current need to develop a more sustainable processes, it would be essential studies that would be able to detect the actual loss of embryos in the IVP. The aim os this reserch was to evaluate the discard of bovine embryos produced *in vitro* from a retrospective study of the embryo surplus production from a commercial database, introducing proposals to reduce production losses. A retrospective study of the IVP of embryos using a historical series of 5 years with 3238 aspirations was used from a data bank of a comercial laboratory. In the laboratory the IVP was performed by the processes of maturation, fertilization and *in vitro* culture. On the seventh day of culture the blastocysts were evalueted through viability for transfer, being part innovated in the recipientes and the surplus discarded. Descriptive statistics was used to perform data analysis. The rates of viable embryos and vable embryos discarded were, respectively: 2013 – 29% (2312/7905) and 7% (167/2312); 2014 – 34.4% (1824/5308) and 11% (195/1824); 2015 – 37.1% (3100/8355) and 21% (559/3100); 2016 – 30.1% (2165/7192) and 22% (598/2624), observing a large variation in the rate of discarded embryos over the series (7%, 11%, 21%, 32% and 22%). In five year historical series the total embryo production was 31.8% (40694/12931) and 16% (2090/12931) of surplus production. This was due the average production of the embryos have exceeded the synchronized receivers available. Analyzing economically, *in vitro* embryo discarding practice generates a loss of product and a lower profitability, consequently. In the environmental focus, the practice of discarding contributes to the waste of materials as well as unnecessary resource and energy consumption. The analyzed series indicates that on average 1/6 of the production of embryos *in vitro* is discarded, being able to reach up to almost 1/3 depending on the year. In short term, aiming at this loss of production it is proposed for these surplus embryos to carry out a planning to measure the number of the recipientes synchoronized by aspiration taking into account the race, folicular population or the history of aspirations of the donor. In long term the idea would be to establish an efficient embryonic cryopreservation technique.

A124 OPU-IVP and ET

Comparison of OPU-PIVE in buffaloes with the use of refrigerated and frozen semen during the unfavorable reproductive period

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The anatomical-physiological specificities of buffalo species such as smaller ovaries, reduced follicular population and quality of recovered oocytes directly affect the efficiency of OPU-PIVE programs. The objective was to verify, in buffaloes, embryo development and cleavage rates in the PIVE with the use of refrigerated semen (RS) in the TRIS extender medium with 10% low density lipoprotein (LDL), 0.5% soy lecithin (LS) and with 10 mM of the antioxidant Acetylcysteine (NAC), compared to frozen semen (FS) with the same medium. The experiment occurred in the state of Minas Gerais (latitude 19°37'05" S and longitude 44°02'35" O). Three bulls (Murrah) were used, each ejaculate being aliquoted in two equal parts (refrigerated at 5ºC/24 hours and frozen). Sperm motility and kinetics were evaluated at CASA and sperm membrane integrity by the hyposmotic test (HOST). The evaluations were performed at 0 hs (post-dilution at 37ºC), 4 hs (equilibrium up to 5°C), 24 hs (refrigeration at 5ºC) and post-thawing. For the OPU, 25 Murrah buffaloes were aspirated. The aspiration sessions occurred from September to November 2017. A total of 239 oocytes were distributed in three sessions: 1st (102), 2nd (80) and 3rd (57). Oocytes were placed in wash medium (TCM 199 Hepes + 10% SFB + 22 µg/mL pyruvate + 83.4 µg/mL amikacin). They were matured in an incubator (38.5°C, 5% CO_2, 95% humidity) for 24 hours. The TALP fertilization medium supplemented with 83 µg/mL amikacin, 44 µL/mL PHE solution (penicillamine 68 µg/mL, hypotaurine 25 µg/mL, epinephrine 8.1 µg/mL), 0.6% BSA, 0.2 mM sodium pyruvate and 10 µg/mL heparin) and subsequently fertilized for 21 hours with 8 µL of semen ($\pm 0.4\times10^6$ SPTZ). The zygotes were stripped and then cultured in SOF medium for 72 hours. The embryo cleavage rates were assessed after 72 hours (D3) and blastocyst rates were observed 148 hours (D6) after IVF. Statistical analysis of the semen was used the package STATA 12.0 (Statacorp, 2012) and Test T (means of two independent samples). For the IVF variables (cultured oocytes, cleaved and produced embryos) the Z-Test was used ($P<0.05$). The SR/24 hours used had progressive motility of 64.3±6.1 and SC 41.3±4.0% and HOST of 89.9±1.5 and 58.6±2.5% for SR and SC, respectively. The results obtained for SR and SC ($P<0.05$) were: a) cultured oocytes = 86.4^a (102/118) and 76.0^b (92/121); b) cleaved oocytes = 34.3^a (35/102) and 25.0^b (23/92) and c) embryos produced = 29.4^a (30/102) and 18.5^b (17/92). It was concluded that in the PIVE of buffaloes, the results obtained with refrigerated semen were superior to those of frozen/thawed semen.