Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Relationship between the time of OPU and in vitro embryo production

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Resumo

The moment of the estrous cycle for ovum pick-up (OPU) in Bos taurus donors may be correlated with in vitro production of embryos. The present study aimed to evaluate the embryonic rate of oocytes aspirated in different phases of the follicular wave. Oocytes were obtained from Wagyu non-pregnant donors (N = 31) with a body condition score (BCS) between 3.5 and 4.25. On a random day of the estrous cycle (D -10) the animals were submitted to a pharmacological protocol for ovulation synchronization, based on the on 2mg of BE IM and intravaginal progesterone (P4) device. On the eighth day of the protocol (D -2), the device was removed and 10 µg of D-cloprostenol, 300 IU eCG and 1 mg EC were applied. The D0, 48 hours after P4 device removal, was considered the day of ovulation. From D0 onwards, the animals were divided into experimental groups: group D4 - OPU on day 04 after ovulation (N = 9), group D8 - OPU on day 8 after ovulation (N = 8), group D14 - OPU on day 14 after aspiration (N = 7) and group D18 - OPU on day 18 after ovulation (N = 7). The oocytes were selected and those with grade I, II and III were destined for IVF and on D7, the blastocyst rate was assessed. The data were analyzed for normality distribution by the Shapiro-Wilk test, transformation with log10 and analysis by ANOVA with Tukey's post hoc test using BioEstat® 5.3 software (p-value ≤ 0.1). Oocytes aspirated on day 4 after ovulation showed a higher rate of blastocysts when compared to animals aspirated on D8 (G4 = 32.17 ± 16.51 vs. G8 = 13.82 ± 16.74; P = 0,0025). In addition, the rate of blastocysts was lower from oocytes aspirated on D8 when compared to those aspirated on D18 (G8 = 13.82 ± 16.74 vs. G18 = 25.44 ± 15.96; P = 0.0025). There was no difference between the oocytes from the group D14 and the others (G14 = 20.76 ± 21.33). Oocytes from beginning of the first follicular wave, beginning and end of the second follicular wave presented the better blastocyst rates. We concluded that oocytes aspirated on D4, D14 and D18 may generate a higher rate of blastocysts.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

CRYOTOLERANCE OF IN VITRO PRODUCED BOVINE EMBRYOS ORIGINATED FROM OOCYTES MATURED IN MEDIUM SUPPLEMENTED WITH RECOMBINANT HUMAN FSH

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Resumo

The recombinant human FSH (rhFSH) has been used during in vitro maturation (IVM) of cattle oocytes as an alternative to the FSH obtained from porcine pituitary (pFSH). The rhFSH show less variation on biological activity, and lacks the sanitary risks associated with the use of protein extracts obtained from other species. However, few studies have compared the efficiency of rhFSH and pFSH considering not only the results of IVM, but also subsequent embryo quality. The aim of this study was to evaluate the cryotolerance of embryos produced in vitro after IVM with rhFSH. Cumulus-oocyte complexes (COC, n=2,040) recovered from slaughterhouse ovaries and morphologically classified as grades I or II were used. The COC were randomly allocated into three groups, which were IVM in TCM199: 1) without FSH (-FSH, n=680); 2) with 0.5 µg/mL pFSH (pFSH, Folltropin-V, Vetoquinol, n=680); or 3) with 0.1 UI/mL rhFSH (rhFSH, Gonal, Merck, n=680), all groups in the same culture conditions (38.5°C, 5% CO2). Cumulus expansion was evaluated at 22 h of IVM and subjectively classified as poor, intermediate, or good. Sperm from a single sire with known fertility was used for in vitro fertilization. The presumptive zygotes were cultured under low oxygen concentration (5% CO2, 5% O2, and 90% N2, at 38.5°C). The cleavage, blastocyst and hatching rates were addressed at days 3, 7, and 10 of culture, respectively. At the day 7, a subset of grade I expanded blastocysts (n=54, 69, and 71 embryos from groups -FSH, pFSH and rhFSH) were cryopreserved by vitrification, stored in liquid nitrogen, and then thawed and in vitro cultured under low oxygen concentrations. Hatching rates were evaluated after 72h. Data were evaluated by the Chi-squared method using the SAS software (SAS Institute). Cleavage and blastocyst rates, were higher in the rhFSH, compared with -FSH and pFSH groups (80.4% and 46.3% vs 71.5% and 34.6%, and 70.0% and 38.0%, respectively, P=0.0069 and P=0.0207). However, there was no difference in hatching rates (74.5%, 79.0%, 70.1% for -FSH, pFSH and rhFSH groups, respectively, P>0.05). There was no difference in hatching rates after vitrification among groups (75.0%, 72.5% and 73.2%, P=0.8875, for groups -FSH, pFSH and rhFSH, respectively). In summary, there is no evidence that the presence or the source of FSH (porcine or recombinant human) during IVM affect subsequent embryo cryotolerance.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Simulated Physiological Oocyte Maturation (SPOM) system application in cattle: A Systematic Review

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Resumo

The Simulated Physiological Oocyte Maturation (SPOM) mimics *in vitro* the physiological events of oocyte maturation. The system uses cAMP modulators in two steps (pre IVM and IVM) and presented promising results for IVF in livestock, generating great interest, many adaptations, and controversies. Thus, this study aimed to systematically analyze the data available in the literature using SPOM in cattle and compared those to the original paper (Albuz et al., Hum. Rep., 25: 2999–3011 2010), classifying them into successful or failed. The PubMed, Scopus, and Google Scholar databases were consulted, and 15 studies were included. Out of those, data from 18 experiments were extracted and evaluated by descriptive statistical analysis. Just experiments that assessed blastocyst rate (BR) were considered for the success parameter, as successful (increase in BR) or failed (neither difference nor reduction in BR). Twenty-five percent (4/16) of the experiments succeeded to improve blastocyst production. From the experiments that used the original base medium (n=3), 33% were successful. Most experiments used TCM-199 as a base medium (13/18) and 27% of them were successful. Considering pre IVM, experiments using either original (14/18), or adapted (4/18) conditions had the same chance of success (about 25%). Regarding IVM, most experiments used cilostamide and, of those, 30% (3/10) had success. All the four successful experiments changed the IVM duration, three of them reduced the duration to 20 h or 24 h, whilst one study increased the duration to 30 h. Four experiments (4/18; 22%) used BSA as the original study concentration (4mg/mL) and only one of them succeeded. Seven experiments (7/18; 38%) used lower concentrations of BSA (0.2 to 1 mg/mL) and 3 of them succeeded. All studies that replaced BSA by FCS failed to increase blastocyst production (n=2). The studies applying the original type and dose of FSH (n=5) had 20% of success, while those promoting adaptions (n=8) reached about 28% of success. Six experiments conducted IVC under high tension of oxygen and most of them (83%, 5/6) failed. The only successful experiment under high oxygen tension also reduced the IVM duration from 28 h to 24 h. Considering oocyte and embryo assessments, two experiments measured cAMP levels and showed an increase; one of them could not be evaluated for BR and the other succeeded. Six experiments assessed nuclear maturation, most of them (83%, 5/6) observed the arrest of meiosis and of those that assessed maturation, 33% (2/6) succeeded to improve blastocyst production. Nine experiments assessed the blastocyst total number of cells and two (29%) showed an increase. Our findings clearly indicate a difficulty in reproducing the SPOM system worldwide and suggest that different supplements used in IVM medium and/or their interaction with modulators for a different time duration, may represent a great bias on the experimental success.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

CIRURGICAL DESCRIPTION OF LAPAROSCOPIC OVUM PICK-UP IN BUFFALO CALVES

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Resumo

The aim of this work was studying the technique of the laparoscopic ovum pick-up (LOPU) in buffalo calves, describing in detail its cirurgical particularities, complications and results. Six lactating Murrah calves aged between 3 and 5 months were used, raised on pasture. Three LOPUs were performed in each animal, with intervals of 15 days between surgeries, totaling 18 procedures. In order to facilitate the analysis of the surgical time, the procedures were divided into four stages: 1) Anesthetic and antisepsis procedure (AA); 2) Dieresis, abdominal insufflation and establishment of laparoscopic portals (DP); 3) Ovarian manipulation and aspiration (OM) and 4) Washing, desinsufflation and synthesis (DS), also making it possible to determine the learning curve of the technique in buffalo calves. After being sedated (Xylazine 0,2 mg/Kg), the calves were placed in a 45° Trendelemburg position. Two 10 mm and one 5 mm trocar were used to establish the three laparoscopic portals, located in the right and left inguinal and central hypogastric positions. The abdomen was inflated with CO2 (5L/min), stabilizing intra-abdominal pressure between 5-8 mmHg two atraumatic forceps (5 mm and 10 mm) were introduced into the inguinal portals for ovarian manipulation. For follicular aspiration, a 20-gauge needle, connected to a vacuum system calibrated to 50 mmHg, was introduced via transabdominal and oocytes were recovered in a 50 mL tube containing saline solution supplemented with 1% FBS and 10 IU/mL of Heparin, kept at 38ºC. Data were submitted to ANOVA and subsequent Tukey test, the descriptive statistics (mean ± SD) was used to present the results. GraphPad Prism 9® Software was used for statistical analysis. The surgical total time (from sedation to removal of the animal from the stretcher) was 49.8 ± 10.1 min, and the step that took the most time was manipulation and ovum pick-up of both ovaries (20.6 ± 9 .7 min) ($p<0.05$) varying according to the number of follicles to be aspirated, there were 22.2% of cases of preperitoneal insufflation resulting in an increase in surgical time by 9.0 ± 1.0 min. The total number of aspirated follicles, recovered oocytes and viable oocytes were 126, 76 and 31, respectively. The same parameters per procedure were 7.17 ± 7.17; 4.22 ± 7.1 and 1.72 ± 4.2; respectively. The recovery rate was 60.3%. The high SD values demonstrated the great individual variation of the ovarian follicular reserve present in this species. The low rate of viable oocytes showed the lowest oocyte competence in prepubertal females. The short surgical time and the good recovery rate demonstrated that this is a safe technique, possible to be performed in farm environment also in the buffalo species, provided that the basic antisepsis procedures are respected, presenting an excellent postoperative response, allowing it to be performed repeatedly, without damage or side effects to the donor.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Does Doppler ultrasonography for recipient selection improves the pregnancy success in equine embryo transfer programs?

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Resumo

Doppler ultrasonography is an emerging technology in equine reproduction and has the potential to assist in the challenge of selecting the best recipient in embryo transfer (ET) programs. We aimed with this study to test the hypothesis that corpus luteum (CL) and uterine blood perfusion determined by Doppler ultrasonography are characteristics more associated with pregnancy success than B-mode accessed characteristics in the CL and uterus of recipient mares. The study was carried out from November/2021 to April/2022 in a commercial farm of Mangalarga Marchador breed in Itaperuna-RJ, Brazil. Recipient mares (n=101), aging from 3 to 16 years old, and on days 4 to 8 after ovulation received a single fresh grade I or II blastocyst (7-10 days after fertilization), recovered by uterine flush from Mangalarga Marchador donors. Before ET, the reproductive system was evaluated by transrectal palpation (uterine tone [0 to 3]), B-mode ultrasonography (CL echogenicity [0 to 6], type of CL [homogenous, trabeculated or with an anechoic center], luteal area [cm2], and uterine echogenicity [0 to 3], edema [0 to 3] and echotexture [0 to 3]) and Color Doppler ultrasonography (CL signals of blood perfusion [0 to 100%], and endometrial and myometrial blood perfusion [1 to 4]). Recipients were split retrospectively in two subgroups according to the mean of CL area [small (≤6 cm2) or large (>6 cm2)], two subgroups according to CL blood perfusion [low (≤55%) or high (>55%)] and five subgroups according to the day related to recipient's ovulation at ET [4 to 8 days]. Pregnancy diagnosis was performed by transrectal ultrasonography based on visualization of a 14- to 16-days embryonic vesicle and Pregnancy/ET (P/ET) was analyzed by logistic regression or GLIMMIX procedure of SAS, considering the effects of all CL and uterine characteristics and recipient's day of ovulation. When CL and uterine characteristics were evaluated according to pregnancy status, a tendency of greater (P=0.09) CL blood perfusion was observed in pregnant (n= 76) than non-pregnant (n= 25) recipients. Among all factors evaluated, P/ET was only significantly (P=0.007) affected by the class of CL blood perfusion (low: 65.5% [38/58] vs. high: 88.4% [38/43)]). Also, P/ET tended to be greater (P=0.1) in recipients with high than low endometrial blood perfusion (83.5% [26/31)] vs. 71.4% [50/70]). When evaluated as a continuous variable, the CL area did not (P>0.1) affect the P/ET, but for CL blood perfusion, a cubic effect (P=0.005) indicated that P/ET is negatively associated to the CL blood perfusion until it reaches 45%, followed by a positive relationship until 75% and then a negative relationship up to 90%. In conclusion, Doppler-US is an innovative tool that has the potential to be used for selection of suitable embryo recipients based on luteal blood perfusion. Selection of recipients that have a greater chance of maintaining pregnancy will increase the success of ET programs in horses.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Immunohistochemical characterization of sensory nerve terminations in the vaginal fornix and cervix of dorper ewes and saanen does

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Resumo

The commercial exploitation of goats and sheep for meat, milk, and skin has represented an important part of the Brazilian livestock economy in recent years. Therefore, aiming at the animal's genetic improvement, the implantation of reproductive biotechnologies in these herds such as embryo transfer is fundamental for an efficient and sustained technical exploitation. Indeed, the overall conditions of animal welfare are of great importance, also when applying reproductive technologies. Thus, this study aimed to investigate the presence of sensory nerve terminations in the mucosa of the vaginal fornix and cervix region of sheep and goat females, seeking for evidence that can improve both embryo collection and transfer techniques in small ruminants. Fragments of the vaginal fornix and cervix of five Dorper ewes and five Saanen does were collected from a local slaughterhouse (latitude 21°46'S, longitude 43°22'W). The histological sections were submitted to the immunohistochemical process, through the reaction of the antibody against the general protein nerve marker product-gene 9.5 (PGP-9.5), focusing on the presence of nerve terminations, their intensity (weak, moderate, intense), and frequency. Overall, a total of 80% of the histological sections of the cervix showed an intense presence of nerve terminations (being 100% (5/5) in goats and 60% (3/5) in sheep). In the histology of the vaginal fornix, goats had a 60% of intense, 20% of moderate and 20% of weak presence. In sheep, there was no intense presence, and the majority (4/5) was moderate (80%), while only one ewe had it weak (20%). In conclusion, the nerve terminations are present in the lamina propria of the mucosa of the histological sections of all animals studied. Their location indicates a sensory function, suggesting a potential "pain sensitivity" in those organs. Therefore, it is recommending the use of anesthetic procedures whenever there is any manipulation of such organs, as in the nonsurgical embryo recovery and transfer in small ruminants.

Keywords: Sheep, Goats, Cervix, Anesthesia.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Embryo recovery rate in oviduct 2.5 days after IFIOT performed with different amounts of CCOs

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Resumo

Many factors may be involved in the low efficiency of IFIOT and to identify these factors, it is necessary to clarify in which point of the process the greatest losses are occurring. The aim was to evaluate the effect of the amount of CCO injected during IFIOT on recovery rate of structures and embryos in the oviduct. Were used 45 non-lactating cows, submitted an ovulation synchronization protocol (D0: P4+2mg EB; D8: 0,5mg PGF and removal P4 device; D9: 1mg EB). At 54.5±2.7h after removal the P4 device, IFIOT was performed: Control Group (CG; n=15): IFIOT with injection of 60µL of medium (TCM199) plus 10% FBS), without the presence of CCO; Group 5 (G5; n=15): IFIOT with injection of 5 CCO in 60µL of medium; Group 50 (G50; n=15): IFIOT with injection of 50 CCO in 60µL of medium. After IFIOT, cows were inseminated and received 10µg of buserelin acetate i.m. (GnRH; Sincroforte®). Between 64 - 66 hours after IFIOT, slaughter was carried out to collect the reproductive tract, with dissection and washing of the oviduct ipsilateral to the ovary that presented ovulation. Oviduct washing was performed with 3 mL of PBS and the content evaluated for the presence of structures. Were considered structures: unfertilized oocytes, zona pellucida or embryos (cleaved oocytes). To assess whether the injected and unrecovered CCO in the oviduct would be retained in the follicle after ovulation, the forming CL was dissected, and internal cavity was washed. The structures recovery rateand embryos were submitted to Pearson's correlation with the follicular diameter (FD); time interval of permanence of CCO in the injection needle and oviduct size. To calculate the recovery rate of total structures and embryos, one structure was removed in order to disregard the physiological structure from the follicle of the "ovulator" cow. The total recovery and embryos rate between groups were compared using the Mann-Whitney test ($P<0.05$). Descriptively, after oviduct washing the CG, 11 (73.3%) cows presented a structure, with 72.7% of cleaved. In G5 and G50 was recovered structures in 10 (66.7%) and 13 (86.7%) cows, respectively. Considering the presence of ≥2 structures, the recovery occurred in 7 (46.7%) and 10 (66.7%) cows, for G5 and G50, respectively. The recovery rate of total structures and embryos was similar between G5 (24%; 9.3%) and G50 (31.6%; 7.0%). Finally, in 2 (13.3%) and 7 (45.6%) animals from G5 (2.7%) and G50 (2.1%) oocytes were recovered after CL dissection. No correlation was found between the recovery rate of total and cleaved structures with any parameters evaluated: FD diameter (R=-0.1 and R=0.32); oviduct length (R=-0.11 and R=-0.17) and length stay of the oocyte on the needle (R=-0.05 and R=-0.05). These results suggest that the capacity of uptake CCO by the oviduct fimbria and/or the release COC at the time of ovulation is being compromised, regardless the amount of COC used.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

EFFECT OF THE ROUTE OF ADMINISTRATION OF RECOMBINANT HUMAN FSH ON OVARIAN SUPERESTIMULATORY RESPONSE IN CALVES

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Resumo

A number of pre-stimulatory protocols using porcine FSH (pFSH) have been proposed to improve oocyte yield and *in vitro* embryo production from calves. However, results are still inconclusive. The pFSH is purified from hypophysis recovered at slaughterhouse, which results in significant variation in biological activity between drug batches. Moreover, due to its short half-life, treatments with pFSH usually require multiple injections over time. Recently, long-acting recombinant human FSH (rhFSH) formulations have been developed to induce superstimulation in women using a single-injection. The aim of the present study was to evaluate the effect of treatment route (IM or SC) on ovarian response to long-acting rhFSH in calves. Prepubertal Nelore (*Bos taurus indicus*) calves (N=10) with 205.6±4.0 days of age and 175.4±5.2 Kg of body weight were enrolled. A preliminary dose-response trial was performed to determine the dose to be used in this study as follows. The calves were randomly allocated to receive 0 to 22.5 mcg, with 2.5 mcg increments of rhFSH (Corifollitropin Alpha, Shering-Plough, Brazil), via im. Ovarian follicular development was monitored daily by ultrasonography (MyLab 30 Gold, Esaote, Italy) for five days. A videoclip was recorded from each ovary and used to measure size of individual follicles. A consistent ovarian response (measure the size of individual mean follicle diameter > 6 mm at 96h after treatment) was obtained with doses greater than 10 mcg, whereas lower doses resulted in a mean follicle diameter similar (P>0.05) to control (0 mcg). A subsequent trial has been performed to evaluate the treatment route. The calves were randomly allocated to receive 10 mcg rhFSH either SC (n=5) or IM (n=5). Follicle development was monitored by ultrasonography as previously described. Data was analyzed using the Glimmix Procedure of SAS. We did not observe an effect of route (P=0.1348) nor an interaction route x time (P=0.8336). Time after treatment affected follicle growth in both groups (P<0.0001), with the average of follicle diameter increasing from 3.6±0.1 to 7.6±0.1 mm from 0h to 96h (growth rate 1.0±0.1 mm/day). Thereafter, follicle development stabilized and no further growth occurred from 96h to 120h (7.6±0.1 vs. 7.8±0.1 mm; P>0.05). In summary, the SC route can be successfully used to induce ovarian superstimulation in calves treated with a single dose of long-acting rhFSH.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Relationship of antral follicle count with reproductive characteristics of embryo recipient mares

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Resumo

This study evaluated the antral follicle count (AFC) in recipient mares to relate the low and high AFC with fertility in an equine embryo transfer (ET) program. Cyclic (n=43) and acyclic (n=34) mares (n=77) aged 5 to 16 years, mixed breeds, between 320 and 460 were used as embryo recipients and evaluated by transrectal ultrasonography (A5V Sonoscape®, Domed, Valinhos, Brazil) to determine the AFC (follicles >2mm). Cyclic recipients were synchronized with the donors (D0 - ovulation day) during follicular control for artificial insemination. Having a dominant follicle (≥35mm), ovulation was induced with histrelin acetate (250µg; i.m.; Strelin®, Botupharma, Botucatu, Brazil) and hCG (1000IU; i.m.; Vetecor® Ceva, Paulínia, Brazil). Then, recipients received a single embryo 4-6 after confirmation of ovulation. Acyclic recipients received i.m. 5mg of estradiol benzoate (EB; Gonadiol®, Zoetis, São Paulo, Brazil) on the day of donor ovulation (D0), 4mg the next day (D-3 recipient; D1 donor) and 3mg (D-2 recipient; D2 donor). On D0 of the recipient (D4 donor) was applied 1500mg P4 i.m. (P4-300®, Botupharma) and repeated more 1500mg P4 on the ET day (D4-6). The parameters of AFC, age, weight, body condition scores (BCS), degree of uterine edema, CL diameter and conception rate were evaluated in all recipients. For data analysis, AFC groups were defined as low (≤11 follicles; n=43; 25 cyclic and 18 acyclic) and high count (>11 follicles; n=34; 18 cyclic and 16 acyclic). Data were analyzed by ANOVA using a mixed-effect model (AFC group, seasonality, and interaction as fixed effects; animals as a random effect and other sources of variation as covariates). The conception rate was evaluated by binary logistic regression, using the same components of the model (5%). Recipients with low and high AFC showed similarities to age (10.6±0.5 and 9.3±0.5; P=0.11), weight (403.7±3.1 and 402.1±4.6; P=0.48), BCS (3.4±0.1 and 3.2 ±0.1; P=0.19), degree of uterine edema (2.5±0.1 and 2.5±0.1; P=0.67) and CL diameter (28.7±0.7 and 26.8±0.9; P=0.23), respectively. None of these variables were affected (P>0.1) by reproductive seasonality or interactions. However, the conception rate was higher in recipients with low compared to high AFC [79.1% (34) vs 61.76% (21); P=0.01], in addition to being higher in cyclic mares (81.4%) compared to those in seasonal anestrus (58.8%; P=0.005). Furthermore, there was a tendency (P=0.06) of AFC*seasonality interaction, revealing that recipients with high AFC in anestrus (37.5%b) showed the lowest conception rate in relation to the other groups (high AFC*cyclic 83.3%a, low AFC*anestrus 77.8%a and low AFC*cyclic 80.0%a). In conclusion, AFC was not related to age, weight, BCS, degree of uterine edema, and CL diameter. However, the low AFC and the presence of reproductive cyclicity determined positive effects on the conception rate of recipients, demonstrating a relationship between fertility and AFC and reproductive seasonality in recipient mares.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

THE USE OF ASTAXANTHIN IN IN VITRO CULTURE EXERTING POSITIVE EFFECTS ON CRYOPRESERVATION OF BOVINE EMBRYOS

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Resumo

During embryo culture and cryopreservation/warming is recognized that the production of reactive oxygen species (ROS) can cause oxidative stress; therefore, the use of antioxidants as astaxanthin, can mitigate these undesirable effects. In this regard, the objective of this work was to evaluate whether the addition of astaxanthin (AST) to *in vitro* culture medium (IVC) could exert positive effects on the cryopreservation of bovine embryos. For this purpose, presumptive bovine zygotes were assigned for IVC in one of the following treatments: CONT (negative control; n=48), DMSO (0.001% of dimethylsulfoxide; n=49), AST 8 (8 ug/L of AST; n=49), AST 2 (2 ug/L of AST; n=52), and AST 0.5 (0.5 ug/L of AST; n=49). On day 7 of IVC, expanded blastocysts were vitrified with medium composed by TCM 199 + 20% FCS (v/v), ethylene glycol (EG), and DMSO. Vitrified embryos were warmed, cultured in IVC medium, and evaluated after 24 h for reexpansion and 48 h for hatching rate. A subset of embryos of each treatment were analyzed, and malondialdehyde metabolites were measured on day 7 of IVC medium drops by TBARS. Data were analyzed by ANOVA (PROC GLM), including treatment as fixed effect (SAS 9.3). TBARS did not have a normal distribution, therefore, was transformed in 1/ square root, although the means ± SEM be shown not transformed. At 24 h, differences ($P \leq 0.05$) between treatments were found for expanded blastocyst rate (CONT: $67.8^{AB} \pm 8.2$; DMSO: $54.4^{AB} \pm 7.9$; AST 8: $64.9^{AB} \pm 8.5$; AST 2: $73.3^{A} \pm 8.2$; AST 0.5: $47.6^{B} \pm 9.0$), hatching blastocyst rate (CONT: 15.0 ± 6.3^{ABC}; DMSO: $11.5^{BC} \pm 4.3$; AST 8: $20.1^{AB} \pm 7.0$; AST 2: $2.9^{C} \pm 2.0$; AST 0.5: $28.4^{A} \pm 7.6$), hatched blastocyst rate (CONT: $17.2^{AB} \pm 5.5$; DMSO: $34.1^{A} \pm 7.1$; AST 8: $15.0^{B} \pm 6.0$; AST 2: $25.1^{AB} \pm 8.1$; AST 0.5: $24.0^{AB} \pm 6.6$), and TBARS (CONT: $236.3^{AB} \pm 44.3$; DMSO: $244.6^{AB} \pm 91.9$; AST 8: $163.4^{A} \pm 21.4$; AST 2: $461.5^{AB} \pm 203.7$; AST 0.5: $303.2^{B} \pm 64.7$). No differences were found among the treatments for other variables (cleavage rate, expanded blastocyst rate, embryo development rate, expanded blastocyst rate at 48 h, hatching blastocyst rate at 48 h, and hatched blastocyst rate at 48 h). In summary, in our experimental conditions, the supplementation of *in vitro* culture medium with astaxanthin does not have an antioxidant effect protecting embryo during vitrification/ warming.

Keywords: Carotenoids, Cryoprotectants, Vitrification, Reactive Oxygen Species

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Oocyte maturation with bisphenol A (BPA) induces oxidative stress and impairs bovine embryo production

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Resumo

Bisphenol A (BPA), a monomer widely used in the plastic industry, is associated with serious effects on reproduction due to its binding to estrogen receptors (ERs). BPA was detected in human urine, saliva, blood, amniotic and follicular fluid. Animal studies have shown that BPA causes meiotic abnormalities, decreases percentage of oocytes that progress to metaphase II and increases oocytes degeneration. Here we aimed to investigate the effects of BPA during oocyte *in vitro* maturation on oxidative stress and the subsequent impact on early *in vitro* embryo development in cattle. For this, five replicates containing 20 cumulus-oocyte complexes (COCs) each were *in vitro*-matured with 1000 µM of BPA (0.1%DMSO) and subsequently submitted to analysis of oxidative stress using CellROX™ Green®, mitochondrial membrane potential using MitoTracker® Red, meiosis progression by Hoechst 33342's analysis; and embryo yield. The control group were *in vitro* matured with basal medium (0.1%DMSO). The effect of 1000 µM BPA was tested by unpaired T-test. Differences were considered significant when $P < 0.05$. We observed that oocytes treated with 1000 µM BPA exhibited toxicity and cell damage due to the high levels of reactive oxygen species and high levels of mitochondrial membrane potential. Also, no oocyte reached to metaphase II. Thereafter, BPA treatment blocked fertilization and embryo development. In general, we concluded that addition of 1000 µM of BPA during oocyte *in vitro* maturation blocks meiotic resumption and increases oxidative stress in bovine oocytes, which leads to impaired *in vitro* development of bovine embryos.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Interspecific embryo production: In vitro fertilization of cow-buffalo hybrids.

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Resumo

Interspecific hybrid embryos are useful models both in research, allowing the study of species-specific maternal-fetal interactions, and in animal breeding for improvements in the environmental adaptation of cattle. The objective of this research is to compare the development of cow-buffalo hybrid embryos (hCxB) and bovine embryos (BxB) by in vitro fertilization (IVF). Three cycles were carried out where cow oocytes (n=84) and buffalo semen were used for hCxB and cow oocytes (n=74) and bull semen for the BxB group. Bovine ovaries were collected from slaughterhouses and transported to the laboratory at 25ºC to 30ºC. Cumulus-oocyte complexes (COCs) were aspirated with 18-gauge needles from follicles with a diameter of 2 to 5 mm and collected in Hepes-buffered Tyrode's albumin (Hepes-TALP). COCs were matured in vitro for 22 h in 100ul drops covered with mineral oil (M8410) of bicarbonate-buffered TCM-199 (31100-035; Gibco, Grand Island, NY, USA), containing 10% fetal bovine serum (013/07; Internegocios, Buenos Aires, Argentina), 10 mg/mL follicle-stimulating hormone (NIH-FSH-P1, Folltropin, Victoria, Australia), 0.3 mM sodium pyruvate (P2256), 100 mM cysteamine (M9768), and 2% antibiotic-antimycotic (ATB, 15240-096; Gibco). For the IVF cycles, the bull and buffalo sperm used were from the same individual, respectively. Frozen semen was thawed in 37ºC water bath 30 seconds. Motility of buffalo and bovine sperm was checked after thawing. Then both spermatozoa were centrifuged twice (490 g, 5 minutes) in Brackett-Oliphant (BO) medium and resuspended in BO supplemented with 5 mM caffeine (C4144) and 20 IU/mL heparin (H3149). Spermatozoa were adjusted to 16 x 10^6 /mL with BO containing 10 mg/mL fatty acid-free bovine serum albumin (A6003). COCs were exposed to the buffalo and bovine sperm suspension for 5 hours in 100 mL droplets at 39ºC under 5% CO_2 in humidified air. Presumptive zygotes were then washed three times in Hepes-TALP. Presumptive hybrid and intraspecific zygotes derived by IVF were cultured in 50 mL droplets of SOF medium supplemented with 2.5% fetal bovine serum at 39ºC under 6.5% CO_2 in humidified air. Cleavage rate was evaluated on Day 2 and blastocysts rate on Day 8. The percentage of blastocysts obtained was evaluated over the total number of oocytes. To evaluate the significance in the development of the hybrids and the control group embryos, a T-Student test was performed for independent samples. There were significant differences ($P<0.05$) in the number of cleaved zygotes (33% hCxB vs 87.3% BxB) and blastocysts (13% hCxB vs 42% BxB) compared to the initial number of oocytes obtained. As a consequence of these results, we can observe that interspecific IVF between cow and buffalo can be carried out successfully. It is noteworthy that the deviations observed between hybrid and bovine blastocyst rate may be due to a poorer buffalo semen quality and a difference of compatibility between the oocytes and sperm of both species.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Transfer of two in vitro produced F1 Angus x Nelore embryos does not improve pregnancy rates: preliminary data

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Resumo

The transfer of two embryos to a single recipient has been proposed to increase calving rates in cattle. In dairy cattle, twin pregnancies are associated with higher incidence of pregnancy losses and dystocia, and the use of double embryo transfer (ET) is controversial. However, this strategy remains to be evaluated in crossbred beef cattle, particularly in programs using large-scale in vitro embryo production (IVP) with Y-sorted semen. The aim of this study was to compare the transfer of a single or two F1 Nelore x Angus embryos to Nelore breed recipients. Cumulus-oocyte complexes (COC) recovered from slaughterhouse ovaries and morphologically classified as grade I were used. The COC were IVM and IVF in the same culture conditions (38,5°C, 5,5% CO2). Sex-sorted sperm from a single Angus sire with known fertility was used for IVF. The presumptive zygotes were cultured under low oxygen concentration (5,5% CO2, 5,5% O2, at 38,5° C). Embryos (n=1,025) were classified according to the developmental stage and transferred at days 6, 7 or 8, according to the availability of synchronized recipients. A single (n=667) or two embryos (n=358, 179 ET) were transferred to 846 recipients. In this estudy only fresh embryos were transferred. The embryos transferred together were loaded in a single straw, and recorded based on embryo classification (i.e., BLBL, BXBX, etc.). Pregnancy diagnosis was performed by ultrasonography approximately 38 days after ET. Data were evaluated by the Chi-squared method using the SAS software (SAS Institute). The overall pregnancy rate was 50,8% (430/846), which did not differ between recipients receiving a single or two embryos (50,5% [337/667] vs 52,0% [93/179]; P=0.0633). In both cases, transfer of embryos that reached the expanded blastocyst stage earlier (i.e., BX at day 6 vs BX at day 7 or BXBX at day 6 vs. BXBX at day 7) resulted in higher pregnancy rates (P<0.0001). When data were pooled, the inclusion of a more developed embryo in double transfers (BXBL vs BLBL at day 7; or BEBX vs BXBX at day 8) did not improve pregnancy rate (63,9% vs. 50,0%, P=0.3681). In summary, the transfer of two embryos does not improve pregnancy rate, which is mostly dependent on individual embryo developmental potential. The further calving rates will be used to evaluate whether this strategy (double ET) is worthy in beef cattle.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Effect of antioxidants on in vitro nuclear and cytoplasmic maturation of bovine oocytes.

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Resumo

Despite all the advances in IVP, approximately 30 to 40% of oocytes that mature in vitro reach the blastocyst stage. Considering that the quality of the oocyte is the key factor for the success of IVP, inadequate conditions during maturation may be responsible for the low efficiency of the technique. One of the alternatives to improve this step is the use of antioxidants, which regulate the production of reactive oxygen species (ROS) from aerobic metabolism. The objective of this study was to evaluate the effect of ITS and Cysteamine (CYS) supplementation on the nuclear and cytoplasmic maturation of bovine oocytes. COCs were obtained from slaughterhouse ovaries and matured in the presence (+) or not (-) of Cysteamine (CYS) and ITS. Therefore, four treatments were used: T1. Control: -ITS -CIS; T2. -ITS+CIS; T3. +ITS+CIS; T4. +ITS-CIS. The CC expansion during IVM, which was determined by the difference between the mean area of all COCs from each treatment before and after IVM. Nuclear maturation was evaluated by staining the oocytes with lacmoid and determing the stage of meiosis. Subsequently, the COCs of the different treatments were subjected to fertilization and in vitro culture. Embryo were evaluated on D2 for cleavage and D6 and D7 for blastocyst formation. Blastocyst of D7 were differentially stained and total cell number and % trophoblast and inner cell mass (ICM) were determined. The number of embryos produced and the expanded area of COC were evaluated by ANOVA, the kinetic of nuclear maturation by Chi-square and cell count by Kruskall-Wallis. No difference ($p>0.05$) was observed in the areas (mm2) of COC's expansion among treatments [(mean ± standard deviation (SD)]: T1= 7.501±1.371; T2= 8.043±1.334; T3= 6,450±1,497; T4= 6.212±1.167. Also no effect ($p>0.05$) of presence of either antioxidant used was detected on nuclear maturation with the majority of oocyte reaching the metaphase II stage: [T1= 89.4% (68/86), T2= 83.5% (71/85); T3= 76.05% (54/71); T4= 67.9% (55/81)]. Similarly, neither cleavage [T1= 72±5% (84/116); T2=74±6% (97/131); T3=72±7% (88/122); T4=72±8% (84/117)] nor blastocyst production at D7 (T1= 35±7% (41/116); T2= 43±7% (56/131); T3= 30±14% (37/122); T4= 35±14% (41/117)] were improved by the treatments. The differential staining showed that total cell number of D7 blastocysts (T1= 179±18; T2= 185±12; T3= 181±11; T4= 186±11) and the percentage of ICM and trophoblast cells of the blastocyst were similar ($p>0.05$) among all treatments. In conclusion, the use of ITS or CYS does not affect nuclear maturation, cytoplasmic maturation or the quality of in vitro produced embryos.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Time of ovulation after intrafollicular injection of cumulus-oocyte complex into dominant follicle of Girolando cows, using two different esters of estradiol as ovulation inducers

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Resumo

Previous studies have indicated that for the intrafollicular oocyte transfer (IFIOT) success, the cumulus-oocyte complexes (COCs) need to remain for 12 to 22h into pre-ovulatory follicle after injection, to complete their maturation process. Thus, the aim of this study was to determine the time of ovulation after IFIOT, using two different estradiol esters as ovulation inducers. 14 non-lactating Girolando cows were used in two replicates, in the cross-over model, previously synchronized (Ourofino®, Cravinhos-SP, Brazil). The ovulation synchronization protocol consisted by the insertion of an intravaginal device containing 1g of progesterone (P4, Sincrogest) and administration of 2mg of estradiol benzoate i.m. (EB, Sincrodiol). Eight days later (D8), the cows received PGF2α i.m. (0.150 mg Cloprostenol sodium, Sincrocio) and the P4 device was removed. At the time of P4 device removal, the animals were randomly assigned in two experimental groups: EC (n=13), received 1mg of estradiol cypionate concomitantly with P4 device removal (EC, SincroCP) and EB (n=10), received 1mg of BE 24h after P4 device removal (Sindrodiol). The intrafollicular injection was performed 48h or 54h after P4 device removal, for EC and EB groups, respectively. The intrafollicular injection was performed using a teflon suction line (WTA, Brazil) coupled to a 27G needle end a syringe (1mL) at the opposite end. The needle was filled with 60µL of the TCM199 supplemented with 10% fetal bovine serum (FBS) for the intrafollicular injection. On day of intrafollicular injection, the cows were examined and only those presenting a single preovulatory follicle ≥10mm in diameter were used. To evaluate the interval from injection to ovulation, ovarian ultrassonographic examinations were performed every 6 hours after intrafollicular injection until ovulation. In each examination, the largest follicles (LF) from ovary where the injection was performed was identified and measured. The time of ovulation was defined as the time of disappearance of a previously identified LF from one ultrassonographic examination to the next. Statistical analysis was performed using the t-test, with a comparison of independent means ($P<0.05$). The diameter (mm) of the LF of the EC (12.36±0.58mm) vs EB (12.33±0.66mm; P=0.98) groups did not differ. Similarly, the time of ovulation (h), in relation to P4 device removal on EC (74.77±1.61h) vs EB (70.20±2.37h; P=0.11) groups did not differ. However, the time of ovulation in relation to intrafollicular injection timing was higher in the EC (26.77±1.61h) vs EB (16.20±2.37h, P=0.001) group. Thus, the intrafollicular injection did not interfere at the time of ovulation, in relation to P4 device removal time. However, the results suggest that intrafollicular injection timing in the EC protocol will need to be delayed in order to prevent the exceeding oocyte ideal maturation time in the IFIOT.

Acknowledgments

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Correlation of two parameters used in the dominant follicle evaluation with the total structures recovery rate from oviduct 2.5 days after intrafollicular oocyte transfer (IFOT).

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Resumo

The objective of this study was to correlate two parameters of dominant follicle (DF) evaluation with total recovery rate from oviduct 2.5 days after intrafollicular oocyte transfer (IFOT). For this, 45 Nellore (Bos indicus) non-lactanting cows were used, with BCS 3.25±0.22 and body weight 585.6±62kg, peviously synchronized with Ourofino® products (Cravinhos-SP,Brazil). The synchronization protocol consisted of the insertion of an intravaginal device containing 1g of progesterone (P4, Sincrogest®) and the administration of 2mg of estradiol benzoate i.m. (EB, Sincrodiol®). Eight days later (D8), the cows received PGF2α i.m. (0.150mg Cloprostenol sodium, Sincrocio®) and P4 device removed. Ovulation was induced 24h later (D9) with a 1mg EB i.m. (Sincrodiol®). At the time of IFOT, the cows were randomly assigned in three experimental groups: Control Group (CG; n=15): submitted to IFOT with 60µL of TCM199 supplemented with 10% fetal bovine serum, without cumulus-oocyte complexes (COCs); Group 5 (G5; n=15): submitted to IFOT with a 5 COCs in 60µL of medium; Group 50 (G50; n=15): submitted to IFOT with a 50 COCs in 60µL of medium. On the day of IFOT (48h after P4 removal), the diameter and vascularization of the DF were evaluated using a B-mode and color-Doppler ultrasonic (MyLab30 Vet Gold, Esaote Healthcare, Italy). Only those presenting a DF≥10mm in diameter were used. The IFOT (at 54h after P4 removal) was performed using a teflon suction line (WTA,Brazil) coupled to a 27G needle on one end and a syringe(1mL) on the other end. The artificial insemination was immediately after IFOT, using a single dose of frozen-thawed semen from a Nellore bull. Subsequently, cows received 10µg of buserelin acetate i.m. (GnRH;Sincroforte®). Slaughter and reproductive system remove, to ipislateral oviduct flushing, were performed 2.5 days after IFOT. Oviduct flushing was performed with 3mL of PBS and the content evaluated for the presence of structures. It was classified as structures: unfertilized oocytes, zona pellucida or embryos/cleaved oocytes. All data were analyzed using Statistical Analysis System software (SAS, 1999). The diameter and vascularization of the DF were evaluate for normality by Shapiro. Subsequentely, ANOVA were used and the means were compared by the Tukey test($P<0.05$). Regarding the total recovery rate, no difference was identified between G5 (24.0%) and G50 (31.6%). The CG had a total recovery rate of 73.3%. The DF diameter (mm) and blood perfusion (%) also did not differ between groups GC (13.5±1.3 and 54.2±0.1), G5 (14.0±2.0 and 48.0±0.2) and G50 (13.7±2.1 and 45.4±0.2), respectively ($p>0.1$). Furthermore, there was no correlation between DF diameter (mm) (R=-0.10) or DF blood perfusion (%) (R=-0.03) with the total structures recovery rate. Thus, we concluded that DF selection based on the DF blood perfusion of the ovulating female does not influence the IFOT success. Acknowledgments: FAPES (Projeto563/2020), Ourofino, Cravinhos-SP.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Human chorionic gonadotropin has long-lasting effects on bovine corpus luteum function.

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Resumo

It was recently proposed that ovulation induction with hCG increases pregnancy rate in dairy cows after embryo transfer (ET) of in vitro-produced embryos, compared to GnRH (55 vs. 26.7% P/ET) (Garcia-Ispierto et al., Reprod Dom Anim, 56: 1145-47). The aim of the present study was to evaluate the effects of hCG on bovine CL function. The procedures were approved by the Ethics Committee for Animal Experimentation from UFPel (CEEA 31587-2020). In experiment 1, to evaluate whether hCG would allow ovulations and P4 synthesis in anestrous cows, Jersey and Holstein cows (n=6) were immunocastrated with two injections of anti-GnRH vaccine (Zoetis, Brazil) thirty days apart. When the cows had only follicles smaller than 4 mm, they received an intravaginal device (IVD) containing 1 g P4 (Agener União, Brazil) on D0 and 830 IU of eCG (Zoetis, Brazil) i.m. on D0 and D2. On D6.5, ovulation was induced by administering 1250 IU of hCG and IVDs were removed. Blood samples were collected on days 6.5, 9, 11 and 14. In experiment 2, to evaluate if hCG treatment would enhance luteal function in cyclic cows, non-pregnant and non-lactating Jersey and Holstein cows were treated with the following protocol: IVD containing 1 g P4 and 2 mg of estradiol benzoate (Agener União, Brazil) i.m on D0. On D8, 482 µg of PGF (Agener União, Brazil) were administered and, on D9, IVDs were removed. After 24 h, 10.5 µg of buserelin acetate (Ourofino, Brazil) (hour 0 = H0) was administered i.m. to cows with follicles ≥ 11 mm. Then, 16 h after GnRH, cows were allocated, according to follicular diameter, to two groups: control (n=5), without any additional treatment; and hCG (n=5), which received 1000 IU of hCG i.m. Ovulation was confirmed and, on days five and seven after GnRH, CL vascularization was assessed using color Doppler and blood was collected for P4 analysis. The data were evaluated using paired Student's T test. In experiment 1, four cows responded to eCG treatment (at least one follicle larger than 10 mm). The four cows responded to hCG treatment and ovulated, which was confirmed by P4 concentrations above 20 ng/ml seven days after hCG administration (D14), indicating functional CL. In experiment 2, hCG treatment did not affect CL vascularization, diameter, and circumference. However, hCG-treated cows presented greater P4 concentration seven days after GnRH treatment, being observed 2.7±0.7 and 4.1±0.9 ng/mL for control; and 3.7±0.8 and 8.5±2.6 ng/mL for hCG, on days 5 and 7, respectively (group: $P<0.05$; day: $P<0.01$; group x day: $P=0.1$). In conclusion, hCG treatment has long-lasting effects on bovine CL function because it induced ovulation and maintained luteal function for seven days in immunocastrated cows and increased P4 synthesis in cyclic cows.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Porcine FSH dose affects the efficiency of in vivo embryo production in Santa Inês sheep

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Resumo

This study compared the effectiveness of two pFSH doses on the in vivo embryo production in Santa Ines ewes subjected to the Day 0 protocol. Estrous cycles of 36 multiparous ewes were synchronized with intravaginal sponges impreganted with medroxiprogesterone acetate (60 mg; Progespon, Syntex, Argentina), and the, superovulatory treatments began 80 h after the intravaginal sponge removal. Ewes received either 133 mg (G133, n=18) or 200 mg (G200, n=18) of pFSH i.m. (Folltropin-V®, Bioniche Animal Health, Canadá), divided into six decreasing doses (25, 25, 15, 15, 10, 10%) every 12 h. Simultaneously with the first dose of pFSH, a intravaginal device of progesterone (P4; 0.36 g; Primer PR, Agener União Saúde Animal, Brazil) was inserted to all ewes, remaining in situ until the fifth dose of pFSH. Simultaneously with the last dose of pFSH, cloprostenol sodium was given (0.24 mg i.m.; Estron, Agener União Saúde Animal, Brasil; and after 24 h the ewes received lecireline (25 µg i.m.; TEC-Relin, Agener União Saúde Animal, Brasil). All animals were checked for estrous behavior and mated naturally every 12 h, from the sixth dose of pFSH until the end of estrus. Ewes previously received a hormonal protocol for cervical dilatation (Leite et al., Arq Bras Med Vet Zootec, 70:1671-1679, 2018), the corpora lutea (CL) were counted by B-mode ultrasonography., and the non-surgical embryo recovery (NSER) was performed on Day 10. The recovered structures were checked regarding their development stage and quality. Data were tested for normality by Lilliefors test. The variables in percentage were evaluated by the Fisher exact probability test, and the other variables were compared with the Mann-Whitney or the student tests; data are presented as mean ± SD. A total of 97.2% (35/36) of the ewes showed estrus and it was possible to transpose cérvix and perform NSER in 80.6% (29/36) of the ewes, with no differences between groups. There were no effects of the treatments on the number of CLs/ewes (G133: 8.5±1.1 vs G200: 10.2±1.2), recovery rate (G133: 47.7% vs G200: 64.2%), embryo viability rate (G133: 50.8% vs G200: 69.1%), and the number of recovered structures/ewes (G133: 4.8±1.1 vs. G200: 7.5±1.7). However, the number of viable embryos per donor was greater in the G200 than in the G133 ewes (G133: 2.29±0.67 vs. G200: 6.47±1.60; P=0.04). In conclusion, the use of 200 mg of pFSH resulted in a greater number of viable embryos, suggesting that this dose should be preferred for ewe superovulation using Day 0 protocol.

Keywords: embryo collection, embryonic quality, follicle stimulating hormone, sheep, superovulation

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Transcriptional profile of lipid metabolism genes in OPU-derived oocytes from Gir (Bos indicus), Holstein (Bos taurus) and 1/2 Holstein × 1/2 Gir (Bos taurus × indicus) cows

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Resumo

Bos taurus taurus (Bos taurus) and Bos taurus indicus (Bos indicus) cattle are subspecies with remarkable differences related to production and reproduction. Moreover, is known that differences in number and development potential of oocytes between breeds affect efficiency and economic viability of in vitro embryo production. Once lipids have a crucial role in oocyte development, we aimed to evaluate the mRNA abundance of genes involved to lipid metabolism in oocytes recovered from dairy breeds: Gir (Bos indicus), Holstein (Bos taurus), and their crossbreed (1/2 Holstein × 1/2 Gir). Cumulus oocyte complexes (COCs) were obtained by ovum pick-up procedure. The oocyte donor cows were from farms in Alfenas in the southern region of Minas Gerais state, Brazil. Part of COCs were submitted to in vitro maturation during 24h. Immature and in vitro matured COCs were processed to remove the cumulus, and denuded oocytes were stored for transcriptional analysis. Total RNA was extracted from pools of 20 immature oocytes (n = 4 pools per breed) and 20 in vitro matured oocytes (n = 4 pools per breed) using the RNeasy® Micro kit (Qiagen). The mRNA abundance of acetyl coenzyme A carboxylase (ACACA), carnitine palmitoyltransferase 1A (CPT1A), fatty acid binding protein 5 (FABP5), fatty acid translocase (CD36) and perilipin 2 (PLIN2), were assessed by real time RT-PCR using Power SYBR® green master mix (Applied Biosystems) and normalized by peptidylprolyl isomerase A (PPIA). Relative mRNA abundance was determined using the ΔΔCt method. The effects of breeds on expression of target genes in oocytes were tested by ANOVA, and means were compared using Tukey-Kramer HSD test. Differences were considered significant when $P < 0.05$. In immature oocytes, CD36 mRNA abundance was higher ($P = 0.022$) in Gir compared to Holstein. Regarding in vitro matured oocytes, CD36 mRNA abundance was higher ($P = 0.001$) in Gir and crossbreed cows compared to Holstein donors. Moreover, CPT1A mRNA abundance was higher ($P = 0.03$) in in vitro matured oocytes from Gir compared to Holstein. In opposite, PLIN2 mRNA abundance was lower ($P = 0.034$) in in vitro matured oocytes from Gir than in Holstein and 1/2 Holstein × 1/2 Gir. In conclusion, we reinforce that the different genetic groups impact expression of genes involved in lipid metabolism. Also, we figure out that differences could be more clearly discriminated between pure breeds: Gir vs Holstein. Furthermore, differential expression of CD36 in immature and in vitro matured oocytes, as wells, CPT1A and PLIN2 in matured oocytes could suggest specifics molecular mechanisms involved with β-oxidation of fatty acids and lipids accumulation during in vitro maturation.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Morphological classification of cumulus-oocyte complexes and in vitro embryo production outcomes

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Resumo

The morphological classification of embryos usually uses as reference the International Embryo Technology Association (IETS) standards. Conversely, there is no consensus on how to evaluate cumulus-oocyte complexes (COC) aspirated from ovarian follicles aiming at in vitro embryo production (IVEP). Different classification scores have been proposed, most of them based on the numbers of layers of cumulus cells and on the aspect of cytoplasm. However, many commercial laboratories prefer to use a less detailed classification system, such as viable or non-viable. Moreover, the use of transvaginal ultrasound-guided follicle aspiration (OPU) subject COC to higher shear stress, compared with aspiration of follicles from slaughterhouse ovaries, and thus result in COC with less average cumulus cells. Therefore, the aim of this study was to evaluate whether the morphological classification routinely adopted really had a predictive value to estimate blastocyst rates. The work was carried out at the in vitro embryo production laboratory - Norte Embryo, located in the Alta Floresta city, Mato Grosso State. COC recovered at slaughterhouse from Nelore cow ovaries were morphologically evaluated and those classified as grades I (GI, n=458), II (GII, n=344) or III (GIII, n=213) were allocated in separated groups and used for IVEP. Data from COC recovered by OPU from Nelore donors during the same period and classified simply as viable were also used for comparisons. IVM and IVF were performed under the same culture conditions (5.5% CO2, 38.5 C). The semen used was from an Angus bull of known fertility at IVF. Presumptive zygotes were cultured under low oxygen tension (5.5% CO2, 5.5% O2, at 38.5 C). The cleavage rate was determined at day 3, and blastocysts rates at days 6 and 7. Data were evaluated by the Proc Glimmix method using the SAS software (SAS Institute). For the COC recovered at slaughterhouse, quality grade affected neither cleavage (72.1%, 65.4% and 65.7% for GI, GII and GIII, respectively; P=0.0838), nor blastocysts rates at days 6 (24.7%, 25.9%, and 26.3%; P=0.5220) or 7 (32.1%, 33.4% and 35.2%; P=0.7231). The average cleavage and blastocyst rates from slaughterhouse COC (pooled grades I, II and III) were not different from those obtained with COC recovered by OPU (68.5% vs 62.8% and 33.2% vs 30.1%, respectively, P>0.05). In summary, the selection of COC by grade, according to the classification currently adopted, does not improve IVEP outcomes.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Effect of the synchronization protocol on embryo recovery after IFOT

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Resumo

The aim of the present study was to compare the total structures recovery rates and embryo recovery rates after IFOT on animals submitted to different synchronization protocols. Multiparous non-lactating Nelore (Bos indicus) cows (n=39) were randomly assigned to one of the following protocols: animals in the Control Group (CG, n=20) received on D-10 a P4 intravaginal device (ReproNeo®, Global Gen) together with an injection of 2 mg of estradiol benzoate (EB; Sincrogen®, GlobalGen); at D-8 the device was removed simultaneously to administration of 0.5 mg of prostaglandin (PG, i.m.; Indocio®, Global Gen) and at D-1, 1 mg of EB (i.m.) on D0 cows received injection of 25 µg of GnRH (TecRelin®, Tecnopec). In the second group cows were submitted to an adapted Ovsynch protocol, (OvS, n=19), on D-8 received a 50 µg of GnRH (i.m) and a P4 device that was maintained untilD-3, together with administration of 0.5 mg of PG, that was repeated on D-2. After 10 hours from the second PG cows received and an injection of 25 µg of GnRH. The heat was recorded based on the Heat Watch® mark, inserted at the time of P4 removal. The IFOT was performed on D0, 52h (CG) or 72h (OvS) after P4 removal. The criteria used to choose a cow to IFOT was the heat detection (CG=95.0%; OvS=73.7%) and the presence of only one dominant follicle larger than 10mm. In total, 10 IFOTs were performed in each group and each injection was performed with 35 COC's (Grade I, II and III) on average, which were obtained by OPU from Nelore donors. The final number of injected COC's were 356 for CG and 314 for OvS. A single dose of semen was used for AI, immediately after IFOT. All animals had their ovulation evaluated 7 days later, and animals that presented a CL were submitted to uterine flushing. For comparison between treatments, continuous variables were analyzed by ANOVA and Tukey's, the percentage of recovered structures, recovered embryos and freezable embryos were analyzed by Chi-square test. Statistical significance was set at 5%. On average, the time from heat to IFOT was shorter (P=0.04) on CG (10.4h) than OvS (18.8h). The dominant follicle diameter (CG=13.6±2.1 vs OvS=11.7±1.2 mm) was similar on D0 (P>0.05). However, the CL diameter was larger (P=0.04) on GC (20.0±1.6mm) compared to OvS (17.5±1.2 mm). The recovery rate of total structures was greater (P>0.01) on CG (11.2%) than OvS (6.0%). However, the recovery of viable and freezable embryos did not differ (P>0.05) between CG (3.9% and 3.4%) and OvS (3.8% and 3.5%). In conclusion, both protocols are able to synchronize the estrous producing a dominant follicle at the time of IFOT. However, despite the very low embryo recovery on both treatments, the EB based protocol still is the best option to IFOT based on the percentage of recovered structures.

Keywords: TIFOI, hormone, oocyte, blastocyst.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

FACTORS AFFECTING INTRAFOLICULAR TRANSFER OF IMMATURE OOCYTES (IFIOT) RESULTS: DOES THE INJECTION MATTERS?

Otávio Augusto Costa de Faria [1,4], Nayara Ribeiro Kussano [1,4], Lucas Costa de Faria [2,4], Danielle Bárbara Pereira de Castro [2,4], Letícia Prates Martins [1,4], José Felipe Warmling Spricigo [3], Margot Alves Nunes Dode [4]

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Resumo

Even though intra-follicular oocyte transfer (IFIOT) represents an interesting alternative for in vivo embryos production, its efficiency is still very low. This study aimed to evaluate whether the injection, the number of oocytes injected and the injection quality affects follicle size and oocyte recovery post injection. Thirty Nellore heifers were synchronized as described by Faria et al., 2021 (Reprod Fertil Dev. 2021 Mar; 33(5):372-380) and 30 h after progesterone device removal (D91/2) the dominant follicle of all animals was measured by ultrasonography. The animals were then distributed into 4 group: 1. control (no injection- NI; n= 6); 2. IFIOT-0 (n= 9): injection of 60µl of PBS; 3. IFIOT 25 (n= 10): injection of 60µl of PBS +25 COCs and 4. IFIOT 50 (n= 11): injection of 60µl of PBS +50 COCs. The quality of injection was classified as grade 1: injection into the center of the follicle and visualization of the entrance of all structures; grade 2: injection in the follicle periphery and the visualization of structures entrance was too fast (causing a vortex effect) or too slow (almost imperceptible); grade 3: more than one perforation on the follicle and no visualization of the structures entrance. After 22 h of IFIOT, all follicles were measured and COCs were recovered by OPU. The results were analyzed by Chi-square and Proc Glimmix (SAS Institute). The injection caused a reduction (P<0.05) on the diameter and volume of follicle regardless the number of COCs injected, being both similar among the injected groups (NI= +1.37%a and +4.16%a; IFIOT-0= - 20.29%b and -49.36%c; IFIOT 25= -11.73%b and -31.23%b; IFIOT 50= -15.34%b and -39.32%b,c. No difference (P>0.05) was observed between IFIOT 25 and IFIOT 50 on the % of recovered COCs (46,53% and 37,50%, respectively). However when the quality of the injection was considered a difference (P<0.05) in recovery rate was observed among grade 1 (53.75%a), 2 (25.75%b) and 3 (6.67%c) injection irrespective of the number of COCs used. The results suggest that the injection itself affects the follicle, being probable involved in the low efficiency of the technique. In addition, more important than the number of oocytes used is the quality of the injection, which and can be considered as one of the factors responsible by the great variability on the results.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Treatment with bovine somatrotopin (bST) improve pregnancy rate in F1 crossbred recipients submitted to FTET

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Resumo

The present study evaluated the administration of bovine somatrotopin (bST) at different moments (at P4 device removal or at time of embryo transfer) on pregnancy to transfer (P/ET) and body weight (BW) in F1 crossbreed recipientes. A total of 751 crossbred (Angus x Nelore) heifers with 14 months of age from farms located in MS and BA (Agropecuária Jacarezinho, Brazil) were used for the study. At the beginning of the ET protocol (D0), heifers received a reused intravaginal P4 device (3th use; CIDR®, Zoetis, Guarulhos, SP-Brazil) and 2 mg estradiol benzoate (EB; Gonadiol®, Zoetis). After 9 days (D9), P4 device was removed and heifers received 2.5 mg of dinoprost and 0.3 mg of estradiol cypionate (EC; ECP®, Zoetis). At the same time, heifers were randomized according to BW (307.9 ± 1.33) and BCS (2.98 ± 0.01) and allocated into four treatments using 2x2 factorial design: 1) Control (n=183): no treatment; 2) bSTD9 (n=187): treatment of 325 mg of bST (Posilac®, Agener União, São Paulo, SP-Brazil) on D9; 3) bSTD18 (n=190): treatment of 325 mg of bST (Posilac®, Agener União) on D18; and 4) bSTD9-D18 (n=191): treatment of 325 mg of bST (Posilac®, Agener União) on D9 and D18. On D18, embryo was transferred only in heifers that had presence of CL (n=628). At the pregnancy diagnosis (20 days after ET), BW was recorded and the size of the embryo (n= 65) was measured using the distance from the crown-rump. Statistical analyses were performed using GLIMMIX of SAS 9.4. No interaction treatment*moment was observed for any variable and data was presented by main effects [bST at P4 device removal (D9) or bST at embryo transfer (D18)]. The proportion of recipients transferred was similar between treatments on D9 [Control=85.0% (317/373) vs. bST=82.3% (311/378); P=0.32]. However, heifers treated on D9 had greater P/ET [Control=27.4% (87/317) vs. bST=38.3% (119/311); P=0.0038]. No effect of treatment on D18 for P/ET was found [Control=30.4% (93/306) vs. bST=35.1% (113/332); P= 0.22]. Furthermore, treatment on D9 did not affect BW at pregnancy diagnosis (Control=381.0±4.66kg vs. bST=382.6±4.48 kg; P=0.78). Nevertheless, treatment with bST on D18 improved the BW at pregnancy diagnosis when compared to control (Control=374.7±4.50kg vs. bST=388.8±4.54kg; P=0.03). The size of the embryo was similar between treatments on D9 (Control=7.7 ± 0.17mm vs. bST= 7.53 ± 0.13mm; P=0.32) and on D18 (Control=7.6 ± 0.16mm vs. bST=7.7 ± 0.15mm; P=0.56). The P/TE at 60 days was greater when bST was administrated on D9 [Control=18.8% (59/314) vs. bST=26.2% (81/309); P=0.02], but no difference was observed for bST on D18 (P=0.52). Also, the pregnancy loss was similar between treatments on D9 (P= 0.80) and D18 (P= 0.66). In conclusion, the administration of bST on D9 (P4 device removal) improved the P/ET in crossbred recipients. Also, the administration of bST on D18 increased BW recorded on pregnancy diagnosis.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Environmental thermal variation on the embryo production of Nellore cows

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Resumo

This study verified the effect of environmental variation on embryo viability and the quality of Nelore cow embryo donors. An embryo production database was got data from 10 years of cows (n= 564) subjected to a traditional superovulation (SOV) FSH-based protocol. The SOV was performed with 4 consecutive days of decreasing doses of FSH. Uterus were flushed 7 days after insemination (n= 1856 embryo recoveries). The quantity and quality of the embryos per flush were registered and the viability rate (VR) calculated. The air humidity and temperature month (tm) were used to calculate the temperature and humidity index (THI): 4 days of SOV(THIsov); 2 days of artificial insemination (THIai); 2 days post AI referring to early embryo development (THIemb); 12 days of the entire embryo transfer protocol (THIet); and THI for each month (THIm). The multiple linear regressions considered VR, VV, total embryos, and the climatic variables for the rainy and dry period. THIsov, THIai, THIemb and THIet did not influence the embryo quantity and quality (P>0.05). The total number of embryos and VR fluctuated based on the tm (TTemb=-5.6087+2.1864xtm-0.0522xtm²; VR=-92.372+12.341xtm–0.2554xtm²; respectively). TTemb and VR were positively impacted by tm when it ranged from 17 to 27 °C (TTemb=16.65±0.50; VR=53.13±2.75%), than under 17 oC (13.41±1.32; 26.34±10.39%; respectively) or above 27 oC presented (11.19±2.20; 33.24±5.52%, respectively). Also, these characteristics were affected by THIm (TTemb= -145.9+4.8137xTHIm-0.0354xTHIm²; VR=-683.2+20.682xTHIm-0.1444xTHIm², respectively; p<0.05). THI values from 64 to 74 resulted in higher embryo viability (TTemb =17.29±0.35; VR= 54.27±2.53%) than under 64 (13.93±1.75; 29.44±9.67%; respectively) or above 74 (11.34±2.76; 34.05±8.82%, respectively. The THI values decreased VR and viable embryos become even worst. These results were very interesting for Nelore cows: The Low temperature activates the endocrine mechanisms for induced thermogenesis, via the hyothalamic-pituitary-thyroid axis. There is also release of adrenaline from the adrenal glands. This process increases overall cellular oxidation and carbohydrate utilization by increasing blood glucose concentration (Silva and Campos Maia, Principles of animal biometeorology, 3:75-106, 2000). 2000). Oocytes from Nellore females suffer from heat and cold stress, impairing embryonic development (Melo-Sterza and Poehland, Int. J. Molec. Sci.,22:3421,2021). Oocytes from donors of the Pantaneira breed, it´s a breed adapted to the tropics, show signs of cold stress as HSP70 were recruited when environment temperatures were below 23 oC (Sousa-Cáceres et al., Theriogenology,130:103-10,2019). Therefore, Nelore embryo donors demonstrate better response to an embryo transfer program (more embryos produced and VR) when the air temperature ranges from 17 to 27 °C and the THI ranges from 64 to 74.

Keywords: beef cattle, environment, thermic stress, embryo transfer, fertility

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Inflammatory cell count in oviducy epithelium in response to injection of different numbers of CCOs by Intrafollicular Transfer of Immature Oocytes – IFIOT

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Resumo

The aim was to evaluate the number of inflammatory cells in different segments of the oviduct epithelium, exposed to different numbers of structures present in the oviduct after IFIOT. Nelore cows (n=45) were submitted to a standard protocol of estrus synchronization (D0: P4 + 2mg EB; D8: 0,5 mg PGF2α and removal P4 device; D9: 1 mg EB). At 54.5±2.75h after removal of the P4 device, IFIOT was performed, according to the groups: Control Group (CG; n=15): females submitted to IFIOT with injection of 60µL of TCM199 medium, without the presence of CCOs; Group 5 (G5; n=15): females submitted to IFIOT with injection of 5 CCOs in 60µL of medium; Group 50 (G50; n=15): females submitted to IFIOT with injection of 50 CCOs in 60µL of medium. After IFIOT, all females were inseminated with semen from the same bull. At 64±2h after IFIOT, slaughter was performed to collect the reproductive tract, and dissection of the oviduct ipsilateral to the ovary that presented ovulation. The oviduct had their interior flushed, and the content evaluated for the presence of structures (non-fertilized oocytes, zona pellucida or embryos-cleaved oocytes). The total number of structures recovered ranged from 0 to 40 per cow. Therefore, regardless of which group the animals belonged to, they were relocated in groups according to the number of structures present in the oviduct: 0 (G0), 1 (G1), 2-9 (G2-9) and 11-40 (G1-40) structures. After flushing, the oviduct was segmented into: proximal isthmus (IP), distal isthmus (ID), proximal ampulla (AP) and distal ampulla (AD), in relation to the uterus-tubal junction. A segment per portion was collected and fixed in 10% formaldehyde to histological processing. For each segment, 5-10 photographs were taken, and inflammatory cells were counted. Data were analyzed using the GraphPad Prism 6.0 program (GraphPad Software, San Diego, CA) using the Kruskal-Wallis test. The number of inflammatory cells from the same oviduct segment exposed to the different numbers of recovered structures, had no difference. However, there was variation in the number of inflammatory cells between segment exposed to the different number of structures. In oviduct with 0 and 11-40 structures, the AP portion (13.6±7.2 and 10.4±6.3) had a higher inflammatory cell compared to the ID segment (4.9±5.5 and 2.9±1.7), with the IP (6.2±6.1 and 5.2±5.4) and AD (11.10±5.9 and 7.3±5.2) segments being similar to the others, in the respective groups (G0 and G11-40). As for the oviduct containing 01 structure, the AP (11.9±5.4) and AD (14.5±8.6) segments had higher cell number than IP (6.2±4.2) and ID (3.4±3.1). The G2-9 group showed no difference between segments. Based on the amount of inflammatory cells, the number of CCO in the oviduct does not generate an increase in the inflammatory response. However, the ampulla was the segment with the highest number of inflammatory cells, especially the proximal portion.

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Animal Reproduction

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OPU-IVF and ET

The first lactation milk performance is higher in offspring delivered from beef recipients than in offspring delivered from lactating Holstein recipients

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Resumo

The objective was to evaluate the effect of lactating Holstein recipient (milk; Bos taurus; 33.0 kg/cow/day) or non-lactating crossbred beef recipient (beef; with a predominance of Zebu breeds; Bos indicus) on first lactation milk production of Holstein offspring generated after the transfer of an in vitro embryo. The study was carried out at Fazenda Santa Rita (Agrindus) in Descalvado, São Paulo, Brazil. The lactating Holstein recipients were kept in sheds with sand-bedded freestall, with adequate ventilation and sprinklers in the trough line during pregnancy. Crossbreed beef recipients were kept on pasture with protein supplementation and water ad libitum during pregnancy. In the statistical model the fixed factors were the breed of the recipient (milk = 483; beef = 472) and the year of birth of the calves (2013 to 2019), as well as the interactions breed recipient*year. The random factors were adjusted milk production for 305 days (MP305) and peak milk production (PMP) in the first lactation of offspring. The Holstein offspring (according to the recipient group) was used as experimental unit and the donor (dam) and bull (father) was used as co-variable. At the moment of the calving, all progenies have the same breeding system until the first lactation. All analyzes were performed using the Statistical Analysis System software (SAS, Version 9.4 for Windows; SAS Inst., Cary, NC) and examined for outliers and missing values using statistics descriptive and box-plot graphics. There was no interaction (P = 0.22) recipient*year for MP305, however there was an increase (P = 0.04) in MP305 for offspring delivered from beef recipients [milk: 10,014.46±168.89kg (199/483); beef: 10,617.40±96.51kg (322/472). Furthermore, there was interaction recipient*year for PMP (P=0.01). Observed that in most years the offspring delivered from beef recipients has higher PMP, between the years 2014 to 2019. Thus, just 2013 offspring delivered from milk recipients has higher PMP. Recent studies report that the epigenetic effect during pregnancy affects the performance of milk production in dairy cattle. The data from the present study support the increase in adjusted milk production for 305 days in the first lactation for Holsteins offspring delivered from beef recipients than Holstein offspring delivered from milk recipients. However, further studies are needed to clarify the effects of breed and management of embryo recipients on potential long-term consequences on offspring performance.

Animal Reproduction

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Neuregulin 1 modulates nuclear maturation during amphiregulin-induced IVM of bovine cumulus-oocyte complexes and improves post-IVF embryo production

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Resumo

Gradual activation of the ovulatory cascade during in vitro oocyte maturation (IVM) has been proposed to enhance nuclear-cytoplasmic synchrony and cumulus-oocyte communication, thus enhancing oocyte developmental competence and post-IVF in vitro embryo production (IVP). In the present study, we assessed the effects of neuregulin 1 (NRG1), an EGF-like factor that modulates EGFR signaling and thus the activation of the ovulatory cascade, on oocyte nuclear maturation dynamics, cumulus expansion, expression of mRNAs regulating these processes and post-IVF embryo development. Bovine cumulus-oocyte complexes (COCs) were aspirated from 2-8 mm follicles of slaughterhouse ovaries, pooled in groups of 20-25, and subjected to IVM in serum-free TCM containing physiological concentrations of FSH, IGF1, steroids, and 100 ng/mL AREG ("IVM Follicular System"); supplemented with 1 ng/mL NRG1 (NRG1 group) or not (Control group) for 6, 9, 12, 20, and 24 h. Oocyte chromatin/meiotic status was assessed by fluorescence microscopy following Hoechst staining at each time-point. Cumulus expansion degree (1 to 3) was assessed after 24 h of IVM and cumulus mRNA expression (real-time RT-PCR) after 9 and 20 h of IVM. Embryo production rates and embryo cell number (fluorescence microscopy/Hoechst) were assessed after standard IVF using frozen semen of a single bull/batch and standard embryo culture for 7 days. All experiments were performed with 5 replicates. Data in percentages were arcsine transformed and all the data were first tested for normality (Shapiro-Wilk test) before assessing treatment effect with the Student's t-test. Data are presented by mean ± SEM and differences were considered significant when $P < 0.05$. NRG1 decreased the percentage of oocytes undergoing germinal vesicle breakdown at 6h of IVM (GVBD; 52.24 ± 4.70 vs. 70.37 ± 5.10; $P = 0.02$), without altering later meiotic dynamics, nor the percentage of oocytes achieving meiosis II at the end of culture. NRG1 did not affect cumulus expansion, but increased the percentage of expanded and hatched blastocysts (39.31 ± 2.56 vs. 32.60 ± 1.03; $P = 0.03$), as well as blastocyst total cell number (202.30 ± 10.52 vs. 169.18 ± 10.55; $P = 0.03$). NRG1 decreased EGFR mRNA abundance (0.82 ± 0.05 vs. 1.00 ± 0.03; $P = 0.02$), while increasing mRNA levels of NPR2 (1.64 ± 0.22 vs. 1.03 ± 0.13; $P = 0.04$) and PTX3 (5.73 ± 2.74 vs. 1.09 ± 0.24; $P = 0.04$) at 9h, as well as those of TNFAIP6 (1.77 ± 0.23 vs. 0.82 ± 0.04; $P = 0.02$) at 20h of IVM. This is the first study to report the regulatory role of NGR1 during oocyte maturation in a mono-ovulatory species, and to demonstrate that this action may be applied during IVM to improve post-IVF embryo development.

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