OPU and ET

Improvement of IVEP program with follicular wave synchronization in cows with high antral follicle count

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We evaluated the effect of the synchronization of follicular wave on the *in vitro* embryo production (IVEP) program according to the antral follicle count (AFC) in cattle. The AFC was evaluated in Nelore (*Bos indicus*) cows (n=16) aged 3 to 10 -year-old and weighing an average of 450kg. These donors were included into one of two treatments, according to the AFC. Donors with high (≥25 follicles, *n*=8) or low AFC (≤15 follicles, *n*=8) were submitted in OPU/IVEP routines in a random (OPU without follicular control) and also synchronized (synch) form. The synchronization was performed by norgestomet implant (Crestar®), estradiol benzoate (2mg, Bioestrogen®) and D-cloprostenol (150µg, Croniben®) on a random day of the estrus cycle (D0) and OPU was performed on D5. We performed a 2X2 factorial design, considering random (n=4) vs. synch (n=4) OPU in donors with high vs. low AFC totalizing 8 OPU procedures in each AFC group, with an interval of 21 days among procedures. After IVEP, blastocysts were transferred into recipients previously synchronized. The pregnancy evaluation was performed 60 days later by ultrasound. Data were analyzed by PROC GLIMMIX or Logistic regression (P≤0.05) and are presented as M±SE or proportion. AFC and synchronization determined an interaction (P=0.01) for viable oocytes revealing that high AFC donors synchronized or not have the best results (low-random 7.88±0.81[b], low-synch 6.27±0.67[b], high-random 16.75±1.84[a] and high-synch 20.40±1.35[a]). The number of embryos was influenced by an interaction (P=0.002) between AFC and synchronization, with the best results to high AFC donors synchronized (low-random 3.14±0.49[c], low-synch 3.24±0.45[c], high-random 6.38±0.74[b] and high-synch 9.77±0.71[a]). An interaction (P=0.05) between AFC and synchronization was also observed for blastocyst rate with best results for high AFC donors synchronized [low-random 41.13%[b] (102/248), low-synch 41.46%[b] (85/205), high-random 38.99%[b] (209/536) and high-synch 49.77%[a] (325/653)]. The number of pregnancy was also influenced by an interaction (P=0.03) between AFC and synchronization, with more pregnancies to high AFC donors synchronized (low-random 1.09±0.21[c], low-synch 1.16±0.26[bc], high-random 2.50±0.39[b] and high-synch 3.84±0.37[a]). In conclusion, selection of donors with high AFC associated with the synchronization of the follicular wave pre OPU are strategies that improve the efficiency of IVEP programs in cattle. Keywords: Antral follicle, synchronization, ovum pick-up, fertility.

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Effect of the use of resveratrol associated with silica nanoparticles during *in vitro* maturation of bovine oocytes

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During the process of *in vitro* embryo production (IVEP), cells go through several challenges that are detrimental to their development. *In vitro* maturation is one of the most important stages of IVEP, in which the oocyte could undergoes a great oxidative stress, which can interfere with maturation process. Thus, the study of supplementation of the medium with antioxidants, becomes important to understand the mechanisms of control and production of reactive oxygen species (ROs). The use of nanoparticles (NPs) as drug carriers is an efficient way for these antioxidants to reach their site of action, due to the sustained release of the drug and its pharmacodynamic capacity, avoiding the toxic action of the active principle with supply peaks and reducing the process of degradation of the drug. The objective was to evaluate the use of resveratrol associated with silica nanoparticles as supplementation of *in vitro* maturation medium of bovine oocytes. The ovaries were collected in a commercial abattoir. Oocytes classified as grade I and II were (n=789), randomly distributed in the treatments during the maturation, the medium was supplemented with resveratrol diluted (Resv) or associated with nanoparticles (Nano): Control (n=159); Resv 0.5 µM (n=158); Resv 1 µM (n=155); Nano+Resv 0.5 µM (n=159); and Nano+Resv 1 µM (n=158). After submitting the oocytes to the treatments, they proceeded normally to the next stages of IVEP. Production rates were evaluated, based on cleavage rates, 48 h after *in vitro* fertilization (IVF) (D2) and blastocyst production, 7 days after IVF (D7). After the maturation period, 95 total oocytes, Control (n=21), Resv 0.5 µM (n=22), Resv 1 µM (n=14), Nano+Resv 0.5 µM (n=23) and Nano+Resv 1 µM (n=15), were stained with the fluorescent probe 4 ', 6'-diamino-2-phenyl-indole (DAPI), to identify nuclear maturation (immature - VG and MI, mature - MII), through the EVOs®FL epifluorescence microscope. The statistical analysis was performed using the Sigma Plot version 11 program. The Resv 1 µM treatment had a reduced ($P<0.05$) cleavage rate (47.9±2.8%) compared to the other groups, which were similar to each other [Control (83.1±3.8%); Resv 0.5 µM (81.1±2.3%); Nano+Resv 0.5 µM (79.1±4.6%) and Nano+Resv 1 µM (84.5±3.2%)]. The blastocyst rates in the Control (39.6±4.1%); Resv 0.5 µM (31.2±4.8%); Nano+Resv 0.5 µM (34.5±3.9%); and Nano+Resv 1 µM (40.2±4.6%) were similar ($P>0.05$). However, a reduction ($P<0.05$) in the percentage of blastocysts was observed in the Resv 1 µM treatment (20.5±2.8%). The average percentage of oocytes in the different stages of the maturation process, were similar between treatments ($P>0.05$). Resveratrol when associated with silica nanoparticles presents a better result for the cleavage rate and blastocysts in relation to Resv diluted form at 1 µM concentration, which may be related to a decrease in the toxicity of the antioxidant, due to the controlled release when the same is associated with these nanoparticles.

Animal Reproduction

OPU and ET

Transcervical embryo recoveries in Santa Inês ewes subjected to short- or long-term superovulatory protocols

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This study compared short- and long-term estrus synchronization in 16 cycling Santa Inês ewes. All ewes were submitted to the two protocols in a cross-over design resulting in replicates #1 and #2 with 16 ewes, each one. Ewes were synchronized with intravaginal sponges containing 60 mg medroxyprogesterone acetate (Progespon®, Schering Plough, São Paulo, Brazil) for 6.5 (G-6.5d) or 14.5 (G-14.5d) days, followed by a superovulation (SOV) of 4 or 3 days with decreasing doses of pFSH (Folltropin®-V, Bioniche Animal Health Canada Inc., Belleville, Canada), respectively. Non-surgical embryo recovery (NSER) was performed 6-7 days after estrus onset with 60 d interval. All ewes were administered i.m. injections of 1 mg estradiol benzoate (Estrogin®, Farmavet, São Paulo, Brazil) and 37.5 mg d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) 16 h plus 50 IU oxytocin (Ocitocina forte®; UCB, Jaboticabal, Brazil) i.v. 20 min before NSER. Blood samples were collected into heparinized tubes for measurements of plasma progesterone (P4) and estradiol (E2) concentrations, quantified respectively using the solid phase radioimmunoassay kits (Beckman Coulter®, Immunotec, Marseille, France) and commercial radioimmunoassay kit (ImmuChem™ Coated Tube, 17β- Estradiol CT, MP Biomedicals, LLC – Orangeburg, NY, USA). Statistical analyses were performed using the SAS software and SigmaPlot®. ANOVA and Tukey test were used for parametric data comparisons and simple linear regression was used for correlation analyses. Proportions were analyzed using a chi-square test. Differences were considered to exist when $P<0.05$. P4 concentrations were greater ($P<0.05$) in G-6.5d than G-14.5d at the time of first pFSH injection, sponge removal and NSER. Estrus onset was delayed ($P<0.05$) in ewes during the #2 compared with #1 replicate by approximately 14 h. NSER could be performed in 11/15 ewes that were in estrus, with an average of 3 viable-embryos/donor. NSER success was similar ($P>0.05$) in G-6.5d (86.7%; 13/15) and G-14.5d (81.3%; 13/16). SOV yields (structures and viable embryos recovered) were similar ($P>0.05$) between G-6.5d (5.8±1.3 and 2.9±1.0) and G-14.5d (7.0±2.0 and 4.1±1.3), but degenerated embryos were only found in G-6.5d animals. Embryo viability was similar ($P>0.05$) between G-6.5d (46.3±12.3%) and G-14.5d (56.2±11.6%) and in replicates #1 (47.1±10.2%) and #2 (65.6±13.6%). In G-6.5d, mean P4 concentrations from sponge removal until NSER were positively correlated with the numbers of degenerated embryos, whereas E2 concentrations at NSER were positively correlated with embryo viability rates. In summary: i. the duration of progestin-priming and multiple-dose pFSH treatment had a limited effect on SOV yields in cyclic Santa Inês ewes; ii. the duration of SOV regimen may alter the influences that endogenous steroids exert on ova/embryo quality in ewes. Acknowledgments: Embrapa (SUPEROV–22.13.06.026.00.03/22.13.06.026.00.04), CNPq (314952/2018-7), Fapemig (CVZ-PPM00042-14).

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Cervical relaxation protocol without estradiol benzoate is efficient to allow transcervical uterine flushing in Dorper ewes

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This study evaluated the efficiency of non-surgical embryo recovery (NSER) with cervical relaxation protocol using different doses of estradiol benzoate (EB) in ewes. A total of 36 pluriparous Dorper ewes received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Progespon®, Zoetis, Brazil) for 9 d plus an injection of 300 IU of eCG (Novormon®, Zoetis, Brazil) i.m. 24 h before sponge removal. Ewes were not mated and were randomly assigned to receive i.m. 37.5 µg of d-cloprostenol (Prolise®, Agener União Saúde Animal, Brazil) and different doses of EB (RIC-BE®, Agener União, Brazil) i.m. at 16 h before NSER: 0.0 mg (0.0EB; *n*=12); 0.5 mg (0.5EB; *n*=12) or 1.0 mg (1.0EB, *n*=12). All ewes also received oxytocin (50 IU; Ocitocina Forte UCB®, UCBVet, Brazil) i.v. 20 min before NSER, which was performed 8 days after sponge removal. Corpora lutea (CL) were counted by transrectal ultrasonography 24 h before NSER. After NSER, ewes were kept in natural breeding for seven weeks. Present data were analyzed using R software (version 3.6.1, The R foundation for Statistical Computing). Fisher Exact Test was used for non-parametric data, while parametric data (mean±SEM) were analyzed by ANOVA and Tukey test, considering P<0.05 as significant. Average CL count (P>0.05) was 2.0±0.3 (0.0EB), 2.1±0.3 (0.5EB) and 1.7±0.2 (1.0EB). NSER was successfully performed in 91.7% [33/36 (0.0EB = 83.3%; 0.5EB = 91.7%; 1.0EB = 100.0%)] of the animals and overall fluid recovery efficiency was over 97% (P>0.05). The successful Hegar transposing rate was 100.0, 91.7 and 100.0% for 0.0, 0.5 and 1.0 EB groups, respectively (P>0.05) and the duration of cervical transposing with Hegar dilator was longer (P<0.05) in 0.0EB (4.2±0.3 min) compared to both 0.5EB (1.7±0.3 min) and 1.0EB (1.5±0.3 min) groups. Similarly, the cervical transposing with mandrel/catheter was longer (P<0.05) in 0.0EB (2.4±0.5 min) than 1.0EB (1.3±0.5 min). Mean duration of uterine flushing was 19.2±1.2, 21.4±1.4 and 18.6±1.1 min and the oocyte recovery rate was 52.6, 39.1 and 40.0% for 0.0, 0.5 and 1.0EB groups, respectively (P>0.05). Ewes with at least one oocyte recovered represented 70.0 (0.0EB), 60.0 (0.5EB) and 58.3% (1.0EB). The post-NSER fertility differed (P<0.05) between 0.0EB (90.0%) and 0.5EB, (36.4%) while 1.0EB was similar to both (58.3%). In conclusion, cervical relaxation protocol without EB could be successfully used for NSER in Dorper ewes without impairing technical viability and the post-NSER fertility. Financial support: Embrapa (22.13.06.026.00.04) and Fapemig (CVZ-PPM 00201-17). KEYWORDS: transcervical, embryo collection, sheep

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Circulating IGF1 and glucose relation to *in vitro* embryo production in young Gyr (*Bos indicus*) dono

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Use of young animals as oocyte donors contributes to genetic gain through reduction of the generation interval. However, *in vitro* embryo production (IVEP) in young animals can be challenging due to low oocyte quality. Studies have shown that IGF1 and glucose are related to oocyte quality and embryo development. Thus, the objective of the present study was to evaluate the relation between blood circulating concentration of IGF1 and glucose to oocyte quality and IVEP of young Gyr donors. For this, 30 Gyr calves (3-10 months) were submitted to blood sample collection and OPU on the same day. The blood samples were analyzed for IGF1 using competitive immunoenzymatic assay (cELISA) in house and for glucose using commercial kits (Bioclin® and CELM®). The results were correlated with OPU/IVEP parameters. Data were analyzed using the CORR procedure of SAS software. The mean of IGF1 was 318.1 45.5 ng/ml and glucose was 104.5±18.5 mg/dL. There were no correlation between IGF1 (P=0.2427; r=0.0484) or glucose (P=0.6863; r=-0,07833) and follicular population, IGF1 (P=0.9428; r=0.0027) or glucose (P=0,5631; r=0,11196) and number of viable oocytes, IGF1 (P=0.1597; r=0.2649) or glucose (P=0,387; r=0,16684) and rate of viable oocytes. Also, there was no correlation between IGF1 (P= 0.9579; r= 0.01007) or glucose (P=0,6635; r=0,08435) and number of cleaved oocytes, cleavage rate (IGF1; P= 0.4594; r= -0.14037; and Glucose; P=0,7979; r=0,0497), number of blastocysts (IGF1; P=0.1160; r=0.0859; and Glucose; P=0,9127; r=0,02129) or blastocyst rate (IGF1; P=0.1059; r=0.0907 and Glucose; P=0,3485; r=0,18059). These data are indicative that circulating IGF1 and Glucose are not related to oocyte quality and IVEP of young Gyr donors.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Effect of astaxanthin supplementation on oocyte quality and *in vitro* embryo development of holstein cows

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Oxidative stress occurs when there is a shift in the balance between oxidants and antioxidants, resulting in accumulation of oxidant factors in the cells. This cellular stress affects follicular and oocyte development and gonadotrophin secretion, negatively impacting oocyte quality, especially in high-yielding Holstein dairy cows. In this manner, this study aimed to determine the effect of the daily supplementation of dairy cows with the antioxidant astaxanthin on oocyte quality and subsequent embryo developmental potential. Lactating Holstein cows (n=45; ± 550 kg of body weight) from a Compost Barn farm located in the Southwest region of Paraná – Brazil received 0 (control), 0.25 (low astaxanthin) or 0.5 (high astaxanthin) mg of astaxanthin PO SID for 75 days before OPU. OPU was conducted twice with a 15 day-interval and oocytes were morphologically classified in viable (Grades 1, 2 or 3 according to cytoplasm homogeneity and cumulus cells layers). Regardless of morphological characterization, all aspirated oocytes underwent IVF to assess their developmental competence. Data were tested for normality of residues (Shapiro- Wilk test) and homogeneity of variances (F max test) for each variable. Data were square root-transformed when premises for analysis of variance were not met and analyzed by one-way ANOVA with Tukey's multiple comparisons test using the GraphPad Prism version 7.0.0 for Mac. Astaxanthin supplementation increased the total number of oocytes (p=0.009), and the total number of viable oocytes (p=0.0257) retrieved per female. More oocytes were obtained from animals supplemented with 0.5 mg/ kg/ day of astaxanthin compared to control and low astaxanthin groups ($p \leq 0.05$). Only cows from high astaxanthin group presented a higher number of viable oocytes retrieved compared to the control group (p=0.0292), but no difference was observed between astaxanthin groups. Interestingly, astaxanthin supplementation affected cleavage (p=0.0408) and blastocyst (p=0.0017) rates unexpectedly. Fewer embryos cleaved at day 3 after IVF from females treated with the highest dose of astaxanthin compared to control group (p=0.0412) while no difference was observed between control and low astaxanthin groups for cleavage rate. Moreover, fewer embryos reached the blastocyst stage at day 7 from high astaxanthin group compared to control and low astaxanthin groups ($p \leq 0.01$). These data suggest astaxanthin supplementation in lactating Holstein cows can improve the number of morphologically viable oocytes obtained by OPU, but at the dose of 0.5 mg/ kg/ day it can affect their *in vitro* developmental potential. Further studies are necessary to better understand how astaxanthin affects the early embryo development in bovine. All animal procedures were approved by the Ethics Committee on the Use of Animals at Universidade Tecnológica Federal do Paraná (CEUA-UTFPR), Paraná, Brazil.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

The use of exogenous progesterone in sheep superovulation protocols influences the percentage of viable embryos

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The success of multiple ovulation and embryo transfer strongly depends on superovulatory protocols (SOV) success which has shown inconstant. SOV protocols have used different sources of progestogen with could impact viable embryo yield. This study compared the effect of different intravaginal devices containing medroxyprogesterone acetate (MAP) or progesterone (P4) during SOV on follicular development and the embryo quality. A total of 36 multiparous Santa Inês ewes received intravaginal sponges containing 60 mg of MAP (Progespon; Schering Plough Animal Health, SP, Brazil) for 6 d plus 300 IU eCG (Novormon 5000; MSD Animal Health, SP, Brazil) and 0.24 mg cloprostenol sodium (Estron, Tecnopec, SP, Brazil) i.m. 24 h before and 0.025 mg lecirelin (Synthetic GnRH, Gestran Plus; Tecnopec, SP, Brazil) 36 h after sponge removal. The SOV began 80 h after sponge removal, using 133mg of FSH (Folltropin-V, Bioniche Animal Health, ON, Canada), administered in six doses every 12 h. In the first dose of FSH, the animals were randomly allocated to three experimental groups (12 ewes/each) inserting of an intravaginal sponge impregnated with 60 mg MAP (G_{MAP}); or a vaginal implant containing 330 mg of P4 (Eazi-Breed CIDR, Zoetis Indústria de Produtos Veterinarios Ltda, SP, Brazil; G_{P4}), or a control group ($G_{Control}$) without treatment. The devices remained in situ until the fifth dose of FSH. With the last dose of FSH, 0.24 mg of cloprostenol sodium was administered, and 12 h later, 0.025 mg of lecirelin i.m. After removing the intravaginal devices, ewes were naturally mated with fertile rams at 12 h interval from the beginning of estrus until 24 h after GnRH application. The ovaries were scanned by transrectal ultrasound (SonoScape, Shenzhen, China, 7.5 MHz linear transducer) every 12 h from the beginning of the SOV until the ovulation was confirmed and the follicles were classified as small (< 3 mm), medium (3-5 mm) or large (> 5 mm). Data were analyzed with a mixed model, including the treatments as a main effect and the repetition as a random effect. During SOV, the follicular population (< 3; 3-5 and > 5 mm) did not differ between treatments, and there was no difference in the number of CLs/treatment (G_{MAP}=7.7±1.2; G_{P4}= 8.2±1.2; $G_{Control}$= 6.0±1.1; P = 0.40). The number of structures recovered/ewe (G_{MAP}= 4.8±0.8; G_{P4}= 4.9±0.8; $G_{Control}$= 2.4±0.9) and the number of viable embryos/ewe (G_{MAP}= 1.9±0.7; G_{P4}= 3.3±0.7; $G_{Control}$= 1.0±0.7) tended to differ with treatments (P= 0.08 for both). The percentage of viable embryos/recovered structures was greater with P4 and MAP than in controls (71.9%[a]; 49.9%[a] vs 24.5%[b]; P= 0.04). In conclusion, the administration of P4 or MAP in SOV protocols did not influence the amount of structures recovered in sheep. However, the greater percentage of viable embryos/recovered structures with the use of exogenous progesterone suggests that the hormonal milieu in which these follicles developed influences the final embryo quality.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Number of oocytes used for intra-follicular transfer of immature oocytes (IFIOT) does not affect nuclear maturation of bovine oocytes

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Many factors may be involved in the low efficiency of IFIOT and, in order to identify these factors, it is necessary to clarify in which point of the process the greatest losses are occurring. One factor that can affect the efficiency of the technique is the number of oocytes injected into the follicle, since the number of oocytes may influence the interaction between oocytes and the follicular environment. Therefore, we aimed to evaluate if different numbers of cumulus oocyte complexes (COCs) injected into the preovulatory follicle by IFIOT, would compromise nuclear maturation of bovine oocytes. To do that, Nellore heifers were synchronized by receiving a progesterone device (P4) associated with an application of estradiol benzoate (2mg; i.m.), on the first day of the protocol (D0). On D8, P4 was removed and 0.5 mg of Cloprostenol was applied (i.m). Twenty-four hours later (D9), ovulators that had a dominant follicle >10 mm diameter, received 25 µg of Gonadorelin acetate (GnRH). Six hours after induction of ovulation, 10 (IFIOT10/n=11 animals), 25 (IFIOT 25/n=5 animals) and 50 (IFIOT50/n=6 animals) COCs were injected on the follicle. Oocytes were aspirated from abattoir ovaries, part of the them were *in vitro* matured (IVM), as described by Leme et al., (2020), and the remained were distributed into the IFIOT groups. Were injected 108, 123 and 300 CCOs in IFIOT 10, IFIOT 25 and IFIOT 50, respectively . Twenty-two hours after IFIOT, the oocytes were recovered by ovum pick up (OPU) and IVM oocyte removed from culture. Recovered COC's were denuded and fixed for 48 hours in ethanol and acetic acid (3:1) and stained with lacmoid 45%. Oocytes were evaluated using a phase contrast microscope (NikonEclipse E200, 1,000X) and classified according to the nuclear stage as: germinal vesicle (GV), metaphase I (MI), anaphase I (AI), telophase I (TI), metaphase II (MII) and abnormal. The results were compared using Chi-square test ($P>0.05$). Before maturation all oocytes were at GV stage (n=94). After 22 hours of maturation, no differences ($P> 0.05$) were found on the percentage of oocytes that reach MII, among groups being 78.6%, 74.6%, 71.4%, 76.4% for IVM (55/70), IFIOT10 (44/59), IFIOT25 = (40/56) and IFIOT50 = (84/110), respectively. In addition, no differences ($P>0.05$) were also found among treatments on the percentage of degenerates oocytes (IVM=5,7; IFIOT10=5,1; IFIOT25=0; IFIOT50=7.3%) It can be concluded that regardless of the number (10, 25, 50) of COCs injected into preovulatory follicle in bovine nuclear maturation is not affected and the majority of the oocytes are able to complete reach MII stage. Acknowledgment: Embrapa; 4Programa Institucional de Bolsas de Iniciação Cientifica (PIBIC) - CNPq

OPU and ET

ZAR1 gene expression is altered in immature oocytes of prepubertal Gyr heifers that do not respond to ovulation induction protoco

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Bos indicus breeds such as Gyr cows present late puberty. An alternative to overcome this particularity is to include prepubertal heifers in breeding seasons. However, prepubertal heifers oocytes shows less developmental competence. Therefore, this study aimed to analyze immature oocyte developmental competence after ovulation induction response by quality marker genes expression (*ZAR1* - Zygote arrest 1; *MATER*- Maternal antigen that embryo requires; *IGF2R*- Insulin Like Growth Factor 2 Receptor). For ovulation induction, nineteen prepubertal Gyr heifers (237.2±32.9 kg and 19.7±0.9 months) created in the same conditions (*Brachiaria Decumbens* pasture and free access to water and mineral salt) received a progesterone vaginal implant (1 g) (Sincrogest®, Ouro Fino Animal Health, Cravinhos, SP, Brazil) for 12 days (D0-12). At D12 were injected of 1.5 mg estradiol benzoate (RIC BE®, Agener União, São Paulo, SP, Brazil). From D12-18, animals were evaluated twice a day by ultrasonography transrectal examination (DP-2200 Vet®, Mindray, China) for ovulation response (absence of the dominant follicle) and grouped as ovulated (OV) or non-ovulated (N-OV). At D18, the follicles (3-8 mm) were aspirated by OPU procedure. After, total RNA was isolated from denuded immature oocytes and analyzed for *IGF2R*, *MATER* and *ZAR1* gene expression by quantitative RT-PCR. The relative transcripts quantification was compared by Mann-Whitney test and considered significant when $p<0.05$ (GraphPad Instat software). Seven heifers ovulated after the hormonal protocol (OV=7 and N-OV=12). An average of 6.5 ± 2.1 and 5.3 ± 1.4 oocytes per animal in the OV and N-OV group respectively were collected. Surprisingly, only the ZAR1 (10.2 ± 1.8 *Vs* 15.6 ± 2.2) transcript was upregulated ($p=0.02$) in immature oocytes recovered from non-ovulated heifers. *IGF2R* (2.2 ± 0.8 *Vs* 1.8 ± 0.5) and *MATER* (2.5 ± 0.6 *Vs* 2.6 ± 0.5) expression was similar between groups (OV *Vs* N-OV, respectively). These transcripts are potential markers of immature oocyte development competence. ZAR1 gene, as an example, play a role in embryonic development before zygotic genome activation. In conclusion, the results suggest that heifers oocytes that do not ovulate after hormonal protocol have a different quality marker transcripts pattern. Such variations provide new information on the ovulation induction effects on the oocyte competence acquisition. Acknowledgments: FAPEMIG, EMBRAPA, CNPq and CAPES finance code 001.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Effect of three-dimensional *in vitro* culture on bovine embryo productio

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Resumo

Reduced competence of the *in vitro* produced embryo to develop to the blastocyst stage is caused largely by disruption of specific biological processes during oocyte maturation, fertilization, and early embryo development. The objective was to evaluate the *in vitro* production of bovine embryos submitted to culture in a three-dimensional system (3D) by magnetic levitation associated with nanoparticles composed of gold, iron oxide, and poly-L-lysine, compared to the traditional two-dimensional system (2D). Thus, 812 oocytes were evaluated, classified as grade I and II (good) and grade III (poor). The 3D system was tested during the *in vitro* culture phase, in which the following medium was used: SOF medium (Synthetic Oviduct Fluid), supplemented with pyruvate solution (0.11 mg / mL), amikacin (83 mg / mL) , BSA free of fatty acids and low endotoxin (6 mg / mL) and fetal bovine serum (2.5%). Oocytes classified as good and poor were treated with concentrations of 50 µL and 75 µL of nanoparticles diluted in the embryonic culture medium, forming the experimental groups: CIV 2D - GOOD (n = 82); CIV 2D - POOR (n = 70); CIV 3D 50 - GOOD (n = 79); CIV 3D 50 - POOR (n = 81); CIV 3D 75 - GOOD (n = 41); CIV 3D 75 - POOR (n = 48). Cleavage rates were evaluated 72 h after IVF (D3) and blastocyst rate 7 days after IVF (D7). Statistical analyzes were performed using Sigma Plot software version 11.0 (Systat Software Inc, San Jose, California, USA). No difference (P> 0.05) in cleavage rates was observed between CIV 3D groups [CIV 3D 50 - GOOD (76.7 ± 5.63%); 3D CIV 50 - POOR (66.0 ± 11.5%); CIV 3D 75 - GOOD (78.63 ± 4.99%); CIV 3D 75 - POOR (68.8 ± 23.2%)], regardless of quality and treatments. However, the cleavage rate was lower for the CIV 3D 50 - POOR treatment (P <0.05), compared to the controls: CIV 2D - GOOD (81.36 ± 7.48%) and CIV 2D - POOR (79.9 ± 5.10%). There was no blastocyst formation in any of the experimental groups. The 3D *in vitro* culture system associated with magnetic nanoparticles did not interfere in the cleavage process, however, it prevented the formation of a blastocyst, probably due to the formation of a magnetic field promoted by the magnetic drive used, since both 2D and 3D treatments were cultured under the same system, probably promoting changes in the processes of division, compaction, polarization, and cell differentiation. Since the cleavage rate of poor oocytes treated with 75 µL of nanoparticles and cultured at 3D system was similar to good oocytes cleavage rate, this method should be tested for maturation of poor oocytes combined with embryo culture in a 2D system. Keywords: *In vitro* embryo production, magnetic levitation.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Ovum pick-up during pregnancy improves the *in vitro* embryo production in Holstein heifers

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The objective of this study was to evaluate if the pregnancy status and period would affect the ovum pick-up (OPU) and *in vitro* embryo production (IVEP) outcomes in dairy cattle. Holstein heifers (n = 9) between 10 and 16 months of age and body condition score ranging from 2.5 to 3.5 (1-5 scale) were selected. The heifers were subjected to OPU and evaluated for oocyte recovery (total and viable) and embryo production (total and rate). OPU procedures were performed at Moment 0 (before artificial insemination; control) and in three-time points during pregnancy: Moment I (0-30 days), Moment II (31-60 days), and Moment III (>60 days of pregnancy). The same technician performed the AI of females after the detection of natural or induced estrus with 25 mg im of Dinoprost (Lutalyse®, Zoetis, Brazil). The AI was performed with commercial semen coming of the same bull. Pregnancy diagnosis was performed at 30 and 60 days after AI. All the OPU procedures were performed by the same technician. The oocytes recovered were matured for 24 hours, fertilized, and presumptive embryos were cultured *in vitro* in a single commercial IVEP laboratory. The statistical analysis was performed using the generalized linear mixed model (GLIMMIX), and a significant effect was compared by the Tukey test (P < 0.05). We found differences in the mean number of viable (13.77 ± 3.63 *vs.* 30.56 ± 3.45; P = 0.02) and total oocytes (17.43 ± 4.52 *vs.* 41.67 ± 3.79; P = 0.001) comparing the Moment 0 (before AI, nonpregnant) *vs.* Moment I (0-30 days of pregnancy), respectively. Pregnancy was also associated with an increase in the embryo rate (%) from Moment II (18.90 ± 4.91) in relation to Moment 0 (7.59 ± 3.25; P = 0.05), although the observed increase in the number of embryos (total) from Moment I (4.55 ± 0.75) to the Moment 0 (1.39 ± 0.85), was not significant (P = 0.07). In conclusion, early pregnancy may be associated with better IVEP outcomes, and the higher indexes were obtained in the first 60 days of pregnancy.

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Reproductive seasonality influences oocyte and embryonic competence but not uterine receptivity in buffaloes*

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Buffaloes are short-day breeders displaying a favorable (FBS) and a non-favorable breeding season (NBS), respectively, in shorter and longer luminosity days. *In vitro* embryo production (IVEP) is an alternative to reduce the impact of seasonality in reproduction. Based on that, we aimed: 1) to evaluate the seasonality effects on oocyte and embryo competence of oocyte donors, as well as on pregnancy establishment (P/ET) of embryo recipients; and 2) to study the effect of two different methods of embryo cryopreservation on P/ET. In Study 1, oocytes recovered from donors in FBS (Jun-Jul) and NBS (Dec-Jan) were used on IVEP. Cryopreserved blastocysts were transferred to recipients in FBS and NBS in a 2 x 2 factorial design (embryo-recipient): FBS-FBS; FBS-NBS; NBS-FBS and NBS-NBS groups. In Study 2, performance of embryos cryopreserved by vitrification (VT) or slow freezing for direct transfer (DT) was compared. Studies were performed at latitude 22º36´22 ” S using puberty heifers and lactating cows as oocyte donors and embryo recipients. OPU sessions were performed on a random day of estrous cycle and recovered COCs were used for IVEP. Recipients received an intravaginal P4 device (0.96g; Progestar, Boehringer) associated with 2mg estradiol benzoate (Estrovulinn, Boehringer) and 265µg sodium cloprostenol (Cioprostinn, Boheringer) on a random day of the estrous cycle (D0). On D9, P4 device was removed and animals received 530µg sodium cloprostenol (Cioprostinn, Boheringer) associated with 400 IU eCG (Novormon, Zoetis). On D11, 10µg Buserelin Acetate (Prorelinn, Boheringer) was administered. Pregnancy diagnosis was done on 30 and 60 days of pregnancy. Oocyte competence was evaluated upon embryo production rate considering the number of structures used to *in vitro* culture. Data were analyzed by chi-square test. Reduced (p=0.02) embryo production was observed in FBS (94/397; 23.7%) compared to NBS (170/551; 30.9%). A total of 162 embryos were transferred to the uterine horn ipsilateral to the ovary containing the CL on D18 and the P/ET were: FBS-FBS (19/38; 50%), FBS-NBS (21/48; 43.7%), NBS-FBS (8/33; 24.2%) and NBS-NBS (9/43; 20.9%). Embryos produced in FBS (40/86; 46.5%) were more competent (p=0.001) for P/ET than in NBS (17/76; 22.4%); however, P/ET was similar (p=0.50) between recipients in FBS (27/71; 38%) and NBS (30/91; 33%). No difference (p=0.14) was observed in P/ET of embryos cryopreserved by VT (28/92; 30.4%) or DT (29/70; 41.4%). No pregnancy loss occurred between 30 to 60 days in the VT group, but was 10.3% (3/29) in the DT group. We conclude that (I) oocytes obtained from buffaloes in NBS are more competent for early embryonic development; (II) embryos produced from donors in the FBS are more competent in establishing pregnancy; (III) the uterine receptivity of recipients in FBS or NBS is similar; and (IV) the cryopreservation method (VT or DT) does not affect P/ET. Acknowledgments: Sesmaria Farm, ABS and Boheringer-Ingelheim.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Risk factors for and impact of inflammatory diseases on maintenance of pregnancy in dairy cows after embryo transfe

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Objectives of this prospective cohort study were to identify risk factors for inflammatory diseases in Holstein-Gyr dairy cows and characterize their impact on reproduction. Diseases and milk yield were evaluated in the first 60 days postpartum in 252 primiparous and 481 multiparous cows. Body condition was scored at calving, 28 and 70 days postpartum. Uterine diseases (UTD) included retained placenta, metritis, and clinical and subclinical endometritis. Non-uterine diseases (NUTD) included mastitis, lameness, pneumonia, and displaced abomasum. Blood was sampled on days 0, 1, and 2 postpartum and analyzed for concentrations of haptoglobin, fatty acids, total Ca (tCa), P, and Mg, and again on day 8 for β-hydroxybutyrate (BHB). All cows were enrolled in a timed embryo transfer (ET) program starting on day 28 and first ET on day 46 postpartum. Pregnancy per ET (P/ET) was evaluated 31 and 59 days of presumptive gestation and days to pregnancy assessed up to 300 days postpartum. Data were analyzed by logistic regression, ANOVA, and the Cox's regression using SAS software. Overall, 63.3% of the cows were diagnosed with UTD and 20.6% with NUTD. The risk factors (P<0.05) for UTD included season of calving (hot = 60 vs. cool = 73% UTD), parity group (1 = 58 vs. 2 = 60.1 vs. >2 = 73.3% UTD), calving problems (no = 61.1 vs. yes = 86% UTD), days with subclinical hypocalcemia (0 = 55.1 vs. 1 = 55.2 vs. > 1 = 73.2% UTD), and increased concentration of haptoglobin or decreased concentration of Mg. The risk factors (P<0.05) for NUTD included parity (1 = 8.7 vs. 2 = 21.0 vs. >2 = 34.3% NUTD) and decreased concentration of Mg. Cows that developed UTD had increased (P<0.05) concentrations of haptoglobin (2.03 vs. 2.42 ± 0.12 AU), fatty acids (0.39 vs. 0.43 ± 0.01 mM), and BHB (0.69 vs. 0.74 ± 0.02 mM), but reduced (P<0.05) tCa (2.04 vs. 1.98 ± 0.01 mM), P (1.39 vs. 1.34 ± 0.02 mM) and Mg (0.96 vs. 0.94 ± 0.01 mM). Similar changes with increases (P<0.05) in serum haptoglobin and fatty acids, but decreases (P<0.05) in serum tCa, P and Mg were observed in NUTD cows. Cows that developed NUTD had a reduction (P<0.05) of 340 kg of milk in the first 60 days postpartum. Inflammatory diseases were associated (P<0.05) with reduced body condition and increased loss of body condition in the first 70 days postpartum. Maintenance of pregnancy based on P/ET was reduced (P<0.05) by UTD following the first (41.7 vs. 25.4%) or all ET (46.4 vs. 36.2%), whereas the negative impact (P<0.05) of NUTD was only observed at the second ET (39.0 vs 25.9%). Uterine diseases reduced (P<0.05) 21-d service rate (61.9 vs. 54.8%) and 21-d cycle pregnancy rate (28.6 vs. 19.9%), thereby resulting in a 35% decrease (P<0.05) in the hazard of pregnancy and an extra 32 days open. Inflammatory diseases depressed fertility in dairy cows receiving ET and the impact was caused primarily by diseases that affect the uterus suggesting that local inflammation in early lactation impairs maintenance of pregnancy.

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Circulating IGF1 and glucose relation to *in vitro* embryo production in young Holstein (*Bos taurus*) donors

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Use of young animals as oocyte donors contributes to genetic gain through reduction of the generation interval. However, *in vitro* embryo production (IVEP) in young animals can be challenging due to low oocyte quality. Studies have shown that IGF1 and glucose are related to oocyte quality and embryo development. Thus, the objective of the present study was to evaluate the relation between IGF1 and glucose to oocyte quality and IVEP of young Holstein donors. For this, 59 Holstein calves (3-10 months) were submitted to blood sample collection and OPU on the same day. The blood samples were analyzed for IGF1 using competitive immunoenzymatic assay (cELISA) in house and for glucose using commercial kits (Bioclin® and CELM®). The results were correlated with OPU/IVEP parameters. Data were analyzed using the CORR procedure of SAS software. The mean of IGF1 was 280.32 39.1 ng/ml and glucose was 96.4 11.35 mg/dL. No correlation were observed between IGF1 (P=0.1498; r=0.18985) or glucose (P=0.9522; r=-0.00844) and follicular population. There was a positive correlation between IGF1 and number of viable oocytes (P=0.0104; r=0.33400), but no correlation was observed for glucose (P=0.9641; r=0.00633). There were no correlation between IGF1 (P=0.6560; r=0.05920) or glucose (P=0.9107; r=0.01579) and viable oocyte rate. There was a positive correlation between IGF1 and number of cleaved oocytes (P=0.0008; r=0.42831) and cleavage rate (P=0.0018; r=0.40077). However, no correlations were observed between glucose and number of cleaved oocytes (P=0.6762; r=-0.05872) and cleavage rate (P=0.3951; r=-0.11923). A positive correlation was found between IGF1 and number of blastocysts (P=0.0124; r=0.32634) and blastocyst rate (P=0.0437; r=0.26589). However, no correlations were observed between glucose and number of blastocysts (P=0.5534; r=-0.08326) and blastocyst rate (P=0.5763; r=-0.07851). These data demonstrate that circulating glucose is not associated with OPU/IVEP efficiency. Nevertheless, circulating IGF1 is related to oocyte quality and IVEP of young Holstein donors and, therefore, may be used as an indicative for IVEP efficiency.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Season and donor breed effects on gene expression in bovine oocytes

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In vitro embryo production (IVEP) is an important biotechnology for genetic improvement of livestock for daily cattle. However, differences between reproductive physiology of *Bos taurus* and *Bos indicus* and on their ability to respond to different stressful situations, could affect IVEP results when donors from different breeds are used. Thus, the aim of this study was to quantify the mRNA abundance of genes related to heat stress (HSPA5), oxidative stress (SOD2), glucose metabolism (SLC2A3) and apoptosis (CASP3 and FOSL1) in oocytes from Gir (n=10), Holstein (n=7) e ½ Holstein-Gir (n=12) donors at the summer and winter seasons. At the end of the both seasons, each donor was submitted to 4 consecutive OPU sessions in a 72-hour interval. After OPU, the cumulus-oocyte complexes (COCs) were classified, and the oocytes from those with at least 3 layers of cumulus cells and homogeneous ooplasm were used to gene expression. Gene expression was quantified by real-time PCR, using GAPDH and ACTB as the constitutive genes. The effect of the season and the donor breed on the relative abundance of the genes were tested by ANOVA, and compared by Tukey's test, considering statistical difference with $P<0.05$. The winter season caused a reduction in the average of total COCs in Zebu (7.5±0.7 vs. 5.9±0.9) and crossbred (17.8±3.4 vs. 11.2±0.5), while the summer was harmful for Taurine donors (2.7±0.9 vs. 4.2±1.9). Regarding the gene expression, the season had no effect on any breed. However, HSPA5 and SLC2A3 genes showed higher expression ($P<0.05$) in Holstein oocytes compared to oocytes from ½ Holstein-Gir donors. In conclusion, there is an effect of the donor breed, but not of the season, on the expression of genes related to heat stress (HSPA5) and glucose metabolism (SLC2A3) in oocytes from Holstein and ½ Gir-Holstein donors.

Animal Reproduction

OPU and ET

Lipid accumulation and mitochondrial activity in *in vitro* matured bovine oocytes supplemented with l-carnitin

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Excessive formation of lipid droplets has been associated with variations in mitochondrial function, which probably affects lipid metabolism. The aim of this study was to measure mitochondrial activity and lipid content *in vitro* matured oocytes supplemented with l-carnitine. For this, the ovaries were collected in a slaughterhouse and transported to the laboratory where the follicles were aspirated and the COCs screened and classified. The oocytes were matured in conventional medium (BO-IVM Bioscience; control) or in the same medium supplemented with l-carnitine at a concentration of 3.03 mM at 38.8°C, 6% CO2 and maximum humidity. total of 158 COCs were collected 0, 4, 8 and 24 hours after IVM, and time 0 was considered immediately after follicle aspiration. Cumulus cells were removed and oocytes stained with MitoTracker® Orange CMTMRos (Mitotracker Orange; Molecular Probes, Eugene, OR, USA) at a final concentration of 300nM in PBS + BSA (0.3%) for 40 min at 38.8°C in 6%CO_2 and Bodipy® 493/503; (Molecular Probes, Eugene, OR, USA) for 10 min in PBS + BSA (0.3%). After washed and fixed, the stained oocytes were analyzed in a confocal microscope coupled with an inverted Axiovert 200M microscope. Fluorescence intensity was quantified using Image J software and statistical analysis was performed using PROC GLIMMIX SAS. There was no difference in mitochondrial activity (p>0.05) between immature and *in vitro* matured oocytes for 4, 8 and 24 hours supplemented or not with l-carnitine. Immature oocytes had lower (p=0.0116) lipid content than oocytes matured for 4, 8 and 24 hours in conventional medium, no differences were observed between these times, suggesting that there is no substantial accumulation during this period. However, oocytes matured for 4 hours supplemented with l-carnitine had (p>0.05) lipid content similar to immature oocytes and oocytes matured for 8 hours supplemented with l-carnitine. Oocytes matured with l-carnitine for 24 hours had higher (p <0.05) lipid content compared to oocytes matured with l-carnitine for 0, 4 and 8 hours, with no difference (p>0.05) when compared with oocytes matured in conventional medium for 4, 8 and 24 hours. Finally, there was a positive correlation (p<0.0001; r=0.66) between lipid content and mitochondrial activity. In conclusion, lipid accumulation occurred largely in the first 4 hours of IVM oocytes, and l-carnitine slows this process without variation of mitochondrial activity, which was positively correlated with lipid content.

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Treatment with GnRH 21 or 22 days after TAI to synchronize non-pregnant crossbred beef heifers to E

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The aim was to evaluate the effect of GnRH administrated 21 or 22 days after TAI to resynchronize non-pregnant heifers for embryo transfer (ET). A total of 503 crossbred (*Nelore x Aberdeen Angus*) heifers with 12 - 18 months old and 3.48±0.38 of body condition score (1-5 scale) were used. On a random day of the estrous cycle (D0), heifers received an intravaginal device with 1 g of P4 (Sincrogest, Ourofino, Cravinhos, Brazil) and 2 mg estradiol benzoate i.m. (EB, Sincrodiol, Ourofino). On D9 the P4 device was removed and 500 µg cloprostenol sodium (PGF, Sincrocio, Ourofino), 0.5 mg estradiol cypionate (EC, SincroCP, Ourofino) and 300 IU eCG (Sincro eCG, Ourofino) were administered i.m. Heifers were submitted to timed artificial insemination (TAI) 48 h later (D11). On D32, heifers were distributed into two experimental groups: (i) GnRH21, heifers (n=251) received 10 µg of buserelin (GnRH; Sincroforte, Ourofino) 21 days after TAI (D32); and (ii) GnRH22, heifers (n=252) received 10 µg of buserelin 22 days after TAI (D33). On the day of pregnancy diagnosis (D40 or D41), heifers diagnosed as non-pregnant and with presence of CL were submitted to ET. Embryo transfer was performed (GnRH21, n=77 and GnRH22, n= 111) in the uterine horn ipsilateral to CL and fresh embryos (grade I) produced *in vitro* were used. The pregnancy diagnosis was performed 30 days after ET. Statistical analyses were performed with PROC GLIMMIX of SAS 9.4 ($P \geq 0.05$). The pregnancy rate per embryo transfer (P/ET) was higher ($P=0.05$) for GnRH21 (53.2%, 41/77) than GnRH22 (38.7%, 43/111). In conclusion, the application of GnRH 21 days after TAI efficiently resynchronizes embryo recipients, with a satisfactory P/ET. Acknowledgments: OuroFino Saúde Animal and Fazenda Santa Tereza - PA.

Animal Reproduction

OPU and ET

Lipid content and mitochondrial activity of bovine embryos with different developmental kinetics

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The kinetics of the first cleavages of the embryos produced *in vitro* was suggested as a tool to select the most viable embryos. Moreover, blastocysts generated from early cleaving embryos showed transcriptional changes, mainly related to lipid metabolism. The aim of this study was to measure lipid content and mitochondrial activity of bovine embryos with different developmental kinetics during development *in vitro*. For this, ovaries were collected in a local slaughterhouse. The follicles were aspirated and the COCs screened and classified. IVM was carried out for 24 hours and IVF for 18 hours at 38.8 ° C, 6% CO2and high humidity. IVC was carried out for 8 days at 38.8 °C, 6% O2, 6% CO2and high humidity. All IVP Media used were from Bioscience (United Kingdom). The cleavage rate and group division were performed 41 hours after IVF, when embryos with 2 and 3 cells were put together into a well and named Slow and embryos with 4 or more cells put together into a well and named Fast. Embryos were collected 41, 48, 72, 144 e 168 hpi in different development stages. The embryos were stained with MitoTracker® (300nM) and Bodipy® (3 µg / ml -1). After washing and fixation, the stained embryos were analyzed in a confocal microscope. The fluorescence intensity was quantified using Image J software and statistical analysis was performed using GLIMIX (SAS). In general, fast embryos have higher mitochondrial activity ($p = 0.0020$) and higher lipid content ($p < 0.0001$) than the slow ones. Morula and blastocyst have higher mitochondrial activity ($p = 0.0465$) and higher lipid content ($p = 0.0036$). When we compared the same embryonic stage between groups we couldn’t see any difference of mitochondrial activity ($p > 0.05$), however, the lipid content was higher ($p = 0.0024$) in fast than in slow blastocysts. In conclusion, lipid content of bovine embryos increase after activation of the embryonic genome, especially in early-cleaving embryos.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Genetic and nutritional influences on reproductive traits of Nelore heifers submitted to OPU-IVEP

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The present study evaluated the effect of genetic trends for precocity and average daily gain (ADG) on reproductive traits and *in vitro* embryo production of heifers. A total of 39 Nelore heifers (initial BW= 218±4.7 kg; age=13.5±0.2 months) with high (HG; n=20) or low genetic value (LG; n=19) for precocity was used. Heifers of each genetic group were randomly assigned to 2×2 factorial arrangement to receive high (HN; ADG=1.0kg/d) or low nutrition (LN; ADG=0.3kg/d) treatment. High nutrition heifers were treated in feedlot (corn silage, ration with concentrated ingredients and mineral salt–2.5% of live weight) and low nutrition heifers were treated in pasture plus protein supplementation (0.5% of live weight). Two months after starting treatment, heifers (pre-pubertal) were submitted to ultrasound guided OPU (EC9-5 Novilha, WTA) in a random day of estrous cycle. The recovered oocytes were sent to a commercial lab for the IVEP. High quality embryos produced were vitrified. At the same day, gynecological evaluation was performed to determine the largest follicle diameter (DF) and uterine horn diameter (UT). Furthermore, heifers were weighed and subcutaneous rump fat thickness (RUFAT) and rib fat thickness (RFAT) were measured by US. Also, blood samples were collected to determine IGF1 and insulin concentration. Statistical analysis was performed by GLIMMIX of SAS®. There was no interaction between genetic and nutrition for the following studied variables (P>0.05). The body weight (BW) at the OPU day was influenced by genetic (HG=329.6±0.4 *vs.* LG=264.1±0.3kg; P<0.001) and also by nutrition (HN=314.1±0.4 *vs*. LN=279.6±0.3kg; P<0.001). Regardless of the nutrition treatment, DF diameter was greater (P=0.0003) for HG heifers (HG= 9.38±0.4 vs. LG=7.42±0.3mm). Moreover, there was only a nutrition effect (P=0.04) on UT (HN= 13.05±0.3 vs. LN=12.20±0.2mm). IGF1 concentration did not differ between genetic (P=0.08) and nutrition (P=0.32) groups. However, HG heifers had higher insulin concentration compared to LG heifers (10.67±2.3 vs. 7.53±0.7 mg/dL; P=0.05; genetic effect). An interaction (P=0.01) between Nutrition*Genetic for RFAT was observed (HG-HN= 3.71±0.4^{a}; HG-LN=0.68±0.3^{bc}; LG-HN=1.83±0.4^{b}; LG-HN= 0.54±0.3^{c}mm). Nevertheless, there was only a nutrition effect (P<0.001) for RUFAT (HN=5.69±0.5 vs. LN=2.65±0.3mm). The number of viable oocytes did not differ between genetic or nutrition (P>0.5), as the number of produced embryos per OPU (P>0.2). However, the number of high-quality embryos was higher for oocyte recovered from HG than LG heifers (5.0±0.7 vs 2.8±0.7; P=0.04), without effect of nutrition (P=0.18). It can be concluded that high nutrition increased BW, UT and RUFA. Heifers with HG and HN had better RFAT than other groups. Also, heifers with high genetic value presented greater BW, DF, insulin, and produce more high-quality embryos than heifers with low genetic. Still, heifers with high nutrition did not increase their embryo production.

Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

OPU and ET

Use of AMH as a marker for selection of oocyte donors in the Gir (*Bos taurus indicus*) bree

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The anti-Mullerian hormone (AMH) is a growth factor produced by growing follicles. AMH concentrations have been proposed as a potential criterium to predict ART outcomes in cattle. Thus, the aim of this study was to evaluate the relationship between plasma AMH concentrations, antral follicle count (AFC), OPU-IVEP outcomes and genomic values for production traits, in the perspective of using AMH as a criterium for selecting oocyte donors. Peripubertal Gir (*Bos taurus indicus*) heifers (n=120), previously selected to be used as oocyte donors in the IVEP program were enrolled. Genomic predicted transmitting ability for milk (GPTAm) and age at first calving (GPTAafc) were determined using GeneSeek-Genomic-Profiler (GGP) Bovine 50 K microchip (Neogen Corporation, USA). At 23.3±0.5 months of age, heifers were examined by ultrasonography to determine AFC without prior synchronization of the follicular growth wave. Blood sample was collected, and plasma was stored for further AMH analysis. Plasma AMH concentrations were determined using an ELISA kit (AL114, AnshLabs, USA) by endocrinology Laboratory (LEAC – Laboratório Especializado em Análises Científicas, SP, Brazil). Data from 506 OPU-IVEP sessions (4.2±0.2 per heifer) performed between 2017 and 2019 were then recorded. Associations between AMH and AFC, IVEP outcomes and genomic value were initially estimated using the Pearson`s correlation method. Correlations when compared using the cocor R package. Heifers were then ranked into quartiles according to AMH concentration (1st: 617.1±80.2; 2nd: 926.9±85.0; 3rd: 966.6±97.2; 4th: 1241.3±98.8) and, for each endpoint, ANOVA was performed and differences among means were determined by the Tukey's test, using the PROC GLM of SAS. On average, heifers presented an AFC of 31.2±1.1 (mean±SEM) and AMH concentrations of 932.9±48.4 ng/mL and produced 32.3±1.5 total oocytes, 25.9±1.4 viable oocytes and 5.3±0.4 embryos per OPU-IVEP session. A moderate, positive correlation was observed between AMH and AFC (r=0.52; P<0.0001), total oocytes (r=0.49; P<0.0001), viable oocytes (r=0.47; P<0.0001), and embryos produced (r=0.34; P=0.0002), but not with GPTAm (r=0.26; P=0.0958) or GPTAafc (r=0.17; P=0.2732). Correlations between AMH and total oocyte yield were similar when calculated using either the results of the first OPU, the average results of all OPU, or results from the OPU with the greatest number of recovered oocytes (r=0.44, 0.49 and 0.46, respectively, P>0.05). The heifers ranked in the 3rd and last quartiles yielded more total oocytes, viable oocytes, and blastocysts, than those ranked in the 1st (P<0.05), but had similar GPTAm and GPTAafc (P>0.05). In summary, AMH can be used as a selection tool to increase oocyte and embryo yield, however, selection of potential donors based on AMH concentrations does not contribute to the increase of genomic values for milk production or age at first calving across generations.
Acknowledgements: Basa, Fapemig CVZ APQ 03430-17.