A043 OPU-IVF and ET

In vitro production and transfer of embryos from 12 and 24 months old Nellore heifers (*B. indicus*) treated or not with FSH

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The present study evaluated the *in vitro* production and transfer of embryos produced by 12 and 24-month-old Nellore (*Bos indicus*) heifers that were treated or not with FSH. For that, 126 heifers from Instituto de Zootecnia de Sertãozinho – SP were submitted to a 2x2 factorial arrangement [12-month-old heifers not treated with FSH (n=31); 12-month-old heifers treated with FSH (n=31); 24-month-old heifers without FSH (n=32) and 24-month-old heifers with FSH (n=32)] and 2 OPU (with 40 days interval; cross over). All heifers were synchronized with 2mg of estradiol benzoate (RIC-BE®, Agener, São Paulo), 0.530mg of sodium cloprostenol (Estron®, Agener, São Paulo) and received an intravaginal P4 device (360mg, Primer PR®, Agener, São Paulo). FSH groups received two injections of 30mg of FSH (Folltropin®, Agener, São Paulo) on day 4 (AM and PM) and two injections of 20mg of FSH on day 5 (AM and PM). On day 7 (after 44 hours of coasting period), the device was removed and all heifers were submitted to OPU (guia EC9-5 Novilha, WTA, Cravinhos, SP; US S8®, SonoScape, China) of all follicles that were counted, classified in small (<5mm), medium (5-8mm) and big (>8mm). Collected oocytes were selected and forwarded to IVP. Semen from 3 sires used for IVF were equally distributed within groups. Produced embryos (n=200) were frozen and transferred in synchronized recipients. Statistical analyses were done by GLIMMIX procedure of SAS®. There was no interaction between age category (12 *vs.* 24) and treatment (Control *vs.* FSH) for the studied variables (P>0.05). Treatment with FSH increased the number of small, medium and big follicles (P<0.001), and the total follicles (GC= 27.8±1.8, GFSH= 31.8±1.6; P=0.04), decreased the recovered oocytes rate (GC= 78.9%, GFSH: 52.5%; P<0.0001), increased viable oocytes rate (GC= 70.2%, GFSH: 78.6%; P=0.0002) and increased blastocyst rate over the total of recovered oocytes (GC: 18.6%, GFSH: 22.0%; P=0.0002), however there was no effect on the number of blastocysts per OPU (GC= 3.9±0.5, GFSH= 3.94±0.5; P=0.96). Still, 12-month-old heifers presented lower total of follicles (G12= 27.7±1.8, G24= 31.9±1.6; P=0.0485), same recovered oocytes rate (G12= 64.8%, G24= 66.6%; P= 0.4632), same viable oocytes rate (G12= 73.5%, G24= 75.4%; P=0.8089), lower blastocyst rate (G12= 16.5%, G24= 24.0%; P<0.0001), and lower number of blastocysts per OPU (G12= 2.8±0.4, G24= 5.9±0.6; P=0.0026). Concerning the ET, there was no effect of age (P= 0.5131) or treatment (P= 0.9623) on pregnancy rate within groups (G12C: 24.1% (7/29); G12FSH: 21.0% (8/39); G24C: 24.3% (17/70); G24FSH: 29.1% (18/62). It is concluded that the treatment with FSH in 12 and 24-month-old Nellore (*Bos indicus*) heifers decreased the recovery rate probably because the size of follicles increased. The treatment improved blastocyst rate, but there was no effect on ET pregnancy rate. Besides that, 12-month-old heifers presented lower efficiency on IVP when compared to 24-month-old heifers.

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Effect of the anticipation of intrafollicular transfer of immature oocytes (IFIOT) in the nuclear maturation of bovine oocyte

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Previous results in our laboratory demonstrated that when intrafollicular transfer of immature oocytes (IFIOT) is performed 52 to 54 hours after the removal of the progesterone implant, the time that CCO´s remain in the follicle is insufficient for them to complete nuclear maturation. Therefore, the objective of this study was to evaluate nuclear maturation of bovine oocytes submitted to IFIOT 30 hours after the removal of the progesterone implant. Twenty-six Nelore ovulators (*Bos taurus indicus*) were synchronized on day 0 (D0) with the insertion of an intravaginal progesterone implant (1g) and 2mg benzoate estradiol. On day 8 (D8), the implant was removed and 500 µg Cloprostenol sodium (PGF) was administered (i.m.). Thirty hours after implant removal ($D9^{1/2}$), grades 1 and 2 COC´s, were injected into the dominant follicle (diameter> 10mm). The CCOs were obtained from slaughterhouse ovaries and in each replicate part of the oocytes were used for IFIOT and part for IVM. All manipulation of the oocytes was performed in follicular fluid. After the IFIOT, the animals were distributed into two groups: Group LH, animals (n = 5) received a dose of an analogue of LH (1.25 mg) after IFIOT or Group GnRH (n = 7), that received a dose of GnRH (50 µg). After 22 hours, oocytes from both groups were retrieved by ovum pick up (OPU). For the IVM group, immature oocytes (CT 0) and oocytes matured *in vitro* for 22 hours (CT 22) were used. Oocytes from all groups were denuded, fixed and stained with Lacmoid for the evaluation of the meiosis stage. The oocytes were classified as: germinal vesicle (VG), germinal vesicle breakdown (VGBD), metaphase I (MI), anaphase I (AI), telophase I (TI), metaphase II (MII) and abnormal. Data were analyzed by chi-square test ($P < 0.05$). The mean size of the dominant follicle at the time of IFIOT was 11.93 (± 0.98) mm. The mean recovery rate (OPU) after 22 hours of IFIOT was 67.25%, being 76% for the LH group and 62% for the GnRH group. A total of 379 oocytes (CT 0, n = 81, CT 22, n = 56, LH 22, n = 106 and GnRH 22, n = 136) were evaluated. At 0 hour, 98.76% of the oocytes were in VG. At 22 hours of maturation, the percentage of oocytes that reached the MII was similar ($P > 0.05$) between the groups (CT 22 = 75%, GnRH = 72.05%, and LH = 67.92). The results demonstrated that the 22 hours intrafollicular maturation period is adequate for oocyte maturation within the follicle. Further studies need to be performed to evaluate the competence of these oocytes. Support: CAPES

A045 OPU-IVF and ET

Pregnancy rate after fixed-time transfer of frozen-thawed Lacaune sheep embryos recovered by transcervical route

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The cervical dilation treatment with d-cloprostenol, estradiol benzoate and oxytocin is an indispensable step for efficient non-surgical embryo recovery (NSER). However, the effects of these hormones on the viability of embryos after cryopreservation and transfer are still questioned. The aim of this study was to compare the viability after fixed-time embryo transfer (FTET) of embryos obtained by NSER and cryopreserved by two techniques. Embryos were recovery by transcervical method after hormonal treatment to induce cervical dilation (Fonseca et al., Reprod. Domest. Anim. 54(1):118-125, 2019) in donors of Lacaune breed (68.3 ± 6.7 kg of body weight - BW and 3.5 ± 0.2 of body condition score) and cryopreserved by either: slow freezing (SF - Fonseca et al., Arq. Bras. Med. Vet. Zootec., 70(5):1489-1496, 2018) or vitrification(VT - Gibbons et al., Theriogenology, 52:1005-1020, 2011). Sixty-three nulliparous ewes (46.7 ± 8.3 kg of BW) received sponges with 60 mg of MAP (Progespon®, Syntex, Buenos Aires, Argentina) for six days, besides 37.5 µg d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) and 200 IU eCG (Folligon 5000 IU®, Intervet, São Paulo, Brazil) intramuscularly on day before sponge removal (Day 0). Ovarian transrectal ultrasonography (Mindray M5VET®, Shenzen, China - 8.0 MHz) was conducted on Day 7 for detecting the corpora lutea (CL) count and side (right or left ovary). The recipients that presented CL (92%, 58/63) were subjected to embryo transfer on Day 8.5 after sponge removal by the semi-laparoscopic technique and received embryos on the uterine horn ipsilateral to the ovary with CL. Straws containing one or two embryos (morulae and/or blastocyst) subjected to SF (*n*=33) or VT (*n*=25) were randomly used. Pregnancy diagnosis was performed on Day 31. Data were analyzed using SAS® software. The PROC GLIMMIX was used with: (1) Poisson distribution for number of embryos/recipient and (2) binomial distribution for pregnancy rate. Recipient BW was used as covariate, and models included fixed effect of cryopreservation technique. The number of embryos/recipient did not differ (P>0.05) between SF (1.9±0.1) and VT (1.8±0.1). The pregnancy rate tended to be higher (P=0.08) in SF (39%, 13/33) than VT (16%, 4/25). The pregnancy rate in SF was similar to the rates observed in commercial FTET programs in cattle, demonstrating the viability of these embryos obtained by NSER. In sheep, the FTET is employed mainly by transfer of embryos produced *in vitro*, but to our knowledge, the present study is the first worldwide reference of FTET with embryos produced *in vivo* and recovered by NSER. In conclusion, embryos recovered by NSER after cervical dilation treatment with d-cloprostenol, estradiol benzoate and oxytocin, and later cryopreserved by either slow freezing or vitrification established pregnancy after FTET, and better rates were observed with the slow freezing technique.
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A046 OPU-IVF and ET

Superovulation and nonsurgical embryo recovery in dairy goats previously affected by hydrometra

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Hydrometra is responsible for sub or infertility reaching over 10% of prevalence in dairy goats. Although treatment exists, its recurrence and the loss of reproductive efficiency caused usually leads the goat to be culled. This study assessed for the first time the efficiency of superovulation (SOV) treatment in dairy goats previously affected by hydrometra (HD). The study was conducted in the anestrus season, in Minas Gerais state (21º 21' S), Brazil. Pluriparous dairy goats diagnosed by transrectal ultrasound (US) with no reproductive disorder (n=11, CONT) or with HD (n=10) were used. All goats with HD were treated before used as donors. The goats aged 1-7 years old, weighted 67.4 ± 7.8 kg and had body condition score between 2.5 and 4.0 (scale 1–5). Intravaginal devices containing 0.33 g progesterone (CIDR-G®, Pfizer do Brazil, SP, Brazil) were inserted for 6 d. For SOV, 133 mg pFSH (Folltropin-V®, Bioniche, Belleville, Canada) i.m. were applied in six decreasing doses, every 12 h, starting 48 h prior to device removal. Three doses of 37.5 µg d-cloprostenol (Prolise®, Tecnopec, SP, Brazil) i.m. were administered, at the fourth and fifth doses of FSH, and 12 h before the nonsurgical embryo recovery (NSER). At 24 h after device removal, 25 µg GnRH (Gestran®, Tecnopec, SP, Brazil) i.m. were given. Moreover, three doses of 1.5 mL flunixin meglumine (Flumax®, J.A. Saúde Animal, SP, Brazil) i.m. were administered (36, 60 and 84 h) after GnRH. After device removal, estrus was checked twice a day and goats were naturally mated. At 6-7 d after estrus onset, the number of corpora lutea (CL) was counted by US (Mindray M5VET®, Shenzen, China - 8.0 MHz) and NSER was performed. Normally distributed data were submitted to ANOVA, whilst non-normally distributed data were analyzed by Mann-Whitney test ($P<0.05$ considered as significant). All goats showed estrus and were subjected to NSER. The interval from device removal to estrus, estrus duration and SOV response rate (positive when >3 CL were observed) were similar ($P>0.05$), respectively, between CONT (29.1 ± 3.9 h; 21.8 ± 2.0 h and 82%) and HD (31.0 ± 2.3 h; 18.2 ± 2.8 h and 90%). The recovery rate, number of retrieved structures and viable embryos per goat were similar ($P>0.05$) between CONT [74% (56/76), 6.9 ± 1.7 and 5.1 ± 1.5] and HD [62% (28/45), 4.5 ± 1.2 and 2.8 ± 0.9), respectively. However, there was a difference ($P<0.05$) in the number of structures in delayed stage (8-16 cells) between CONT [1% (1/76)] and HD [29% (13/45)]. This can be a result of a poor oviduct/uterine environment to promote embryo development, suggesting a possible mechanism for the reproductive failure after HD-treatment. In conclusion, although the SOV response and NSER technique were not affected in HD-goats, the quality of retrieved embryos is questionable, and caution should be taken before indicating SOV in these animals. Financial support: CNPq (479826/2013-7), FAPEMIG (CVZ-PPM 00201-17) and FAPERJ (E-26/202.268/2018).

A047 OPU-IVF and ET

Effect of FSH treatment on the IVEP of Gyr (*Bos indicus*) calves, pubertal heifers and adult cows and pregnancy rate of the ET

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For this study, 90 Gyr donors were used: 30 calves (3-10 months), 30 pubertal heifers (16-22 months) and 30 cows (44-88 months), distributed into: Calves - control (CC, n=15), calves with FSH (FC, n=15); Heifers - control (CH, n=15), heifers with FSH (FH, n=15); Adult animals – control (CA; n=15), adult animals with FSH (FA; n=15). All animals received an intravaginal P4 device (calves – Primer PR, Agener União – Saúde Animal, Brazil; heifers and cows – Prociclar, Ceva Saúde Animal, Brazil) and estradiol benzoate (calves and heifers - 1 mg, cows - 2 mg; Fertilcare Sincronização, MSD Saúde Animal, Brazil) on D0. The treated groups received 80mg (FC), 100mg (FH) or 140mg (FA) of FSH (Folltropin, Vetoquinol, Brazil), split into 4 injections given twice a day in decreasing doses (coasting period: Calves - 24h; Heifers and Cows - 48h). The control animals of each category received no additional treatment. On D7 the P4 devices were removed and all animals underwent transvaginal ultrasound-guided OPU (EC9-5 Heifer, WTA, Brazil; ultrasound S8®, SonoScape, China). The recovered oocytes were sent to a commercial lab for the IVEP. The produced embryos (280 embryos) were transferred to crossbred heifer recipients. The obtained data were analyzed by the GLIMIX procedure of SAS®. Treatment with FSH increased (P=0.03) the number of medium sized follicles on D7 of all animal categories when compared to the same animal category without treatment (CC: 0.9±0.5; CH: 1.1±0.9; CA: 1.6±1.2 vs. FC: 8.3±7.3; FH: 11.8±7.0; FA: 7.4±5.3). Heifers yielded more oocytes (P=0.02) when compared to calves and cows (heifers: 19.9±0.8; calves: 12.3±0.6; cows: 11.2±0.6). The effect of FSH on the number of viable oocytes varied (P<0.001) according to animal category (CH: 15.2±10.0^{a}; FH: 12.9±10.4^{ab}; FA: 9.7±7.6^{bc}; CC: 8.1±6.9^{cd}; FC: 8.7±6.9^{cd}; CA: 6.5±4.2^{d}). The number of cleaved oocytes was greater (P<0.001) for heifers (11.3±0.6) than for calves and cows (5.8±0.4 and 7.1±0.4), the cleavage rate was greater (P=0.01) for heifers (56.7%; 11.3/19.9) and cows (63.3%; 7.1/11.2) when compared to calves (47.1%; 5.8/12.3). The number of blastocysts per OPU showed a tendency to increase (P=0.06) when calves (FC: 2.0±1.7; CC: 1.1±1.3) and cows (FA: 4.9±4.6; CA: 3.1±2.2) were treated with FSH. Regarding the ET, no difference was observed for pregnancy rate at 30 [CC: 52.9% (9/17); FC: 30.7% (8/26); CH: 47.6% (31/65); FH: 42.3% (24/58); CA: 55.5% (25/45); FA: 57.9% (40/69); P=0.38] and 60 days [CC: 41.1% (7/17); FC: 30.7% (8/26); CH: 40.0% (26/65); FH: 36.2% (21/58); CA: 51.1% (23/45); FA: 49.2% (34/69); P=0.88]. No difference was observed for pregnancy loss [CC: 22.2% (2/9); FC: 0.0% (0/8); CH: 16.1% (5/31); FH: 12.5% (3/24); CA: 8.0% (2/25); FA: 10.0% (4/40); P=0.69]. These results demonstrate that treatment with FSH increases de IVEP of Gyr calves and cows and has no effect on pregnancy rates.

A048 OPU-IVF and ET

Oocyte quality evaluation of 14 months old Nellore heifers that became pregnant or not after breeding season

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It was aimed to investigate the influence of oocyte quality on 14 months old Nellore heifers submitted to TAI. For this study, 75 Nellore (*Bos indicus*) heifers of Fazenda Campina – Nelore CV (Caiuá, SP) were aspirated then synchronized for TAI. On day 0 of the experiment, heifers were submitted to transvaginal US guided OPU (DP2200Vet, Mindray, China) on random day of the estrous cycle. The oocytes obtained were selected and forwarded to IVF/IVP in the laboratory (Bovitran, Cuiabá, Brasil). It was used semen from 9 sires for oocytes fertilization, and the same mates were maintained for TAI. On the day of OPU (D0), all heifers were synchronized receiving an ear norgestomet device (Crestar®, MSD, São Paulo). On day 9, the ear device was removed and were administered 0.530mg of sodium cloprostenol (Ciosin®, MSD, São Paulo), 0.5mg of EC (Fertilcare Ovulação®, MSD, São Paulo) and 200IU of eCG (Folligon®, MSD, São Paulo). On day 11, heifers were artificially inseminated. Pregnancy diagnosis was done 22 days later by US Color Doppler (M5®, Mindray, China). Statistical analyses were performed by GLIMMIX procedure of SAS®. Pregnancy rate of heifers submitted to TAI was 40.5% (there was no effect of BCS, weight, womb diameter, FD diameter and ciclicity previously to experiment; P>0.05). Total oocytes (23.9±2.3 *vs*. 21.3±2.2; P=0.51), viable oocytes (15.3±2.1 *vs*. 13.0±1.1; P=0.45), cleaved number (10.3±0.9 *vs*. 11.0±1.1; P=0.39) and embryo produced by OPU (4.6±0.5 *vs*. 4.7±0.6; P=0.73) did not differ between heifers that became pregnant or non-pregnant after TAI. Pregnancy rate of embryos produced by pregnant and non-pregnant heifers did not differ as well [36.8% (28/76) *vs.* 33.0% (36/109), P=0.6155). It is concluded that heifers that became pregnant to its first TAI presented similar efficiency in OPU/IVP when compared with heifers that became non-pregnant. Acknowledgements: Nelore CV, Fertiliza, Bovitran.

A049 OPU-IVF and ET

Use of phenazine etosulfate (PES) on the *in vitro* production of bovine embryos

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The aim of this study was to evaluate the effect of different doses of the drug phenazine etosulfate (PES) during *in vitro* maturation of bovine oocytes from slaughterhouse. Consequences for embryo production and survival after vitrification were evaluated. The effects of supplementation of IVM medium with PES at 0; 0.16; 0.4; 1.0 and 2.5 μM and of replicate were evaluated on the proportion of bovine embryos produced, survival, hatching and expansion rates after thawing from vitrification. Data were submitted to analysis of variance (PROCGLM) and, when binomially distributed, to the generalized linear model procedure (GENMOD), following normality tests under the SAS® UNIVARIATE procedure. The dose of PES 2.5 μM was toxic - no blastocyst production after 7 days of culture, and only one grade I embryo at day 8 (2 replicates, n= 70 oocytes). The other groups had a minimum of 550 cumulus-oocyte complexes matured for each dose tested on 12 replicates, resulting in 400 vitrified embryos. The proportions of embryos produced between the replicates ($P < 0.0001$) ranged from 23.5% to 51.0%. The proportion of embryos produced after addition of PES during *in vitro* maturation was higher ($P < 0.0035$) for the control group (C = 41.5 ± 1.8%, n = 237) than for 0.16 (32.5 ± 1.7%, n = 182) and 1.00 (32.6 ± 1.7%, n = 186) but did not differ from the 0.4 group (35.6 ± 1.7%, n = 210). All PES-treated groups did not differ. The survival rate after 48 hours post thawing ($P = 0.123$) did not differ (C = 52.3 ± 7.4%, n = 52, 0.16 = 49.2 ± 7.6%, n = 36; 0.4 = 48.8 ± 7.3%, n = 49; 1.0 = 52 ± 7.3%, n = 44). The hatching rate ($P = 0.104$) in this period tended to be better for groups 0.4 and 1 (0.4 = 32.4 ± 5.6%, n = 38, 1 = 34.3 ± 5.6%, n = 32) than control and 0.16 (C = 27.2 ± 5.7%, n = 13; 0.16 = 22.4 ± 5.8%, n = 17). The rate of expansion ($P = 0.029$) at 48 hours after thawing was the same for the control group (27.1 ± 5.2, n = 20), 0.16 (24.3 ± 5.6%, n = 20) and 1 (17.7 ± 3.5%, n = 10). The 0.4 (10.3 ± 2.0%, n = 12) and 1.0 groups did not differ from each other. Even with the careful selection of grade I and II oocytes only, because they were from slaughterhouse, possible differences between groups of animals for each replicate (subspecies, nutritional status, parity, reproductive status) may have led to this replicate effect in the embryo production results. The low rate of expansion in the embryos at the highest doses was possibly due to the high hatching rates of these treatments, which did not mean that the doses 0.4 and 1 were harmful or toxic to the expansion of the thawed embryos. The PES treatment of 0.4 μM had no deleterious effect on embryo production, 77.5% of the blastocysts that survived after 48 hours of thawing hatched whereas for the control group 25% hatched. Therefore, more studies are needed to evaluate the use of PES in maturation for vitrification of bovine embryos.

A050 OPU-IVF and ET

In vitro production of embryos from Holstein females treated with propylene glycol

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The objective of the present study was to evaluate the effects of 500 mL propylene glycol (PPG) supplementation every 12 hours for 5 days in vitro embryo production (PIVE) of Holsteins (Bos taurus). We used 323 females belonging to different animal categories randomized, into control group (CTL) and PPG group: cows at the beginning of lactation (CTL: n = 41; PPG: n = 37), repeat breeder cows (CTL: n = 38; PPG: n = 36), dry cows (CTL: n = 45; PPG: n = 49) and prepubertal heifers (CTL: n = 39; PPG: n = 38). The OPU was performed on day 0 for follicular ablation and for synchronization of the emergence of the follicular growth wave, and then the OPU was performed for PIVE on day 5. The aspirated oocytes were matured for 24 hours, fertilized with the same set of sexed semen and cultured in vitro. Statistical analyzes were performed using Statistical Analysis System (SAS, Version 9.4) software. The results suggest that there was an increase (P = 0.0583) in the rate of embryonic development in prepubertal heifers supplemented with PPG when compared to the control group (16% vs. 26%). In dry cows, a trend (P = 0.0580) of increase in the cleavage rate was observed in the PPG treated animals when compared to the control group (44% vs. 47%). For the category of lactating cows, there was a significant increase (p = 0.0189) in the rate of blastocysts in cows treated with PPG compared to the control group (18% vs. 37%). In this way, an improvement in the oocyte quality was demonstrated by the increase in the rate of blastocysts in cows at the beginning of lactation. Also, unexpectedly, there was a reduction in the number of oocytes recovered by OPU in PPG treated animals when compared to untreated animals (5.7 ± 0.69 vs. 3.7 ± 0.53, P = 0.0042). At the end, there was no difference in the number of embryos produced by OPU between the groups (0.98 ± 0.21 vs. 1.14 ± 0.19, P = 0.8349). For cows at the end of lactation (repeat breeder cows) no effects of treatment with PPG on embryo production efficiency were observed. This animal category has high levels of circulating glucose, which may explain the lack of response. In addition, repeat breeder cows may exhibit peripheral insulin resistance. Thus, it is concluded that supplementation with 500 mL of PPG every 12 hours for 5 days increases the rates of PIVE in heifers and cows at the beginning of lactation, and may improve oocyte competence, resulting in higher rates of embryo development and quantity of blastocysts produced by OPU.

A051 OPU-IVF and ET

Association between antral follicle count and *in vitro* embryo production in Holstein calves

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There is an increasing interest in anticipating reproducing of high genetic value cattle. Antral follicle count (AFC) is an important tool for the selection of potential oocyte or embryo donors in adult cattle. However, little is known about AFC and in vitro embryo production from prepubertal calves. Our objective was to compare the association between antral follicle count and in vitro embryo production (IVEP) in *Bos taurus taurus* calves. Holstein donors (n = 135) between 7 and 9 months of age were retrospectively classified as Low (n = 67) or High (n = 68) AFC, according to the number of oocytes recovered by OPU (Low = 1-10 and High = 20-78). The oocytes recovered were matured for 24 hours, fertilized and cultured in vitro. The statistical analyses were performed using statistical software Minitab, version 18.1, adopting $p<0.05$, and analysis of variance was performed using the generalized linear model. After 264 procedures of OPU, the mean number of oocytes recovered by High and Low AFC was 34.60±1.78 and 6.10±0.23, respectively; $p<0.001$. Corroborating previous data from adult Holstein cattle, embryo production between the High and Low AFC differed (3.66±0.38 vs. 0.70±0.09, respectively; $p<0,00$). In conclusion, AFC is a useful criterion for selecting Holstein calves for in vitro embryo production.

A052 OPU-IVF and ET

Productive and reproductive performance of female Holstein calves born from different reproductive biotechnologies (AI, ET and IVF)

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The objective of this study was to evaluate the effect of different reproductive biotechnologies (AI, ET and IVF) on reproductive and productive performance of females Holstein calves born from lactating Holstein cows. 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 (during the years of 2013 to 2018) these three biotechnologies (AI=3439, ET=722 and IVF=333) were analyzed. The animals were submitted to the same farm management conditions. In the female calves, the weight at birth (WB), the mortality from birth to weaning (MBW) and the weight at weaning (WW) were analysed. Also, age at first calving (AFC) and milk production on the first lactation (MP1L) were partially analyzed in the calves born by these different biotechnologies. The data were analyzed by the PROC GENMOD and GLIMMIX of SAS. The WB [AI=39.2^{B} (n=1546), ET=39.8^{AB} (n=587) and IVF=39.4^{A} (n=306) kg; P<0.001] and the WW [AI=101.6^{B} (n=1292), ET=99.4^{A} (n=583) and IVF=101.4^{B} (n=204) kg; P=0.013] differed according to the biotechnology. However, the MBW [AI=24.1% (256/1062); ET=9.8% (33/336) and IVF=25.8% (16/62), P=0.248] and the AFC [AI=445.5 (n=2207), ET=466.1 (n=496) and IVF=444.1 (n=83) days; P=0.981] did not differ according to the biotechnology. The MP1L did differ according to the biotechnology [AI=$9,860.7^{AB}$ (n=1350), ET=$10,296.5^{A}$ (n=290) and IVF=$10,856.13^{A}$ (n=46) kg; P=0.0031]. As a conclusion, even though there was difference according to the biotechnology used on the WB and WW, it could be equalized during the calves' development. The AFC did not differ between the biotechnologies. However, the MP1L was higher in the calves born by ET and IVF comparing to AI, probably due to the improve in the genetic merit. More data is still needed to conclude the influence on the productive and reproductive performance of Holstein females born from different biotechnologies.

A053 OPU-IVF and ET

Lipid profile of embryos produced *in vitro* of Nelore cows with low and high numbers of antral follicles

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The quantity of lipids available for the initial development processes of in vitro embryos produced can reflect directly on the embryonic quality and consequently on pregnancy rates. However, the objective of the present study was to investigate the lipid profile of in vitro embryos produced (IVP) from females *Bos taurus indicus* with different antral follicle counts (AFC). For in vitro culture, ovaries (n = 498) of 249 Nelore females were collected from local slaughterhouse and transported in saline solution at 30-35 °C to the laboratory. AFC number was determined by visual counting of the surface of both ovaries of each animal. The animals were classified as low (≤31; mean less SD) and high number of AFC (≥92; mean plus SD). Oocytes were matured, fertilized and cultured in vitro. The cleavage and blastocyst rates were evaluated in D3 and D7, respectively. On day 7 (D7) blastocysts (n = 18 per group) were collected and submitted to lipid profile analysis by desorption electrospray ionization-mass spectrometry (DESI-MS). The cleavage and blastocysts rates were evaluated by the logistic regression test using the Car statistical package "R (R Development Core Team – 2008), and the differences were considered significant if P <0.05. For the lipid profile analysis, the principal component analysis (PCA) was used, followed by Fisher's test. There were no differences between groups (78.9% and 41.7% in High AFC; n = 419 oocytes; 79.5% and 40.3% in Low AFC; n = 357 oocytes). The lipid profile of embryos derived from cows with higher AFC presented a higher concentration of lipids in the category of Triacylglycerols (TAGs) when compared to the embryos of cows with a low AFC. In contrast, embryos with cow low AFC had higher concentrations of cholesterol and its derivatives and Diacylglycerol (DAG). In conclusion, our results confirm differences in lipid profile when analyzing different amounts of AFC, nevertheless it was not possible to evaluate its significant interference in the cleavage and blastocysts rates. Our results may support studies related to the influence of lipid content on embryonic development.

A054 OPU-IVF and ET

OPU and IVEP from non-stimulated 2-4 and 8-10 months old Nelore (*Bos taurus indicus*) donors

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In vitro embryo production (IVEP) is a powerful tool for cattle herds' genetic improvement. The Nelore breed and its crossbreeds are predominant in Brazilian beef herds, but are lesser precocious than most taurine breeds. Thus, the inclusion of prepubertal Nelore calves as oocyte donors in IVEP programs could shorten the generations interval and accelerate herds' genetic gains. However, it is controversial whether oocytes obtained from prepubertal cattle are less competent, generate fewer embryos or are less able to establish pregnancies than those obtained from adult ones. Thus, the aim of the present study was to evaluate the capacity of Nelore calves of 2-4 and of 8-10 months of age in IVEP compared to pubertal females. On that context, 8 Nelore calves had their follicles aspirated every other 15 days, from 2 to 4 months old by laparoscopic ovum pick-up (LOPU) and from 8 to10 months age by ovum pick-up (OPU). Nelore cows were used as a control group, and underwent OPU at the same times. The calves were raised on pasture (*B. decumbens*) and had *ad libitum* access to suckling and to water. The LOPU was performed by laparoscopy (Storz®, Xenon 300W) and under sedation and anesthesia induced with Xylazine 2% (IM, Anasedan, Ceva, Brazil), Atropine Sulfate 1% (UCB Brazil), Ketamine Hydrochloride 10% (IM, Dopalen, Ceva, Brazil), and Lidocaine Hydrochloride 2% (SC, Bravet, Brazil). OPU was performed with a portable ultrasound equipped with a transvaginal, 8MHz probe (MyLab 30 VetGold, Esaote®). The recovered oocytes were morphologically evaluated and their diameters were measured with the aid of a Motic camera. Viable cumulus-oocyte complexes (COC) were used for IVEP. The oocyte diameter and blastocyst rates were evaluated by ANOVA, while the oocyte morphological quality was evaluated by Kruskal-Wallis. As expected, adult cattle produced more grade I and less grade III COC than had prepubertal calves (12.9% and 30.1% in cows vs. 1.7 and 49.0% in calves [2-4 months] and 4.1% and 44.5% [8-10 months], respectively, $P<0.05$). Oocyte diameter of 8 to 10 months old calves were similar to those in cows but greater than in 2 to 4 months old calves (124.8±8.5μm and 126.0±7.5 μm vs. 121.3±7.5 μm, respectively, $P<0.05$). Coherently, blastocyst rates on day 7 of oocytes recovered from non-stimulated 8 to 10 months old calves were similar to those recovered from cows (42.0% [50/119] vs. 48.1% [130/270], respectively, $P>0.05$), while blastocyst rates of 2 to 4 months old calves were lower than those in cows, in simultaneous IVEP batches (31.0% [53/171] vs. 71.6% [177/247], respectively, $P>0.05$). In conclusion, oocyte diameter is a potential marker of acquisition of development potential throughout puberty. Moreover, oocytes recovered from non-stimulated 8 to 10-months old Nelore calves had similar competence of those from cows, suggesting that calves of that age can be used as donors in IVEP programs. Support: FAPDF (193.001.393/2016), EMBRAPA (SEG MP3 03.17.00.066.00.00)

A055 OPU-IVF and ET

The use of synthetic oviduct fluid in the *in vitro* maturation of bovine oocytes alters the expression of genes related to lipid metabolism and bovine oocyte maturation

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The objective of this work was to investigate whether the use of synthetic oviduct fluid (SOF) with or without conjugated linoleic acid (CLA) during the *in vitro* maturation of bovine oocytes modulates the expression of genes linked to lipid metabolism and oocyte maturation. The *cumulus*-oocyte complexes were aspirated from ovaries obtained at slaughterhouse and distributed into four groups: standard for *in vitro* maturation (IVM), IVM with 100 μM CLA (IVM+CLA), SOF and SOF with 100 μM CLA (SOF+CLA). After maturation, the gene expression of oocytes and *cumulus* cells was evaluated. Total RNA samples were treated with DNAse before being submitted to the reverse transcription protocol. We performed qPCR analyzes of the FADS2 (Fatty Acid Desaturase 2), SCD (Stearoyl-CoA Desaturase), SREBP1 (Sterol Regulatory Element Binding Transcription Factor 1), GREM1 (Gremlin 1), AREG (Anphiregulin) e PTGS2/COX2 (Prostaglandin-Endoperoxide Synthase 2/Cyclooxygenase 2). The PPIA Gene (Peptidylprolyl Isomerase) was used as a reference, and the efficiency correction ΔΔCt method was used to calculate the relative expression values (target genes / PPIA) for each target gene using a control sample as a calibrator. The data were submitted to analysis of variance (ANOVA) and Tukey test, being transformed into logarithm when they did not present normal distribution. Analyzes were performed using JMP software (SAS Institute Cary, NC) and data are presented as mean ± standard error of the mean. Differences were considered significant at the 5% level of significance ($p<0.05$). FADS2 (1.03 ± 0.17^{a}) and SCD1 (1.04 ± 0.15^{a}) presented higher mRNA expression in the oocytes of the IVM group. GREM1 was more expressed in groups SOF (2.26 ± 0.08^{a}) and SOF+CLA (2.68 ± 0.31^{a}). PTGS2/COX2 was more expressed in groups SOF (2.19 ± 0.14^{a}) and SOF+CLA (1.96 ± 0.41^{a}). SCD1 expression was higher in the *cumulus* cells of the IVM (0.76 ± 0.16^{a}) and IVM+CLA (0.55 ± 0.06^{a}) groups. The use of SOF or SOF+CLA in the maturation of bovine *cumulus*-oocyte complexes decreases the expression of the FADS2 and SCD1 genes related to lipid metabolism and, in addition, induces greater expression of PTGS2 / COX2 and GREM1 related to the process of oocyte maturation, specifically linked to the expansion of *cumulus* cells.

A056 OPU-IVF and ET

Effect of FSH and/ or rBST treatment on the *in vitro* embryo production of Holstein (*Bos taurus*) calves

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A total of 62 Holstein females were used in this study: 49 prepubertal (3 to 9 months of age) and 13 pubertal. The prepubertal animals were distributed in four groups: Control (CTLG, n=13); Treated with rBST (BSTG, n=11); Treated with FSH (FSHG, n=13); and Treated with rBST+FSH (BFG, n=12). The pubertal heifers (PHG) were included as a positive control. All the animals received a P4 device (Primer PR, Agener União – Saúde Animal, SP, Brazil) and 1 mg IM of estradiol benzoate (Fertilcare Sincronização®, MSD Saúde Animal, SP, Brazil) on D0. The animals of CTLG received no additional treatment. The animals of BSTG received 500 mg IM of rBST (Boostin®, MSD Saúde Animal) on day -2. The animals of FSHG received 140 mg IM of FSH (Folltropin®, Vetoquinol - SP, Brazil), performed 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 the BFG received 500 mg IM of rBST on D-2 and the same FSH protocol mentioned above. The PHG received no additional treatment. On day 7 the P4 devices were removed and the animals of all groups were submitted to ovum pick-up guided by transvaginal ultrasound (guide EC9-5 Heifer, WTA, SP; ultrasound S8®, SonoScape, China). The recovered oocytes were sent to an IVEP commercial lab. The oocytes were fertilized with sexed semen from three Holstein bulls (*Bos Taurus*), balanced between the experimental groups. Data were analyzed by the GLIMIX procedure of SAS®. No statistical differences were found between groups regarding total number of oocytes on D7 (CTLG: 9.8±1.1; BSTG: 14.7±5.5; FSHG: 13.5±3.8; BFG: 13.0±2.7; PHG: 9.8±2.4; P=0.7), number of viable oocytes (CTLG: 6.1±1.0; BSTG: 9.9±4.9; FSHG: 9.1±2.6; BFG: 9.0±1.7; PHG: 5.0±1.1; P=0.2), cleaved oocytes (CTLG: 4.5±0.9; BSTG: 7.45±3.0; FSHG: 9.1±3.1; BFG: 6.7±1.8; PHG: 5.6±1.4; P=0.7) and blastocyst rate [on total oocytes; CTLG: 2.3% (0.23/9.85); BSTG: 4.9% (0.73/14.73); FSHG: 4.5% (0.62/13.54); BFG: 8.3% (1.08/13); PHG: 14.0% (1.38/9.85); P=0.2]. Regarding the number of blastocysts produced/OPU, no statistical difference was found (P=0.9) between the experimental groups (CTLG: 0.23±0.1; BSTG: 0.73±0.3; FSHG: 0.62±0.2; BFG: 1.08±0.4; PHG: 1.38±0.4). The number of medium follicles (5-8mm) on D7 was higher (P=0.024) for the FSHG and BFG (9.0±2.38 and 7.83±2.42, respectively) when compared to the other groups (CTLG: 1.23±0.46; BSTG: 0.91±0.25; PHG: 2.08±0.52). The cleavage rate (on total oocytes) was higher (P=0.0002) for animals treated only with FSH [68.1%; (9.2/13.5)] when compared to the other groups [BSTG: 51.0% (7.5/14.7); BFG: 52.3% (6.8/13); CTLG: 45.9% (4.5/9.8) and PHG: 58.1% (5.7/9.8)]. These data show that treatment with FSH improved the cleavage rate of young Holstein donors, however, treatment with BST or the association of both treatments had no influence on the IVEP of prepubertal heifers.

A057 OPU-IVF and ET

Effect of application moment of FSH-LH during superovulation protocols on oocyte quality in Blanco orejinegro cows (BON)

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The objective of this study was to evaluate the effect of the application moment of FSH-LH hormones in a superovulation (SOV) protocolo on oocyte quality in Blanco orejinegro cows (BON). Twenty non-lactating cows were used, in a cross design, randomly distributed in 5 experimental groups. For groups 1 to 4, day 0 was considered the moment of removal of all follicles ≥5mm [follicular ablation (FA)], which was performed by ultrasound-guided transvaginal aspiration (UGTA). Groups were assigned as Group 1 (G1) cows received a single dose of 60 IU im of FSH-LH (Pluset ®) 27 hours (h) after follicular FA, and 27 h later the cumulus-oocyte complexes (CCOs) were collected by UGTA; Group 2 (G2) 27 h after FA cows received a single dose of 60 IU im of FSH-LH, and 48h later the UGTA was performed; Groups 3 and 4 (G3 and G4) received 60 IU im of FSH-LH divided into two doses, at 24 and 48 h after FA, and UGAT was performed 27 and 48 h later, respectively. Control cows (G5) were submitted to UGAT in a random phase of the estrous cycle. The COCs were collected, counted and classified into four categories, according to the compaction and transparency of cumulus cells, homogeneity and transparency of the ooplasm, as follows: Grade 1, >4 layers of cumulus cells; Grade 2, three or four layers of cumulus cells; Grade 3, one or two layers of cumulus cells; Grade 4, denuded oocytes or oocytes with expanded cumulus. CCOs from grades 1 to 3 were considered viable and grade 4 were considered not viable. The data was analyzed using the MIXED MODELS procedure from SAS 9.1 software. The means were compared using Tukey test. For the total number of collected oocytes, number of viable oocytes and oocytes of grade 2 and 4 no significant differences were found among groups (P> 0.05). A lower (P0.05). In addition, a greater proportion (P = 0.02) of grade 3 oocytes was observed in the control group cows (G5: 53.98%, 122/226) when compared to G1 (38.98%, 69/177). The use of FSH-LH in SOV protocols in BON donor cows did not increase the number of total oocytes or number of viable oocytes obtained; however, the proportion of grade 1 oocytes recovered was increased, and this can be reflected in a greater embryos production in *in-vitro* programs.

A058 OPU-IVF and ET

Embryo production and future fertility of heifers after superovulation

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The Multiple Ovulation and Embryo Transfer (MOET) technique for bovine genetic multiplication has been used commercially since the 1960s. In the last years, this technique has gained a great differential, with the possibility of selection of donors by genomic evaluation. However, the use of these technologies is far below the possibilities, since some farmers and technicians believe in information, usually without proper scientific evidence, that the MOET technique can affect the fertility of heifers. Thus, the objective of this study was to evaluate if the MOET is harmful to the future fertility of heifers. It was used Holstein heifers (n=1783) between 312 and 387 days old and weighing between 273 and 307kg from two commercial farms. These animals were divided among donors (446) or non-donors (G1=1327) according to the result of genomic evaluation. The donors were always super ovulated with 180mg of Folltropin (Vetoquinol-Brazil). Of these, 337 were submitted to MOET once (G2) and 109 twice (G3). The second MOET was made only in heifers who produced more than eight viable embryos in the 1st MOET. In those super ovulated twice, the interval between MOET was 45 days. Non-donor females (G1) were in seminated as of the date that reached 320kg of body weight. The donors (G2 and G3) were inseminated from 15 days after the last embryo harvest and when they reached the same weight. The age at 1st insemination, age at conception and age at birth were evaluated by anova and compared by Tukey's test. The number of services per conception was compared by chi square, considering significance at 5% probability. The first MOET in 446 donors provided 6.8±4.6 total embryos and 3.9±2.8 viable embryos. The second MOET, selecting the best donors, provided 12.6±5.3 total embryos and 8.5±3.8 viable embryos. The age at 1st insemination was 381.5±3.6b; 387.4±15.8ab and 412±19.7a days, age at conception 438.3±29.8^{b}; 449.1+32.3^{ab} and 470±31.8^{a} days and age at partum 720.3±33.8b; 730.1±35.8ab and 749±34.3a ($P<0.05$) for G1, G2 and G3, respectively. The number of services per conception was 2.3±0.8; 2.4±0.6 and 2.3±0.7-($P>0.05$) for G1, G2 and G3 respectively. It is concluded that one or two MOET processes, prior to the beginning of the insemination phase, does not interfere with the heifer fertility. A MOET before the start of the breeding phase does not interfere with age at the 1st insemination, conception and 1st calving. In addition, two MOETs delays conception by 32 days. On the other hand, heifers submitted to two MOET produced 16.4±5.3 viable embryos before the 1st conception. Acknowledgment: Biotran, Unifenas, CNPq and CAPES

A059 OPU-IVF and ET

Superovulation protocols for *in vivo* embryo production using sexed semen

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The objective of this study was to compare the efficiency of two using two or three doses of sexed semen for donor insemination at fixed time (SOVFT) to produce bovine embryos in vivo with sexed semen. Twenty girolando heifers donors, aged between 16 and 52 months, were used in a crossover scheme, where all the animals participated in both treatments. Each donor was superovulated twice. Females were superovulated using 180 mg Folltropin™ (Vetoquinol-Brazil) in a decreasing dose schedule. The superovulation protocol was as follows: D0 - progesterone device insert (Primer™ – Brazil); D1 - 2 mg of estradiol benzoate (Ric Be™ – Brazil) IM, D5 to D8 - Folltropin every 12 hours. In D7 the afternoon 0.5 mg of cloprostenol (Estron™ – Brasil) was applied and in D8 in the morning the device was removed. In D9, donors were divided into two groups: G2AI (N = 23), received 0.05 mg of Gonadorelin (TecRelin™ – Brazil) at 2pm and was inseminated in D10 at 7am and 7pm. G3AI (N = 23) received 0.05 mg of Gonadorelin at 7am and was inseminated at D9 at 7pm and at 10 at 7am and 7pm. All inseminations were made using sexed semen from Holstein bull (ABS Pecplan-Brazil). For the same donor, the same semen was used in both treatments. Uterine flushings were made using DMPBS™ (Reprodux-Brazil) on D16. The ovaries were evaluated by ultrasonography (Mindray - M5™-China) on the day of flushing to measure the corpora lutea (CL). The number of CLs and embryonic production was compared using Anova at 5% probability. The number of CLs on the day of collection was 14.2±6.4 and 12.4±6.2 ($P > 0.05$) for G2IA and G3IA. The mean total embryos were 8.7±4.6 and 9.9±7.9 ($P<0.05$) and viable of 5.6±3.2 and 6.9±4.0 ($P<0.05$) for G2IA and G3IA. These results show that there is no difference in SOV, indicating that the design study used was correct, since this response to SOV has great individual variation. However, even with a similar ovarian response, G3IA donors produced more total and viable embryos, indicating that an insemination protocol with an additional insemination has better results, justifying the use of an additional dose of semen. In conclusion, the protocol of SOV using three doses of sexed semen to produce bovine embryos in vivo is superior to that using only two doses. Acknowledgments: Vetoquinol, ABS Pecplan, IMV, Reprodux, Biotran, Unifenas, CNPq and CAPES.

A060 OPU-IVF and ET

Embryo production and recovery in naturalized Brazilian ewes

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This study hypothesized and tested if the same superovulation protocol and non-surgical embryo recovery (NSER) technique would be efficient to produce and recovery embryos naturalized Brazilian sheep breeds managed in different production systems. A superovulation protocol were used in Morada Nova (MN; n=20); Santa Inês (SI; n=20) and Somalis donors (SO; n=20) consisting in progesterone-based intravaginal device (0.33g; Eazi Breed CIDR®, Zoetis, São Paulo, Brazil) maintained for nine days and administration of six decreasing doses (25-25-15-15-10-10%) of p-FSH (133 mg; Folltropin V®, Vetoquinol, Brazil) injected i.m. every 12 h starting -60 h before device removal. Concomitant to the 5th and 6th p-FSH dose, were administrated i.m. d-cloprostenol (37.5 µg; Prolise®, Agener União, Brazil) and females were submitted monitored for estrous response and natural mating with fertile rams. To prevent corpus luteum (CL) regression, ewes also received three administrations i.m. of flunixin-meglumine (24.9 mg; Banamine®, MSD, São Paulo, Brazil) on Days 3, 4 and 5 after first mating. NSER (Fonseca et al., Reproduction in Domestic Animals, 54:118-125, 2019) was performed seven days after device removal only in ewes mated and embryos collected were frozen. CL was counted by transretal Doppler mode ultrasonography one before NSER. Data were presented in a descriptive form. Estrous responses were 80 (16/20), 95 (19/20) and 90 (18/20) % respectively to MN, SI and SO ewes. Successful NSER was done in 94 (15/16), 95 (18/19) and 94 % (17/18) in MN, SI and SO ewes respectively. The average number of corpora lutea found (considering only ewes collected) by breed was 8.7±0.1, 13.9±1.4 and 9.9±0.7 for Morada Nova, Santa Inês and Somalis ewes, respectively. Embryo recovery (structures/ CL counted x100) were 95.4% (125/131), 89.9% (195/217) and 77.4% (130/168) and viability rates (viable embryos/total structures x 100) were 96.0% (120/125; 104 morulae, 16 blastocyst and five unfertilized eggs), 87.2% (170/195; 140 morulae, 30 blastocyst, one 4-7 cell embryos and 24 unfertilized eggs) and 90.0% (117/130; 59 morulae, 58 blastocyst, one eight-cell embryo and 12 unfertilized eggs) for MN, SI and SO ewes, respectively. It is concluded that the *in vivo* embryo production is feasible in naturalized Brazilian ewes with the same superovulatory protocol. The results showed (1) the repeatability and efficiency of the superovulatory protocol for ovarian stimulation irrespective to breed, (2) high success of NSER related to both successful transcervical uterine flushing and total and viable structures recovered in Morada Nova, Santa Inês and Somalis ewes and (3) elevated embryo viability for embryos recovered after estradiol-cloprostenol-oxytocin based protocol for cervical relaxation and transposing in sheep.
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A061 OPU-IVF and ET

Bull individual effect is determinant to *in vitro* embryo production regardless of seminal profile

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Bull fertility is an intriguing issue of animal reproduction and so far, different groups have tried to characterize which sperm traits contributes to fertilize and sustain embryo development. In addition, the difference between *in vivo* and *in vitro* system turns difficult to extrapolate the results from the field to the laboratory. Then, the aim of this study was to create a model with flow cytometry and computer assisted sperm analysis (CASA) to identify which sperm traits contribute and how much they contribute to create a predictive model of *in vitro* fertility. For that, we produced a database of in vitro embryo production (IVP) of 51 semen batches from 23 Nelore bulls. For each batch, at least 3 IVP manipulations were performed, in total of 184 IVP manipulations, collecting sperm traits analysis by the *in vitro* fertilization step, after Percoll® gradient selection and embryo production rates (cleavage and blastocyst rates). Sperm traits evaluated by flow cytometry were acrosome and membrane integrity (FITC-PSA/PI), mitochondrial membrane potential (JC-1) and chromatin integrity (modified SCSA) and CASA parameters related to total and progressive motility, movement kinetics and velocities were recorded. Twenty-two sperm traits, cleavage rate and bull individual effect were included to build a mathematical model, considering the blastocyst rate as predictor of the in vitro bull fertility. Statistical analysis was performed using the SAS 9.4 Software with the GLM model, and the selection of the variables was performed through the Forward Selection. The adjusted coefficient of determination (ADJRSQ; R2) and p-value $\leq$ 0.5 were used as criteria for model acceptance. Between all variables included to predict blastocyst rate, bull and cleavage rates were those, which presented higher F values and then better indicators of embryo production. The best predicted model achieved for blastocyst rate included the percentage of total motility, the percentage of static sperm, cleavage rate and the bull individual effect ($p<0.0001$ and Adj R-Sq = 0.6319): Blastocyst rate (%) = - 1.2382(Intercept) + Bull + 0.1229*(total motility) + 0.0667*(Static) + 0.4494*(cleavage rate). Our results indicated that the bull individual effect is one of the most determining factors for IVP outcome. This effect, carried by the sperm produced by these animals will directly affect blastocyst rate. The assessment of sperm traits is an attempt to explore such bull individual effect. However, none of the 22 sperm attributes analyzed alone or in combination can explain a significant part of this bull effect on blastocyst rate. Then, our results indicate that bull individual effect, which is probably independent from the different sperm traits analyzed, is the strongest effect to define the IVP rates. Further studies focusing on sperm nuclear proteins, micro RNAs and metabolism may enlighten the knowledge on such bull individual effect. Financial Support: FAPESP (n° 2016/15147-5)

A062 OPU-IVF and ET

The oviduct fluid supplementation in the *in vitro* fertilization medium does not reduce polyspermy incidence during the non-breeding season in goat species

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Polyspermy is one limiting factor of IVP in several species. In goats, this pathologic condition affects up to 35% of *in vitro* embryos. The action of oviduct fluid (OF) on monospermic modulation has been already demonstrated in swine and bovine species. This study assessed the OF effect on polyspermy occurrence using different inseminating doses for IVF in goats. The study was performed during the non-breading season at Nouzilly, France. Goat genital tracts in late follicular phase of estrous cycle were obtained at a local slaughterhouse. At the laboratory, tracts were dissected, and the oviducts lumen were flushed with 500 µL of IVF medium. The flushing containing OF was centrifuged (10,000□*g*) two times, aliquoted and stored at -80 °C for later use. After follicular aspiration, *cumulus* oocyte-complexes (COCs) were submitted to IVM (TCM199, 10 ng/mL EGF, 100 µM cysteamine, 10% FCS) for 22 h. Then, COCs were randomly distributed in six groups, co-cultured with three different sperm concentration (1.0, 2.0 and 4.0 x 10^6 cells/mL) in SOF medium supplemented with 5 µg/mL heparin, 4 µg/mL gentamycin and 10% estrus sheep serum with 10% OF (OF groups; OF1, OF2 and OF4) or without (control groups; CTRL1, CTRL2 and CTRL4). After 18 h of IVF, presumptive zygotes (n=628) were denuded, fixed, stained with Hoechst and evaluated under fluorescence microscopy. The normality of variables was verified by Kolgomorov-Smirnov test and data were compared by Mann-Whitney test. The results are shown in mean±SE. In six runs, 1.576 COCs were used for IVP: there was no difference (P>0.05) for OF1 *vs*. CTRL1, OF2 *vs.* CTRL2 and OF4 *vs.* CTRL4, respectively, for: cleavage rate 65±7(96/148) *vs.* 70±7(109/154), 77±2(119/155) *vs.* 72±5(114/156), 75±7(114/152) *vs.* 74±4%(112/149); blastocyst rate at day 8, 41±5(61/148) *vs.* 38±7(59/154), 39±6(59/155) *vs.* 32±6(50/152), 38±4(60/156) *vs.* 33±6%(50/149); penetration rate (74±5 *vs.* 67±4; 71±6 *vs.* 69±7 and 67±7 *vs.* 72±6%) and monospermy rate (60±5 *vs.* 46±7; 52±4 *vs.* 46±5 and 38±2 *vs.* 31±4%), although OF has shown a propensity to increase monospermy at the three sperm concentrations. The production efficiency of monospermic zygotes (monospermy/penetration) tended to be higher (P=0.06) for OF2 than CTRL2. The absence of an OF positive effect could be a consequence of the strong positive photoperiod blocking the hypothalamic-pituitary-gonadal axis and inhibiting the ovulation. Ovarian steroids (progesterone and estradiol) play roles on the modulation of oviduct activity, including secretory function. The OF composition is influenced by estrous cycle phase; the oviduct-specific glycoprotein (OVGP1) reaches its peak when estradiol concentration increases at preovulatory period; and it is well known that OVGP1 has a role on zona pellucida hardening after fertilization and thus modulation of penetration. In conclusion, the OF supplementation on IVF medium was not capable to modulate the incidence of polyspermy at the non-breeding season in goats.

A063 OPU-IVF and ET

Effect of age at first calving epd on bovine *in vitro* embryo production

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Individual bulls differ in their ability to fertilize oocytes and/or to develop to blastocyst stages following *in vitro* fertilization (IVF) procedures. The objective of this experiment was to evaluate the effect of Age at First Calving - Expected Progeny Difference (AFC EPD) of the bull in the *in vitro* embryo production (IVP). The data of this study were obtained from the commercial laboratory of IVP - MÚLTIPLA EMBRIÕES LTDA (Ji-Paraná - Rondônia - Brazil), between November 2017 and November 2018. The ovum pick-up procedures were performed in six farms from Rondônia and Roraima states. A total of 1.579 Cumulus Oocyte Complexes (COCs) were collected and submitted to *in vitro* maturation (IVM), followed by *in vitro* fertilization (IVF) and culture (IVC), at 38.5ºC, 5,5% CO2 and high humidity. Seventeen bulls were used in this study. Only bulls used in more than 2 IVP procedures were included in this study. Therefore, 17 bulls were used. Considering the EPD AFC, bulls were categorized into two groups: TOP+ (n=14; DECA 1 bulls for AFC), and, TOP- (n=3, DECA 2, 3 and 4 for AFC). Chi-square test was used to compare the cleavage and blastocyst rates between bull groups. From 1.579 COCs subjected to IVP, 1.309 were inseminated with semen of TOP+ bulls and 270 with semen of TOP- bulls. The TOP+ bulls had higher cleavage (76.3%, 999/1309 x 69.3%, 188/270; P = 0.01) and blastocyst rates (53.7%, 703/1309 x 47.03%, 127/270; P = 0.04) than TOP- bulls. The results of this study may provide evidences that AFC, as a genetic parameter, is useful to select more fertile bulls to IVP. However, more detailed studies are necessary to better understand the association between specific EPDs and *in vitro* embryo production.

A064 OPU-IVF and ET

Evaluation of the pregnancy rate of poor embryos produced *in vivo* submitted to culture prior to the-transfer

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One of the most important factors associated with the success of the embryo transfer (ET) techniques is the morphological embryos evaluation before cryopreservation and / or transfer to recipients. The relation between the morphological evaluation and the developmental capacity of the embryos is highly associated with pregnancies rates. The objective of this study was to evaluate the prior transfer culture of poor quality embryos produced in vivo, developmental rate on the culture, embryo transfer rates with single or doubly embryo transfer. Date were analyzed from ET of Angus beef cattle of a central of ET, located in the city of Santiago, Rio Grande do Sul, Brasil. The embryos used in this study were those that showed low quality, classified as poor (IETS, 1998) these embryos were submitted to a culture in an enriched medium (Holding Plus ®, Vitrocell, Campinas, São Paulo, Brasil) on the heating table at a temperature of 36ºC and the developmental stage was observed during some hours (2 to 12 hours). The embryos that were developed were transferred to recipients and according to availability, and in some, two embryos were transferred, so the inovulation were divided into two groups, group I: single transfer and group II: double transfer. From the 127 embryos submitted to culture, 71 (55.9%) were transferred, obtaining a developmental rate greater than 50%. Of total transfers (71), 36 pregnancies were diagnosed (pregnancy rate 50.70%). On group I, 48 transfers were performed, with 20 pregnancies (pregnancy rate 41.66%); On group II, 23 transfers were performed, confirming 16 pregnancies (pregnancy rate 69.57%), a higher pregnancy rate when two embryos were inovulated together. It was concluded that the use of lower quality embryos kept in culture before the transfer was shown to be a viable alternative, since it obtained a good developmental rate as well as a good pregnancy rate. Double inovulation can also be a viable alternative, since they significantly increase the pregnancy rate. However, should be evaluated the percentage of twin births. In conclusion, the use of prior transfer culture for poor embryos is an alternative for their use as well as a decrease in costs with recipients.

A065 OPU-IVF and ET

Addition of melatonin to the maturation medium of bovine oocytes subjected to heat shock: effects on nuclear maturation, apoptosis, reactive oxygen species, mitochondrial activity, and gene expression

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The effects of melatonin addition to IVM medium of bovine oocytes under heat shock (HS) on nuclear maturation, apoptosis, mitochondrial activity, reactive oxygen species (ROS) and *GDF9* gene expression were evaluated. Cumulus-oocytes complexes (COCs) were recovered from 3-8 mm follicles of crossbreed *Bos indicus ovaries* collected at a slaughterhouse. COCs were matured under HS (12h at 41.5ºC and 7% CO_2 followed by 12h at 38.5ºC and 5% CO_2) in medium with 0, 10^{-12}, 10^{-9}, 10^{-6} and 10^{-3} M melatonin (Sigma-Aldrich, St. Louis, USA). In the non-stress (NS) group oocytes were matured for 24h at 38.5ºC and 5% CO_2 without melatonin. Oocytes were processed for TUNEL assay (Promega, Madison, USA) and stained with DAPI (Vector Lab., Burlingame, USA) to evaluate apoptosis and maturation rates (six replicates, 140±36 CCOs/replicate). For mitochondrial activity (three replicates, 133±18 CCOs/replicate) and ROS (four replicates, 130±20 CCOs/replicate) oocytes were stained in MitoTrackerRed CMX-Ros (Thermo Fisher Scientific, Waltham, USA) and DCFDA (Sigma-Aldrich) and analyzed under a fluorescence microscope. Images were analyzed by the software Image J 1.49. The *GDF9* gene expression was evaluated by RT qPCR (Applied Biosystems 7300 Real-Time PCR System, Thermo Fisher Scientific, Waltham, USA; three replicates, 10 CCOs/replicate). It was considered a randomized block design. Data were analyzed by the GLIMMIX procedure (SAS® 9.3), using binomial (maturation and apoptosis rates) or gamma (mitochondrial activity and ROS) distribution. The *GDF9* gene expression was analyzed by the software REST® and the results expressed regarding the calibrator NS. Melatonin did not improve (P>0.05) the maturation rate under HS (67.8±0.6; 75.2±0.2; 59.5±0.3; 67.6±0.2 and 55.8±0.5% in the 0, 10^{-12}, 10^{-9}, 10^{-6} and 10^{-3} M, respectively). The maturation rate did not differ (P>0.05) between 0, 10^{-12}, 10^{-6} M and NS (76.6±0.14%). Apoptosis rate in the NS group (0.6±0.6%) was lower (P<0.05) than in the groups 0, 10^{-12}, 10^{-9} and 10^{-6} M (4.4±1.0; 3.9±0.9; 4.0±1.2; 3.2±0.9%, respectively) and did not differ from 10^{-3} M (2.1±0.4%). Mitochondrial activity was lower (P<0.05) in the 10^{-3} M (42.9±0.1 arbitrary units - AU) than in the other groups (0 M: 63.9±0.1; 10^{-12} M: 62.4±0.1; 10^{-9} M: 59.8±0.1; 10^{-6} M: 58.0±0.1 AU) and it was greater in 0 M than in NS (57.1±0.1 AU). ROS production was lower (P<0.05) in the 10^{-3}, 10^{-6} and 10^{-9} M (13.5±0.2; 16.2±0.2 and 16.1±0.2 AU, respectively) than in 10^{-12} M (32.5±0.2 AU) and 0 M (31.2±0.2 AU). ROS was greater in 10^{-12} M and 0 M than in NS (25.0±0.2 AU). *GDF9* gene expression was greater in the 10^{-3}, 10^{-6} and 10^{-9} M (5.8±1.6; 2.5±0.8; 1.7±0.4 folds) compared to NS. Melatonin at 10^{-6} M in the IVM protects oocytes from the damage caused by HS, as demonstrated by maturation rate similar to that observed on oocytes from NS, lower ROS production, and greater *GDF9* gene expression. Financial support: CNPq (427476/2016-0), FAPEMIG and CAPES (Financial code 001).

A066 OPU-IVF and ET

Cytoplasmic granules in oocytes do not influence embryonic and early fetal development in bovine

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Morphological oocyte evaluation is imprecise and subjective. The aim of this study was to test the hypothesis that oocyte with cytoplasmic granules can be included in IVP routine. Two experiments were performed using control cytoplasm (CC- homogeneous cytoplasm) and granulated cytoplasm (GC- cytoplasmic dimorphism). Both presented intact cumulus cells (more than three layers of cells, nonatretic, with no signs of expansion). The first experiment evaluated cleavage (%Cleav), blastocyst (%Bl), pregnancy (%Pre) rates and early fetal development (Crown-rump lenght-CRL) of oocytes obtained from cow donors (CEUA / EGL-3956180316). The second experiment aimed characterization of oocyte quality by Active caspase 3 levels-C3, Gap junction activity-GJA (Calcein-AM), percentage of lipid content-LC (Oil red) and transcription profile of oocyte (P1A, IGF2R, ZAR1), cumulus cells (BMP15, IGF2R) and blastocyst (P1A, IGF2R) obtained from slaughterhouse ovaries (results were analyzed as expression of the target genes relative to the GAPDH endogenous gene using the standard curve method). Results when necessary are presented by number/mean ± SE. The %Cleav and %Bl were compared by T-Test and pregnancy rate by Fisher's Exact Test. The C3, GJA, LC and percentage of transcripts evaluated were compared by the Mann-Whitney Test. All analyses were performed using the GraphPad Instat Software. The CRL was submitted to ANOVA using the Minitab Software ($P<0.05$). In Experiment 1, none of the analyses performed differed statistically (%Cleav, CC: 813/68.8±4.8; GC: 469/74.4±5.8; %Bl, CC: 368/12.1±2.9; GC: 209/11.3±4.1; %Pre, CC: 60/24.2±10.8; GC: 30/26.3±8.0; CRL: CC/GC, n.40/25: 31d- 9.2/9.8; 37d- 16/17; 43d- 23.3/24; 49d- 31.4/33.9; 55d- 46.3/47.2). In Experiment 2, C3 of the GC group (39/172.1±16.9) was higher when compared to CC group (21/66.2±11.6), other structural analyses did not differ between groups (GJA: CC: 38/5.6±0.4, GC: 57/6.2±0.2, LC: CC: 9/21.9±7, GC: 9/19.9±6.6). In the transcription profile, only ZAR1 was higher in CC group (178.2±151.6) when compared to the GC group (0.8±0.8). All other transcripts analysis did not show significant difference in oocytes (P1A, CC: 1.140±1, GC: 0.09 ±0.04, IGF2R, CC: 0.09±0.09, GC: 0.55±0.47), embryos (P1A, CC:0.07±0.05, GC: 0.016 ± 0.02 IGF2R, CC: 0.16±0.16, GC: 0±0) and cumulus cells (BMP15, CC: 0.03±0.01, GC: 0.02±0.04, IGF2R, CC:0.14±0.03, GC: 0.31±0.1). Despite granulated oocytes presented particularities in C3 levels and expression of the ZAR1 transcript, the results demonstrate that they have the same potential for development of control oocytes, suggesting that cytoplasm heterogeneity does not reflect oocyte competence in bovine. Acknowledgments: EMBRAPA, CAPES, CNPq.

A067 OPU-IVF and ET

Does the continuous ovum pick up (OPU) routine in donors affect the characteristics of *in vitro* embryos?

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In the commercial practice of in vitro embryo production (IVP) in cattle, losses in the characteristics of IVP after successive OPU in donor cows for prolonged periods are reported. The aim of this study was to evaluate the effect of the number of consecutive OPU during several years on the characteristics of IVP in Gyr dairy cows. The experiment has evaluated 19 Gir donors and they were classified according to their embryo rate (ratio of number of viable oocytes to embryos produced) in classes: 1(> 50.1% n = 7, OPU = 239, average age = 9 years old), 2 (44.1 to 50% n = 5, OPU = 144, average age = 10 years old) and 3 (<44% n = 7, OPU = 197, average age = 11 years old). A total of 580 OPU sessions were held from 2013 to 2018. The donors were kept at Embriza Farm (Campo Grande, Mato Grosso do Sul, Brazil) and were maintained in Brachiaria decumbens pastures, with mineral supplementation and water at libitum. The standard reproductive management immediately after OPU consisted of a first-use progesterone device (Cidr®-Zoetis) insert in vagina on day zero (D0). On day eight (D8) the implant was taken out and 25 mg of prostaglandin (Lutalyse®-Zoetis) was injected. The blastocyst rates were evaluated 168 hours post-insemination (performed only with sexed semen of Holstein breed), and the IVP procedures were performed in the Embriza laboratory (Campo Grande, Mato Grosso do Sul, Brazil) with media produced by Embriotec laboratory (Anápolis, Goiás, Brazil). The performance over the years was evaluated through the following characteristics: total oocytes (TotOv), viable oocytes rate (TxVia - relation between TotOv and total viable oocytes) and embryo rate (TxEm). Differences between OPU along the years and within the classes were evaluated using Kruskal Wallis test with significance level of 5%. The averages were compared in pairs through post hoc analysis of the same statistical test using SPSS software v.22. In the 1 and 3 classes, there was a decrease in the total number of oocytes in year 6 ($p < 0.05$). The average category 2 did not show different performance for this variable due of collection years (4 only). TxVia showed a positive effect from the year of collection in the three categories, with increase in the last two years of collection. The TxEm presented a negative effect in category 3 only in year 5 ($p = 0.037$), but in year 6 the result was similar to years 1 to 4. In classes 1 and 2 there was no effect from the year for this characteristic. The aspects related to the expertise of those involved in the field and laboratory routines, as well as in the general and reproductive management of oocyte donors, should be considered in the evaluation of this experiment. Finally, the main characteristics of in vitro embryo production were not negatively affected by the continued use (4-6 years) of Gyr donors cows.