Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Effects of early weaning on fetal programming of gonadal development characteristics in precocious Nelore females

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We aimed to evaluate the effect of early weaning on fetal programming of characteristics associated with gonadal development in Nelore females. 55 Nelore heifers used in this experiment passed by fetal programming when they were in the uterus of primiparous (PRI) or pluriparous (PLU) Nelore cows through two weaning moments of their suckling calves: early (EW) (n=25; 150 days) or conventional (CW) (n=30; 240 days) weaning. At 14-15 months of age, heifers were evaluated by ultrasonography for antral follicle count and were treated with 150 mg of long-acting progesterone (Sincrogest injetável, Ourofino Saúde Animal) to induce puberty. Twenty days after puberty induction, a subgroup of 23 heifers (11 pubertal and 12 prepubertal) were submitted to an *ovum pick-up* procedure for *in vitro* embryo production (n=6 daughters of EW PLU cows; n=6 of CW PLU cows; n=4 of EW PRI cows; n=7 of CW PRI cows). Data were first analyzed as a 2 x 2 factorial (weaning strategy and dam's parity) and then separated according to pubertal status. A split-plot ANOVA was performed using the MIXED procedure of SAS. No significant (P>0.10) interaction of weaning strategy and parity was detected for antral follicle count and the *in vitro* embryo production variables analyzed. Also, a significant main effect of weaning time or parity was not detected for antral follicle count (total mean: 24.5±1.4, P=0.60), number of viable cumulus-oocyte complex (COCs) recovered (23.1±2.6, P=0.68), cleavage rate (70.6±3.8%, P=0.66), number of blastocysts (9.0±2.1, P=0.90), number of grade 1 and grade 2 blastocysts (7.8±1.9, P=0.88) and hatched blastocysts on day 9 after fertilization (6.9±1.6, P=0.57). However, the total number of COCs tended to be greater (P=0.08) in heifers gestated in PLU cows (50.5±5.9) than in PRIM cows (34.5±5.7). At the same time, the number of non-viable COCs was lower (P=0.03) in heifers gestated in PRIM cows (14.5±2.7) than in PLU cows (24.6±3.3). Comparing prepubertal and pubertal heifers, no differences were observed in total viable COCs recovered (21.3±3.5 vs. 25.2±4.1, respectively; P=0.47), cleavage rate (69.2±3.8 vs. 72.0±6.9%, respectively; P=0.39), total blastocysts (9.1±3.1 vs. 8.7±3.0, respectively; P=0.81), grade 1 and grade 2 blastocysts (8.0±2.7 vs. 7.6±2.8, respectively; P=0.70) and hatched blastocysts (6.8±1.9 vs. 7.0±2.6, respectively; P=0.79). In conclusion, the anticipation of weaning during fetal programming or the dam's parity order does not impact on the antral follicle count and effectiveness of *in vitro* embryo production of Nelore heifers. In addition, Nelore heifers have reasonable *in vitro* embryo production, regardless of their response to puberty induction.

Acknowledgments: FAPESP (2017/18937-0; 2022/13791-5); ABS-In vitro; and Biogenesis Bagó.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Pregnancy rate of *in vitro-produced* embryos from prepubertal Nelore heifers treated with injectable progesterone

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Embryo quality is one of the factors that affects pregnancy rate results, once embryos with higher quality have lower mortality rate and faster development after transfer than poor ones. Embryos from prepubertal heifers have a lower pregnancy rate than pubertal or cows, due to the low competence of oocytes. Prepubertal heifers have an immature hypothalamic-pituitary axis that affects the competence and quality of oocytes, reflecting on cytoplasmic and nuclear maturation. Therefore, aiming to improve the quality of oocytes and embryos from prepubertal females, the objective of the study was to evaluate the effect of injectable progesterone use in prepubertal Nelore heifers, 7 days before ovum pick-up (OPU). The study was conducted at the Centro Avançado de Pesquisa e Desenvolvimento de Bovinos de Corte, Sertãozinho, SP. Were used 23 Nelore females, 13 ± 0.8 months of age, allocated into two groups: progesterone group (PG) with 1ml (150 mg/ml) of injectable progesterone (Sincrogest injetável® Ourofino, Cravinhos-SP) 7 days before OPU and control group (CG) without progesterone application. After 30 days, the second OPU was performed (crossover between heifer groups). After OPU, the oocytes were selected for IVF and the vitrified embryos were subsequently transferred to recipients. Data were analyzed by the MIXED procedure of SAS®. No differences were observed between PG (37.75%; 111/294) and CG (35.35%; 111/314) in blastocyst rate (blastocyst / COC's *100), (P=0.58). However, the pregnancy rate of embryos from PG was higher (42.1%; 32/76) than CG (28.4%; 23/81) (P=0.02). In our experimental conditions, the use of injectable progesterone before OPU increases the pregnancy rate of *in vitro-produced* embryos from prepubertal Nelore heifers. Further studies with a large number of embryo transfers are required to confirm our results.

Acknowledgment: The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship granted. FAPESP (Processo nº 2017/50339-5), Vitrogen®, Cravinhos, SP, Brasil e Ouro Fino®, Cravinhos, SP, Brasil.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Characterization of chromatin condensation, quantification of lipids and mitochondrial activity in calf oocytes

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In IVP routine it is well established that prepubertal females' oocytes are less competent and have poorer ability to develop into embryos compared to pubertal females. The aim of the first study was to characterize chromatin condensation and to quantify the lipid content and mitochondrial location and qualification on immature calves and heifers' oocytes. Moreover, to evaluate an in vivo maturation system using intrafollicular oocyte transfer (IFOT). Calves (6~8 months old; n=10) and pubertal heifers (18~22 months old; n=6) were submitted to OPU. Gardes I or II COCs were used and part of oocytes (Calves, n= 24 and Heifers, n= 33) were denuded, fixed, stained with lacmoid for evaluation on a phase contrast microscope (Nikon E200). The chromatin configuration was classified based on Germinal Vesicle (GV) condensation morphology and grouped into low [LC (GV0 and GV1) or high [HC (GV2 and GV3) condensation. Another subset of immature oocytes (Calves, n= 10 and Heifers, n= 19) were fixed and evaluated using a LSM Leica (New Orleans, USA) confocal for measurement of lipid droplet area (Bodipy) and mitochondrial qualification and location. For the mitochondrial assessment, oocytes were equilibrated for 15 minutes in culture media supplemented with 5% FCS and incubated for 30 minutes at 38,5°C in 400 nM of the fluorescent dye MitoTracker Deep Red, data is presented as pixel average. The relative area of lipid droplets was quantified using the ImageJ® (N. I. H., USA). The remaining oocytes from the same OPU were either in vitro (IVM-C; n=21 and IVM- n=29) or in vivo (IFOT-C; n= 36 and IFOT- n=9) matured. After 22 hours of maturation, denuded and fixed oocytes were submitted to nuclear maturation evaluation and chromatin was classified into the different meiosis stage. The data obtained were analyzed by Chi-squared test. The lipids relative area and number of pixels in mitochondrial assay were analyzed by ANOVA, and Tukey's test. Significance level was set at 5%. Calves (70.8%; n=17) had a higher percentage of oocytes classified as LC ($p>0.05$) compared to heifers (27.3%; n=9). Heifers' oocytes (72.7%; n=24) showed higher percentage ($p>0.05$) of HC, compared to Calves (29.2%; n=7). No differences ($p>0.05$) were observed between heifers and calves' immature oocytes regarding the percentage of lipid droplets area (8.8%±5.3 vs 10.8%±4.5) or mitochondria number of pixels (35847±21895 vs 40215±14857). No difference ($p>0.05$) of oocytes reaching MII was observed among groups IVM-C (n=21; 80.9%); IVM-P (n=29; 75.6%); IFOT-C (n=9; 80.1%) and IFOT-P (n=36; 89.1%). In summary, calves' oocytes have similar patterns of lipid droplets and mitochondrial number of pubertal heifers. However, the chromatin of oocytes from calves is less packed and this can be related to its lower fertility in IVP programs. Finally, the use of IFOT as an in vivo maturation system proved to be efficient, since the proportion of MII oocytes was similar compared to in vitro maturated ones.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Effect of alpha lipoic acid on maturation of cumulus-oocyte complexes, on *in vitro* production of bovine embryos

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The inclusion of antioxidant supplements during *in vitro* maturation (IVM) of bovine embryos has been explored as a strategy to improve embryo quality and development. Alpha-lipoic acid (ALA), a powerful antioxidant, has attracted interest due to its protective properties against oxidative stress. Ovaries (n = 809) from *Bos taurus* female cattle were obtained from a local slaughterhouse and transported to the laboratory in 0.9% saline solution at 36°C. Cumulus-oocyte complexes (COC) were aspirated from antral follicles (2-8 mm), and grade I and II oocytes were selected for use. The COCs (N = 2,935) were matured in TCM-199 medium (ABS Global Brasil®, Mogi Mirim, SP, Brazil) without or with supplementation of ALA (CAS 1077-28-7, Sigma Chemical®, St. Louis, Missouri, USA) at different concentrations (2.5, 5, 10, or 25 µM) for 24 hours. After maturation, COC were transferred to TBM medium (Tris-buffered medium; ABS Global Brasil®, Mogi Mirim, SP, Brazil) and fertilized with semen from a single bull. The first feeding (SOF medium) was performed on the third day of IVC, and the second feeding (SOF+Glucose medium) was performed on the fifth day of culture. The entire embryo production process was carried out at a temperature of 38.5°C, 5% CO2 atmosphere, and 95% humidity. We evaluated the cleavage, blastocyst formation, and hatching rates on days 2, 7, and 9, respectively. Statistical analysis was performed using ANOVA with a generalized linear model, with treatment as a fixed factor, FIV routine as a random factor, and the number of COC as a covariate. Tukey's test was used as a post hoc test. The descriptive analysis presented mean values and standard error of the mean. All statistical analyses were performed using Minitab software, version 18.1, with a significance level of 5%. There was no difference ($p \geq 0.05$) between the treatments and control groups considering all compared variables. For the control group, the rates were 74.4 ± 4.8 for cleavage, 32.3 ± 5.8 for blastocyst, and 58.8 ± 8.7 for hatching. For the 2.5 µM ALA group, the rates were 72.6 ± 4.8 for cleavage, 32.1 ± 4.9 for blastocyst, and 63.1 ± 7.9 for hatching. For the 5 µM ALA group, the rates were 74.8 ± 6.0 for cleavage, 31.9 ± 6.3 for blastocyst, and 54.7 ± 9.3 for hatching. For the 10 µM ALA group, the rates were 72.7 ± 4.5 for cleavage, 36.1 ± 5.4 for blastocyst, and 55.2 ± 5.9 for hatching. For the 25 µM ALA group, the rates were 76.8 ± 3.6 for cleavage, 34.5 ± 1.9 for blastocyst, and 64.5 ± 3.8 for hatching. Although our results indicate that ALA supplementation during IVM did not result in significant differences in embryonic development rates, it is believed that more studies are needed to investigate the antioxidant capacity of ALA on embryonic metabolism when included in the IVM stage.

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SOF media nutrients reduction on bovine IVF embryo development, pregnancy, and birth rates

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Nutrient reduction on culture media may provide new perspectives on embryo metabolism (Herrick et al., Reproduction and Fertility,1:1, 51-65, 2020). This study aimed to evaluate if bovine embryos can successfully develop in vitro with significantly reduced nutrient concentrations (carbohydrates and amino acids) and if the produced embryos would result in full-term pregnancy development. Total of 1539 viable COCs were recovered from 92 Gyr donors and fertilized with female sex-sorted semen from six Holstein bulls. Presumptive zygotes were split into two treatment groups: control – standard Embryotec's (Embriotec Reprodução Animal, Anápolis, GO, Brazil) SOF media (851 COCs); RED: Embriotec's SOF media with reduction of 75% on components concentration (688 COCs). Embryo development was checked at day 7 and 454 embryos were transferred to recipients (control: 280; RED: 174). Pregnancy diagnosis was determined at 30 and 60 days (D30 and D60) after embryo transfer. The number of recipients that had pregnancy to term and delivered offspring was recorded. Data were analyzed by chi-square test in contingency table in Jamovi 2.3.21 software. The nutrient reduction on SOF media did not affect the embryo development rate at day 7 (control: 43.36% vs RED: 42.00%; P>0.05), the pregnancy rate at day 30 (control: 47.10% vs RED: 52.29%; P>0.05), the pregnancy rate at day 60 (control: 44.28% vs RED: 48.27%; P>0.05) nor the pregnancy loss between D60 and D30 (control: 6.06% vs RED: 7.69%; P>0.05). As well, there were no differences for the birth rate per transferred embryos (control: 39.64% vs RED: 41.95%; P>0.05), birth rate per pregnancy at D30 (control: 84.09% vs RED: 80.21; P>0.05) nor for birth rate per pregnancy at D60 (control: 89.50% vs RED: 86.90%; P>0.05). In conclusion, the reduction of nutrients on SOF media composition did not affect embryo development competency and viability, as determined by full-term pregnancy development.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Livestock-Forest integrated system attenuates deleterious heat stress effects in bovine oocytes

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The objective of this study was to analyze molecular markers associated with heat stress during the tropical summer in oocytes from Girolando and Holstein heifers kept in an integrated system (IS) or a conventional Full Sun (FS) system. The study was conducted between November and February and the analyses were made at the same time for all groups. Based on temperature-humidity index (THI) data, we found an intense heat stress condition during the experiment. IS used was a prototype modeled by Embrapa Dairy Cattle (*Urochloa decumbens*.; 200 eucalypt trees per hectare; basal area (area of the cross-section of trees at chest height) of 1.33 m2 ha-1) and FS was composed of a similar pasture without trees. Dairy heifers (16 Girolando 3/4 Holstein and ¼ Gir and 16 Holstein) were allocated in four experimental groups: Girolando IS (8), Girolando FS (8), Holstein IS (8) and Holstein FS (8) (1.8 animal units/ha). Vaginal temperature was assessed using iBotton data loggers every 15 minutes during 2-4 days in 15-day intervals along the experiment, and the number of hours vaginal temperature exceeded 39.1ºC and maximum vaginal temperature were compared among groups. Oocytes were obtained on random day of the estrus cycle by OPU at the beginning of experiment (D0), and after 30 (D30) and 60 (D60) days. Oocytes were denuded with hyaluronidase, fixed in 4% PFA and immunostained for caspase3 and IGFBP2, oocyte quality markers previously validated by our group (de Silva, M. O. et al. 2022, Reprod Dom Ani 57(9), 980–988. https://doi.org/10.1111/rda.14164). Results were transformed by Johnson and means were compared among groups in each breed using ANOVA and Tukey posttest. IVF-grade and oocyte recovery rates were compared among groups in each breed using Fisher Exact test. T Test was used to compare the number of hours above 39.1ºC and maximum vaginal temperature. Girolando and Holstein heifers showed an increased ($p<0.001$) number of hours above 39.1ºC when kept at FS (14.1±5.6) compared to IS (11.2±4.9). Meanwhile, Holstein (14.8±5.4) heifers also showed an increased number of hours above 39.1ºC compared to Girolando (10.7±5.1). Oocyte diameter decreased during summer in FS and IS groups, but more drastically in FS for Girolando, which was lower than IS at D60 (100.66±1.10 vs 81.93±1.53*). Caspase 3 and IGFBP2 proteins, involved in apoptosis and IGF negative regulation, were increased ($p<0.05$) from D0 to D30 and D60 in FS but not in ILF oocytes. Caspase was higher in FS oocytes at D30 in Girolando (154.42±1.91 vs 175.70±2.05*) and Holstein (126.97±6.77 vs 141.54±1.00*) and D60 in Girolando (144.08±1.21 vs 163.86±1.33*). IGFBP2 was increased in Girolando FS in comparison to ILF, at D30 (140.46±3.49 vs 166.28±3.2*) and D60 (119.79±1.53 vs 136.81±1.64). In conclusion, ILF system attenuated maternal hyperthermia and its effects on oocytes throw caspase-3 and IGFBP2 associated pathways for both Holstein and Girolando heifers.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Case report: Birth of first twin calves produced by the intrafollicular transfer of immature oocyte

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In the present report, we describe an alternative method for the production of twins based on the intrafollicular transfer of immature oocytes (IFIOT) that resulted in the birth of the first twins reported using this technique. On day 0 (D0), thirteen multiparous Nellore cows received a vaginal device containing 1 g of progesterone (Primer®, Tecnopec, São Paulo, Brazil) and 2 mg i.m. injection of estradiol benzoate (Estrogin®, Farmavet, São Paulo, Brazil). On D8, the progesterone device was removed, and i.m. injections of prostaglandin analog (150 µg sodium cloprostenol; Prolise®, Tecnopec, São Paulo, Brazil), eCG (300 IU; Novormon®, MSD Animal Health, São Paulo, Brazil) and estradiol cypionate (1 mg; E.C.P®, Zoetis, São Paulo, Brazil) were applied. Timed artificial insemination (TAI) was performed 48-52 hours later. Two hours before TAI, two of the synchronized cows were selected to serve as donors and subjected to follicular aspiration of visible antral follicles. Two COCs recovered from the preovulatory follicles of the two donors and nine COCs from follicles > 3 mm recovered from one of the donors were transferred individually to the preovulatory follicle of the remaining 11 cows at the time preceding TAI. Forty days later, three cows that received COCs from 3-8 mm follicles were diagnosed by ultrasound as pregnant, one of which had twins. After 264 days of gestation, the cow-carrying twins gave birth to two female calves. The weight of the calves at birth was 29.1 kg and 27.4 kg, respectively. Genotyping results showed that one of the twins was the mother's biological daughter, while the other was unrelated to the mother. In conclusion, to our knowledge, this is the first report of twins produced using IFIOT. The expectation is to significantly improve the performance of the technique in the near future, making it another reproductive tool to be applied in the production and research sectors.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Superovulation response using high and low dose of recombinant FSH (bscrFSH) in different cattle breeds: effects on ovarian structures and in vivo embryo production

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The ovarian superovulation protocols performed in cattle require the use of follicle- stimulating hormone (FSH). The aim was to test the effects of high and low doses of recombinant FSH (bscrFSH) on ovarian structures and in vivo embryo production in different Bos taurus cattle breeds. A total of 20 individuals [breeds: Angus (ANG; n=10); German Red Pied (GRP; n=10); BC: 3-3.5] were randomly allocated to 4 groups [ANG-H (n=5); GRP-H (n=5); ANG-L (n=5), and GRP-L (n=5)]. Two SOV protocols (H and L) were applied: Day 0: intravaginal progesterone (P4) device (CIDR: 1.38 g) + 2.5 mg intramuscular (IM) estradiol benzoate E2B + 100 mg P4 (IM); Day 4: total dose (H) = 160 µg of bscrFSH divided in 4 day/24 h intervals/4 decreasing doses: 60 + 50 + 30 + 20 µg; Day 6: third bscrFSH dose + two PGF2α i.m. doses (500 µg of D-cloprostenol each); Day 7: CIDR removal at the fourth bscrFSH dose application); Day 8: estrus detection + 1st AI; Day 9: 2nd AI; Day 15 (embryo collection). The L protocol was applied in the same way with dose modifications (total dose (L) = 135; 4 day/24 h intervals/4 decreasing doses: 50 + 40 + 30 + 15 µg). Ovarian structures [follicles (FL; Day 8), corpora lutea (CL; Day 15), and non- ovulated follicles (NOFL; Day 15)] were assessed by ultrasonography. Embryo-derived traits were evaluated: total structures (TS), viable embryos (VE), degenerated embryos (DE), and unfertilized oocytes (UFOs). The data were analyzed by GLMM (SPSS® 25, IBM Corp., USA). Differences were detected in CL (16.0±1.9 vs. 13.6±0.9) being greater in ANG when only breeds were compared ($p<0.05$). Differences were observed in DE (0.4±0.2 vs. 1.0±0.4) and UFOs (0.7±0.3 vs. 1.6±0.7) being greater in L when only superovulation protocols were compared ($p<0.05$). No differences were observed among groups regarding ovarian- and embryo-derived parameters when both breeds (VE: 10.8±1.2 vs. 9.8±0.9 for ANG and GRP, respectively; $p> 0.05$) and protocols (VE: 11.20±1.0 vs. 9.40±1.1 for H and L dose, respectively; $p> 0.05$) were considered. In conclusion, although the ANG breed showed a greater CL number and the H protocol exhibited a lower number of DE and UFOs, no differences were observed among the interaction of breed and protocol regarding ovarian structures and in vivo embryo production using high and low bscrFSH dose in both cattle breeds. Regarding the VE no differences were observed between doses or breeds.

This research was partially supported by ANID 21201280 and DIRGI-CP2022-005.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Differential superovulation response in dairy and beef heifers using recombinant FSH (bscrFSH): effects on ovarian structures and in vivo embryo production

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In cattle, the superovulation response depends on different factors such as breed aptitude (dairy vs. beef). The aim of the present study was to assess the effects of a superovulation protocol using a recombinant FSH (bscrFSH) on ovarian structures and in vivo embryo production in dairy and beef heifers. A total of 18 heifers (Age: 12-15 mo.; BC: 3–3.5) were divided into two groups [Holstein heifers (H; n= 9) and Red Angus heifers (RA; n= 9)]. All heifers were subjected to the same superovulation protocol: Day 0: intravaginal progesterone device (CIDR: 1.38 g) + 2.5 mg of estradiol benzoate and 100 mg of progesterone (IM); Day 4: bscrFSH (150 µg total dose) once daily in decreasing doses over 4 days (50, 40, 35, and 25); Day 6: third bscrFSH dose + 500 µg of D-cloprostenol (a.m. and p.m.); Day 7: fourth bscrFSH dose + CIDR removal (a.m.). Day 8 (a.m.); Ovarian structures [follicles (FL)] were recorded by ultrasonography; Day 8 (p.m.): AI (36 h post-CIDR removal); Day 9 (a.m.); 2nd AI (48 h post-CIDR removal); Day 15: Corpora lutea (CL) and non-ovulated follicles (NOF) were recorded by ultrasonography and embryo were collected. Moreover, embryo-derived parameters such as the number of total recovered structures (TS), transferable embryos (TE), degenerated embryos (DE), and unfertilized oocytes (UFOs) were assessed. Data were analyzed by GLMM (SPSS® 25, IBM Corp.). Overall, ovarian-derived (FL and CL) and embryo-derived (TS, DE, and UFOs) parameters were greater in RA compared to the H group. A greater number of CL (10.5±1.1 vs. 8.8±0.6; p=0.05) and DE (2.9±0.7 vs. 1.1±0.5; p= 0.05) were observed in RA compared to H; however, no differences were observed regarding TE between breeds (4.5±0.5 vs. 5.6±0.7, for RA and H, respectively; p=0.07). Finally, the number of NOF was lower in RA compared to H (0.4±0.2 vs. 1.8±0.3; P= 0.01). No differences between breeds were observed in any other ovarian-derived (FL) or embryo-derived (TS and UFOs) parameters (p> 0.05). In conclusion, bscrFSH was successful in inducing superovulation response in both dairy and beef heifers; however, differences were observed in ovarian response (CL and NOF) and embryo production traits (DE) between breeds. These differences may be related to genetic factors (breed differences) due to all individuals being subjected to the same SOV protocol.

This research was partially supported by ANID 21201280 and DIRGI-CP2022-005.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Cows with larger vulvar width have greater antral follicle count, viable oocytes, and higher circulating AMH

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Owing to the low heritability of reproductive traits, the search for markers that can indicate reproductively superior individuals is important in the selection process for reproductive efficiency. This study aimed to assess the effect of antral follicle count (AFC), vulvar-width (VW), anti-Müllerian hormone (AMH) concentrations on reproductive efficiency indices in *Bos taurus* and *Bos indicus* females. The mean age of zebu females was 4.8 ± 2.7 years (2–9 years) and that of taurine females was 5.7 ± 3.2 years (2–16 years) and had body condition scores (BCSs) from 3 to 8 (1–9, lean to obese). Zebu cows weighed 317–774 kg (mean of 531.00 ± 98.62 kg), while the taurine cows weighed 258–803 kg (mean of 533 ± 95.72 kg). To determine the AFC, the population of follicles ≥3 mm in diameter in both ovaries was assessed using transrectal ultrasonography (Aloka SSD 500; Mure, Japan) with a B-mode linear transducer (5.0 MHz) for OPU (Walmur®) on a random day of the estrous cycle. *Bos indicus* cows were classified as having high AFC (≥50 follicles, *n* = 37), intermediate AFC (30–49 follicles, *n* = 70), or low AFC (<30 follicles, *n* = 76). *Bos taurus* cows were classified as having high AFC (≥25 follicles, *n* = 39), intermediate AFC (16–24 follicles, *n* = 16), or low AFC (≤15 follicles, *n* = 44). Brahman (*Bos taurus indicus*, *n* = 126) and Simmental and Angus (*Bos taurus taurus*, *n* = 155) cows were classified as having large (≥86 mm) and small (≤86 mm) VW. To determine the AMH serum concentrations blood samples were collected by coccygeal venipuncture. Serum AMH concentrations were determined by an enzyme-linked immunosorbent assay kit (AnshLabs, Webster, Texas, USA). Three assays were performed with a sensitivity of 0.011 ng/mL and variability from 1.8 to 2.8 intra-assay and from 0.4 to 0.8 inter-assay. From the respective frequency distributions, the animals were classified as having high AMH (>0.81 ng/mL) and low AMH (≤0.81 ng/mL). The assays were conducted in the IgAc (Instituto Genese de Análises Científicas, São Paulo, BR) laboratory. Reproductive efficiency was evaluated from the reproductive history of females, provided by the property records: age at first calving, calving to first service interval, calving interval, number of services per pregnancy, and number of viable oocytes (VO). The GLIMMIX procedure in SAS® (Cary, NC, USA, 2016) was used to evaluate the effects of breed, vulva width classification (VWC), and AMH on these variables. Brahman, compared to taurine cows, had greater AFC (36.30 ± 1.34 vs. 22.09 ± 1.67), VW (106.94 ± 15.83 vs. 69.78 ± 14.11 mm), and AMH (1.18 ± 0.07 vs. 0.42 ± 0.05 ng/mL, respectively). AFC (36.10 ± 1.90 vs. 22.78 ± 1.64, for large VWC vs. small VWC), AMH (1.17 ± 0.07 vs. 0.48 ± 0.007 ng/mL), and VO (18.86 ± 1.76 vs. 10.15 ± 1.49) were greater ($P < 0.05$) in the large VWC than in the small VWC. In conclusion, VW was an efficient predictor of AFC and AMH concentrations in both genetic groups.

Animal Reproduction

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Determination of intraoocyte polyamine concentration during *in vitro* maturation of bovine oocytes

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Polyamines (PAs) are a group of molecules that are ubiquitous in prokaryotic and eukaryotic cells, with putrescine (PUT), spermine (SPM), spermidine (SPD), and cadaverine (CAD) being the PAs of scientific interest. Composed of two or more amine groups that carry a positive charge, PAs can interact with important cellular compounds such as DNA, RNA, ATP, and phospholipids, all of which carry a negative charge. Despite their important reproductive functions, the intracellular concentration of PAs is only known in Xenopus. The aim of this study was to measure the intracellular concentration of PAs during in vitro maturation (IVM) of bovine oocytes. The oocytes were obtained from a local abattoir, matured in four-well plates containing 30 COCs per well. The oocytes were removed from IVM at 0 h (immature), 3 h, 6 h, 9 h, 12 h, 15 h, and 22 h, and were subsequently denuded and grouped into samples of 200 oocytes and stored at -18 °C in triplicates, totaling 21 observations. Statistical analysis was performed by analysis of variance, with means compared using the SNK test at a 5% probability level using SAS software version 9.4. The samples were dipped three times in liquid nitrogen, macerated between cycles, and subjected to ultrasonic sonication for five cycles of 20 seconds, followed by maceration in 100 µL of perchloric acid. After 1 h of incubation at 4 °C, the samples were centrifuged for 20' at 20,000 × g at 4 °C. The supernatant was collected and the free PAs were analyzed by dansylation with dansyl chloride. The PAs were then identified and quantified by HPLC. A fluorescence detector at 340 nm (excitation) and 510 nm (emission) was used to detect PA peaks. Peak areas and retention times of PAs were measured by comparison with standard PAs PUT, SPD, and SPM. The intracellular concentration of putrescine showed a slight peak at 3 h (0.55±0.32 ng/oocyte), but only differed from the concentration observed at 22 h (0.06±0.03 ng/oocyte) ($P<0.05$). Spermine started IVM with high concentration (0.56±0.09 ng/oocyte), with a decrease in its concentration at 3 and 6 h, an increase at 9 and 12 h, and a decrease at subsequent times, reaching a concentration of 0.14±0.05 ng/oocyte at 22 h ($P<0.05$). Cadaverine showed very low concentrations with a small peak at 12 h of culture ($P<0.05$). The increases in PA concentration occurred around 6 to 12 h, which suggests the use of these molecules during the resumption of meiosis in bovine oocytes. This is the first study to determine the concentration of polyamines during mammalian oocyte maturation, and this analysis can be used as an important tool in studies on signaling pathways involved in oocyte maturation.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Effect of prostaglandin administration concomitant with GnRH in a fixed-time superovulation program in super early Nelore donors

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In modern livestock, most reproductive programs seek animals with greater sexual precocity. Therefore, in super-precocious donors, this study evaluated the effect of the injection of Prostaglandin F2α (PGF2α) concomitant with GnRH for ovulation induction in fixed-time superovulation protocols, in order to verify the effect of the drug on the final growth phase of the dominant follicle, thus inducing the ovulation. To that end, 10 young Nelore donor heifers (13 months) with high antral follicle count (AFC) were submitted to the superovulation protocol (SOV). On day 0 (D0), all animals received the insertion of an intravaginal progesterone (P4) device (1g, Repro neo®, Biogénesis Bagó, Curitiba, Paraná, Brazil), and between the fourth and the seventh day (D4 to D7), they received 7 administrations of 200UI FSH (Pluset®, Biogénesis Bagó), at intervals of 12 hours. On the sixth day (D6), all animals received a single administration of 150µg of D-Cloprostenol (Croniben®, Biogénesis Bagó), as part of the SOV protocol. In addition, on D7, the P4 implant was removed. On the eighth day (D8), all animals received the administration of 10,5µg of buserelin acetate (Gonaxal®, Biogénesis Bagó, Curitiba, Paraná, Brazil), and only the animals in the treatment group received 300µg of D-Cloprostenol (Croniben®). The artificial insemination was performed 12 and 24 hours after GnRH administration, using frozen semen from 9 bulls, by direct mating. Each cow received semen from the same bull and lot in the different routines (control and treatment). On D15, the embryos were collected and evaluated, in addition to the subsequent transfer of viable embryos to the recipients. The methodology was designed in a 2x2 factorial so that all animals passed through the control and the treatment groups. The effect of the PGF2α application was evaluated by ANOVA using the Adjusted Mixed Effects Model. For model composition, donors were considered as a random effect, and PGF2α treatment (treatment vs. control) and sires were considered as fixed factors. For descriptive analysis, data are presented as mean (M) and standard error (SE). A significance level of 5% was adopted. In general, there was no difference between the control and treatment groups for the total number of embryos (10.40 ± 1.52 vs. 9.60 ± 1.36; P = 0.63), number of viable embryos (6.30 ± 1.22 vs.4.30 ± 0.71; P = 0.16); non-fertilized structures (3.50 ± 0.75 vs. 4.60 ± 1.24; P = 0.33) and degenerated embryos (0.45 ± 0.21 vs. 0.55 ± 0.20; P = 0.72), respectively. For super-precocious donors, the PGF2α treatment did not affect embryo production in fixed-time superovulation protocols by stimulating ovulation concomitantly with GnRH. However, more studies are needed in order to elucidate the action of PGF2α as a possible factor involved in ovulation.

Acknowledgments: Biogénesis Bagó.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Does the quantity of antral follicles in prepubertal Nellore heifers influence the diameter of the largest follicle?

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The antral follicle count (AFC) is a non-invasive method used to estimate ovarian reserve in female bovine. AFC exhibits high variability among individuals and repeatability within the same animal, allowing for a single AFC measurement throughout the lifetime of a female bovine. However, the relationship between AFC and fertility in *Bos indicus* is not yet well understood. Therefore, the objective of the present study was to evaluate the follicular diameter of prepubertal Nellore females allocated into groups of low, medium, and high AFC. For this study, 100 heifers with an average initial age of 17±0.8 months and a final age of 24±0.8 months were used. At the beginning of the study, the mean weight of the heifers was 243±44 kg, and the body condition score (BCS) was 5.0±0.88 for the low AFC group (N=28), 237±44 kg and BCS of 5.0±0.84 for the medium AFC group (N=46), and 218±38 kg and BCS of 5.0±0.70 for the high AFC group (N=26). To perform AFC, the heifers underwent ultrasound examination using a Mindray DP50® device with a linear transrectal transducer operating at a frequency of 7.5 MHz. Ultrasonographic evaluations were conducted once a month on random days of the estrous cycle for 8 months (March to October), totaling 8 assessments. For AFC, the ultrasound recordings were carefully reanalyzed by the same technician to map the location of each follicle, and antral follicles measuring ≥3mm were counted. To assess the relationship between follicular diameter and AFC, the largest follicle observed in both ovaries at the time of ultrasound evaluation was considered (the mean follicular diameter was calculated from the average of two linear transverse measurements of the follicular antrum captured on the ultrasound monitor). The groups were defined based on quartiles of the AFC distribution and classified as low (n≤30 follicles), medium (between 31 and 43 follicles), and high AFC (≥43 follicles). The data were analyzed using the Statistical Analysis System (SAS) software with the MIXED procedure. Comparing follicular diameter among the AFC groups, it was observed that the low AFC group exhibited a larger follicular diameter (8.82±0.18 mm) compared to the medium AFC group (8.37±0.15 mm) and high AFC group (8.16±0.19 mm). AFC can be a useful tool as a potential fertility indicator in bovine herds, as follicle has been linked to success in timed artificial insemination (TAI), given that follicular diameter is associated with corpus luteum diameter and subsequent progesterone production. Therefore, this study concluded that prepubertal Nellore heifers classified with a low antral follicle count present a larger follicular diameter compared to heifers classified with a medium and high antral follicle count.

Acknowledgment: The Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted. Grant 2022/08182-0 São Paulo Research Foundation (FAPESP).

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Ovarian superstimulation establishes differential histological phenotypes and opposite outcomes in isthmus and ampulla from bovine oviduct

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Estradiol and progesterone are essential for the control of morphological changes during each phase of the estrous cycle, and ovarian superstimulation (OVS) increases E2 concentration in the bovine oviduct. In addition, the extracellular matrix (ECM) plays an essential role in regulating reproductive function and is associated with morphogenesis and tissue remodeling. Therefore, we aimed to gain insight into the morphological changes in the bovine oviduct caused by OVS. Nelore cows (Bos taurus indicus) were subjected to OVS with FSH (n=5), FSH/eCG (n=5) or synchronized only (control group, n=5). Cows were slaughtered 12h after progesterone-releasing vaginal inserts had been removed. After slaughter, oviducts were harvested, fixed in methacarn for 24 hours, and stored in 70% ethanol. Tissues were dehydrated in graded ethanol and embedded in paraplast. The blocks were cut into 4-µm-thick sections, and the slides were stained with hematoxylin and eosin (HE), Picrosirius Red (PSR), and Alcian blue according to the laboratory's standard histological protocol. This project was approved by the Ethics Committee for the Use of Animals (Protocol 6182). Slides were analyzed, and images were acquired using a digital photomicroscope at 40× magnification. Analyses were performed using Image J software. Ampulla and isthmus were analyzed separately for morphometric analysis, fractal dimension (FD), total collagen, collagen subtypes, and mucin quantification. The effect of OVS on stereological features, fractal dimensions, collagen, and mucins were assessed by one-way ANOVA. Mean values were compared using the Tukey-Kramer test. Differences were considered significant when $P< 0.05$. In isthmus, both OVS treatments decreased mucosal height ($p=0.006$), epithelial area ($p=0.01$), luminal area ($p=0.004$), and increased total collagen ($p=0.0001$). Notably, FSH/eCG treatment increased collagen type I ($p=0.0001$) and collagen type III ($p=0.0001$) compared to the control group. In addition, both OVS treatments using PSR staining, but not HE, decreased FD in the isthmus ($p=0.0001$). In the ampulla, the application of FSH/eCG increased the area of the muscle layer ($p=0.04$) and the height of the mucosa ($p=0.02$). Unlike in the isthmus, both OVS treatments decreased total collagen in the ampulla ($p=0.01$). Regarding FD with HE staining in the ampulla, the use of FSH/eCG increased DF ($p=0.0116$), while with PSR staining, there was a decrease in FD ($p=0.0019$). Moreover, FSH treatment in combination with eCG increased the relative abundance of mucins in the isthmus and ampulla ($p<0.05$). In conclusion, we have shown that OVS impacts collagen modulation and tissue remodeling. Also, we found that the isthmus and ampulla show opposite pathways and the FSH/eCG approach has a major impact on these phenotypes. In summary, these findings could suggest a huge impact on oviduct functionality caused by high levels of oviductal E2 demonstrated in our previous studies.

Funding: FAPESP (2018/06674-7).

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Estimation of repeatability of antral follicle count in prepubertal Nellore females

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The antral follicle count (AFC) is a tool used to predict the ovarian reserve, and this characteristic is important for the efficiency of reproductive biotechnologies. The AFC presents great variability in the bovine herd, and high repeatability in the same individual. However, there are few studies regarding the repeatability of the AFC during the reproductive development of prepubertal Nellore heifers on random days of the estrous cycle. Therefore, the objective of the present study was to determine whether the AFC is repeatable in prepubertal Nellore heifers. One hundred Nellore heifers from 17 to 24 months of age with and initial body weight of 234±43 were used. To perform the AFC, heifers were submitted to ultrasonography using Mindray DP50® equipment (Shenzhen, China) coupled to a linear transrectal transducer at a frequency of 7.5 MHz. The count was performed once a month for 8 months (March to October), with images and videos of both ovaries on random days of the estrous cycle. The material obtained was carefully analyzed by the same technician, and all antral follicles larger than ≥3mm were counted. The repeatability coefficient was calculated as follows: r = σm^2 / (σm^2 + σe^2), where r is the repeatability of the feature, σm^2 is the variance of repeated measurements of the feature, σe^2 is the variance of the measurement error of the characteristic. The data were analyzed with the aid of the "Statistical Analysis System" (SAS) program using the MIXED procedure. The repeatability of the AFC in pre-pubescent Nellore heifers was 0.76, which is considered high. The high repeatability in the cattle herd can help in the selection of females with high AFC at the beginning of reproductive life, contributing to the efficiency of reproductive biotechnologies such as in vivo and in vitro embryo production and FTAI. Thus, it was concluded that the AFC can be performed on random days of the estrous cycle without synchronization, regardless of the female's age, and can be used as a possible parameter of reproductive superiority in the bovine herd. In addition, the measurement can be performed only once, as the high repeatability indicates that the AFC does not change in the same individual regardless of age.

Acknowledgment: The Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted. FAPESP (Process nº. 2022/08182-0).

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Metabolic profile of individual follicular fluid of overweight women

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Infertility is defined as the inability of a person to become pregnant during a period of 12 months of continuous attempts, using no contraceptive method. Female infertility is associated with endometriosis, anatomy conditions, advanced age, diminished ovarian reserve, genetic as well as other conditions such as lifestyle and obesity. It is well-known that high body mass index (BMI) may impact the retrieval and quality of oocytes resulting in poor embryo development. One of the hypotheses for the negative effect of high BMI on embryo development is that an altered metabolic profile of the follicular fluid (FF) may impact oocyte metabolism and nutrition. The objective of this work was to characterize the metabolic profile of individual follicular fluid from women presenting different BMI. Twenty women were voluntarily recruited (13 Control BMI (CB – BMI: 18.5 – 24.9) and 7 high BMI (HB - BMI: 25.0 – 29.9 Kg/m2). Each follicle (>14mm) was aspirated individually through transvaginal ultrasound-guided follicle aspiration, and immediately frozen at -80°C. Only FF with no blood contamination was included. Oocytes from each follicle were identified, fertilized by ICSI, and kept up with the embryo development until day 5, 6 and 7 when biopsy, embryo transfer and/or cryopreservation is made. (data not included). For each FF, lactate (Colorimetric assay - Bioassay Systems), glucose (Fluorometric assay - Invitrogen), insulin (Immunoassay – Abcam), and lipid peroxidation (MDA by TBARS method) were quantified. Results were verified for the presence of outliers and normality, transformed if necessary, and compared by Student's t test (Control BMI vs High BMI), considering alpha = 0.05. The mean BMI of the CB group was lower (22.49 kg/m2) than that of HB (26.60 kg/m2) (P= 0.0001). There were no differences in glucose, lactate and insulin concentration in the follicular fluid between CB and HB groups (glucose p=0.83, lactate p=0.2 and insulin p=0.72). Interestingly, women with high BMI had a decrease in MDA amount in follicular fluid when compared to control BMI: 17,80 ± 7,75 vs 49,69 ± 28,77, (P=0.0013). We hypothesize that this result may be because of the attempt of the patient to have a healthier life recovery following the medical recommendation to lose weight aiming for a better result in the treatment. In conclusion, altered BMI may impact the follicular metabolic profile, which may be associated with the success of the assisted reproduction cycles, however, the patient lifestyle seems to be more impactive than the increase of BMI.

Acknowledgements: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Superovulatory parameters and embryo yield in donor goats subjected to different durations of progestogen treatment and non-surgical embryo recovery

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This study checked the effect of the duration of progestogen treatment on the reproductive parameters and embryo quality of superovulated (SOV) goats undergoing non-surgical embryo recovery (NSER). This study was approved by Ethics and Animal Care Committee of Embrapa Gado de Leite nº 6789240322. Dairy goats (n=24) received intravaginal devices containing 0.3 g of progesterone (P4; Eazi-Breed CIDR®; Pfizer, São Paulo, Brazil) for six (G6) or nine (G9) days. The SOV consisted of 133 mg of porcine FSH (pFSH; Folltropin-V; Bioniche Animal Health, Belleville, Canada) administered i.m. twice daily at 12 h intervals in six decreasing doses (25, 25, 15, 15, 10, and 10%), starting 48 h before device removal. Three doses of 131.5 µg of cloprostenol (Sincrocio®, Ouro Fino, Cravinhos, Brazil) were administered i.m. simultaneously with the fourth and fifth doses of pFSH and 12 h before the NSER. Goats also received 25 µg of GnRH (Gestran®, Tecnopec, São Paulo, Brazil) i.m. 24 h after device removal. Donors were mated while in estrus at 12 h intervals with fertile bucks (n=4). Three doses of 75 mg flunixin meglumine (Flumax®, J.A. Saúde Animal, São Paulo, Brazil) i.m. were given at 3, 4, and 5 days after the onset of estrus and NSER was performed seven days after the first mating. Data were analyzed using Bio Estat 5.0 software (Belém, Brazil) and subjected to the Shapiro-Wilk test for normality. Parametric data were submitted to the t-test, while non-parametric data to the Mann-Whitney test and Fisher's exact test for frequencies. The results are presented as mean ± SEM and differences were considered significant when $P \leq 0.05$ and tendency when $P \leq 0.10$. There were no differences ($P > 0.05$) in G6 and G9, respectively, for estrus response rate (100% and 100%), the interval from device removal to estrus (21 ± 2.2 h and 18 ± 2.3 h), estrus duration (29 ± 4.0 h and 42 ± 7.2 h), and percentage of females successfully flushed (100% and 91.7%). All goats (100% for both groups) responded to the superovulation protocol (≥ 4 CLs). The CL number per flushed female tended ($P = 0.09$) to be smaller in G6 (12.6 ± 1.2) than in G9 (16.6 ± 2.1), but the recovery rate was higher ($P < 0.05$) in G6 (64.9%) compared to G9 (30.6%). However, the average of recovered structures (8.2 ± 1.6 and 5.1 ± 1.4) and unfertilized oocytes (0.4 ± 0.2 and 1.0 ± 0.5) was similar ($P > 0.05$) between G6 and G9, respectively. Of note, the G6 tended ($P = 0.07$) to have a higher number of viable (Grades 1, 2, or 3) embryos per flushed female when compared to G9, respectively (7.3 ± 1.4 vs 3.9 ± 1.3). Based on these results, we can conclude that although treatment with a progestogen for 9 days provided a higher ovulation rate, the protocol with 6 days of treatment tends to improve embryo viability. Therefore, we recommend using the 6-day treatment.

Financial support: FAPEMIG (PPM-00201-17 and APQ-00148-23) and CAPES.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Ovarian morphophysiology correlated to body weight at parturition in Tabapuã cows

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Studies on fertility biomarkers such as antral follicle count (AFC) associated with ovarian follicular reserve can be effective in the selection of animals with better fertility and longevity. Thus, the aim of this study was to correlate ovarian morphophysiological aspects (ovarian area and follicular population) with postpartum body weight of beef cattle, in addition to offspring weight gain. The body condition of 32 Tabapuã females (Bos taurus indicus) kept in pasture was evaluated on the day of parturition and, on average, at 33, 55 and 72 days postpartum (dpp). Considering the parity (primiparous and multiparous), cows were classified and separated into two groups (1 e 2, respectively), according to body weight on the day of calving. At approximately 55 dpp, all cows initiated a FTAI protocol and four days after initiation, were measured ovarian area and AFC by ultrasonographic examination. Postpartum weight and body condition score (BCS, scale 1-9) variations of cows, in addition to the weight of their respective calves were analyzed in a statistical model containing group, day and interaction effects using the Proc Mixed of SAS (on demand, SAS studio). And the average area of the two ovaries and AFC were verified by ImageJ and VirtualDub, respectively. These variables were statistically analyzed in a model containing group effect, performed by Proc Glimmix of SAS. The average weight of the cows at parturition was (458.9±67.3 vs. 549.5±80.0 Kg live weight, P<0.0004) for groups 1 and 2, respectively. For weight variation, the significant group effect (P<0.0004) indicated that differences between groups were maintained in the postpartum period, and other non- significant effects (P>0.05) suggested weight maintenance. The group effect (P<0.04) for BCS was due to the higher score for cows in group 2 (5.9±0.8 vs. 5.6±0.8) and the day effect (P<0.0004) was due to an increase in the score after 55 dpp, observed in both groups. The weight of calves at birth did not differ (P>0.05) between groups and averaged 36.4±5.9 kg, with no difference in growth rate between groups. Analyzing all animals, the average area of the ovaries was 4.5 cm2 (ranging from 2.2 to 8.5 cm2) and the average AFC was 31 antral follicles (ranging from 13 to 53). The area of the ovaries was larger (P<0.0003) in the group of animals that calved with greater weight (5.0±1.2 vs. 4.0±1.2 cm2). However, AFC did not differ (P=0.4) between groups (29.7±11.2 and 31.5±8.0 for groups 1 and 2, respectively). Therefore, ovarian correlates with body development of the animals, however, the same does not occur with the AFC, which is an intrinsic characteristic to the animals and associated with ovarian reserve and does not necessarily follow ovarian development. These ovarian morphophysiological characteristics may be important to assist the selection of females during the breeding season.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Impact of postpartum uterine diseases on re-establishment of ovarian function in dairy cows: preliminary results

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Uterine diseases in dairy cows negatively impact productivity and reproductive performance. The objective of this *cohort* study was to evaluate the impact of the severity of these diseases on the reestablishment of ovarian activity. As preliminary results in a total of 45 healthy Holstein cows that will be followed from 14 days before the expected date of parturition (D-14) until the at the end of the voluntary waiting period (D40), 22 animals have already been clinically examined around days (D-14), (D7) and, (D21). On (D0), date of parturition, history and zootechnical information were recorded. Around D7, uterine contents were analyzed using the Metricheck® device. Ultrasound examinations to verify the presence of corpus luteum were performed on about D21. The variables were statistically analyzed in a multinomial logistic regression model using the RStudio software. Data obtained 7 ±2 days before parturition showed that the animals were within the expected physiological parameters, except for the fact that they anticipated parturition by 6 ±1 days. The duration of pregnancy did not interfere with the intensity of the inflammatory process caused by uterine diseases in the postpartum period ($P>0.05$). According to the clinical picture and analysis of the uterine content, at 7 ±1 days after parturition, the animals were characterized into two experimental groups: Absence of Reproductive Disease (ARD), defined by animals that did not present changes at parturition, clinical signs and vaginal discharge or had clear non-fetid mucus and Clinical Uterine Disease (CUD) divided into three levels: Grade 1 (mild), determined by some alteration during parturition, absence of clinical signs and vaginal discharge or presence of clear non-fetid mucus; grade 2 (moderate), indicated by the presence of cloudy mucus with blood stains or mucopurulent discharge <50% pus non-fetid, which may show clinical signs; and, grade 3 (severe), with systemic involvement, presence of white, yellow, or reddish-brown mucopurulent vaginal discharge ≥50% pus or thin, serous or watery, reddish-brown fetid mucus, with or without patches necrotic tissue. Prevalence were high for the CUD group with 54.54%, 18.18% and, 13.64% in grades 3, 2 and, 1, respectively, compared to 13.64% for the ARD group. The classification of the inflammatory process was decisive for the resumption of cyclic activity at 22 ±1 days after parturition, defined by the presence of corpus luteum, that is, the ARD group presented (3/3) of the animals compared to the CUD group: Grade 1, grade 2 and, grade 3 with (2/3), (2/4) and, (1/12), respectively ($P<0.01$). Therefore, the classification of animals in relation to the intensity of clinical uterine diseases allows greater flexibility in reproductive protocols, better control and prevention programs, minimizing postpartum uterine contamination and, more rational and effective treatments, in addition to stimulating further studies in the area.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Anethole and Croton grewioides essential oil reduce oxidative stress and improve growth of bovine preantral follicles during in vitro culture of ovarian tissue

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This study aims to evaluate the effects of anethole and Croton grewioides essential oil (CGEO) on primordial follicle activation and growth, follicular survival and ovarian stromal cell density. The activity superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), the concentration of thiol, and the levels of mRNA for SOD, CAT, GPX1, perirredoxin 6 (PRDX6), and nuclear factor erythroid 2-related factor 2 (NRF2) were also evaluated in cultured tissues. To this end, fragments of ovarian cortex (3x3x1 mm) from ovaries of 16 cows were fixed in 10% paraformaldehyde (uncultured control) or cultured in vitro in 500 µL of control medium alone or supplemented with 1, 10, 100 or 1000 µg/ml CGEO or 1, 10, 100 or 1000 µg/ml anethole at 38.5°C with 5% CO2 in the air for 6 days. The control medium was α-MEM supplemented with BSA, glutamine, penicillin/streptomycin, hypoxanthine, insulin, selenium and transferrin (α-MEM+). At the end of the culture period, the tissues were fixed and processed for classical histology. The follicles were classified as primordial or developing follicles, as well as morphologically normal or degenerated. Ovarian stroma cell density was assessed by calculating the number of stromal cells in 10 areas of 100 µm^2, in each treatment. Evaluation of the activity of SOD, CAT and GPX1, concentration of thiol, as well as the levels of mRNA SOD, CAT and GPX1, PRDX6 and NRF2 were performed in tissues cultured in control medium alone or supplemented with anethole 1 µg/ml or CGEO 1 µg/ml. The percentage of primordial and developing follicles, as well as normal or degenerated follicles were analyzed by Chi-square test. Data of stromal cell density, activity on antioxidant enzymes and mRNA expression were analyzed by Kruskal-Wallis test ($P<0.05$). The results show that tissues cultured in the presence of 1 µg/ml anethole or 1 µg/ml CGEO had significantly higher levels of morphologically healthy follicles than those cultured in control medium ($P<0.05$). In addition, tissues cultured with 1 µg/ml CGEO had a higher number of stromal cells per area, when compared to uncultured control. Ovarian tissue culture with 1 µg/ml CGEO showed significantly higher thiol levels than those cultured with control medium alone or supplemented with anethole. SOD activity was reduced in tissues cultured in control medium alone or supplemented with anethole or CGEO. On the other hand, anethole increased CAT activity, while CGEO increased GPX activity when compared with the control group. The presence of 1 µg/ml anethole reduced the levels of mRNA for CAT, PRDX6 and NRF2 when compared to α-MEM+ and CGEO ($P<0.05$). In addition, 1 µg/ml CGEO reduced mRNA for CAT, PRDX, GPX1 and NFR2 when compared to α-MEM+ ($P<0.05$). CGEO 1 µg/ml reduced mRNA for GPX1 when compared with the anethole 1 µg/ml ($P<0.05$). In conclusion, 1 µg/ml anethole or CGEO promotes follicle survival and regulates the levels of thiol, the expression of mRNA and activity of antioxidant enzymes.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Progesterone role in the ovarian follicular microenvironment: effects on the cumulus-oocyte complexes

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The present study aimed to evaluate the progesterone (P4) role in the acquisition of cumulus-oocyte complexes (COCs) developmental competence. For that, the follicular fluid (FF) was separated according to corpus luteum (CL) relative position (ipsilateral and contralateral to the CL) from cows (n=6) previously synchronized to be on day 11 of the estrous cycle. The FF was analyzed for P4 concentration, and used to characterize the groups, ipsilateral follicles- high P4 (iFF) and contralateral follicles- low P4 (cFF). Cumulus cells pools (CC) from immature COCs (n~5 per replicate) were collected for RNA sequencing (RNAseq) analysis. Total RNA extraction from CC (iFF=4 and cFF=4) was done using miRNeasy Mini Kit (QIAGEN). The RNA library preparation was performed using the SMART-Seq HT kit (Takara Bio). Based on RNAseq results, COCs from contralateral follicles (3-6mm; slaughterhouse ovaries) were divided into three groups for P4 supplementation during IVM to test the P4 effects during the in vitro acquisition of competence. The IVM (500µL) medium (TCM199 based) was split into 1) Control (without P4); 2) 30 ng/mL (Low P4); and 3) 190ng/mL (high P4). After 21 hours of IVM, COCs were collected to assess metaphase II (MII) rate (first polar body extrusion, n=6 replicates containing ~100 COCs each) and CC expansion (images of COCs before and after IVM, n=5 replicates). The CC expansion was measured using the Image J software. The intrafollicular P4 concentration, MII rate, and CC expansion were tested for outliers, and normality, and were compared using the Student's t-test or ANOVA (GraphPad Prism; $p<0.05$). The intrafollicular P4 concentration was higher in iFF (high P4 - 188.9 ± 24.20 ng/mL) than cFF (low P4 - 27.24 ± 5.57 ng/mL; $p=0.0002$) group. RNAseq analysis of CC detected 177 differentially expressed genes, 142 upregulated in iFF CC and 35 upregulated in cFF CC. Cellular components analysis from both groups shows that cFF CC genes regulate cell projection membrane, ruffle, and actin-based cell projection, while iFF CC genes regulate oxidoreductase complex, mitochondrial respiratory chain complex I, and NADH dehydrogenase complex. Our functional experiment to investigate the in vitro effects of P4 demonstrated that high P4 concentration increases MII rate in comparison to low P4. Nevertheless, no difference was observed in CC expansion. In conclusion, high intrafollicular P4 concentration modulates CC transcriptome and cellular components related to communication processes. Besides, during IVM, high P4 concentration enhances oocyte nuclear maturation. Based on that, we postulated that during follicular development and IVM, intrinsic P4 mediates developmental competence of COCs. Therefore, understanding the mechanisms affected by P4 can help to improve oocyte quality during both in vivo and in vitro development.

Funding: FAPESP 2021/06645-0, and CAPES finance code 001.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Distinct *in vitro* maturation media composition modifies lipid metabolism genes expression in bovine cumulus cells

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IVM alters the lipid accumulation on the cumulus-oocyte complexes (COC), modifying the oocyte (OO) metabolism. Conventional IVM medium (MC) uses fetal calf serum (FCS) in its composition, which is known to be one of the major factors driving oocyte lipid accumulation. Herein, we aimed to investigate the effects of different IVM media compositions on OO lipid accumulation and the expression of lipid metabolism genes in cumulus cells (CC). For this, bovine COCs obtained from follicles (3-6 mm) slaughterhouse ovaries were submitted to two different IVM medium conditions: (i) MC (control group) composed by a base medium (TCM 199 Bicarbonate (Gibco), 100 mM sodium pyruvate, 16.67 µg/mL gentamicin) supplemented with 1 µg/mL FSH Folltropin® (Vetoquinol), 5 µg/mL LH and 10% FCS (Gibco); or (ii) modified IVM medium (MF) composed by base medium supplemented with 0.4% BSA, 100 ng/mL amphiregulin (AREG), 10 ng/mL insulin- like growth factor 1 (IGF-1), 50 ng/mL 17β-estradiol, 150 ng/mL progesterone and 10-2 UI/mL FSH recombinant human. After 9 hours of IVM, the COCs were denuded, OO were fixed for lipid droplet (LD) analysis, and CC samples were snap-frozen for transcripts analysis. For LD analysis (MC=72; MF=72), we followed a staining protocol with 20 µg/mL of BODIPY 493/503, where LDs from the OO' central slice were captured in a Confocal Leica TCS SP5 Microscope and analyzed through ImageJ software. The ratio between the LD area and the OO area (LD/OO ratio) was calculated, and the results were submitted to ANOVA, followed by Tukey's test with a 5% significance level. CC samples were pooled for the gene transcription analysis (20 COCs/pool; 5 biological replicates), and their RNA was extracted with RNeasy Plus Micro Kit (QIAGEN®). Library preparation was performed with PCR- cDNA Sequencing Barcoding kit #SQK-PCB109 and sequenced on MinION Mk1B (Oxford Nanopore Technologies®) using as reference the Ensembl Bos_taurus.ARS-UCD1.2.99 genome. Transcripts per million based analysis was performed considering p value <0.05 for our target genes (PPARγ, RXRα, PGC-1α, SIRT1-3, PEX14, ABCD3, FABP3, FABP5, CD36, ACACA, ELOVL3, SCD, PLIN2, LPL, ACS, ACADS, and ACADVL). LD analysis in OO showed no difference between the groups (p=0.4329). In the CC, FABP5, SCD, and ACLS3 were upregulated in the MF group, while PPARγ and FABP3 were downregulated in the MF group compared to the MC group. The other genes had no difference in expression. Taken together, the differentiated expression of lipid metabolism genes in CC without difference in oocyte lipid accumulation suggests that the CC could have a protective role, avoiding the excessive accumulation of intraoocyte lipids. In conclusion, this study reveals that IVM medium composition alters molecularly COC lipid metabolism by modifying the expression of related genes in bovine CC.

Funding: FAPESP 2021/09123-4, CNPq 407223/2021-5, and CAPES finance code 001.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Antral follicle count throughout the estrous cycle in *Bos indicus* and *Bos taurus* cows

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There are many physiological and metabolic differences between *Bos indicus* and *Bos taurus* that may impact reproductive outcomes. The antral follicle count (AFC) is a highly repeatable variable for the same individual and it is reported to be higher in *Bos indicus* than *Bos taurus* cattle. The aim of this study was to evaluate ovarian function and AFC throughout the estrous cycle in non-lactating Nelore (*Bos indicus*) and Holstein (*Bos taurus*) cows. With this purpose, 10 Nelore (508 ± 17 kg body weight [BW]; 3.1 ± 0.1 body condition score [BCS]); and 7 Holstein cows (575 ± 20 kg BW; 3.1 ± 0.1 BCS) were housed in pens, exposed to the same nutritional and environmental conditions, and were submitted to the same synchronization protocol. On D -7, cows received an intravaginal progesterone (P4) device (1.9 g) and 100 µg gonadorelin acetate (GnRH) im. On D -2, concomitant with P4 device withdrawal, cows received 0.5 mg cloprostenol (PGF) im, followed by a second dose on D -1. From D0 (determined as the beginning of the estrous cycle), ultrasound evaluations were performed daily, until confirmation of the next ovulation. All cows had the AFC evaluated daily and follicles were grouped into three classes: small (SF: ≤ 5.0 mm); medium (MF: > 5.0 ≤ 9.9 mm) and large follicles (LF: ≥ 10 mm). Statistical analyses were done by PROC GLIMMIX of SAS 9.4 (P ≤ 0.05). There was an effect of genetic group on AFC, in which Nelore cows had greater total AFC throughout the cycle compared to Holstein cows (40.4 ± 1.9 *vs* 18.7 ± 0.9). When evaluated by the classes, the number of SF was greater in Nelore than in Holstein cows (38.1 ± 1.8 *vs* 15.0 ± 0.9). However, there was no difference in number of MF (2.2 ± 0.1 *vs* 2.5 ± 0.2). In Holstein cows there was an effect of time, in which the number of SF on D0 was greater than on D15 and D21, and de number of SF on D1 was greater than on D21. Moreover, the number of MF on D3 and D4 was greater when compared with D0 and from D11 to D14. Nevertheless, the total AFC in Holstein cows only differed between the first 2 days of the cycle and D15. In Nelore cows, the effect of time was observed only for the number of MF, which was lower on D0 than from D4 to D6 and from D15 to D22. However, there was no effect of time on total AFC in Nelore cows. In conclusion, Nelore cows had greater follicular population during the entire estrous cycle compared to Holstein cows. In addition, regardless of the genetic group, there was slight variation in total AFC throughout the cycle.

Acknowledgments: FAPESP#2009/05547-2;2008/04188-8; CAPES.

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THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Heat stress reduces the amount of serum estradiol and follicular fluid in dairy cows

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This study aimed to evaluate the effects of heat stress in vivo in Holstein cows, on estradiol concentration and quality of cumulus oocyte complexes (COCs). Twenty-seven Holstein cows in the first lactation were used, which remained for seven days in climatic chambers, distributed as follows: Control (C) - with ad libitum feeding and THI = 60; Heat stress (HS) - with ad libitum feeding and THI = 76; Pair-feeding (PF) - same amount of feed ingested by the HS group and THI = 60. Ovaries were obtained after slaughtering the cows, and COCs after follicle aspiration using 18 G needles. For the viability of the COCs, the homogeneity of the cytoplasm was considered and with at least 5 compact layers of cumulus cells, the viability rate was calculated using the following formula: number of viable COCs / number of total COCs x 100. Only COCs and follicular fluid (FF) from 2 - 8mm follicles were used. Blood was collected immediately before slaughter from the jugular vein. Estradiol (E^2) was measured in serum and FF by RIA (Beckman Counter ® RIA Kit). COCs were denuded and cumulus cells (CC) and oocytes were subjected to gene expression by RT-PCR using the Fluidigm platform. For statistical analysis, we used analysis of variance using proc glimmix (SAS ondemand). For all analyses $P<0.05$ was used. The average recovery of COCs per animal did not differ statistically among the groups (HS=75.6 (±17); PF=55.3 (±17.07); C=68.3 (±18.25) P=0.67), however the viability was lower in HS (53% - 301/605) in comparison with the other groups (p-value=0.0142), while PF (59% - 242/410) and C (54% - 241/443) were similar to each other. E^2 concentration in serum was lower in HS (HS=7.3 pg/mol; C=10.7 pg/mol; PF=14.18 pg/mol; p-value=0.04). This pattern was repeated in the FF (HS=43.6 pg/mol; C=267.8 pg/mol; PF=79.0pg/mol; p-value=0.037). In HS cumulus cells and oocytes, we observed lower expression of 7-desidrocolesterol reductase (DHCR7), a gene related to cholesterol synthesis, a precursor of E^2. Other genes that participate in the synthesis of steroid hormones were tested, such as CYP19A1 and Star, but the expression of these genes did not differ among groups. We conclude that the lower concentrations of E^2 in serum and FF, as well as the lower expression of DHCR7 are involved with the lower viability of COCs obtained from dairy cows under heat stress.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Lipid metabolism of COCs subjected to IVM obtained from dairy cows under *in vivo* heat stress

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Heat stress alters not only the thermoregulatory control pathways, but also affects numerous pathways and mechanisms that can reduce reproductive rates in cattle. This study aimed to evaluate the effects of heat stress in vivo in dairy cows on the lipid metabolism of cumulus oocyte complexes (COCs) submitted to IVM. Primiparous, non- pregnant Holstein cows were kept in a climate chamber with ad libitum feeding and constant temperature of 28°C and temperature-humidity-index (THI) of 76 for 7 days (Heat stress - HS, n = 9) or under constant temperature of 16°C, THI = 60 and receiving the same amount of feed as heat-stressed cows (Pair-feeding - PF, n = 9) or ad libitum feeding (Control – C, n = 8), for the same period. COCs were obtained by slicing the ovaries. Samples were prepared for analyses before (T0) and after (T24) IVM (IVF Bioscience®, Falmouth, England - without BFS). T0 and T24 COCs were denuded, and CC and oocytes (Oo) were subjected separately to analyses of lipid content (LiCo; Bodipy®) and mitochondrial activity (MA, MitoTracker®) by fluorescence and confocal microscopy (oocytes n: HS=24; C=92; PF=87), lipid profile by LC-MS (4 repetitions with 5 oocytes per group); and gene expression by RT-PCR using the Fluidigm platform (3 replicates with 5 oocytes per group). Metaboanalist 5.0 (heatmap and volcano plot) software were used to statistically evaluate the LC-MS results. For fluorescence and gene expression, data were designed in a 2x3 factorial model (factorial design with 3 factors (stress groups), each with two levels (T0 X T24)) and analyzed by proc GLM (SAS ondemand). For all analyses, $P<0.05$ was considered. Oo and CC from T0/HS showed lower MA and lower LiCo, compared to the other groups, and an increase in both characteristics after maturation was observed. Oo and CC from T24/C showed reduced MA and maintained LiCo compared to T0/C. Despite the increased LiCo there was an overall reduction of TG in HS and PF groups after IVM, in Oo and CC. The greatest reduction was observed in TG consisting of the oleic, stearic, and linolenic fatty acids (FA). MTCH2 (repressing mitochondria metabolism) and SOD1 (act against oxidative stress) were more expressed in HS before and after IVM in Oo and CC. CC of HS cows showed lower expression of genes related to lipid metabolism, ELOVL6 before IVM and ELOVL5 after IVM. Apoptosis-related genes were more expressed in CC (BCL2) before and after IVM and in Oo (BAX) after IVM. It is concluded that lipid metabolism is affected by heat stress in dairy cows. There was a higher consumption of lipids via mitochondria, in response to HS in Oo and CC during the stress period. Apparently, this fact generates compensation during IVM, since the accumulation of lipids in this group was higher than the others after IVM. In addition, the lipid profile changed, reducing the amount of FA that are related to oocyte quality.

Animal Reproduction

THEMATIC SECTION: 36th ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Effect of heat stress on cell membrane integrity markers of oocytes and cumulus cells of dairy cows

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This study aimed to evaluate the effects of heat stress in vivo in dairy cows on cell membrane integrity markers of cumulus oocyte complexes (COCs). Twenty-seven Holstein cows were used, which remained for seven days in climatic chambers, distributed as follows: Control (C) - with *ad libitum* feeding and temperature-humidity index (THI) = 60; Heat stress (HS) - with *ad libitum* feeding and THI = 76; Pair-feeding (PF) - same amount of feed ingested by the HS and THI = 60. COCs were obtained after slaughtering the cows by slicing the ovary. COCs were denuded, and cumulus cells (CC) and oocytes (Oo) were subjected separately to analyses of lipid profile (LC-MS) and gene expression by RT-PCR using the Fluidigm platform. Heatmap, PCA and volcano plot test (metaboanalyst 5.0) were used for statistical analysis of the LC-MS results. For statistical analysis of gene expression analysis of variance was used through Proc Glimmix (SAS ondemand). For all analyses, $P<0.05$ was considered. The most predominant lipid class in the constitution of cell membranes are the phospholipids (PL). The regulation of PL varied between HS and PF groups, and most of them were downregulated in HS: 80% (16/20) in CC and 100% (8/8) in Oo. Additionally, in Oo and CC of HS, we observed lower expression of SLC2A1 gene, a gene that aids in glucose and fructose transport across the membrane. The expression of Sirt1 gene, a gene related to cell cycle regulation and proliferation, and Sirt 2 gene, a gene related to the control of cytoskeleton dynamics, were similar between CC and Oo in HS. However, Sirt1 gene showed higher expression in HS compared to PF, and Sirt 2 gene showed lower expression in HS compared to PF. In conclusion, heat stress *in vivo* for 7 days can negatively affect the membrane functionality and the cytoskeleton dynamics of oocytes and cumulus cells from dairy cows.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

The LH effects on PI3K/AKT pathway: Gene expression analysis *in vitro* cultured granulosa cells

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The role of PI3K/AKT and their regulation during ovulation in monovulatory species is not completely understood. In this study, we aimed to analyze if LH can regulate PI3K/AKT pathway genes in granulosa cells from pre-ovulatory follicles. For this, slaughterhouse bovine ovaries containing follicles with a diameter between 8.5 to 15 mm were used to obtain granulosa cells through repeated flushing with DMEM/F12 and heparin. Granulosa cells were washed three times by centrifugation at 900×g for 10 min each and filtered through a 150 mesh (Merck; Darmstadt, Germany). Cell viability was estimated with Trypan blue. Cells were seeded into 24 well culture plates at a density of 1×10^6 viable cells per well in 1 ml of culture medium and cultured for 24h at 37.0 °C and 5% CO2 in DMEM/F12 supplemented with 0.1% BSA, insulin (10 ng/ml), penicillin (100 IU/ml), streptomycin sulfate (100 µg/ml), FSH (1 ng/ml) and SFB (2%). The medium was replaced with DMEM/F12 supplemented with 0.1% BSA, penicillin (100 IU/ml), and streptomycin sulfate (100 µg/ml). After 18h, the LH (400 ng/mL) was added to the treatment group for 6h. At the end of culture, granulosa cells were collected (n=6), submitted to total RNA extraction according to Qiazol® (Qiagen) protocol, and followed by reverse transcription using High-Capacity Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The RT-qPCR analysis of EREG was performed in order to exclude unresponsive replicates. Only replicates that presented higher EREG expression in LH treatment compared to the control group were used to analyze the other genes ($P \leq 0.05$). RT- qPCR analysis was performed to evaluate selected genes: *PTEN, PI3K, AKT1, BAX, BCL-2, CREB, BRCA, CYP19A1, CYP17A1, 3BHSD,* and XIAP. The relative expression of PI3K/AKT pathway genes (*PTEN, PI3K, AKT1, FOXO3a, BAX, BCL-2, CREB, BRCA*) and genes related to the granulosa cells physiology (*CYP19A1, CYP17A1, 3BHSD, XIAP*) were analyzed in order to evaluate cells response to LH. Expression levels were calculated using the $2^{-\Delta Ct}$ method and normalized by the geometric mean of ACTB and H2A as reference genes. Relative gene expression data (mean ± SEM) were tested for outliers' presence, normality (Shapiro-Wilk test) and were analyzed by the student´s test ($P \leq 0.5$) (JM Software). The results demonstrated higher expression of *CREB* (P=0.0285) and *CYP19A1* (P=0.0173) in granulosa cells treated with LH compared to control. Based on these results, the LH surge may regulate the PI3K/AKT pathway since a gene such as CREB is altered. Thus, PI3K/AKT could be involved in mediating LH surge-induced gene expression in granulosa cells during growth of ovulating follicles in cattle.

Funding: FAPESP 2019/15491-6; 2021/06645-0; 2020/13075-2 and CAPES finance code 001.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

TNF-alpha on the viability of cumulus-oocyte complexes

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Elucidating the effects of pro-inflammatory cytokines during cellular differentiation processes, particularly in oogenesis and folliculogenesis has proven to be a challenging task largely due to its pleiotropic nature. Follicular fluid (FF) concentration of tumor necrosis factor-alpha (TNF-α) is regulated by infection diseases and environmental stressors experienced by females. It has already been documented that Covid-19 reduces the TNF-α levels 1.3-fold compared to the FF physiological concentration whereas an overdose causes a cytotoxic effect. This study aims to investigate the effects that the TNF-α plays on the quality of cumulus-oocyte complexes (CoCs). Homogenous bovine oocytes with more than three layers of cumulus cells were subjected to IVM medium supplemented with TNF-α at different concentrations and submitted to the IVM procedure. Oocytes were divided into four groups according to the concentration of TNF-α added to the maturation medium (n=25/group): Oocytes cultured in medium containing 2.9 ng/mL (physio; physiological concentration of TNF-α found in FF); oocytes cultured in medium containing 2.0 ng/mL (sub-dosage; 1.3 times less than the physiological level of TNF-α); oocytes cultured in medium containing 200 ng/mL of TNF-α (overdose); oocytes cultured in maturation medium without the addition of TNF-α (control). For statistical analysis, data were subjected to analysis of variance (ANOVA) and logistic regression with a significance level of 5% ($P<0.05$). To measure the expansion area of the cumulus cells of the COCs during IVM (n=8/group), images of oocytes before and after IVM were captured. Thus, it was possible to obtain the cumulus cell expansion area based on the difference in the cell area before and after IVM period. The Image J tool was used to determine the area of the cumulus cells. The results indicate that the capacity of cumulus cell expansion was not affected ($P>0.05$) by TNF-α treatment in the maturation medium compared to the control group. To measure cytoplasmic maturation, after IVM, oocytes from all groups were subjected to Lens culinaris agglutinin conjugated with FITC and the pattern of cortical granules distribution was analyzed under fluorescence microscopy. The results indicate that the cytokine did not interfere ($P>0.05$) with the morphospatial distribution of oocyte cortical granules. Finally, to assess nuclear maturation, the extrusion of the first polar body was considered. Oocytes from all groups were subjected to nuclear staining with Hoechst 33342 solution and analyzed under fluorescence microscopy. The results indicate that the extrusion of the first polar body was affected ($P<0.05$) by TNF-α overdose (33.3%) in the maturation medium, and the other groups did not differ ($P>0.05$) from the control group (75.0%). It is concluded that the treatment of the maturation medium with TNF-α overdose impaired oocyte nuclear maturation while sub-dosage did not trigger perceptible effects on the variables analyzed.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Functional genomics unveils the effects of heat stress during mouse oocyte growth

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Heat stress (HS) is an inability to thermoregulate under chronic exposure to elevated temperatures. To explore the impact of HS on oocyte developmental programming, we developed a novel mouse model which exposes mice to this environmental challenge during the first wave of oocyte growth. Here, we describe the impact of such HS model on the transcriptional landscape of mouse full-grown oocytes. Swiss mice (EPM2 substrain, UNIFESP, Brazil) at six-eight weeks were randomly mated and placed in individual cages before delivery. Lactating females with litters on postnatal day 9 (P9) were randomly allocated to control (CTL) (21°C/24h) or HS (35°C/12h/light and 21°C/12h/dark) from P10 until weaning on P21. The HS was carried out in an automated climatic chamber (Alesco, Brazil) inside the mouse facility. The climatic chamber-initiated heating daily from 6:00 until 18:00. Weaned HS and CTL females were kept at 21 °C until puberty at P35. To collect germinal vesicle (GV) oocytes, females at P35-42 were subject to an intraperitoneal injection of 10 IU PMSG. Females were euthanized 46h after the PSMG. Pools of 200 oocytes were subject to total RNA extraction (two replicates per group). RNA libraries were prepared with the Zymo-Seq 3' mRNA Library Kit. Initial quality control of sequencing results was performed with FastQC (v0.11.9). Sequencing errors were identified with UMI-tools (v1.1.1). The initial sequence analysis was done with Trim Galore! (v0.6.6), and the mapping to the mouse genome (GRCm38) was done with STAR (v2.6.1d). The processing read alignments was carried out with SAMtools (v1.9). The estimation of the complexity of genomic sequencing libraries was performed with Preseq (v2.0.3). The quality control of alignment sequencing data was done with RSeQC (v4.0.0) and Qualimap (v2.2.2-dev). Reads were summarized by featureCounts (v2.0.1). The differential expression was determined by DEseq2 (v1.28.0). Differentially expressed genes (DEG; Log2 fold change cutoff of 0.585) were enriched by Gene Ontology (GO) and KEGG pathway analysis. The functional enrichment was done with g:Profiler (FDR cutoff 0.05). A total of 16,379 and 20,664 unique transcripts were mapped in CTL and HS samples, respectively. Further, there was a limited representation of rRNA in samples (CTL: 0.93% and HS: 1.28% of mapped transcripts). A total of 85 transcripts were differentially expressed between groups, of which 41 were upregulated and 44 downregulated under HS. Of those, 16 were pseudogenes and 15 of them (93.7%) were downregulated in the HS group. The DEGs were mainly part of the inhibin and FSH-mediated pathways, oxidative stress, and developmental competence, among other cellular processes. DEGs were epigenetic regulators, nuclear receptors/transcription regulators, long non-coding RNAs, membrane receptors, and so on. These findings unveil novel genes affected by HS and further suggest actionable (druggable) targets for mitigating HS-mediated damage.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

Relationship between antral follicle count and IGF-1 concentrations with conception rate of Nelore heifers

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The aim of this study was to evaluate the pregnancy rate and hormone levels of IGF-1 and E2 in heifers with different AFCs (antral follicle count). The experiment was conducted on a farm in Mato Grosso do Sul and for this purpose 73 Nelore heifers were used. They had an average age of 11.5±1.3 months, average weight of 306.15±29.86 kg and median BCS 3 (2.75-4). The heifers were submitted to a hormonal protocol for pre-synchronization of the estrous cycle, with two applications of P4 i.m., 24 (D-24) and 12 days (D-12) before the beginning of the FTAI protocol. On Day 0, 2 mg of EB (Gonadiol®, Sintex AS, Argentina) was injected i.m. and the insertion of an intravaginal device with 0.5 g of progesterone (P4) (DIB 0.5®, Sintex AS, Argentina). On Day 8, the P4 device was removed, and the females received 12.5 mg of dinoprost tromethamine (Lutalyse®, Zoetis, São Paulo, Brasil), 1 mg of estradiol cypionate (EC) (ECP® Zoetis, São Paulo, Brasil) and 300 IU of eCG (Novormon®, Zoetis Buenos Aires, Argentina), both i.m. On Day 10, FTAI and ultrasound-guided follicular aspiration (OPU) were performed to obtain follicular fluid (FF) for all visualized follicles, except dominant follicle. The determination of AFC was performed on D0 by evaluating a single ovary of each female and multiplying the number found by two (Oliveira Junior et al., 2015). Blood samples were collected from females on Day 10 and Day 40. IGF-1 and E2 concentrations were determined by RIA kit (Beckman Counter®), in duplicate. Conception rate was evaluated on Day 40 by transrectal ultrasonography. Data were analyzed by the SAS University program, using PROC GLIMMIX, PROC LOGISTIC and PROC CORR, for comparison of means, logistic regression and Pearson correlation, respectively. For all tests, a statistical difference was considered when $P<0.05$. The AFC ranged between 10 and 46, with a mean of 22.1 ± 8.2 follicles, and thus the mean AFC subtracted from one standard deviation was considered low, and the mean AFC plus one standard deviation was considered high, as follows: low (≤14; n=22) and high (≥30; n=20). A positive correlation ($P=0.028$; $R=0.66$) of IGF-1 with AFC was observed, but not with conception rate, while serum E2 showed no correlation with AFC and conception rate. The mean concentration of E2 in serum (5.535µg) at Day 10 did not vary between the groups. Mean serum concentration of IGF-1 was higher ($P<0.01$) in high AFC heifers on Day 10 (418.7µg x 269.2µg) and on DG (387µg x 306.9µg). Although the IGF-1 concentration in FF did not differ between groups, it showed a high correlation ($P=0.0049$; $R=0.77$,) with serum IGF-1 collected on the same day. The overall pregnancy rate was 43.8%, being 45.5% for low AFC heifers and 41.2% for high AFC heifers ($p>0.05$). We conclude that, under the conditions of this experiment, AFC did not interfere with the conception rates of Nelore precocious heifers, even in the case of higher IGF1 concentrations in the high AFC group.

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FOLLICULOGENESIS, OOGENESIS, AND SUPEROVULATION

The AFC does not influence conception rate, oocyte quality and carcass traits in crossbred (Nelore x Angus) heifers

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The aim of this study was to evaluate the relationship between AFC and conception rate after FTAI, oocyte quality and carcass ultrasound characteristics in crossbred heifers. For this purpose, 140 confined heifers (½ Angus x ½ Nelore), with a mean age of 10 ±0.54 months, mean weight of 295.1 ±32.6 kg and mean body condition score (BCS) of 3.44 ±0.41 were used. The heifers were submitted to a hormone pre-synchronization protocol of the estrous cycle, followed by a three-management FTAI protocol based on progesterone and estrogens. FTAI was performed with a single Brangus Bull. Ovary evaluation and AFC determination was performed on D0. Loin eye area, fat thickness and rump fat thickness were obtained by ultrasound on D0 and DG. The non-pregnant heifers on DG30 were slaughtered and the ovaries were collected. The pairs of ovaries from each female were collected individually after evisceration, and placed in properly identified packages, made with permeable material. In the laboratory, the verification of ovarian morphology was carried out by measuring the diameter of the ovaries, diameter of dominant follicles and diameter of corpus luteum. COCs of each ovary were placed into a petri dish for selection and classification in accord to the cytoplasm integrity and the amount of cumulus cells layers and its compactness (Grade I, II, III, IV and degenerated). Data were analyzed by the SAS University program, using PROC GLIMMIX, PROC LOGISTIC and PROC CORR, for comparison of means, logistic regression, and Pearson correlation, respectively. AFC of heifers varied between 4 and 52 and corresponded to an average of 22.7±8.5 follicles. Thus, considering the methodology for group separation by AFC, the mean AFC subtracted from one standard deviation was considered low, and the mean AFC plus one standard deviation was considered high, as follows: low (n=29) and high (n=22) groups contained ≤14 and ≥31 follicles, respectively. No correlations of carcass traits and AFC were identified. The overall pregnancy rate was 55%, being 44.8% for low AFC heifers and 57.1% for high AFC heifers (p>0.05). No correlations were observed among ovary morphology characteristics and AFC. The number of COCs grade II was higher in the high AFC group (P=0.0004). We conclude that, under the conditions of this experiment, AFC did not interfere with the conception rates and carcass traits of precocious crossbred heifers. However, better quality of COCs were observed in heifers with high AFC. New experiments must be conducted with a higher number of heifers to confirm these results.