Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Endometrial and luteal ultrasonographic characteristics during estrous cycle in Brown Swiss cows – preliminary results

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Abstract

Ultrasonographic examination (US) contributes to understanding physiologic characteristics of cow's reproductive tract. The present study aimed to assess endometrial (EN) and corpus luteum (CL) US characteristics during the estrous cycle in Brown Swiss cows. Post-partum estrous of seven healthy cows were monitored (Herd Navigator™, DeLaval International, Sweden). After heat alarm (second estrous cycle), US was performed daily until ovulation (Day (D) 0), followed by every two days until the next ovulation. Ovaries and uterine horns were scanned using the same image settings with an ultrasound device (MyLabOne, Esaote, Italy) equipped with a linear transducer (6-10 MHz). Selected images from videos saved in the ultrasound device memory were analyzed using ImageJ (Version 1.52a, NIH, USA). EN thickness was measured about 1 cm beyond the bifurcation. CL size was the average diameter of the two largest perpendicular CL cross-sections. EN (four square-shaped spots, 0.25 cm^2) and CL (disregarding cavity area) echogenicity and echotexture were determined using mean pixel intensity and pixel heterogeneity, respectively. CL blood flow (CLBF) was evaluated both subjectively (0-3) and objectively (ratio of CL blood flow area to CL area). Spearman's correlation coefficients were calculated, and mean differences were assessed using Friedman test followed by Wilcoxon test. CL size enlarged from D 0-8, steadied till D14, and reduced from D16 on; besides it was positively correlated with both subjective and objective CLBF ($r=0.7$, $P<0.001$). Subjective and objective CLBF were correlated ($r=0.84$, $P<0.001$), increased until D4 ($P<0.05$), steadied during D6-16, and decreased at D18 and 20 ($P<0.05$), compared with anterior measure. EN thickness had a numerical U-shape pattern, was similar between D0-16, but at D20 was higher ($P<0.05$) than D0, being correlated with CLBF ($r =-0.4$, $P<0.001$) and EN echogenicity ($r=-0.31$, $P<0.05$). No differences in CL or EN echogenicity and echotexture during the estrous cycle were detected ($P>0.05$). In conclusion, CL diameter and CLBF have a concurrent development during the estrous cycle; subjective CLBF is simpler and as effective as objective CLBF. Furthermore, EN thickness apparently reflects hormonal changes during estrous cycle.

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Reproductive losses in beef cattle rearing on extensive system production: Effects of genotype, and parity

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Abstract

Reproductive losses are a major cause of reproductive failure in cattle, resulting in delayed pregnancy and fewer calves born, with a concomitant financial loss to the beef industry [1]. Crossbreeding has been used to increase the weaning weight of calves per cow [2]; however, little information is available on the effect of heterosis on reproductive losses. It was hypothesized that in extensive beef cows Campos grassland production, crossbreeds' cows had lower reproductive losses than purebreds' cows independently of parity. A second hypothesis was: the primiparous cows had the highest reproductive losses independently of the genotype. The objective of this work was to compare the reproductive losses of purebred [Hereford (HH) and Angus (AA)] and crossbred (HA and AH) cows and the effect of the parity (Nulliparous, Primiparous and Multiparous). This work is a retrospective studied of the reproductive losses of breeding cows of two different purebreds (HH and AA) and their F1 crossbred (AH and HA) used in a diallelic design. The dataset contained 2959 records of pregnancy diagnosis, calving and weaning of multiparous (cows with more than two calving), primiparous (first calving cows) and nulliparous (heifers without calving), registered over a 12 years (1994 – 2006). Multiparous and primiparous cows were mated with andrologically evaluated bulls during 80 days beginning on December 1st (summer, SH) following the most frequent mating period in the region. Each bull was placed with different genotypes of cows assigned to be mated for it (paternity identification). Three bulls of each breed were used per year, repeating one of them the following year, in order to connect the information between years. The nulliparous cows were bred at 24 months of age, and at least 280 kg of body weight. The mating period lasted 45 days (late November to mid-January, end of spring – summer, SH). The diagnosis of gestation was conducted 45 days after the mated period ended. The total reproductive losses (number of weaned calves/total exposed cows * 100) were studied in three Periods: I) from the mating period to pregnancy diagnoses (PD, number of cows diagnosed as non-pregnant/ total exposed cows * 100); II) from PD to calving (number of calving cows/ number of cows diagnosed as pregnant * 100); III) from calving to weaning (number of weaned calves/numbers of calving cows * 100). Data were analysed using SAS Academic Edition (SAS OnDemand for Academics, SAS Institute Inc.), including in the model the effect of the genotype and parity of the cows and their interaction as fixed effect, and the year and the bull as random effect. The means were compared with Tukey test when the main effect was significative. Data were expressed as least square means ± sem. Statistical differences were considered significant when P ≤0.05, and as tendencies when 0.05 < P ≤0.10. The total reproductive losses were affected by the genotype of the cows ($P < 0.01$). In average purebreds presented greater losses than their crosses (0.45 ± 0.03 vs. 0.28 ± 0.02; $P < 0.01$) without differences between HH and AA, or HA and AH. Parity also affected total reproductive losses ($P < 0.01$). Primiparous cows presented greater losses ($P \leq 0.02$) than nulliparous and multiparous cows without difference between the last two parity (Primiparous: 0.46 ± 0.03 vs Nulliparous: 0.30 ± 0.02 vs. Multiparous: 0.33 ± 0.02). No interaction was detected. The only period affected genotype and parity was Period I ($P < 0.01$). The purebred cows had greater losses ($P < 0.01$) from mating to pregnancy diagnoses than the crossbred, without differences between the purebred (HH and AA) or between the crossbred (AH and HA). Primiparous cows showed the highest losses ($P \leq 0.01$) during Period I without difference between nulliparous and multiparous. No losses were detected from pregnancy diagnoses to calving. The losses during Period III were not affected by any of the effect studied; the total mean was less than 4%. It was concluded that the animal crossing is a tool to decrease the reproductive losses in beef meat breeding herds.

References

[1] Shorten, P.R., Morris, C.A., Cullen, N.G., 2015. The effects of age, weight, and sire on pregnancy rate in cattle1. J. Anim. Sci. 93, 1535–1545. https://doi.org/10.2527/jas.2014-8490

[2] Gregory, K.E., Cundiff, L.V., 1980. Crossbreeding in Beef Cattle: Evaluation of Systems1. J. Anim. Sci. 51, 1224–1242. https://doi.org/10.2527/jas1980.5151224x

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Effect of epidermal growth factor concentrations on *in vitro* maturation of red-rumped agouti's (*Dasyprocta leporina* Linnaeus, 1758) oocytes

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Financial support: CNPq and CAPES.

Abstract

One of the limitations of *in vitro* embryo production in red-rumped agoutis is the low rates of *in vitro* maturation (IVM) oocytes. In different mammals, epidermal growth factor (EGF) has been proposed as an important supplement for oocyte development. Nevertheless, results studying the EGF on IVM and oocyte competence are variable. Moreover, this response of EGF can occur in a dose-dependent manner. Therefore, the aim was to evaluate the EGF concentrations (10 and 100 ng/mL) on the IVM rates of red-rumped agouti's oocytes. These wild rodents have often been studied for their ecological, economic, and scientific role in the ecosystems they inhabit. Then, twelve red-rumped agoutis were used for ovarian recovery. The *cumulus*-oocyte complexes (COCs) were recovered by slicing, classified under stereomicroscope and only oocytes with more than one layer of *cumulus* cells and homogeneous cytoplasm were used for IVM. These COCs were transferred to the IVM medium composed by TCM-199 with 0.23 mM sodium pyruvate, 10% FBS, 100 µM cysteamine, 10 mIU/mL FSH, and 10 ng/mL (EGF10 group) or 100 ng/mL (EGF100 group). Oocytes were divided randomly in both groups and matured *in vitro* for 24 h at 38.5°C in a humidified atmosphere with 5% CO_2. Immediately after the IVM, oocytes were evaluated for expansion of *cumulus* cells using a stereomicroscope, and the *cumulus* expansion index (CEI) was quantified, considering CEI ~ 0 as reduced expansion and CEI ~ 5 as full expansion. After, oocytes were denuded and assessed for the presence of first polar body (1PB) using a stereomicroscope. Additionally, viability of *cumulus* cells was evaluated using trypan blue. Finally, data were expressed as mean ± standard error and means were compared using Fisher exact test with $P < 0.05$. Thus, after three repetitions (eight ovaries per repetition), 208 viable immature oocytes were recovered, and 96 used in this experiment. No difference ($P > 0.05$) was observed between EGF10 and EGF100 in terms of qualitative parameters for 1PB presence [52.1 ± 13.6% (25/48) *versus* 37.5 ± 8.7% (18/48)]. Nevertheless, 100 ng/mL EGF during IVM resulted in a low viability of *cumulus* cells [88.2 ± 6.5% (255/289)] when compared to 10 ng/mL EGF [94.0 ± 3.4% (156/166)]. Regarding the expansion of *cumulus* cells, oocytes matured with 100 ng/mL EGF had a lower CEI when compared to those matured in the presence of 10 ng/mL EGF. Probably, high concentrations of EGF alter the viability of *cumulus* cells without affecting oocyte quality during IVM. In summary, in terms of qualitative parameters observed in the red-rumped agouti's COCs, 10 ng/mL EGF ensures better quality of *cumulus* cells of oocytes matured *in vitro* when compared to 100 ng/mL EGF.

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EFFECT OF THE ASSOCIATION OF ESTRADIOL BENZOATE AND INJECTABLE PROGESTERONE ON THE OVSYNCH PROTOCOL

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Abstract

The aim of this study was to improve the Ovsynch protocol by inducing follicular recruitment with injectable association of progesterone and estrogens. The research was performed in the west of Santa Catarina, south of Brazil. Twenty-two Holstein-Friesian cows at 30 to 45 days postpartum, with BCS between 2.5 and 3.5 and a healthy uterus were selected. The Control group (n=11) was synchronized with the Ovsynch protocol, where was applied 10µg of buserelin acetate (GnRH) on day 0 (D0), 500µg of sodium D-cloprostenol on D7 and 10µg of GnRH on D9. The FTAI occurred 20 hours after the last dose of GnRH administered. The cows in the Treatment group (n=11) started synchronization (D0) with the application of 2mg of estradiol benzoate and 300mg of long-acting injectable progesterone, and the continuation of the protocol was the same as the control group. On day D9 markers were placed at the sacrocaudal region for estrus identification in both groups. On D0 and D4 of the hormonal protocol, the presence and diameter of follicles and their respective locations in the ovary were noted. On D9, the measurement of the largest follicle was carried out and blood was collected to measure progesterone and estradiol levels. Pregnancy diagnosis was performed 30 days after insemination. For variables analysis: diameter of the largest follicle in the first evaluation and diameter of the ovulatory follicle, the Kruskal-Wallis test was used followed by one-way ANOVA. Pearson's Correlation test was used to assess serum levels of progesterone and estradiol and also pre-ovulatory follicle diameter; for all, differences with $p<0.05$ were considered statistically significant. On D4, 80% (n:n) of the cows in the control group and 20% (n:n) of the treatment group started to recruit a new follicular wave. On D9, only 20% (n:n) of cows in the treatment group had a pre-ovulatory follicle greater than 16mm, indicating that this treatment was not efficient to induce follicular recruitment and atresia during the hormonal protocol period. The pregnancy rates were 55% (6/11) for the Control group and 9% (1/11) for the Treatment group, a highly significant difference ($p=0.001$). The diameter of the pre-ovulatory follicle in the control group was larger ($p=0.045$) than the treatment group. However, there was no difference in the circulating serum levels of progesterone ($p=0.86$) and estradiol ($p=0.95$) between the groups. The preliminary results of the present study suggest that the use of injectable progesterone at a dosage of 300mg in dairy cattle for estrus synchronization is not indicated and, based on data analysis, there was a delay on growth and development of follicles throughout the treatment.

Acknowledgements

Federal Institute of Santa Catarina – Campus Concórdia, FAPESC 20/2020 and Resmini Farm.

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Structural organization of collagen and elastic system fibers in the cervix of bitches with closed and open pyometra

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Abstract

Pyometra has been extensively studied over the years. However the cervical opening phenomenon plays an important role role in pyometra prognosis. However, little is known about the structural changes in the cervical wall that lead to increased compliance, allowing the purulent secretion expulsion. Considering previous evidences that cervical extracellular matrix remodeling is associated with its opening during parturition, this study aimed to compare cervix uterine collagenous fibers and elastic system fibers structure obtained from bitches with open or closed cervix pyometra. Study approved ethics committee (number 012/2020-State University of Londrina) Seven cervix of each status of pyometra obtained during ovariohysterectomy were studied. Samples were processed for light and electron microscopy. In cervices of bitches with closed cervix pyometra, the Picrosirius-polarization method evidenced bundles of thick fibers, strongly birefringent, which corresponds to a population of thick fibrils (mean=64.0±10.5nm) identified by electron microscopy. In contrast, in the cervices from dogs with open cervix pyometra, the Picrosirius-polarization method showed the presence of fragmented, thin and weakly birefringent collagen fibers that correspond to finer loosely packed fibrils (mean= 46.6±8.4nm) at ultrastructural level. Morphometric analysis showed that fraction area occupied by collagen is smaller in cervices of bitches with open-cervix pyometra (50.8% vs 69.7%, $p < 0.0001$). Elastic system fibers were studied by Weigert's Resorcin Fuchsine stain with previous oxidation, and also by ultrastructural analysis. Mature elastic fibers and elauninic fibers are present in the cervix of animals with closed cervix pyometra and rarely appear in the cervix of animals with open cervix pyometra. In contrast, a plexus of fine oxytalan fibers predominates in the open cervix pyometra. The results suggest that cervical relaxation in pyometra is related to structural changes in collagen fibers and that collagenolysis may be the main cause of cervical opening. Eosinophils presence in degranulation process in open cervix pyometra cervices indicates that these cells may play a relevant role in pre-existing collagen degradation. In conclusion, due to the small number of samples, more studies are still needed to confirm the collagenolysis role and eosinophils importance in biche's cervix opening.

Acknowledgements

CAPES scholarship and Laboratory of Cell Biology of Faculty of Medicine at the University of São Paulo.

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Yolk sac development in dogs: from morphology to functional aspects

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Abstract

Reproduction in mammals is directly related to the evolution of extra-embryonic membranes (chorion, amnion, allantois and yolk sac), and to the relation between embryonic and maternal tissues during placentation, in order to promote maternal-fetal exchanges. The yolk sac (YS) is the only fetal membrane that is present in all vertebrates, in addition to having unique morpho-functional characteristics. In mammals for example, the YS is responsible for the nutrition of the embryo during the first trimester of pregnancy, that is a period in which vascular communication has not yet been established. Thus, the aim of this research was to study the development and morphology of canine YS from early (up to the 20th day of gestation, n = 6), mid (21-30 days of development, n = 6) and late (31-60 days, n = 6) gestational stages. Samples were collected during caesarean sections, or in spay/neuter programs (Protocol approved by Ethics Committee – 029.2022). After sampling, YS membranes were fixed in 10% buffered formaldehyde and analyzed for gross morphology (location, shape, color, and vascularization) and histology. In dogs, unlike other animals (ruminants, horses, primates, and humans) the YS does not undergo regression during the course of development, remaining present and fully developed until the end of gestation. On the other hand, morphological changes occur during pregnancy. Approximately, on 20th day of pregnancy, the YS is well-vascularized and has a prominent size (average length 3.5 cm) in relation to the embryo (crown-rump average 0.8 cm). After the 24th day of pregnancy, the YS is large, reddish and has an inverted "T" shape, with a central region connected to the embryo's abdominal region, and two long membranous projections reaching 7.5 cm in embryos with crown-rump average of 3.0 cm. Commonly, the ends of the projections connect with the amnion that covers the zonary placenta surface. Near term, the YS has 12 cm in length, and despite their walls being more compressed, vitelline vessels are still well distributed along the whole membrane. During all stages of development the YS are located in a space limited by the zonary placenta and amniotic membrane. For this reason, the YS plays a fundamental role in the development of a temporary choriovitelline placenta, which is derived from the fusion of the YS with the chorion, which are juxtaposed to the uterine endometrium. Structurally, the YS wall consists of three layers: an inner endodermal epithelium with cuboid to columnar cells, an outer lining epithelium, which corresponds to the mesothelium that maintain direct contact with the exocoelom, and an intermediate mesenchymal layer, containing blood vessels and macrophages. Vascularization that is prominent throughout pregnancy is established by numerous vascular islets filled with blood cells, which correspond to the first hematopoietic site of the embryo. In summary, the maintenance of the YS in carnivores represents an important evolutionary condition, which is associated with the different functions presented by this membrane during the entire pregnancy, i.e. transport and synthesis of proteins and minerals, as well as the absorption of amino acids and vitamins by endodermal cells; essential functions for embryonic growth and development. From now, these morphological knowledge regarding to the YS components will be useful for identification of undifferentiated and pluripotent cell populations, which may be present in specific phases of pregnancy, and therefore, may be isolated for the establishment of stem cell lineages. This research is supported by Fundação Araucária - Paraná.

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Study of the maturation of bovine oocytes in plates and microfluidic

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Abstract

The study of oocyte maturation is significant as it can provide answers about oocyte quality, which is the ability to be fertilized and to develop a healthy embryo. However, it is difficult to understand the oocyte maturation process individually on the macroscale. Furthermore, on macroscale culture system is static and does not correspond to what occurs *in vivo*. Thus, microfluidics can help to understand cell behavior in microenvironments and dynamic systems. This study aimed to compare maturation rates in plate and microfluidics culture systems. Firstly, was developed a reversible and reusable microfluidic device in laminated polydimethylsiloxane ($PDMS_{LAM}$). The oocytes from bovine ovaries were aspirated, and we selected oocytes with one or two layers of cumulus cells and homogeneous cytoplasm. Oocytes were matured in TCM119 B medium supplemented with 22 µg/ml sodium pyruvate, 50µg/ml gentamicin, 0.4% BSA, 10 ng/ml IGF-1 (insulin-like grown factor), 100ng/ml AREG, 10^{-2} IU /ml human recombinant FSH, 50 ng/ml 17β-estradiol, 150 ng/ml progesterone, and 25mM sodium bicarbonate. Oocytes were cultured in groups in plates and microfluidic devices (20 oocytes per drop plate and 10 oocytes per chamber in a microfluidic device). The oocytes cultured in plates were cultured with 90µL of medium (groups) and 20µL medium 1 oocyte only), and the oocytes cultured in microfluidic were cultured with 6µL of medium (group) and 4µL of medium (1 oocyte only). We performed 8 routines with a pool of 100 oocytes per routine. We analyze the maturation in groups and isolated using booth culture systems. Statistical analyses of the 8 replicates were carried out by ANOVA followed by the Tukey test, using the ORIGIN PRO 8.5. The data were expressed as means ±SEM, and $p < 0.05$ was considered significant. The maturation rate in microdevices and plates was compared regarding the expansion of cumulus (by image) cells and the presence of the polar body. Oocytes cultured in microdevice showed a higher expansion of cumulus cells than oocytes matured in plates. The oocytes cultured in a microfluidic system presented the first polar body at the end of the oocyte maturation process, confirming the lack of adverse effects. The maturation rates were 79% and 75% in plates and microfluidic device, with no statistical differences. Preliminary results show the potential of microfluidics for studying and understanding oocytes since there was no difference in oocyte morphology and first polar body extrusion. In addition, the microdevice allows the recovery of oocytes and the reuse of the microdevice more than once. The results demonstrate that the material used for the construction of the microdevice does not interfere with the oocyte maturation process, being an alternative for future studies of embryos in a less invasive way.

Financial support: FAPESP grants 2021/06645-0 and 2022/02701-5

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Galectin-1 as a compound of *pharmacos* used in assisted reproduction – safety and toxicology studies

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Abstract

Galectin-1 has been cited as a mediator involved in preventing early embryonic death in mammals, implicated in maternal-fetal tolerance, and associated with regulating, and modulating the development and embryo elongation, migration, trophoblastic invasion, and adherence to the endometrium, and placentation, all essential events in early gestational development in mammals. The authors already demonstrated that an exogenous recombinant galectin-1 (eGAL1) single dose (with intrauterine - IU administration) could improve the pregnancy rate in beef cows inseminated by 8,68 percentage points and that innovation can be disseminated to another mammal's species, with just a little dose and administration way adjustments. To enforce the security of using this protein as a *pharmacos* compound we are spending efforts to comprehend its pharmacokinetics, toxicology, and pharmacological safety, all based on ICH Guidelines (S5-R2 and S6R1), all summarized here. (i) Pharmacokinetic Studies in rodents - no clinical signs were observed after intravenous (IV) intraperitoneal (IP) and intrauterine (IU – surgery method) routes of administration of 1mg.kg^{-1} of eGAL1. The eGAL1 concentrations (by ELISA Assay) were observed in plasma and uterus homogenate samples. In plasma samples, higher C_{max} (2.774 ng.ml^{-1}) was observed in IV, higher AUC0/t (12.746 hr*ng.ml^{-1}) was observed in IP, and higher T_{max} (2.167 hr.) was observed in IU. After IV administration, the content of eGAL1 in uterus at 0.5 h, 2.0 h, 4.0 h, 8.0 h, 24.0 h, 48.0 h observed were 10.92, 15.25, 17.92, 12.48, 20.50, and 0.00 ng.gm^{-1}, respectively. After I.P. administration the contents observed were 29.72, 15.40, 54.25, 23.53, 6.70, and 0.00 ng.gm^{-1}. After I.U. administration the contents observed were 128.22, 131.97, 134.43, 136.05, 94.40, and 0.00 ng.gm^{-1}; (ii) Pharmacokinetic Studies in swine - no clinical signs were observed after IU administration (dose 1mg.kg^{-1}). The plasma eGAL1 concentration (by ELISA Assay) were 16.1 ng.ml^{-1} (0h), and 20.5, 31.7, 22.4, 16.5, 14.2, 13.0, 13.4, 12.7, 12.7, 17.4 ng.ml^{-1} at 1, 2, 3, 4, 5, 6, 7, 8, 12 and 24hs after administration. (iii) Acute Toxicity Study in rodents (IU and IV) and Swine (IU) where no toxicity effects were observed, during 14 days after a single dose administration (maximum dose = 5mg.kg^{-1}) in both species. No toxicity effects were observed in female Sprague Dawley Rats and *Afrodite* Female Swine's, considering hematological (HEM[1]), biochemical (BIO[2]), urinalysis (URI[3]) parameters, and weighing and histopathology evaluation (HIS[4]) after IU administration. In female rats, after, IV administration, no signs of toxicity were observed (by HEM, BIO, URI, and HIS) and the LD50 would be greater than 5 mL/kg b.wt. (iv) Dermal Toxicity Study – with no toxicity effects observed during 14 days after (clinical signs observation and by HEM, BIO, URI, and HIS parameters) independently of the dose tested (0.1, 0.25, and 0.5mg.kg^{-1}); (v) Immunogenicity Studies – 0, 7, 14 and 21 days after eGAL1 administration, cow serum (after a single dose, 0.0005µg,kg^{-1}) and in Swine serum (groups with 0, 0.5, 1 and 5mg.kg^{-1}) were submitted for ELISA Assays to quantify IgG-anti-GAL1, and both studies demonstrated no evident IgG production after eGAL-1 intrauterine administration; (vi) Toxicity to Reproduction for Medicinal Product Study - using eGAL1 supplementations on the mediums, for *in vitro* production of bovine embryos. No effects on embryo development parameters were observed (7-8th day blastocysts, expanded or hatched blastocysts taxes) until 40ng.mL^{-1} of eGAL1 supplementation (detailed presented in another abstract). A 2nd reproductive study is still in progress, with the objective to evaluate the effect of eGAL1 in three stages of fetal development in fetuses' rats. With the all results present until now, eGAL-1 is safe until 5mg.kg^{-1} as a compound of *pharmacos* products indicates in assisted reproduction procedures.

Keywords: Tolerana®, Proteins related to fertility, Galectin-1, Pregnancy, Clinical trial.

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[1] Hematological Parameters = Hematocrit, Hemoglobin, Differential Leucocyte Count, Total Leucocyte count, Total Erythrocyte count, Reticulocyte count, Platelet count, Blood Clotting Time/Potential.

[2] Biochemical Parameters = serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP), Total Protein, Albumin, Blood Urea Nitrogen, Creatinine, Glucose, Globulin (Calculated value), Triglycerides, Total Cholesterol, Total Bilirubin

Calcium (Ca), Sodium (Na), Potassium (K), Phosphorus, Low-Density Lipoproteins (LDL);

[3] Urinalysis Parameters = Appearance, Volume, pH, Specific gravity, Glucose, Proteins, Blood cells

[4] Weighing and Histopathology of organs like Brain, Spleen, Lungs, Heart, Thymus, Adrenals, Liver, Kidneys, Urinary bladder, Uterus, and Ovaries.

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EVALUATION OF FRUCTOSE ON IN VITRO BUFFALO EMBRYO DEVELOPMENT

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Financial support: FAPESPA, UFPA.

Abstract

Despite the great advances achieved in the efficiency of in vitro production (IVP) of bubaline embryos, this biotechnology still needs improvements, especially according to pre-implantation embryo development. Thus, questions regarding the composition of the culture media appropriate for the species remain unclear. In many species, glucose is the main energy provider in embryonic development; however, studies have shown other energy sources, such as fructose, with excellent results in terms of embryo quality. It is interesting to investigate alternatives for energy support in the culture of bubaline IVP. Therefore, this work aims to evaluate the substitution of glucose for fructose during bubaline IVP. For this purpose, 128 cumulus-oophorus complexes (COCs) from a local slaughterhouse were selected and matured in vitro. After 24 hours of maturation, the COCs were activated by parthenogenesis using ionomycin, then were treated with 6- Dimethylaminopurine (6-DMAP) for 4 hours. On the 1st day of culture, the probable zygotes were randomly distributed between the experimental groups: Group culture in SOF medium with 1mM glucose and group culture in SOF medium with 1mM fructose. Statistical analysis was performed using program Sigma Plot 14.0, ANOVA test with 5% significance level. Embryo development was analyzed on the 8th day of culture by evaluating the blastocyst rate, hatching rate, and counting the number of embryonic cells. According to the results obtained there was no statistical difference regarding the blastocyst rate between the glucose group (28.6±14.2) and the fructose group (26.3±11). Regarding the kinetics of embryonic development, in both groups there was a predominance of hatched embryos ($p>0.05$), with an average rate of 58.3% (±41.9) in the glucose group and 74.4% (±37.7) in the fructose group. In the evaluation of embryo quality, 17 embryos were analyzed in the Fructose Group and 16 in the Glucose Group, the number of cells in embryos cultured with fructose (173.45±16.9) was similar to those exposed to glucose (152.16±45.4). Therefore, we can affirm that the culture of embryos in the concentration of 1mM of fructose did not improve the pre-implantation embryonic development in bubalines but could be a substitute for glucose in a culture media. A complementary study would be the evaluation of more concentrations of fructose added to the culture medium as a substitute for glucose, and further analysis of the expression of important genes of carbohydrate metabolism comparing with embryos in vivo.

Keywords: Buffalo; in vitro culture; glucose; fructose.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

USE OF PLATELET-RICH PLASMA IN THE IN VITRO PRODUCTION OF BOVINE EMBRYOS

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Abstract

One of the most important aspects to consider for the success of in vitro production (IVP) of embryos is the nature of the culture medium that makes up the stages of reproductive biotechnology. The medium must meet the metabolic needs of both the gametes during oocyte maturation and sperm capacitation and the embryo during the first cell divisions. Most IVP protocols use some blood serum as a source of hormones, proteins, growth factors, and nutrients in the composition of the medium. On the other hand, serum contains many substances that may interfere with maturation, fertilization, and embryonic development, and it has a high lipid content that may negatively affect the quality of the embryos produced. Several studies have reported the use of platelet-rich plasma (PRP) as an alternative to the use of fetal serum in cell culture, particularly for stem cells. Therefore, the aim of this work is to evaluate the use of PRP as a substitute for fetal bovine serum (FBS) during oocyte maturation for in vitro production of bovine embryos. Cumulus Oocyte Complexes (COCs) were distributed to the following experimental groups during in vitro maturation (IVM): Group G1 (IVM medium with addition of 5% PRP); Group G2 (MIV medium with addition of 5% PRP plus 5% SFB); Group G3 (MIV medium with addition of 5% FBS); and Group G4 (MIV medium without addition of PRP and/or FBS). After 20 hours of maturation, the COCs were transferred to the fertilization plate containing the serum-free TALP FERT medium. Approximately 24 hours after fertilization, the probable zygotes were transferred to droplets containing synthetic oviduct fluid (SOF) medium and 10% FBS. Rates of cleavage and blastocyst formation were assessed at day 2 and 7 of embryonic development, respectively. All statistical analyzes were performed using SigmaPlot® software (version 12.0). Analyzes of variance were performed for comparison of means, using Tukey's posttest where appropriate, always at the 5% significance level ($p > 0.05$). No significant differences were found between experimental groups in the rate of cleavage on the second day of culture. However, a significant difference ($p < 0.05$) was detected with respect to group G4 (group without addition of any type of blood supplement), which showed a lower rate of blastocyst formation on the seventh day of culture compared to the other treatments (G1: 35.180 ± 5.424^{a}; G2: 40.819 ± 5.691^{a}; G3: 41.329 ± 8.514^{a}; G4: 17.354 ± 8.190^{b}). Studies have shown that PRP is rich in growth factors such as FGF, TGFβ, PDGF, IGF and EGF, as well as binding factors such as fibrinogen and serotonin, which are essential for cell culture and folliculogenesis Based on the results of in vitro embryo production, it was concluded that PRP can be used as a viable and less expensive alternative to FBS for in vitro maturation of bovine oocytes, as there were no significant differences in the rates of cleavage and blastocyst formation between treatments (except for group G4 without addition of a blood component), including the group in which both preparations were used together (experimental group G2).

Keywords: Embryo; Fetal bovine serum; In vitro maturation; Platelet Rich Plasm

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Extracellular vesicles from superstimulated cows carry diferential molecular signatures and drives gene expression of oocytes and blastocysts

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Funding: FAPESP grant #2012/50533-2, #2013/11480-3.

Abstract

Ovarian superstimulation (OVS) can affect cellular and molecular events in ovarian follicular cells, cumulus-oocyte complexes (COCs), oviduct and embryo, as well as cell-to-cell communication through secretion and uptake of extracellular vesicles (EVs). The EVs are secretory membranous elements used by cells to transport proteins, lipids, mRNAs, and microRNAs (miRNAs) and they are gaining increased attention for their function in transporting RNA extracellularly for wide-ranging effects. Moreover, cells have the ability to selectively sort miRNA into EVs for secretion to nearby or distant targets. To gain insight about the effects of OVS on miRNAs expression in EVs from follicular fluid (FF) and transcriptional profile of COCs and blastocyst co-cultured with EVs, Nelore cows were submitted to ovarian superstimulation with FSH (n=10; FSH group) or FSH combined to eCG (n=10; FSH/eCG group); or not (synchronized cows; n=10; NS group). In experiment 1, EVs were submitted to the evaluation of 384 precursors and mature miRNAs by RT-qPCR. In experiment 2, EVs were added during oocyte in vitro maturation (IVM). COCs were obtained from ovaries in a local abattoir. The matured oocytes were fertilized, and on day 7, blastocyst was collected. Blastocysts and matured COCs were submitted to a low-density array for 96 genes. The mRNA abundance was quantified with Biomark HD® assay. The effect of OVS on EV's miRNAs profile and further impacts on transcriptional modulation of blastocysts and COCs was tested by ANOVA. Means were compared by Tukey test. Differences were considered significant when P≤ 0.05. As result of experiment 1, using TargetScan® tool, we found 239 miRNAs present in EVs, among them, 110 miRNAs were exclusively detected in the NS group. A total of 1738 target genes were predicted and gene ontology (GO) analysis showed regulation of biological processes, nucleus, multicellular organismal development, cellular differentiation, protein binding, and intracellular pathways. Four miRNAs were exclusively detected in FSH and six in FSH/eCG groups which predicted 273 e 258 target genes, respectively. Furthermore, when we analyzed miRNAs detected in the three groups, 13 miRNAs were differentially expressed and 12 of them were up-regulated in NS group. Moreover, 138 target genes were predicted, and GO analysis infers alterations in protein binding, and intracellular e cytoplasm pathways. Taken together, we reinforce there is miRNAs regulation on follicle microenvironment and specifically, that superstimulatory treatments down-regulate follicular miRNAs in fluid-derived EVs. In experiment 2, no differences were found on mRNA abundance in cumulus cells, however, in oocytes, EVs promoted differential expression of *CASP9, CPT1B, GREM1, NLPR5, BMP15, and MAPK1,* among groups. In blastocysts, *HSPD1, ARO, HAND1, HSP90AA1, PFKP,* and *SLC2A3* were altered by EVs treatment during oocyte IVM. In conclusion, we demonstrated that OVS modifies miRNAs profile of EVs present in bovine FF, which consequently, drives gene expression of oocytes and in vitro-produced blastocysts in cattle.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Supplementation of bovine *in vitro* embryo mediums as Reproductive Toxicity Study Model for Galectin-1

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Abstract

Galectin-1 (GAL1), a 14KDa lecithin, is a modulator/regulator of early pregnancy in mammals, involved in events like maternal-fetal tolerance, development and embryo elongation, trophoblastic migration, invasion and adherence to the endometrium, and placentation. The authors already demonstrated that an exogenous recombinant galectin-1 (eGAL1) single dose (with intrauterine administration) could improve the pregnancy rate in beef cows inseminated. To enforce the security of using eGAL1 as a *pharmaco* compound, we are spending efforts to comprehend its toxicology and pharmacological safety. As recommended in the ICH Guidelines (S5-R2 and S6-R1), and based on bioethical concerns, we design two experiments to evaluate *in vitro* embryo development parameters, considering different amounts of eGAL1, as supplemented mediums (maturation - IVM on experiment #1 or SOF mediums on experiment #2). The development parameters evaluated were the percentage of Cleavage on the 2nd day (CLE) and blastocysts on day 7th (BlD7), expanded blastocysts (BxD7) and hatched blastocysts (BhD7), and hatching blastocysts on day 8th of culture (BhD8) on both experiments and using oocytes aspirated from slaughterhouse ovaries, and divided equally per groups per each IVP batches. All *in vitro* embryo production (IVP) mediums were acquired by *Bioklone Reprodução Animal*®. The IVP (*in vitro* embryo production) design was done with 3 batches per experiment and distributed the oocytes in four groups of treatment (G1 - 0µg.mL^{-1}, G2 - 2µg.mL^{-1}, G3 - 20µg.mL^{-1} and G4 - 40µg.mL^{-1} buffered eGAL1 solution) as supplementation of the mediums. For statistical analyses, it was used the Chi-Square method by Jamovi 2.2.5. In experiment #1, a total of 962 oocytes were aspirated and submitted for 3 batches of IVP with IVM medium supplementation. No statistical differences were found in CLE parameters between groups (means ± SD obtained were 79.47 ± 3.05% for G1, 88.66 ± 4.38% for G2, 66.73 ± 43.50% for G3, and 81.66 ± 17.84% for G4); or by the BD7 parameters (26.0 ± 7.6% for G1, 33.9 ± 9.2% for G2, 18.5 ± 8.8% for G3, and 18.5 ± 10.6% for G4) even considering the embryo development stage (Bl, Bx, and Bh at D7). No statistical differences were found considering the BD8 parameters too (29.3 ± 12.3% for G1, 37.7 ± 11.1% for G2, 24.0 ± 12.4% for G3, and 23.2 ± 6.7% for G4). In experiment #2, a total of 1,213 oocytes were aspirated and submitted for SOF medium supplementation. No statistical differences were found in CLE parameters between groups (means ± SD obtained were 79.47 ± 3.05% for G1, 88.66 ± 4.38% for G2, 66.73 ± 43.50% for G3, and 81.66 ± 17.84% for G4); or by the BD7 parameters (26.0 ± 7.6% for G1, 33.9 ± 9.2% for G2, 18.5 ± 8.8% for G3, and 18.5 ± 10.6% for G4), but when we slip the results by embryo stage, considering BxD7it was demonstrated statistical difference ($p<0.05$) between groups (G1 and G2 with better results compared with G3 and G4, being 12.7 ± 3.0 and 11.5 ± 2.70 for G1 and G2 versus; 3.4 ± 3.2 and 5.0 ± 5.1 for G3 and G4 respectively. No statistical differences were found considering the BD8 parameters too (29.3 ± 12.3% for G1, 37.7 ± 11.1% for G2, 24.0 ± 12.4% for G3, and 23.2 ± 6.7% for G4), independently of the stage of development of the embryos, although the results demonstrated a discrete improvement of BD8 when the SOF medium was supplemented with 2µg.mL^{-1} of eGAL1. Among that, some Immunohistochemistry Assays, based on D8 embryos, treating fixed embryos with antibody anti-humanGAL1 and DAB/Substrate kit stain, made it possible to comprehend if eGAL1 penetrates the embryo structure and interferes with the development of then. The immunohistochemistry assay with D8 embryos cultivated with eGAL-1 supplementation on the culture medium (SOF medium) could demonstrate the presence of exogenous GAL-1, distributed in mass cell and trophoblastic cells, and the profile of stained observed is dependent on the amount of the supplementation and it was more evident in hatched embryos. The findings reassure the use of a reasonable amount of eGAL-1 (at the maximum dose tested 40µg.mL^{-1}) does not compromise the *in vitro* embryonic development and makes using eGAL-1 in assisted reproduction mammals more reliable and safer.

Keywords: Tolerana®, Proteins related to fertility, Galectin-1, Pregnancy, Clinical trial.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Vitrification of buffaloes cumulus-oocyte complexes with follicular fluid

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Financial Support: PROPPG – IFPA and FAPESPA

Abstract

The cellular cryopreservation allows the conservation of live biological material in liquid nitrogen (LN_2) for long periods, having in mind the reduced number of female gametes obtained in the processes of follicular aspiration in buffaloes, which hinders the studies and improvement of reproductive biotechnologies in the species. This work aimed form a bank of immature cumulus-oocyte complexes (COCs) evaluating the effect of the addition of buffalo follicular fluid (BFF) in the vitrification solution. Ovaries from local abattoir that presented ovarian follicles with diameter between 8 and 10 mm were aspirated and the obtained follicular fluid deposited in 15 mL tubes and subjected to centrifugation for 25 minutes at 3000 rmp. The supernatant formed was filtered on a 0.22µm membrane and stored in freezer. Ovarian follicles between 2 and 8 mm were aspirated and the selected COCs were distributed among the following experimental groups: Control (not vitrified); Toxicity (submitted only to the cryopreservation protocol, without going through liquid nitrogen (LN_2) and Vitrified (submitted to Medium 199 + 20% bovine follicular fluid (BFF) + 10% DMSO and 10% EG for 3 minutes, then to Medium 199 + 20% BFF + 20% DMSO + 20% EG and 0.5M sucrose, filled in glass tube (300µm diameter) and submerged in LN_2 in a maximum period of 20 seconds. After 1 hour the glass tubes were removed from LN_2 and the COCs were subjected to two solutions with decreasing sucrose concentrations (0.125 and 0.031M, respectively). Immediately after warming all groups were subjected to 24 hours of *in vitro* maturation before being fixed in methanol:acetic acid (3:1) for evaluation of oocyte nuclear progression and stored in RNA® for analysis of hypoxia-inducible factor 1-alpha (HIF1-alpha) gene expression in oocytes and *cumulus oophorus* cells in Control and Vitrified groups. Regarding nuclear progression, metaphase II evaluation of oocytes stained with 1% acetic orcein solution, rates of 80% were observed for the Control group (16/20) and 73.68% for the Toxicity group (14/19), being significantly ($P<0.05$, Chi-square test) higher compared to 6.5% in the Vitrified group (3/46). In this experiment it was observed that approximately 60% of vitrified and heated oocytes showed morphological signs of cryo injury, such as cryo fracture of zona pellucida and loss of cytoplasmic membrane integrity. As expected, our results demonstrate higher expression level of HIF1-alpha in vitrified oocytes when compared to Control ($P<0.05$, ANOVA post Tukey's test). Despite the inefficiency of the protocol evaluated in this study for oocyte cryopreservation, the *cumulus oophorus* cells showed an excellent response to the vitrification protocol used. When analyzing these cells under light microscopy it was possible to observe nuclear material apparently intact and with an active aspect. Corroborating with the results of HIF1-alpha expression that showed no difference in relation to the Control *cumulus oophorus* cells. This demonstrates that the main challenge lies precisely in the complexity of the female gamete, which, being a large cell, presents a greater abundance of water and cytoplasmic components, characteristics that are very relevant for the success of cryopreservation.

Keywords: Buffaloes, Vitrification, Follicular fluid, Immature oocyte.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Factors involved with the reproductive sucess in the Wagyu cattle raised in Brazil

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Abstract

The Wagyu cattle breed is originally from Japan, where its is known for the marbling deposition capacity between the muscle fibers. Wagyu cattle got in Brazil in 1992, however until now this breed is not fully disseminated, possibly because the cost and time involved to raise this cattle. Nevertheless the market demand has been increasing due to the incomparable quality and flavor of the meat. Due to the lack of knowledge about the reproductive capacity of this breed in Brazil, the present study aimed to compare different Fixed Time Artificial Insemination (FTAI) protocols in a Wagyu herd in the reproductive seasons of 2021-2022. The work was carried out on a farm in the state of Rio Grande do Sul that has been raising Wagyu cattle since 2002. The work was conducted in n=55 Pure Breed Wagyu Kuroge cattle from Tajima line. A comparison between the reproductive success was conducted among the years 2021 and 2022. Cattle were raised in the native field in the summer and oats associated with Tarumã, Tarumaxi and Pastoreio grazing wheat in the winter within the rotational grazing system. Management in season 21 was with a group of 60 females, average weight 385 and ECC 3, on day zero (D0) deworming was performed with levamisole 4.5mg/kg (SC), implantation of intravaginal progesterone device (P4) monodose 0.5g(IV), and IM injection of estradiol benzoate (EB) 2mg/animal, on day seven (D7) prostaglandin (PgF2) 0.52mg/animal, day nine (D9) removal of the P4 implant, PGF 0.52mg/animal plus injection of estradiol cypionate (ECP) 2mg/animal (IM), after 48h artificial insemination (AI) was performed. Animals were fed with energy supplementation of 150g/animal/day. After insemination, the females were led to the paddocks with oat and wheat pastures until the pregnancy check (PC) which was carried out at forty-five days, with a result of 75% of pregnancies with an FTAI. In season 22, the animals were on a millet pasture during part of the summer and wheat grazing in the winter, animals were fed with energy supplementation of 150g/animal/day. In the 22 season there were some changes in the FTAI protocol, starting with 55 females single cattle, average weight 402Kg and ECC 3.1, on D0, deworming was performed with levamisole 4.5mg/kg (SC), vitamin A 1,000,000 IU/animal single dose (IM), IV implantation of P4 device, monodose 0.5g, BE 2mg/animal, on D7 0.52mg/animal of PGF, D9 removal of the P4 implant, PGF 0.52mg/animal plus ECP 2mg/animal and chorionic gonadotropin equina (eCG) 300 IU/animal. Estrus were detected using the Estrotect devise glued to the sacral bone, after 48 hours it was observed that 100% of the females had estrus and AI was performed using a sanitary AI sheet. The DG was performed with 51 days with a result of 92.72% of pregnancies. This work indicates that for the success of the reproductive performance in the Wagyu cattle raised in Brasil, it is needed an additional nutritional support of energy and vitamins to compensate its high metabolism.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

A new approach using a 3D Matrix to improve the bovine in vitro embryo production

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Abstract

Despite commercial progress of in vitro embryo production (IVP), current conditions available in the human medicine and animal production industry are still below ideal. Innumerous abnormalities in the oocyte matured under in vitro maturation (IVM) conditions can lead to negative effects on the embryo. In cattle, more than 80% of the IVM oocytes routinely undergo normal fertilization and complete the first cell cycle resulting in cleavage to the 2-cell stage. However, only 30 to 40% of these structures reach the blastocyst stage. Much of the evidence has shown that in vivo matured oocytes produce more and better embryos. These differences between in vivo and in vitro appear at the level of morphology, metabolism, and gene expression. Thereby, culture environment shows a dramatic effect on the development of bovine blastocysts. Although three-dimensional (3D) system has gained attention in recent years, little progress in animals as human medicine has been made. A way to approximate the in vitro conditions to in vivo environment is by changing the approach. Here, we suggest an innovative and promising technology for 3D maturation (IVM-3D). Using a 3D Matrix our approach improves oocyte maturation, as it reduces the flattening of the cumulus-oocyte complex (COC) and preserves its structural and functional integrity. We investigated the blastocyst rate and the viability to produce embryos under the 3D Matrix. For this, COC cattle were recovered from ovaries obtained in a slaughterhouse (n= 50 COC/group with 4 replicates) and were matured in vitro using the new 3D Matrix system or in vitro or control group using 2D maturation for 24 hours in a basic medium. After that, the matured COC was followed by regular in vitro fertilization (IVF) and in vitro culture (IVC). In our model, COC did not adhere to the surface of the plate, a condition that increases their exchange surface area with the culture medium. Although, 3D Matrix system did not improves (p= 0.2506) blastocysts yield (45.27%) compare to in vitro 2D system (43.77%) may have a beneficial effect on early embryonic development. In summary, we suggest that the 3D Matrix system proposed here is capable of simulating the mechanical force exerted by the extracellular matrix, a condition that positively influences the development and cellular interactions of the embryo Finally, we hope that this will be an ideal commercial model for the evaluation of new products and bioprocesses that can maximize the fertility of mammalian species in assisted reproduction programs.

Keywords: three-dimensional (3D) cultive, oocyte maturation, biotechnology, embryo, innovation.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Impact of Endometritis on Specific Oxidative Stress Parameters in Mares.

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Abstract

Oxidative stress results from an imbalance between the reactive oxygen radicals and antioxidants and has adverse effects on animal health and production yields. It has been well described in bovine endometritis and characterized by an elevation in free radicals (1). However, few papers discussing the relationship between mare endometritis and oxidative stress are available (2). The purpose of the present study was to investigate the relationship between the uterine infection and specific oxidative stress parameters from serum and low volume uterine flush in mares. Forty-two mixed-breed mares were divided into control (n=20) and endometritis groups (n=22) according to reproductive history and endometrial cytology results. All mares were in estrus phase during the experiment. Each underwent a low volume uterine flush with 250 mL of saline 0,9%. The recovered fluid was centrifuged for 15 minutes at 3000 rpm and stored at -20ºC for further oxidative stress analysis. A blood sample was withdrawn from the jugular vein of each mare. Serum tubes were used. The samples were centrifuged at 3000 rpm for 10 minutes, and the serum aliquoted and stored at -20ºC. Oxidative stress was assessed by measuring superoxide dismutase (SOD), catalase (CAT), nitric oxide (NO), total antioxidant capacity (FRAP) and malondialdehyde (MDA). Distribution of the data was tested and the parameters analyzed by Student's T-test or by Mann Whitney. Differences were considered significant when $P < 0.05$. Higher SOD (66,51 ± 11,30 U mg/protein) and FRAP (3,55 ± 0,32 µM) means were obtained in serum samples of control group. No differences between endometritis and control group were observed in low volume uterine flushes. In agreement with previous studies, the analyzed results indicates that mares with endometritis undergo an oxidative process (2). Free oxygen radicals produced in presence of the uterine disease can damage cells by disrupting the mechanism of the antioxidant system (2). It seems reasonable that in mares affected by endometritis the increase in free radical production reduces systemically the antioxidant capacity, which is evidenced in this study by the lower serum SOD and FRAP concentration in endometritis group. We also emphasize that few descriptions of local oxidative stress parameters in mares with endometritis are available. More studies in the field are necessary, thus contributing for the knowledge related to reproductive pathophysiology of the specie.

References

(1) KAYA S, OGÜN M, OZEN H, KURU M, SAHIN L, KÜKÜRT A, KAÇAR C. The impact of endometritis on specific oxidative stress parameters in cows. Journal of the Hellenic Veterinary Medical Society, v.68, p.231-6, 2017.

(2) ABDELNABY, E. A., EMAM, I. A., SALEM, N. Y., RAMADAN, E. S., KHATTAB, M. S., FARGHALI, H. A., ABD EL KADER, N. A. Uterine hemodynamic patterns, oxidative stress and chromoendoscopy in mares with endometritis. Theriogenology, v.158, p.112-120, 2020.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effect of heat stress on reproductive performance and its association with the occurrence of periparturient disorders in dairy cows

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Abstract

Heat stress (HS) is a serious concern in the dairy industry because it affects health status and is one of the main causes of low productivity. This study aims to assess the effect of HS on I) the occurrence of periparturient diseases and II) the reproductive performance of dairy cows. A total of 1,825 animals were used and diagnosed for puerperal metritis (PM) and subclinical ketosis (SK) on day five postpartum (pp), and for clinical/subclinical endometritis (CE/SE) on day 28 pp. After a voluntary waiting period of 50 days, cows detected in heat were bred by artificial insemination (AI). Pregnancy checks were done 42 and 93 days after AI, and reproductive parameters, such as days to first and further inseminations, pregnancy outcome, and inseminations per pregnancy were recorded or calculated. Body condition score was assessed at calving and at day 28 pp. Ambient temperature and relative humidity were recorded every 30 min by digital loggers (Plus 2, Tinytag-Gemini Datalogers Ltd., the UK) placed in the barns. To evaluate the effect of HS, the temperature humidity index (THI) was calculated, and the comfort level was set at THI ≤ 68. HS level was calculated as accumulation area between the THI threshold and the amplitude approximated by the Riemann sum. Afterwards, accumulated THI values above the threshold were summed, and a median value was defined. Accordingly, HS levels were scored into 'no-HS' (without HS, n=491), 'low-HS' (below median, n=665), and 'moderate-HS' (above median, n=669). No differences were found between HS levels and the occurrence of PM, CE, and SE ($P > 0.05$). However, cows experiencing HS before the diagnosis had a higher risk of SK than non-HS cows ($P < 0.05$). There was a lower chance of pregnancy after first AI in heat stressed animals compared to non-heat stressed animals (Odds Ratio: 0.83; CI: 0.72–0.96; $P<0.05$). Conversely, HS did not affect the interval from calving to either or first AI or conception ($P > 0.05$). In summary, these results show that HS is a risk factor for impaired fertility and increased the risk for SK. Further studies need to assess more thoroughly the relations between HS and fertility.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effects of anethole supplementation on bovine embryo production and quality

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Abstract

To further expand the potential of *in vitro* embryo production (IVP), improvements are necessary. Anethole, a natural antioxidant, was shown to be a low-cost alternative to decrease oxidative stress during *in vitro* culture and improve embryo production. The objective of this study was to evaluate the effect of anethole on oocyte maturation during *in vitro* maturation (IVM) and/or *in vitro* culture (IVC) on the production and quality of bovine embryos. For this, *cumulus*-oocyte complexes (COCs) were matured in IVM medium supplemented with 300 µg/ml anethole (M300) or not (MC) for 24 hours. Subsequently, the COCs were submitted to *in vitro* fertilization (D0) and IVC, and divided into four groups: MC-CC (IVM and control IVC); MC-C300 (control IVM and IVC with 300 µg/ml); M300-CC (IVM with 300 µg/ml and control IVC); and M300-C300 (IVM and IVC with 300 µg/ml anethole each). Cleavage was evaluated on D3 of the culture and the production and classification of blastocysts were assessed on D8. Furthermore, the expression of genes involved in oxidative stress and embryo quality were observed in blastocyst. The present study showed that the combination of 300 µg/ml anethole during IVM and IVC (M300-C300) increased the percentage of cleaved embryos when compared to the IVM experimental groups without anethole (P = 0.025), suggesting that anethole promotes beneficial effects on early embryo development. Regarding embryo production, there was a difference between the M300-CC and M300-C300 groups (P = 0.037), increasing the percentage of blastocysts when used anethole during IVM and IVC. Regarding gene expression, the genes *GPX1*, *IFNT2*α, and *HSAPA1A* were differ expressed in the presence of anethole. *GPX1* gene was higher in groups of oocyte maturation with 300 µg/ml of anethole (M300-CC and M300-C300; P=0,0056). The expression of *IFNT2*α decreased in the presence of anethole treatments during IVM and IVC (MC-C300, M300-CC, and M300-C300), differing from the control group (P=0,0074). *HSPA1A* presented lower expression in the anethole-treated group, only in the IVC (MC-C300), when compared to the control (P=0,0218). It is concluded that the addition of 300 µg/ml anethole during IVM and IVC improved embryo cleavage and production, as well as modulation of genes related to oxidative stress and embryo quality. Then, anethole was an important role in the gene modulation of oxidative stress and it may be used for the production of good-quality embryos.

Keywords: anethole, antioxidant, ROS, cattle, IVP, gene expression

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Role of antifreeze protein type I (AFP I) on feline immature oocyte vitrification

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Abstract

The domestic cat has been used as an experimental model for wild felines, particularly in studies focused on gamete cryopreservation. Reducing the damage created by cryopreservation is one of the main challenges to disseminate this biotechnology. Antifreeze proteins (AFPs) have been used in cryopreservation protocols due to their ice-binding properties that can promote cell protection at negative temperatures. The aim of this study was to investigate whether the addition of AFP I to feline oocyte vitrification solution affects post-warming oocyte morphology and dissolution of zona pellucida (ZP). To achieve that, *cumulus* oocyte complexes (COCs) were recovered from feline ovaries obtained in elective surgeries. Only COCs presenting homogeneous cytoplasm and surrounded by at least one layer of *cumulus* cells were selected, and only oocytes graded as I and II were vitrified. For vitrification, COCs were allocated into three experimental groups: 1) G0 (negative control group, 0 µM AFP I), 2) G0.5 (0.15 µM AFP I), or 3) G1 (0.3 µM AFP I). After vitrification, 123 COCs (G0, n=40/G0.5, n=42/G1, n=41) were warmed, morphologically evaluated, and then, ZP's resistance to 0.5% pronase solution was measured in seconds. The statistical analyses were performed in the GraphPad Instat. Morphology was evaluated by Fisher´s exact test and ZP by ANOVA followed by the Tukey test. A value of $P<0.05$ was considered statistically significant. For morphology, COCs were evaluated immediately (0 h) and 28 h after warming. Only the COCs that kept the same parameters of selection (Grade I and II) were categorized as good-quality COCs. The good-quality COCs rate at 0 h and 28 h, respectively, was: 75% and 65% (G0), 85.7% and 78.3% (G0.5), and 78% and 65.9% (G1), with no differences ($P>0.05$) between both timepoints and/or among groups. Regarding ZP's dissolution, the G0 needed a longer time ($P<0.05$) compared to the G0.5 group (460.2 ± 24.5 vs 346.1 ± 12.99 s and a shorter ($P<0.05$) time of dissolution than G1 (765.2 ± 33.3 s. In conclusion, AFP I did not affect COCs morphology after vitrification, but in a concentration of 0.15 µM was able to reduce the ZP dissolution time, showing a possible strategy to reduce ZP hardening caused by the cryopreservation process. ACKNOWLEDGMENT: CAPES and FAPERJ.

Keywords: cat, COCs, cryopreservation

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effect of needle immersion vitrification and a tissue cryosystem on morphology and viability of canine ovarian preantral follicles

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Financial Support: National Council for the Scientific and Technological Development - CNPq.

Abstract

Nowadays, vitrification appears as an effective alternative for the conservation of ovarian tissues, contributing to the preservation of valuable female germplasm. Among the different types of vitrification techniques, needle immersion vitrification (NIV) and the use of a device called ovarian tissue cryosystem (OTC®, LAMOFOPA – UECE, Brazil) stand out. Our aim was to evaluate the effect of different vitrification techniques (NIV and OTC) on the morphology and viability of preantral follicles enclosed in canine ovarian tissues. Six ovarian pairs from adult females were collected and washed in isotonic saline solution at 37° C and in Hank's Minimum Essential Medium (MEM). Then, the ovaries were fragmented (3x1x1mm³) and exposed to an equilibrium solution (ES) composed of 20% fetal bovine serum, 3.75% dimethylsulfoxide (DMSO), 7.5% ethylene glycol (EG) and 1.25% polyvinylpyrrolidone (PVP). Fragments were then exposed to the vitrification solution (SV) 20% FBS, 7.5% DMSO, 15% EG, 2.5% PVP and 0.5 M sucrose, and finally cryopreserved through NIV technique, in which the fragments were embedded in a 30 G needle, in the proportion of four fragments per needle, exposed to immersion in both solutions, and stored in cryotubes in cryobiological cylinders (-196° C). In the OTC device, the fragments were exposed to the solutions inside the device, and after their removal, the device was closed and stored in liquid nitrogen (-196° C). After one week, the samples were heated and the fresh (control) and vitrified fragments were evaluated for morphology through classic histology where follicles were classified primordial, primary and secondary according to their development stage, as well as morphologically normal or degenerated. Also, follicle viability was evaluated through a Trypan blue (0.4%) assay. For both parameters, treatments were compared by ANOVA test followed by PLSD Fisher ($P < 0.05$). As the main results, the OTC technique provided a most efficient ($P < 0.05$) preservation of the morphology of primordial follicles (81.81 ± 305%) in comparison to NIV (66.92 ± 9.21%), which differs to the fresh control group (77.34 ± 3.40%). For the morphology of primary and secondary follicles, there were no differences between NIV and OTC ($P > 0.05$). Regarding follicle viability, similar values ($P > 0.05$) were found for NIV (80.55 ± 4.58%) and OTC (74.39 ± 4.28%). In view of the results, we can suggest that both techniques are suitable for the vitrification of the canine ovarian tissue, as they both provide efficient preservation of preantral follicles morphology and viability. These results can be useful for the preservation of germplasm from females of valuable genotypes, or extrapolated to endangered close-related species, enabling the formation of germplasm banks.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effect of beta-NGF supplementation in *in vitro* oocyte maturation medium on bovine oocyte cytoplasmic structure

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This study was financed in part by CAPES (Finance Code 001) and CNPq (310049/2018-0)

Abstract

Cattle farming and milk are two of the main activities of Brazilian agribusiness. Therefore, biotechnological alternatives are being sought to reduce the cost and improve the quality of these activities, such as in vitro embryo production (IVEP). An important phase for IVEP is the *in vitro* maturation of oocytes because it is the moment when transformations occur that directly influence fertilization and early embryonic development. Recently, it was described that the addition of Beta-NGF (Nerve growth factor) in the oocyte maturation medium increases the efficiency of IVEP in cattle. Thus, the present study aimed to evaluate how Beta-NGF supplementation in the *in vitro* oocyte maturation medium take effect on the cytoplasmic maturation of oocytes. Cumulus oophorus complexes (oocyte plus cumulus cells) (CCO) were used, part of which was matured in vitro supplemented with 100ng/mL of Beta-NGF and part was not supplemented (control). COCs were aspirated from ovaries collected in a slaughterhouse and those classified as grade I and II according to Stojkovic (1) were selected. The selected COCs were washed seven times in 100µL drops of TCM – 199 medium. A final wash was performed in a 100µL drop of TCM 199 maturation medium with bicarbonate. Finally, the COCs were transferred to the maturation plate containing 3 drops control and 3 drops supplemented, in which 15 COCs were placed per drop of 100µL of maturation medium, covered with mineral oil and kept for about 22 h in an incubator at temperature of 38.5°C, 100% humidity and atmosphere of 5% CO2. Confocal microscopy was used to evaluate the cytoplasmic maturation of COCs after the in vitro maturation period. Mitochondrial activity and reactive oxygen species (ROS) in oocytes and apoptosis and cell death in COCs were evaluated, using MitoTracker, DCF, YOPRO-01 and propidium iodide, respectively. The quantitative evaluation of fluorescence was performed by computational image analysis using an algorithm developed in a Scilab programming environment. To verify the difference between the groups, the student's t test was used ($p<0.05$). For the quantification of mitochondrial activity, 11 oocytes without supplementation and 7 with supplementation were analyzed. Oocyte fluorescence was measured, and the fluorescence value observed in the Beta-NGF group was higher when compared to the control group. In the evaluation of ROS production, 6 oocytes without supplementation and 14 with supplementation were analyzed. Fluorescence emission was higher in the control group than in the group supplemented with Beta-NGF. In assessing the rate of apoptosis and cell death, the fluorescence of 12 control oocytes and 17 oocytes from the Beta-NGF group was evaluated. Both stains showed higher fluorescence emission in the control group when compared to the Beta-NGF group. Studies show that the high concentration of ATP in oocytes leads to a significant increase in fertilization rates and blastocysts (1) and the greater production of ATP is due to the increase in mitochondria and /or mitochondrial activity. However, mitochondrial activity leads to the formation of ROS, which in excess can increase lipid peroxidation, protein aggregation and degradation, and DNA damage (2). However, in the present work, Beta-NGF supplementation increased mitochondrial activity and decreased ROS production, apoptosis and cell death. When oocyte maturation occurs in the follicular fluid, there are enzymatic antioxidants that will transform ROS into less harmful molecules. However, when *in vitro*, in addition to not having antioxidants in the follicular fluid, there is still exposure to oxidative factors (3). Therefore, supplementation with Beta-NGF probably controls, activating antioxidant mechanisms, the production of ROS, which would provide better cell development and embryonic conversion. We conclude that Beta-NGF supplementation positively affects the production of embryos in vitro, increasing mitochondrial activity, without increasing the amount of ROS, leading to a lower rate of apoptosis and cell death.

References

(1) STOJKOVIC, M. et al. Mitochondrial Distribution and adenosine triphosphate content of bovine oocytes before and after in vitro maturation: correlation with morphological criteria and developmental capacity after in vitro fertilization and culture. *Biol. Reprod.*, 94(3), 904-909, 2001.

(2) NAGANO, M., et al. ATP content and maturational/developmental ability of bovine oocytes with various cytoplasmic morphologies. *Zygote*, 14(4), 299–304, 2006.

(3) AMBEKAR, A. S. et al. Proteomic analysis of human follicular fluid: A new perspective towards understanding folliculogenesis. *J. Proteomics*, 87, 68–77, 1 jul. 2013.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Generation of induced pluripotent stem cells (iPSCs) from camelids: preliminary results on *Camelus dromedarius*

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Abstract

Camelids are domestic animals with diverse social and economic applications due to their adaptive capacity to adverse conditions and the diversity of tasks to which they are submitted. Therefore, the production of induced pluripotent stem cells (iPSCs) has become a desirable technological tool for regenerative veterinary treatments in these species, as well as to enable new advanced reproductive technologies such as the possibility of generating embryos and gametes *in vitro*. The present study aimed, in an unprecedented way, the reprogramming of camelid fibroblasts (*Camelus dromedarius) into pluripotency* using protocols previously established for other domestic ungulates. Fetal cells were obtained from a cell bank (DUBCA, ATCC), grown in Iscove's Modified Dulbecco's Medium (IMDM - Life Technologies) supplemented with 10% FBS (Hyclone) and 1% antibiotics (pen/strep, Gibco). The reprogramming process was performed using lentiviral transduction with human or murine OCT4, SOX2, KLF4, and C-MYC factors (hOSKM or HS and mOSKM or MS, respectively). Transduction with the FUGW vector was performed to validate the transduction protocol and generate GFP-positive cells for further experiments. Six days after the transduction with human or mOSKM, the cells were replaced onto a monolayer of mitomycin-treated MEFs and cultured in iPSC media (DMEM/F12 knockout, 20% KSR, 1% GlutaMAX, 1% NEAA, 1% pen/strep, 3.85 uM β-mercaptoethanol (Gibco) and 10ng/mL bFGF (Peprotech)) for at least 30 days. Discrete morphological alterations were observed, and the cell culture was collected for molecular analysis by qPCR analysis regarding the expression of endogenous and exogenous pluripotency-related factors on the 6th and 15th day after reprogramming. When compared to fibroblasts, experimental groups HS and MS showed an increased relative abundance of mRNA for OCT4, SOX2, and NANOG compared to fibroblasts, confirming the reprogramming process in vitro. In addition, the HS group expressed the hOSKM, and the MS expressed the mOSKM as expected. These results confirm the expression of exogenous and endogenous pluripotency factors in this species, although in vitro culture adjustments to maintain pluripotency are still required. These initial results may greatly contribute to future regenerative medicine and animal reproduction research. This study was supported by FAPESP (# 2015/26818-5, # 2020/07921-8).

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

The use of a progesterone device for four days after superovulation promotes a greater number of ova/embryos recovered

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Financial support: Embrapa (Project 22.13.06.026.00.04)

Abstract

This study aimed to assess the efficacy of using a progesterone device from the fourth to the eighth day after superovulation on the luteal count and number of ova/embryos recovered in crossbred Dorper/Santa Inês ewes. A total of 20 multiparous ewes received an intravaginal progesterone device (P4; 0.36 g; Primer®, Agener União Saúde Animal, Brazil) for nine days, inserted (D0) and removed (D9) in the afternoon (18:00 h), as well as six decreasing doses (25, 25, 15, 15, 10, 10%) of 133 mg pFSH i.m. (Folltropin®, Vetoquinol, Brazil) at 12 h intervals, starting 60 h before device removal and finishing concomitantly to its removal. All ewes received 131.5 µg cloprostenol i.m. (Sincrocio®; Ourofino, Brazil) at the fifth and sixth pFSH doses, and 50 µg gonadorelin i.m. (Gestran®, Tecnopec, São Paulo, Brazil) at D10, and were subjected to natural mating every 12 h while in estrus. On D13, the ewes previously detected in estrus and with viable corpora lutea (CL; n=19) were equally allocated for receiving their own intravaginal implants used a few days before (G-P4; n=10) or not (G-Control; n=9). On D17, the devices were again removed, and all females received the cervical relaxation protocol including 131.5 µg cloprostenol i.m. and 0.5 mg estradiol benzoate i.m. (Gonadiol®; Zoetis, Brazil) both 16 h before, as well as 50 mg oxytocin i.v. (Ocitocina Forte, UCB, Brazil) 20 min before non-surgical embryo recovery (NSER). CL count and viability were performed by transrectal B-mode and color Doppler ultrasonography before implant re-insertion (D13) and at its removal (D17). Blood samples were done before implant removal or insertion on D9, D13, and D17 for plasma progesterone (P4) analyses. Non-parametric and parametric data were analyzed by Mann-Whitney test and ANOVA, respectively. Frequencies were assessed by Chi-square or Fisher exact test. Differences were considered as significant when $p<0.05$. The estrous response was 95% (19/20) and natural breeding was carried out at two (2/19; G-control and G-P4), three (16/19; G-control and G-P4), or four (1/19; G-P4) times. All ewes in estrus showed at least one viable CL at D13. P4 was similar ($P>0.05$) on D9 (1.0±0.2 *vs.* 1.9±0.7 ng/mL) and D13 (1.1±0.4 *vs.* 1.6±0.5 ng/mL) but differed on D17 (4.0±1.3 *vs.* 9.2±2.8) for G-Control and G-P4, respectively. CL regression was similar ($P>0.05$) in G-Control (44.4%) and G-P4 (33.3%) ewes. The difference in average recovery rate was not significant ($P>0.05$) and the number of ova/embryos recovered was inferior ($P<0.05$) in G-Control (24.5 ± 10.6% and 3.7 ± 2.0) than in G-P4 (52.7 ± 10.5% and 11.6 ± 2.9) ewes, respectively. The embryo viability rate was similar ($P>0.05$) to G-Control (9.1% or 2/22) and G-P4 (23.5% or 19/81). Altogether, these data suggest that the reinsertion of the intravaginal progesterone implant for four days after superovulation promotes greater P4, resulting in a greater number of ova/embryos recovered.

Keywords: corpus luteum, estrous synchronization, NSER

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Ovum Pick Up and In Vitro Production of bufallo embryos on Marajó Island

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Abstract

Reproductive biotechnologies, when applied to the buffaloes species, do not present the same efficiency observed in cattle and with little commercial application when compared to other animal species. These facts can be explained by the characteristics of this species, such as seasonal reproduction, difficulty in detecting estrus, and difficulty in selecting bulls to obtain semen for in vitro fertilization, from which arises the need to seek improvements for the application of these biotechnologies in this species. The objective of this study was to investigate the reproductive seasonality in buffaloes in Marajó during the favorable and unfavorable reproductive periods and its effects on oocyte uptake (OPU) and in vitro embryo production (IVP). The experiment was conducted at the Laboratory of In Vitro Embryo Production, EETEPA, Salvaterra- PA. The collections were performed in a farm located in the municipality of Cachoeira do Arari- PA, both located in Marajó Island, from January 2021 to January 2022. For the OPU sessions, 40 Murah breed bubaline females of reproductive age were selected and divided into two groups according to their Body Condition Score (BCS): G1 (BCS ≤ 3) and G2 (BCS > 3). The reproductive-related parameters of the animals (number of follicles aspirated and oocytes collected) were evaluated according to the season (favorable-rainy and unfavorable-dry). The collected cumulus oocyte complexes (COCs) were used for IVP (maturation, fertilization, and in vitro culture), and the blastocyst formation rate was evaluated on the seventh day of culture. In the rainy season, we obtained mean values of 11.23±1.39 follicles and 5.96±1.56 COCs per animal, which were not statistically different from those in the dry season ($p > 0.05$): 11.74±1.04 and 7.27±0.83, respectively. In the wet season, we found that the mean number of aspirated follicles, aspirated COCs, COCs for IVF, and viable COCs were not statistically different in terms of the presence of corpora lutea and dominant follicles ($p > 0.05$). With respect to BCS, there was no significant difference ($p > 0.05$) between the variables analyzed (number of aspirated follicles, number of aspirated COCs, number of COCs for IVF, and number of viable COCs). The blastocyst formation rate was higher in the favorable rainy period (25.25%±0.64%) than in the unfavorable dry period (19.37%±14.06%), although we observed a large individual variation reflected in the standard deviation. This suggests that bubaline females are not affected by dry and rainy periods in their reproductive variables, but that variation in blastocyst formation rate may occur and that reproductive biotechnologies can be applied to bubaline species in the Marajó region.

Keywords: buffalo, reproductive seasonality, buffalo embryo.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Comparison of the antioxidant role of cysteamine and *Citrus sinensis* essential oil during *in vitro* maturation of bovine oocytes

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Financial support: CNPq and CAPES.

Abstract

During *in vitro* maturation (IVM) of bovine oocytes, cysteamine (CYS) has been used to reduce oxidative stress by reducing or inhibiting reactive oxygen species (ROS), aiding in the synthesis of glutathione (GSH). Although CYS is promising, the use of natural antioxidants in IVM medium may be a lower-cost alternative for reducing the negative effects of ROS. Biochemical assays have shown an antioxidant action of the essential oil of *Citrus sinensis* peel (EOCS), and its effect on gametes has not yet been evaluated. Therefore, the aim of the study was to compare the role of EOCS and CYS during IVM of bovine oocytes regarding their effects on MII rates, bioenergetic status and GSH levels. EOCS was extracted by hydrodistillation of the peels using a Clevenger-type apparatus. The chemical composition of EOCS was performed using a gas chromatography coupled to mass spectroscopy. EOCS presented as constituents D-limonene (48.5%), α-terpineol (40.2%) and other compounds (11.3%). Then, ovaries were recovered from a local slaughterhouse. The *cumulus*-oocytes complex (COCs) was recovered by follicular aspiration, and oocytes were categorized by stereomicroscope for the number of *cumulus* cell layers and cytoplasmic homogeneity. The COCs were transferred to an IVM medium composed of TCM199 with 0.2 mM of sodium pyruvate, 10% of fetal bovine serum, 1% of antibiotic-antimycotic and 20 µg/mL of FSH/LH. Furthermore, 100 µM CYS or 10 µg/mL (EOCS10) or 30 µg/mL (EOCS30) EOCS was added to the IVM medium. Finally, the groups were matured for 24 h at 38.5 °C and 6.5% CO_2. After IVM, oocytes were evaluated for MII, bioenergetic status and GSH levels. In the MII rates, oocytes were stained by Hoescht 33342 in ten repetitions. For bioenergetic status and GSH levels, oocytes were stained with MitoTracker Red® (CMXRos) for mitochondrial membrane potential and Cell Tracker Blue (CMF_2HC), respectively, in eight repetitions. All data are expressed as the mean ± standard error and analyzed using the StatView 5.0 software ($P < 0.05$). Mitochondrial membrane potential and GSH levels were altered with arcsine and analyzed by ANOVA followed by a Tukey test. MII rates were compared with a chi-squared test. A total of 258 ovaries were used to acquire 1053 viable immature oocytes (4.0 viable oocytes/ovary). No difference was observed in the IVM rates obtained from MII among EOCS10 (75.7% ± 3.3; 112/146), EOCS30 (74.2% ± 4.0; 108/143), and CYS (75.5% ± 6.8; 125/163). Moreover, EOCS10 (1.24 ± 0.77) and EOCS30 (1.12 ± 0.27) maintained GSH levels similar to CYS (1.00 ± 0.22). Probably, EOCS did not stimulate GSH synthesis because it required a higher concentration of the essential oil than what was used in this study. No difference was observed in the bioenergetic status evaluated by mitochondrial membrane potential among EOCS10 (0.86 ± 0.25), EOCS30 (0.90 ± 0.37), and CYS (0.87 ± 0.22). This parameter shows that although the EOCS did not lower mitochondrial membrane potential, EOCS did not have any toxic effect on the oocytes. In summary, bovine oocytes matured in the presence of EOCS at the concentrations evaluated showed a similar response to MII and an antioxidant role when compared to oocytes matured with CYS. Although we observed no difference among the groups evaluated, this demonstrates that EOCS did not act as a toxic component, as it did not interfere with the final development of these cells.

Animal Reproduction

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PI3K/AKT pathway evaluation in granulosa cells from antral follicles

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Abstract

The PI3K/AKT pathway is modulated during primordial follicle activation until to reach the primary stage (1). During the antral phase, gonadotropins are important for follicular growth, and in particular LH is involved with stimulates steroid release, dominant follicle selection, ovulation and luteinization process in granulosa cells (GCs). However, information about how the PI3K/AKT pathway acts during folliculogenesis in the antral follicle and if LH activates this pathway are scarce. We aimed identify the effectors' role of the PI3K/AKT signaling pathway in the antral follicle and during ovulation process on GCs from antral follicles. For this, in the experiment 1 follicles of different sizes (3-5; 5.1-7 and 7.1-9 mm) were collected from bovine ovarian obtained from a slaughterhouse in stages 2 and 3 according (2). Follicles were individually measured and dissected in Petri dishes. GCs were centrifuged at 300g during 10 min and the pellet was frozen (-80°C) for gene expression analysis. In the experiment 2, GCs were collected from follicles with ≥10 mm through repeated flushing with DMEM/F12 (Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham) supplemented with 0.1% bovine serum albumin (BSA), penicillin (100 IU/ml), streptomycin (50 µg/ml) and heparin (Sigma). GCs were washed twice by centrifugation at 600×g for 10 min each and filtered through a 150 mesh (Sigma). Cell viability was estimated with 0.4% Trypan blue. Cells were seeded into 24 well tissue culture plates (KASVI) at a density of 0.5×10^6 viable cells per well in 1 ml of culture medium and cultured for 6 h at 38.5 °C and 5% CO_2 in DMEM/F12 supplemented with 0.1% BSA, insulin (10 ng/ml), penicillin (100 IU/ml), streptomycin sulfate (50 µg/ml), FSH (1 ng/ml) and androstenedione (10^{-7}mM). Cells were culture for 6 h with DMSO (0.25µg/mL), DMSO (0.25µg/mL) and LH (100 ng/mL), DMSO (0.25µg/mL) and LH (400 ng/mL) using a serum-free non-luteinizing GCs culture system as describe by (3) with modifications. The GCs from experiment 2 (n=3) were collected and frozen (-80°C). GCs from the two experiments were submitted to total RNA extraction according to Qiazol® (Qiagen) protocol, with an RNA co-precipitant (GlycoBlue®; ThermoFisher Scientific), treated with DNase Amplification Grade (Invitrogen, Brazil) followed by the reverse transcription using the High-Capacity Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). For experiment 1, the RT-qPCR analysis of *PTEN*, *PI3K*, *AKTT*, *BAX*, *BCL-2*, *CREB*, *BRCA*, *CYP19A1*, *LHR*, *FSHR*, and *ADAMTS1* were performed. For experiment 2, the same genes were performed except for *BAX*, *BCL2*, *FOXO3a*. The transcripts were performed using the GoTaq® qPCR Master Mix (Promega), according to the manufacturer›s instructions for experiment number 1 and 2. The relative expression of the genes related to PI3K/AKT pathway (*PTEN*, *PI3K*, *AKTT*, *FOXO3a*, *BAX*, *BCL-2*, *CREB*, and *BRCA*) and genes related to the GCs steroidogenesis (*CYP19A1*, *LHR*, *FSHR*, and *ADAMTS1*) was analyzed. 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 ANOVA followed by Tukey test (P<0.5) (JMP; Software). The results from experiment 1 demonstrated that the relative expression differed for antral follicle sizes for *PI3K*, *CYP19A1*, and *LHR*. The PI3K relative expression test increased in GCs from larger antral follicles (5.1-7 and 7.1-9 mm) compared to the GCs from small antral follicles (3-5mm; P = 0.031). As expected, mRNA abundance of *CYP19A1* (P <0.001) and *LHR* (P = 0.002) increased in GCs from large antral follicles with (7.1-9 mm) compared to GCs from smaller follicles sizes (3-5 and 5.1-7mm). In the experiment 2, the relative mRNA abundance of all evaluated genes was similar between groups. Based on these results, PI3K pathway seems to be differently modulated throughout follicular growth in cattle. Also, surge LH may have no influence in modulating PI3K/AKT pathway in GCs from pre-ovulatory follicles culture in vitro within 6 h of culture. However, further investigation is required to understand if PI3K/AKT is directly or indirectly modulated by LH.
Funding: FAPESP 2021/06645-0; 2020/13075-2 and CAPES finance code 001.

References

(1) MAIDARTI, M.; ANDERSON, R.A.; TELFER, E.E. Crosstalk between PTEN/PI3K/Akt Signalling and DNA Damage in the Oocyte: Implications for Primordial Follicle Activation, Oocyte Quality and Ageing. ***Cells*** 2020, *9*, 200. https://doi.org/10.3390/cells9010200. (2) IRELAND, J. J.; MURPHEE, R. L.; COULSON, P. B. Accuracy of Predicting Stages of Bovine Estrous Cycle by Gross Appearance of the Corpus Luteum. Journal of Dairy Science, 1980. https://doi.org/10.3168/jds.S0022-0302(80)82901-8 (3) FERST, J.G.: ROVANI, M.T.; DAU, A.M.P.; GASPERIN, B.G.; ANTONIAZZI, A. Q; BORDIGNON, V.; OLIVEIRA, D. E.; GONÇALVES, P. B.D.; FERREIRA, R. Activation of PPARG inhibits dominant follicle development in cattle, Theriogenology,v. 142,2020,p. 276-283. https://doi.org/10.1016/j.theriogenology.2019.10.032.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

EVALUATION OF GENES EXPRESSION OF CARBOHYDRATES METABOLISM CUMULUS-OOCYTE BUFFALO'S COMPLEXS MATURED IN HIGH AND LOW OXYGEN CONCENTRATION

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Financial support: FAPESPA, UFPA.

Abstract

Buffalo breeding has become an important activity in Brazilian livestock, a fact well related to technological advances, such as *In vitro* Embryo Production (IVP). However, there are still some limitations of IVP in the buffalo species, such as the quality of oocytes produced and the low rates of blastocysts. In this sense, ideal conditions to achieve a cellular metabolism more similar to *in vivo* conditions drive studies on metabolism in oocyte maturation. It is known that *in vitro* culture (IVC) at low oxygen tension (5%) improves the quality of bovine embryos *in vitro*, which may be related to a regulation of genes involved in anaerobic glycolysis, where glucose is converted to lactate (Warburg effect), as lactate dehydrogenase A (*LDHA*). Another gene related to this hypoxia condition is the hypoxia-inducible factor 1 (*HIF1A*), already observed in porcine and mouse oocytes. In this sense, the present work aims to evaluate the expression of some genes involved in carbohydrate metabolism and that are related to the Warburg Effect during IVM of buffalo *cumulus*-oocyte complexes (COCs), under high and low concentrations of O_2. For this, 120 COCs were divided into the following experimental groups: G1: COCs matured in 20% O_2; G2: COCs matured in 5% O_2; and G3: immature COCs. After 24 hours of maturation, the oocytes were separated from the *cumulus* cells and stored in RNA later in pools of 40 cells for analysis of gene expression and later processed for extraction of total RNA by TRIzol®. The cDNA was obtained using the High Capacity kit (Applied Biosystems). The gene expression analysis was performed by rt-PCR, using SYBR Green through the ΔΔCT method, to analyze the *HF1A* and *LDHA* genes and the citrate synthase (*CS*) gene, an enzyme of the Krebs, in addition to the endogenous gene glyceraldehyde-3-phosphate (*GAPDH*). Data were analyzed by ANOVA and Fisher's post-test, adopting a significance level of 5%, using SigmaPlot® 11.0 software. According to the data obtained, expression of target genes could not be observed in oocytes. Regarding gene expression in *cumulus* cells, there was no significant difference for the expression of the *HIF1A* gene between the groups ($p>0.05$), but a significant difference was found for the expression of the *CS* gene ($p<0.05$), with more expressed in the group under low O_2 tension. We also verified that the expression of the *LDHA* gene ($p<0.05$) was higher in the group matured under high O_2 tension. Based on our results, it is suggested that the activity of *CS* and *HIF1A* gene expression acts on the energy maintenance of buffalo COCs from *cumulus* cells, which capture glucose and trigger a series of reactions to maintain oocyte homeostasis. The expression of *LDHA* seems to be related to oxygen tension during reactions important for oocyte maintenance, since the increase in its expression may be related to the production of biomolecules, such as nucleic acids. However, we also need to evaluate the rates of blastocyst formation referring to different oxygen concentrations during IVM.

Keywords: Buffalo; carbohydrate metabolism; oocytes.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

THE EFFECT OF AI SHEATHS ON THE PREGNANCY RATE OF DAIRY CATTLE

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Abstract

The profitability of cattle farming depends on the success of reproductive performance among the animals, when an adequate reproductive performance is not achieved, it can result in great economic losses to the producers. The search for tools is constant to increase pregnancy rates in cattle, one of them is the use of sanitary Sheaths in the Artificial Insemination (AI), to reduce the probability to bring to the uterus external contamination that might compromising the conception rate. The objective of this study was to evaluate the pregnancy rate of dairy cows inseminated with and without the use of AI Sheaths. For that, we used 823 multiparous cows distributed in five farms located in the state of RS/Brazil from April/August of 2022. The animals were randomly separated into two experimental groups. G1 - Insemination without the use of AI Sheaths (N= 449); and G2 inseminated with the use of AI Sheaths (N= 374), all the animals were inseminated by the same veterinarian. Both groups underwent the FTAI protocol with the use of intravaginal implant, and pregnancy detection was conducted on day 30, using the ultrasound (EASISCAN GO - IMV Imaging Scothland); Statistical analyzes were performed using the X^2 test in the R statistical environment. The pregnancy rate in G1 was 34% while the animals from G2, the pregnancy rate was 44%. No statistical difference was observed among the treatments at 30 days of pregnancy at the first AI ($P>0.05$). However, even not observing an statistical difference among treatments, an increase of 10% of pregnancies were achieved, representing 30 additional calves being born as a result in use of the sanitary Sheaths, which alone justifying its use.

Acknowledgements

INOVAVET, IMV – Technologies do Brasil, University of São Paulo, University of Passo Fundo

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

MACROSCOPIC AND MICROSCOPIC ANALYSIS OF PLACENTA FROM CATS 36 TO 48 DAYS OF GESTATION

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Abstract

Cats have an incomplete placenta zonaria (1), composed of the placental girdle or ring where the chorionic villi are located and remain attached to the endometrium. In the establishment of trophic relations, the chorion invades the endometrium until it reaches the uterine vessels to establish trophic relations. Adequate placental vascularization allows proper fetal development and is crucial for gestational success. Fourteen animals at gestational stages between 36 and 48 days were used. Macroscopic and microscopic morphological analyses were performed, including photographic documentation, processing and Hematoxylin and eosin, Picrosirius, Toluidine blue and Schiff's periodic acid staining. The samples were analyzed for differences in gestational phases based on the literature described. Between 36 and 48 days, respectively, hemorrhagic areas were observed along the uterine surface and placenta, with a higher frequency at the final stage of development. Macroscopically, the hemorrhagic areas increased proportionally with advancing gestation, indicating that blood leakage intensifies throughout fetal development. Biochemical analyses of the hemophagic region of the cat placenta, characterized by intense erythrophagocytosis showed low acid phosphatase enzyme activity (44%) and high values of cathepsin D activity (> 300%) relative to non-phagocytic areas of the placenta (2). In this study, erythrophagocytic activity was also observed in the cells bordering the allantoid, in addition to intense blood vessel formation in the region where the syncytiotrophoblast advances toward the maternal vessels. Tertiary chorionic villi, with well-defined syncytiotrophoblast and cytotrophoblast layers and increased vascular caliber in the chorionic villi, were observed in the placenta of cat between 36 and 48 days of gestation. Together these data indicate increased erythrophagocytic activity and greater blood supply to the placenta in late gestation, emphasizing the presence of different mechanisms to meet the fetal metabolic demands that intensify throughout gestation.

References

1 – AMBRÓSIO, C.E.; MIGLINO, M.A.; LIMA, A.R.; MARTINS, A.S; ASSIS-NETO, A.C.; BONATELLI, M.; Flávia Thomaz VERECHIA, F, T.V.; CARVALHO, A.F.; Fissura placentária de gatas SRD, *Felis catus* – Linnaeus, 1758. Aspectos macro e microscópicos. Brazilian Journ al of Veterinary Research and Animal Science, 2004. v. 41: p. 81-85.

2- MINAZAKI, C.K., GAGIOTI, S., ZAGO, D., TERRA, W., ARAUJO, V.C., OLIVEIRA, R.A. and BEVILACQUA, E. Acid phosphatase and cathepsin D are active expressed enzymes in the placenta of the cat. Research Veterinary Science, 2008. v. 84: p.326-334.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Gene-editing in bovine mammary gland cells as a model for recombinant protein production

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Abstract

The production and commercialization of recombinant proteins is an ever-expanding multi-billion dollar market. Due to the limitations of production models in bacteria and yeast, alternative methods, such as those based on mammalian cells, have emerged. One of the most promising approaches in this sense is to produce recombinant proteins in the mammary glands of bovines taking advantage of all the molecular machinery of protein expression in the milk of these animals to produce proteins of commercial and biomedical interest. In this context, the present study has the knockout (KO) of the *CSN2* and *LGB* genes in a bovine model by CRISPR/Cas9 technology as the primary objective. The cells being edited are a commercial immortalized cell line from bovine mammary gland alveolar tissue, called MAC-T (1), which are cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, insulin (5 ug/mL) and penicillin-streptomycin (0,5%) at 37° C and 5% CO_2. All the CRISPR constructions are being done in the plasmid pSpCas9(BB)-2A-GFP (PX458) from Addgene, the guide RNAs (gRNAs) were designed with the CRISPOR online tool (2) and all transfections done by Lipofectamine 3000 (Invitrogen) (3). Initially, standardization of all steps of gene editing strategies - design of the guide gRNAs, construction of the plasmid vectors, transfection tests, cell sorting - were performed. After the plasmids sequencing results, MAC-T cells will be transfected with a combination of plasmids containing CRISPR constructions aiming at different introns and exons of our target genes' and sorted by flow cytometry to generate clones. These clones will be cultured, selected, and evaluated by Sanger sequencing for subsequent indels frequency analysis by the Synthego ICE tool as KO validation. With this KO lineage established and tested we intend to introduce a short non-coding sequence in the *CSN2* and *LGB* genes' loci to validate the technique and as a proof of concept. This proposal can potentially generate a new model of recombinant protein production by allowing the introduction of transgenes of commercial and therapeutic interest in bovine mammary glands.

References

(1) Huynh H.T., Robitaille G., Turner J.D. Establishment of bovine mammary epithelial cells (MACT): an in vitro model for bovine lactation. Exp. Cell Res. 197:191-199 (1991).

(2) Concordet JP, Haeussler M. CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. Nucleic Acids Res; 46(W1):W242-W245. (2018).

(3) Oliveira, V.C.D., Moreira, G.S.A., Bressan, F.F., Junior, M., Gomes, C., Roballo, K, C. S. Ambrósio, C. E. Edition of TFAM gene by CRISPR/Cas9 technology in bovine model. PLoS One, 14(3), 1-14. (2019).

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Influence of heat stress on bovine luteal cells culture treated with embryo conditioned medium at Day 7 of embryonic development

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Abstract

Heat stress (HS) directly influences the reproductive performance of cattle herds. It is characterized by the inability of animals to thermoregulate. HS is an important factor that impairs ovulation, fertilization, and early embryonic development (1). *In vitro* produced embryos secrete miRNA cytokines, vesicles in the culture medium that may influence maternal-embryonic communication (2,3). Furthermore, our previous studies revealed *in vitro* produced embryos under HS have less IFNT when compared to non-HS embryos (4). Consequently, HS alters embryo IFNT secretion in the conditioned medium. Thus, our hypothesis is that conditioned medium from HS produced embryos alters the signaling in bovine luteal cells culture. We are investigating the influence of HS on the communication of *in vitro* produced embryos to luteal cell culture. Therefore, our objective is to evaluate the ISGs, angiogenesis, cell survival and steroidogenic genes on luteal cells primary culture treated with heat stressed embryo conditioned medium. The primary culture of bovine luteal cells was performed using corpus luteum (CL) collected in slaughterhouses. The luteal cells were dissociated and seeded in 60mm plates (24 wells) in culture for 24 hours at 37ºC. After this period, the cells were treated with 60% DMEM medium and 40% embryo conditioned medium for 6 hours. Six groups with different treatments were assessed. The embryo conditioned medium was collected from stressed embryos at different timepoints of embryonic development: control group without embryos, embryo control group without HS, group of Oocyte Cumulus Complex (OCC's) stressed at maturation; group of OCCs stressed in fertilization; group of zygotes stressed on the first day of *in vitro* embryo culture, and the group stressed at all stages of *in vitro* embryo production (IVP). After 6 hours of culture, the medium and cells were collected and stored at –80ºC. Total RNA was then extracted using PureLink™ RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (100ng) was reverse transcribed (RT) using the iScript™ cDNA Synthesis Kit (Bio Rad, Des Plaines, IL, USA). It was evaluated the expression of steroidogenic enzymes (P450sCC, StAR and 3βHSD), interferon stimulated genes (ISG15, OAS1, MX1 and MX2), angiogenesis-related gene VEGF, and cell survival genes AKT and XIAP. Luteal cells presented 75% viability (trypan blue). Also, the expression of genes related to steroidogenesis (StAR, 3βHSD, P450sCC) presented no difference in all treated groups, these results validate the luteal culture model. The relative expression of ISG15 mRNA increased in the embryo control when compared to the no embryo control. All the heat stressed treatments had the is expression similar to the no embryo control group ($p<0.05$). The expression of OAS1 presented no difference in the negative and positive embryo control and the IVM heat stressed group. However, the IVF, IVC and IVM+IVF+IVC heat stressed groups had a decrease in the expression. Interestingly, the MX1 and MX2 presented the same pattern as the ISG15 expression. The expression of VEGF, XIAP and AKT had no difference in the no embryo control and embryo control groups, and the same happens on the heat stress groups. In conclusion, luteal cells in culture for 6 hours do not alter their steroidogenic, angiogenic and survival genes when treated with conditioned medium from HS produced embryos. However, interferon stimulated genes decrease to the same levels as the no embryo control group in the HS-embryos groups. Further studies are ongoing to evaluate luteal cells culture response at 12 and 18 hours.

References

1. de Rensis F, Saleri R, Garcia-Ispierto I, Scaramuzzi R, López-Gatius F. Effects of heat stress on follicular physiology in dairy cows. Vol. 11, Animals. MDPI; 2021.

2. Bridi A, Bertolin K, Rissi VB, Mujica LKS, Glanzner WG, de Macedo MP, et al. Parthenogenetic bovine embryos secrete type I interferon capable of stimulating ISG15 in luteal cell culture. Anim Reprod. 2018;15(4):1268–77.

3. Bridi A, Andrade GM, del Collado M, Sangalli JR, de Ávila ACFCM, Motta IG, et al. Small extracellular vesicles derived from in vivo- or in vitro-produced bovine blastocysts have different miRNAs profiles—Implications for embryo-maternal recognition. Mol Reprod Dev. 2021 Sep 1;88(9):628–43.

4. Amaral CS, Koch J, Correa Júnior EE, Bertolin K, Mujica LKS, Fiorenza MF, et al. Heat stress on oocyte or zygote compromises embryo development, impairs interferon tau production and increases reactive oxygen species and oxidative stress in bovine embryos produced in vitro. Mol Reprod Dev. 2020 Aug 1;87(8):899–909.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Pregnancy rate, serum progesterone, and volume luteal tissue of beef cows treated with hCG 5 days after FTAI

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Abstract

This research aimed to evaluate the effect of administration of Human Chorionic Gonadotropin (hCG) 5 days after fixed-time artificial insemination (FTAI), on pregnancy rate, progesterone (P4) serum concentration, and the volume of corpus luteum (CL) on Days 18 and 25 of pregnancy. It was hypothesized that the hCG contributes to the supply of P4 during early pregnancy and luteal characteristics during the pre-attachment period. For this, non-lactating cross bread cows (n=65) from a beef farm were used in the study. All cows were submitted to estrous cycle synchronization protocol. At to the moment of FTAI, on day zero (D0), cows were randomly assigned to the experimental groups: the hCG group (hCG; n=37) and Control group (C; n=28). Five days after FTAI (D5), the cows of hCG group received a single dose of 2.500UI of hCG (Chorulon®, MSD Animal Health, I.M). Half of the cows in each group were slaughtered on two different days: at 18 (D18) and 25 days (D25) of gestation. After slaughter, the uteri were dissected, and both ovaries were removed. The luteal tissue was dissected free from the stroma and weighed, and measurements of height, width, and depth of the CL were taken. The volume of CL was calculated by $V=4/3\times\pi\times R^3$, and R was obtained through the circumference and area measurements of the CL. The concentration of P4 was determined in serum by chemiluminescent assay kit (ADVIA Centaur, Siemens) on Days 5, 14, and 22 following FTAI. The administration of hCG resulted in greater serum progesterone concentration in hCG compared to C on D14 (14.57 ± 1.09ng/ml and 22.36 ± 1.38ng/ml) and D22 (7.18 ± 1.96ng/ml and 14.17 ± 1.85ng/ml; C and hCG, respectively). There was no difference between the pregnancy rate of the cows in C (46.42% - 13/28) compared to the hCG (51.35% - 19/37). The weight (wCL) and volume (vCL) of the CL also did not differ between the groups, on D18 and D25 of pregnancy (wCL D18: 3.59 ± 0.40g and 3.46 ± 0.34g, D25: 3.77 ± 0.51g and 3.58 ± 0.42g; vCL D18: 2.83 ± 0.43 cm^3 and 3.07 ± 0.34cm^3, D25: 3.46 ± 0.48 cm^3 and 3.49 ± 0.39 cm^3; C and hCG, respectively). The increase in serum P4 is associated to the accessory CL, as the cows in the hCG had a 59.45% accessory CL formation rate (22/37), while none of the cows in the C had accessory CL (0/28). Furthermore, accessory CL of hCG cows had greater weight on D18 than on D25 (4.15 ± 0.43g and 2.64 ± 0.46g, D18 and D25, respectively). This is also confirmed by the fact that the volume of the original CL did not differ between groups in both days. However, when we evaluated the total mass of luteal tissue, on D18, the cows of the hCG had greater luteal mass compared to the C (3.48 ± 0.61g and 6.73 ± 0.52g; C and hCG, respectively). It was concluded that administration of hCG on Day 5 after FTAI was efficient to increase the concentration of serum progesterone through inducing accessory corpus luteum and an increase in total luteal mass but had no effect on the conception rate. Future analysis will be carried out to verify the molecular and morphological characteristics of the original CL and the accessory CL after the administration of hCG.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effect of heat stress on expression of genes involved in double-stranded DNA repair from in vitro-produced bovine embryos

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Abstract

Heat stress (HS) represents one of the most important causes of bovine productive and reproductive losses, especially in dairy industry. It also impairs reproduction rates, immune defenses and induces the occurrence of oxidative stress. In vitro-produced bovine embryos demonstrated developmental inhibition when exposed to temperatures considered as heat-stressed cows in vivo, reducing cleavage and blastocyst rates. Our hypothesis is that heat stress alters expression of genes involved in double-stranded DNA repair from in vitro-produced bovine embryos. Therefore, the aim of this study is to evaluate induced heat stress effects on mRNA expression of *BRCA1, KU70* and *RAD51* from in vitro-produced bovine embryos. The experiment was divided into five groups: (1) Control; (2) Oocytes matured under HS conditions (IVM HS); (3) Oocytes fertilized under HS conditions (IVF HS); (4) Zygotes cultured on the first day under HS conditions (IVC HS); and (5) Embryos submitted to HS during 3 days of embryo production (IVM+IVF+IVC HS). For HS treatments, the temperature was gradually increased until 40.5ºC, and remained for 6h. Bovine ovaries were obtained from slaughterhouse. Oocytes were aspirated from follicles 3-8mm in diameter and matured for 22h under 5% CO2 atmosphere. Matured oocytes were fertilized using spermatozoa selected with Percoll® gradient. Spermatozoa and matured oocytes were co-cultured in Fert medium supplemented for 18h under 5% CO2 atmosphere. IVF day was considered as day 0 of embryo production. After 18h of IVF, zygotes had cumulus cells removed by 2 minutes of vortex. Zygotes were cultured in SOF medium under 5% CO2, 5% O2 and 90% N2 up to 54 hours post insemination (hpi) and then divided in 2-cell, 4-cell or 8- to 16-cell embryos. The study was completed in three replicates. Data were statistically analyzed using JMP Software (13.1.0; SAS Institute Inc.). There is no difference in cleavage rates among groups ($p>0.05$). However, when mRNA was analyzed, our data revealed the findings: only on IVM+IVF+IVC HS group, *BRCA1* and *RAD51* were downregulated on 8- to 16-cell, compared to 4-cell embryos ($p<0.05$). The *KU70* was not different among groups ($p>0.05$). The first approach of our study led us to know that although we had no differences in the development rates of embryos cleaved at 54 hpi, embryos submitted to HS for 3 days have a diminished DNA repair mechanism. Within the IVM+IVF+IVC HS group, comparing the number of cells, it was expected that the 8- to 16-cell group had higher expression of *RAD51* and *BRCA1*, but the expression of these genes was lower in this group. We believe that the damage caused by exposure to HS in this group was severe to the point that these embryos were unable to activate DNA repair pathways. Another possible explanation is that the higher expression of *RAD51* and *BRCA1* in the 2-cell and 4-cell groups is of maternal origin, since the 8-to 16-cell group is already in the zygotic maternal transition. More studies will be carried out to try to elucidate these results. It was concluded that when the embryos were submitted to HS during 3 days of initial development, DNA repair mechanisms are decreased, although they should be increasing due to HS.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

FIBROBLAST GROWTH FACTOR 18 (FGF 18) MODULATES THE EXPRESSION OF GENES OF THE HIPPO SIGNALING PATHWAY IN BOVINE GRANULOSA CELLS IN VITRO

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This work was funded by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Abstract

Fibroblast growth factor 18 (FGF18) is a protein expressed in the ovarian follicle, more specifically in theca cells (TC) and granulosa cells (GC) [1]. The Hippo signaling pathway is an intracellular signaling pathway involved in the regulation of key cellular processes such as proliferation, apoptosis and differentiation, which results in organ size. It has been widely studied associated with follicular dynamics. Its action occurs through the protein, Yes-associated protein 1 (YAP). When the pathway is active, they phosphorylate and are retained in the cytoplasm of cells, and when deactivated, they translocate to the cell nucleus, where they bind with transcription factors of the TEAD family and express the main target genes of this pathway: connective tissue growth factor (CTGF) and cysteine-rich angiogenic inducer 61 (CYR61). Recent data suggest that, like FGF18, the Hippo signaling pathway is also linked with estrogen secretion, as when GCs are treated with follicle stimulating hormone (FSH), estrogen secretion increases due to follicular growth and decreases CTGF expression. Thus, the present study aimed to evaluate whether FGF18, through the reduction of steroidogenesis, regulates the expression of the Hippo signaling pathway effectors in bovine's GC *in vitro*. To understand the regulation of Hippo pathway genes and gene related to the apoptosis process in bovine granulosa cells, we used a serum-free granulosa cell culture system, which maintains a phenotype of steroidogenic cells with minimal luteinization [2, 3] [2, 3]. The GCs were isolated from follicular fragments obtained by dissection of the follicles and subsequent washing in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F12). Then, these cells were centrifuged and seeded in a 24-well culture plate containing 1mL per well. The culture was maintained at 37°C in an atmosphere of 5% CO2 for 5 days, with two changes of 70% of the medium. On the fifth day, treatments at doses of 0,1, 10 and 100ng/mL of FGF18 were added and collected after 24 hours. To measure mRNA expression, total ribonucleic acid (RNA) extraction and qPCR were performed. The addition of FGF18 decreased the abundance of mRNA encoding CTGF and CYR61, while steroidogenic acute regulatory protein (STAR), YAP and ankyrin repeat domain 1 (ANKRD1) showed no significant difference in their expression. The presence of FGF18 also significantly decreased the expression of glutamine-fructose-6-phosphate transaminase 2 (GFPT2), BCL2-associated protein x (BAX), B-cell lymphoma 2 (BCL2) and aromatase cytochrome P450 (CYP19). Treatment with different doses of FGF18 reduced the expression of the target genes of the Hypo signaling pathway, CTGF and CYR61. This fact may be due to the duration of treatment with FGF18 (24h), which implies the phosphorylation and translocation of YAP that is stimulated by FGF18, as observed in recent studies[4]. In conclusion, FGF18 decrease mRNA of genes induced by the Hippo pathway and genes related to the apoptosis process in cultured granulosa cells.

References

1. Portela VM, Machado M, Buratini Jr J, Zamberlam G, Amorim RL, Goncalves P, Price CA. Expression and function of fibroblast growth factor 18 in the ovarian follicle in cattle. Biology of reproduction 2010; 83:339-346.

2. Sahmi M, Nicola ES, Silva JM, Price CA. Expression of 17beta- and 3beta-hydroxysteroid dehydrogenases and steroidogenic acute regulatory protein in non-luteinizing bovine granulosa cells in vitro. Mol Cell Endocrinol 2004; 223:43-54.

3. Machado MF, Portela VM, Price CA, Costa IB, Ripamonte P, Amorim RL, Buratini J, Jr. Regulation and action of fibroblast growth factor 17 in bovine follicles. J Endocrinol 2009; 202:347-353.

4. Tong G, Chen X, Lee J, Fan J, Li S, Zhu K, Hu Z, Mei L, Sui Y, Dong Y, Chen R, Jin Z, et al. Fibroblast growth factor 18 attenuates liver fibrosis and HSCs activation via the SMO-LATS1-YAP pathway. Pharmacol Res 2022; 178:106139.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Blocking YAP-TEAD transcriptional activity alters mRNA expression of ISGs in luteal cells *in vitro*

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Abstract

The Hippo signaling pathway is highly conserved between species and presents well-defined roles in regulating organ size, cell differentiation, proliferation, and apoptosis in different tissues (1,2). However, previous studies suggested the effect of IFNT-related signaling in the YAP activity in pregnant ruminants (3,4). The corpus luteum (CL) is responsible for progesterone synthesis, maintaining endometrial functions, early embryonic development, and successful fetal and placental development (5). In ruminants the secretion of Interferon tau (IFNT) by the trophectoderm is necessary during the peri-implantation period of pregnancy for maternal recognition of pregnancy signaling. The expression of interferon stimulated genes (ISGs) during early pregnancy was shown also in extrauterine tissues, like CL (6). Therefore, the main hypothesis of this study is the Hippo signaling pathway is involved in the IFNT signaling during early pregnancy to the CL. For this reason, the objective was to determine the effect of roIFNT on Hippo pathway target genes in the absence or presence of Verteporfin (VP) - inhibitor activity of YAP–TEAD interactions on luteal cells (LC). The primary culture of bovine LC has been performed from CL collected in a slaughterhouse. The cells were dissociated and placed in 60mm plates (24 wells) in culture for 24 hours at 37ºC with DMEM-F12 presenting 75% viability (Trypan blue). After this period the medium was replaced and LC were treated according to the following groups: Control group (CG – 500uL DMEM-F12), group 1 (G1 – 1ng/mL of roIFNT), group 2 (G2 - 1ng/mL of roIFNT + 0,1 uM of veterporfin (VP)), group 3 (G3 – 1ng/mL of roIFNT + 1,0 uM of VP), group 4 (G4 – 0,1 uM of VP) and, group 5 (G5 – 1,0 uM of VP). All treatments were done for 6 hours. After 6 hours of culture, the medium and cells were collected and stored at –80ºC for further analyses. Total RNA was extracted using PureLink™ RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (100ng) was reverse transcribed (RT) using the iScript™ cDNA Synthesis Kit (Bio-Rad, Des Plaines, IL, USA). It was evaluated the expression of steroidogenic enzymes (*P450sCC, StAR* and *3βHSD*), interferon-stimulated genes (*ISG15, OAS1, MX1* and *MX2*), and the Hippo signaling pathway target genes (*CTGF, CYR61, ANKRD1* and, *BIRC5*). The relative expression of *P450sCC* and *3βHSD* presented no difference ($P > 0.05$) in all groups, this result validates the luteal culture model. Surprisingly, the relative expression for *StAR* was greater ($P < 0.05$) in only VP-treated groups (G3 and G5). According to other studies in cumulus and granulosa cells (7). The treatment with roIFNT stimulated the expression of ISGs in the LC. The relative expression for all ISGs was greater in the G1 ($P < 0.05$), this result validates the pregnancy model. Surprisingly, when LC was treated with the VP the ISG's relative expression was lower, this result is interesting because shows that YAP-TEAD interaction is blocked the ISG's expression are altered according to the pattern of ISG's expression demonstrated in this study. As expected, *CTGF* relative expression was different among all groups ($P < 0.05$). The *CTGF* relative expression was altered by depending-dose of VP demonstrates that the treatment changed the transcriptional activity of YAP-TEAD on LC. The other target genes of the Hippo pathway did not present difference ($P > 0.05$) amongst treated groups. We conclude that blocked YAP-TEAD transcriptional activity decreases ISGs mRNA expression.

References

1. Yu FX, Guan KL. The Hippo pathway: Regulators and regulations. Vol. 27, Genes and Development. 2013. p. 355–71.

2. Meng Z, Moroishi T, Guan K-L. Mechanisms of Hippo pathway regulation. Genes Dev [Internet]. 2016 Jan 1;30(1):1–17. Available from: http://genesdev.cshlp.org/lookup/doi/10.1101/gad.274027.115

3. Kusama K, Bai R, Sakurai T, Bai H, Ideta A, Aoyagi Y, et al. A transcriptional cofactor YAP regulates IFNT expression via transcription factor TEAD in bovine conceptuses. Domest Anim Endocrinol [Internet]. 2016;57:21–30. Available from: http://dx.doi.org/10.1016/j.domaniend.2016.05.002

4. Aires K de V, dos Santos EC, da Silva AP, Zappe IG, de Andrade LG, Amaral C dos S, et al. Energy balance and hippo effector activity in endometrium and corpus luteum of early pregnant ewes. Reprod Fertil Dev [Internet]. 2022; Available from: https://doi.org/10.1071/RD22081

5. Spencer TE, Ott TL, Bazer FW. -Interferon: Pregnancy Recognition Signal in Ruminants. Exp Biol Med [Internet]. 1996 Dec 1;213(3):215–29. Available from: http://doi.wiley.com/10.1111/j.1600-0897.1997.tb00253.x

6. Godkin JD, Bazer FW, Roberts RM. Ovine trophoblast protein 1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein synthesis. Endocrinology. 1984;114(1):120–30.

7. Sun T, Diaz FJ. Ovulatory signals alter granulosa cell behavior through YAP1 signaling. Reprod Biol Endocrinol. 2019;17(1):9–12.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Does Phenazine Ethosulfate improve early embryo development?

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Abstract

Early porcine embryos need glucose through a correct balance between the pentose-phosphate pathway (PPP) in early cleavages and in subsequent glycolysis (1). However, *in vitro* procedures causes a shift in energy production towards glycolysis. The Phenazine Ethosulfate (PES) is an electron acceptor related to glucose production through the PPP by NADPH/NAD+ oxidation. Since the PPP is temporarily required during early cleavages, we hypothesized that a time-dependent exposure to PES would enhance embryo development. Sow ovaries were collected in a local abattoir and transported to the laboratory in warm saline solution within one hour, washed in the same solution, and kept warm during aspiration. Follicles with 3-6 mm diameter were collected with an 18G needle coupled in a syringe and deposed in a 50 mL tube. After settling for 10 min, the supernatant was removed, and the pellet was resuspended in TCM-HEPES containing 50 IU/mL of gentamycin, 10% FCS, and 3 mg/mL of BSA. After another 10 min-settling, cumulus oophorous-oocyte complexes were resuspended and selected in the same medium. Oocytes with homogenous cytoplasm and at least three layers of compacted cumulus oophorous cells were selected and rewashed in maturation media. Groups of 20 to 25 oocytes were matured in 90 µL droplets of TCM-199 (Thermo Fischer Scientific® 11150059) supplemented with 3.05 mM of D-glucose, 0.57 mM of cysteine, 0.91 mM of $C_3H_3NaO_3$, 50 IU/mL of both EGF and gentamycin, and 10% of porcine follicular fluid under mineral oil during 44h at maximum humidity, with 10 IU/mL of both eCG and hCG during the first 22h. After maturation, cumulus oophorous cells were removed by gentle pipetting, with oocytes being parthenogenetically activated by 5 min exposure to 15 µM of ionomycin, 15 min exposure to 200µM of TPEN, and four-hour exposure to 7.5 µg/mL of cytochalasin B. After activation, presumptive parthenotes were cultured in PZM 5 (2) until day 7 (D7), with feeding at day 5 (D5), by supplementing the media with 3 mg/mL of BSA. Treatments with 0.05 µM of PES (3) were conducted in five replicates (20 to 25 embryos per group). Structures were randomly distributed in four groups: Control (PZM 5), PES (0–48 h), PES (24–48 h), and PES (0–24 h). Embryos were evaluated for cleavage at 24 h and 48 h and the percentage of blastocyst stages was recorded at 168 h after activation. Data were analysed by ANOVA, followed by Tukey test for multiple comparisons. We found no difference among any evaluated treatments and stages ($p > 0.05$) as cleavage rates at 24h, at 48h, and total blastocysts for Control were 61,0%, 73,1% and 58,0%; for PES (0–48 h) were 54,5%, 70,0% and 43,6%; for PES (24–48 h) were 53,1%, 70,4% and 39,8%; and for PES (0–24 h) were 61,0%, 73,0% and 53,3%, respectively. Interestingly, porcine embryos produced *in vivo* and cultured *in vitro* from 24 h up to 120 h in the same PES concentration had better blastocyst development, fewer TUNEL positive cells, and higher survival after freezing and thawing procedures (4). Nevertheless, more studies are necessary to asses if our PES dose and time of exposure may influence embryo quality.

References

1. Prates EG, Nunes JT, Pereira RM. A Role of Lipid Metabolism during Cumulus-Oocyte Complex Maturation: Impact of Lipid Modulators to Improve Embryo Production. Mediators Inflamm [Internet]. 2014;2014:1–11. Available from: http://www.hindawi.com/journals/mi/2014/692067/

2. Yoshioka K, Suzuki C, Onishi A. Defined system for in vitro production of porcine embryos using a single basic medium. J Reprod Dev. 2008;54(3):208–13.

3. Braga JVC, Bohn AP, Soares LP, Komninou ER, Lucia Júnior T, Vieira AD, et al. Docosahexaenoic acid and phenazine ethosulfate are not efficient lipid modulators for porcine in vitro maturation systems. Vet Res Commun [Internet]. 2022;47(0123456789). Available from: https://doi.org/10.1007/s11259-022-09889-5

4. Gajda B, Romek M, Grad I, Krzysztofowicz E, Bryła M, Smorg Z. Lipid content and cryotolerance of porcine embryos cultured with phenazine ethosulfate. Cryo-Letters. 2011;32(4):349–57.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Evaluation of corpus luteum blood flow by color Doppler ultrasound and gene expression of ISGs in recipients of vitrified bovine embryos

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Abstract

The embryo vitrification process has greater efficiency for in vitro produced bovine embryos (IVP) when compared to slow freezing (1). In vitrification, the presence of elevated concentrations of permeable cryoprotective agents, is essential to make the vitrified solution in liquid nitrogen and to avoid crystallization during warming, thus avoiding damage by the formation of ice crystals, but the chemical toxicity of cryoprotectants is the main negative factor (2). There are several controversial studies about the pregnancy rate of vitrified embryos, however, the improvement of techniques, similar rates of IVP embryos cryopreserved in different ways are observed (3), making it important to evaluate pregnancy losses when using cryopreserved embryos by vitrification. Therefore, the aim of this experiment was to evaluate corpus luteum (CL) blood flow and IFNT-stimulated genes (ISGs) on Day 18 of gestation as an early indicator of pregnancy status in recipients of vitrified bovine embryos. For this, 18 crossbred beef cows were submitted to gynecological examination before and after synchronized with a standard protocol for fixed-time embryo transfer (FTET). Before performing the FTET, the presence of CL side was identified by transrectal B-mode ultrasound (US) to inovulate the embryos ipsilateral to the CL. Embryos were obtained from commercial companies vitrified and devitrified according to the protocol established by the company and transferred to previously synchronized recipients. On Day 18, blood samples were collected to determine blood concentration of progesterone (P4) and isolation of polymorphonuclear cells (PMNs) and peripheral blood mononuclear cells (PBMCs). Cervical cells samples were obtained by cervical cytology using a cytological brush (cytobrush) and the CL was examined using color flow Doppler US. Total RNA was extracted from blood and cytobrush samples and mRNA abundance of target genes (ISG15 and OAS1) was quantified by RT-qPCR and normalized to reference genes. Pregnancy diagnosis was performed on Day 28 by B-mode transrectal US, and cows were classified as non-pregnant (NP) (n = 10) or pregnant (P) (n = 8). CL diameter was different ($P < 0.05$) in the NP (17.05 ± 1.25) and P (20.6 ± 0.58) cows. On 18 days, 72.2% of the cows presented the CL vascularization by Doppler compatible to pregnancy (4). These cows were classified as Positive Doppler (PD), consequently the cows presenting a low vascularization were classified as Negative Doppler (ND). The expression of target genes in PMNs was evaluated, ISG15 and OAS1 mRNA expression were different ($P <0.05$) between the ND (0.83 ± 0.14) (0.63 ± 0.20) and PD (2.36 ± 0.48) (6.28 ± 1.7), respectively. Difference was also presented in Cytobrush samples ($P < 0.05$) for ISG15 and OAS1, ND (1.20 ± 0.40), (1.82 ± 0.69) and PD (29.64 ± 14.42) (13.16 ± 5.24) respectively. The concentration of P4 on Day 18 presented difference ($P < 0.05$) between ND (3.32 ± 1.84) and PD (18.24 ± 2.40). At 28 days, the pregnancy rate was 44.4% diagnosed by B-mode US, an indication that 27.78% of the cows could have lost pregnancy between Days 18 and 28. Pregnancy losses in cattle are very frequent in first thirty days of gestation, thus the gene expression of ISGs in PMNs and Cytobrush can as a biomarker to detect early embryonic mortality (4, 5), as well as the use of color Doppler ultrasound to monitor blood flow in the CL for around 20 days after insemination is widely used to accurately diagnose non-pregnant cows (4). The results obtained in the present study associated with concentration of P4 suggest that there was a pregnancy loss in this period. It is concluded that the association between CL blood flow and ISGs on Day 18 of gestation can be used as an early indicator of pregnancy status in recipients of vitrified bovine embryos and further analyzes should be performed to confirm a possible early pregnancy loss in these animals.

References

1. Yodrug T, Parnpai R, Hirao Y, Somfai T. The effects of vitrification after equilibration in different concentrations of cryoprotectants on the survival and quality of bovine blastocysts. Animal Science Journal. 2020 Jan 1;91(1).

2. Vajta G, Holm P, Kuwayama M, Booth PJ, Jacobsen H, Greve T, et al. Open Pulled Straw (OPS) Vitrification: A New Way to Reduce Cryoinjuries of Bovine Ova and Embryos.

3. Sanches BV, Lunardelli PA, Tannura JH, Cardoso BL, Colombo Pereira MH, Gaitkoski D, et al. A new direct transfer protocol for cryopreserved IVF embryos. Theriogenology. 2016 Apr 1;85(6):1147–51.

4. Dubuc J, Houle J, Rousseau M, Roy JP, Buczinski S. Short communication: Accuracy of corpus luteum color flow Doppler ultrasonography to diagnose nonpregnancy in dairy cows on day 21 after insemination. J Dairy Sci. 2020 Feb 1;103(2):2019–23.

5. Kunii H, Koyama K, Ito T, Suzuki T, Balboula AZ, Shirozu T, et al. Hot topic: Pregnancy-induced expression of interferon-stimulated genes in the cervical and vaginal mucosal membranes. J Dairy Sci. 2018 Sep 1;101(9):8396–400. 6. Melo GD, Pinto LMF, Rocha CC, Motta IG, Silva LA, da Silveira JC, et al. Type i interferon receptors and interferon-t-stimulated genes in peripheral blood mononuclear cells and polymorphonuclear leucocytes during early pregnancy in beef heifers. Reprod Fertil Dev. 2020 Jul 1;32(11):953–66.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

EMBRYONIC DEVELOPMENT OF QUAIL EGGS (*COTURNIX COTURNIX JAPONICA*) IN AN HOMEMADE INCUBATOR

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Abstract

Knowledge of the embryonic development of species such as quail is important for its production and reproduction. Quail is a practical solution to the animal protein shortage problem in developing countries and is an excellent alternative to replacing chicken (Shanaway, 1994). The study evaluates quails embryonic viability, comparing the main morphological changes that occur over the days of development with data articles using a homemade incubator created with easily accessible and low-cost materials, which allows small producers and communities to develop quail farms as an activity to increase income or even to produce a source of animal protein for the community from Brazilian Amazon area. The homemade incubator measures were 40 cm x 42 cm x 32 cm and its constructed with wooden boards, digital thermostat, an incandescent lamp. A total of 24 fertile eggs were incubated at a temperature of 37.5°C and relative humidity of 60%, age "0" was set at this time. 2 eggs were opened for each day of development, which starts from the fifth day to the 16th day of incubation. After gently opening the eggs, the embryos were removed, separated from the placenta and amniotic fluid, washed with running water, and weighed on a digital scale (SHIMADZU AUY 220 brand and UNI BLOC model). The "crown-rump", and other lengths were measured with a digital caliper (DIGITAL CALIPER brand and inch/F model) and the morphological structures were visualized in a stereomicroscope (NOVA brand and NOVA ZTX-E model). All eggs opened contained live embryos, which indicates homemade incubator temperature, relative humidity, and manual turning were adequate to maintain the quail's viability. The morphometric data from the embryos in different periods of development are similar than described at literature. Embryos' weight average was 0.0069 g at 5 days and 4.7863 g at 16 days and crown-rump (CR) length´ means were 0.368 cm and 3.657 cm for the same dates. Weight and CR increase of 0.434 g and 2.593 cm respectively per day of incubation. In conclusion, after 16 days of incubation in a homemade incubator, the embryos presented all the phases of the development cycle, with proportional relation of weight and height according to the days of incubation, and no anomaly or external interference, proving that the homemade incubator does not alter embryonic development of quails embryos in Brazilian Amazon area.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Determination of embryonic age in the first third of gestation in free-ranging capybaras by ultrasound

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Abstract

The capybara, *Hydrochoerus hydrochaeris,* is an endemic mammal of South America, being considered the largest rodent in the world. This mammal has been migrating from its natural habitat to the urban environment, approaching areas inhabited by humans, increasing the cases of spotted fever in Brazil. (1). This public health problem demands population control management that requires a deeper and more comprehensive knowledge of the reproduction of this species. Taking this into account, gestational ultrasound may be a promising tool for such management, however, this technique has been widely used to assess human pregnancy, but has not been very common in studies of gestational follow-up and age determination in wild animals (2, 3). In addition, there are no reports using the technique in capybaras. Thus, the objective of this work was to follow, for the first time, the sonographic and morphological changes during embryonic development until the end of the first third, to establish the gestational age in capybaras. To this end, ultrasound examinations were performed on a female previously conditioned to receive the procedure without pharmacological restraint, with a frequency of 2 times a week, using an ultrasound device (LOGIQ E VET GE ®). In addition, population control campaigns were also carried out on free-ranging capybaras authorized by DeFAU n. 8689 /2019 /SP. Of these, four embryos of 2 cm of crown rump from one pregnancy were collected. Embryos were preserved in 4% paraformaldehyde and submitted to routine histological processing, paraffin embedding and hematoxylin/eosin staining for organogenesis evaluation. The gestational age of a embryo of 2cm crown rump was compatible with approximately 51 days of gestation, according to the mating date and the ultrasound evaluation used in this study, considering that the capybara has a gestation of 150.6 + 2.8 days (4). The biometric measurements obtained by ultrasound were confirmed with the morphometric findings of the embryos collected in the field. In this final stage of the first third of gestation, macroscopically, the embryo presents a cervical flexure, primary vesicles (forebrain, midbrain and hindbrain), optic vesicle with dark pigmentation, pharyngeal arches (auricular ridges), thoracic and pelvic limbs, with all the organs already trained interns. In addition, through microscopy, it was possible to observe cardiac atrial cavity, lung, diaphragm, liver, stomach, pronephros, mesonephros, undifferentiated gonads, somites, medullary cervical flexure, midbrain and oral cavity. Ultrasound as a tool for gestational monitoring and age determination proved to be effective, which can be used in future studies to prove alternative techniques of chemical interruption in early pregnancy as a method of population control. Acknowledgements: FAPESP (processes 2019/03138-0)

Keywords: Rodent, Population Control, Embryology, Pregnancy and Ultrasound.

References

1. Pacheco, C. R. et al.Rickettsial infection in capybaras (Hydrochoerus hydrochaeris) from São Paulo, Brazil: serological evidence for infection by Ricekttsia bellii and Rickettsia parkeri. Biomédica, v.27, p.364-71, 2007.

2. Cootney, RW. Ultrasound imaging: principles and applications in rodent research. ILAR J. 2001;42(3):233–47

3. Francisco C.A. Sousa et al. Doppler ultrasound of the placenta and maternal and fetal vessels during normal gestation in captive agoutis (Dasyprocta prymnolopha, Wagler, 1831).Theriogenology. 2016; 86; 1921–1930.

4. Pinheiros, S. M. A criação em sistema semi-intensivo e o Manejo Sustentável de capivaras na natureza. Sitio Argentino de Produção Animal. Embrapa. 2008. ISSN 1806-9193

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Palmitic acid affects epigenetic markers in bovine endometrial epithelial cells

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Financial support: FAPESP grants 2014/22887-0, 2020/13075-2, 2021/02458-0 and 2021/06645-0.

Abstract

The postpartum negative energy balance (NEB) is an important risk factor in the establishment of reproductive failure in high producing dairy cows. There is an excessive mobilization of body reserves in this period, which in turn increases serum concentrations of non-esterified fatty acids (NEFAs), such as palmitic acid (PA). Previous studies indicate that NEFAs, especially PA, have an additive effect on endometrial cells of cattle by altering epigenetic markers (1). However, the complete mechanism has not been fully elucidated. Epigenetic mechanisms are known to regulate essential cell functions and modulate cell adaptation responses including cell metabolism and stress. Moreover, microRNAs (miRNAs) are a class of non-coding RNAs that play important roles in regulating gene expression. MiRNAs biogenesis is regulated at multiple levels, including at the level of miRNA pre and post – transcription processing and their dysregulation is associated with many diseases. Based on that, the aim of the study was to investigate the effect of PA on epigenetic changes and miRNAs biogenesis in bovine epithelial cells. Non-pregnant uteri (n=5) were selected in early luteal phase, based on the corpus luteum morphology, and dissection was performed from the ipsi lateral horn in the intercaruncular region. The tissue was incubated in 5 mL of digestion solution for 3 h at 37°C, subsequently cells were centrifuged twice at 3.000 g for 8 min. The cell precipitate was resuspended in 1 mL of culture medium Advanced DMEM/F12 supplemented with 10% fetal bovine serum, streptomycin (50 µg/mL) and penicillin (50 IU/mL). The culture medium was replaced every 2 days until reaching purified epithelial cells and than reached 85–90% confluence. Subcultures were prepared by treatment with 0.05% trypsin-EDTA for 1–2 min. Cells were seeded at density of 10×10^4 viable cells per well of 24-well tissue culture plates (Corning Incorporated, Costar® 3524, USA) using 1 mL of culture medium, and treatments started 48 h after cell seeding. Epithelial cells were exposed for 24 h to the following conditions: Control (basal PA concentration – 50 µM) and High PA (PA equivalent to that measured in follicular fluid during NEB – 150 µM). The PA was dissolved in ethanol according to the recommended solubility and the concentrations used in this study are based on bovine circulating concentrations during a period of NEB (2). Epithelial cells were submitted to total RNA extraction using QIAzol, followed by DNase treatment and cDNA synthesis using High Capacity cDNA Reverse Transcription Kit. The relative abundance of mRNA for genes involved in epigenetic changes (*SUZ12, SP1, HDAC1, HDAC4, RBL2, DNMT1, DNMT3A, TET1, TET2, MECP2, MAT2A, MAT2B, AHCY, BHMT, SHMT1, SHMT2* and *EZH2)* and miRNA biogenesis (*DGCR8, DROSHA, XPO5, DICER1*, *TARBP2, PRKRA* and *AGO2*) were determined using two genes (*GAPDH* and *PPIA*) as reference. Expression levels were calculated using the $2^{-\Delta Ct}$ method and differences in continuous data between treatments were assessed by Student's *t* test. A level of 5% significance was used. The relative mRNA abundance of all genes related to miRNA biogenesis was similar in control cells and those treated with High PA in epithelial cells. In the cells treated with High PA, the relative abundance of histone deacetylase 1 (*HDAC1)* and retinoblastoma like protein-2 (*RBL2)* mRNA was lower compared to control cells. HDAC1 provides a tag for epigenetic repression and plays an important role in transcriptional regulation and cell cycle progression (3). Also, RBL2 is related with arrest of cell cycle through its repressive effects on gene expression (4). Previous studies showed that epithelial cells treated with PA for 6 h increased immunofluorescence signal for H3K9me3 compared to the control, which is associated with promoter regions of transcriptionally repressed genes (1). Based on that, we suggest that at 24 h after High PA exposure genes related to repression of gene transcription was lower because it's was already been translated into protein, which may not be happening at 6 h. In conclusion, exposure to High PA appears to alter epigenetic markers in bovine epithelial cells.

References

(1) Ferst et al., Animal Reproduction Science, 223: 106851, 2021. (2) Leroy et al., Reproduction, 4:485-95, 2005. (3) Qiu et al., Molecular Cell, 22: 669-679, 2006. (4) Bremner et al., Molecular Cell Biology, 15: 3256-65, 1995.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Vitamin E reduces the reactive oxygen species production in dominant follicle during the negative energy balance in cattle

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Financial support: CAPES, CNPq, FAPERGS, and FAPESC

Abstract

In postpartum of cattle there is an increase in non-esterified fatty acids (NEFA) and ketone bodies in both serum and follicular fluid (FF). The increase in fatty acids concentration during the negative energy balance (NEB) period results in increased production of reactive oxygen species (ROS) and oxidative stress that can compromise bovine fertility. Furthermore, the oxidative stress has been related to the depletion of antioxidant defenses, as the vitamin E (α-tocopherol). Therefore, the objectives of this study were to characterize the lipid profile of the FF of cows in induced NEB and to evaluate the effect of parenteral α-tocopherol in the prevention of oxidative stress in the serum and FF of cows in NEB. Twenty-nine *Bos taurus* beef cows were submitted to a hormonal protocol to synchronize a new wave of follicular growth. This was accomplished by using a progesterone-releasing intravaginal device (D-4; IVD; Primer, Tecnopec, São Paulo, Brazil; 1g progesterone) and an intramuscular (IM) injection of 2mg estradiol benzoate (EB; Gonadiol, Zoetis, Sao Paulo, Brazil). Four days after prostaglandin F2α analogue (D0, PGF2α; 500μg cloprostenol, Estron, Agener União Saúde Animal, São Paulo, Brazil) was administered intramuscularly. At D0, saline solution or α-tocopherol (Monovin E, Bravet, Rio de Janeiro, Brazil) at a single dose of 1,000 IU was administered intramuscularly in cows that made up the groups: 1) control (n = 9); 2) Fasting + VitE (n = 10); and 3) Fasting (n = 10). The IVD was removed 8 days after the time of its insertion (D4). Between D0 and D4 blood samples were taken daily to assess circulating concentrations of NEFA, ROS production, total antioxidant capacity, lipid peroxidation and α-tocopherol (Vitamin E). On D4, the diameter of the dominant follicle was analyzed, and ultrasound-guided follicular aspiration was performed for analysis of FF. Our results demonstrate that fasting was effective in causing increased fat mobilization in animals. During the fasting, concentration of C18:1n9 (oleic acid) increased in serum and FF ($P < 0.05$). There was no difference in the proportion of palmitic (C16:0) and stearic (C18:0) acids in FF among the groups. Serum α-tocopherol concentration was higher in the control and Fasting + VitE groups compared to the Fasting group. In FF, there was an increase of α-tocopherol in the Fasting + VitE in comparison to Fasting cows. There was an increase of ROS production in the serum of fasting cows in D4 ($P = 0.04$). ROS production in FF was higher in the Fasting compared to Fasting + VitE group ($P < 0.05$). Total antioxidant capacity and lipid peroxidation did not differ among groups in serum and FF ($P > 0.05$). The mean diameter of the dominant follicle and estradiol concentration in the FF did not differ among groups ($P > 0.05$). NEFA concentrations in FF reflect blood concentrations which in turn causes an increase of ROS production in FF. Moreover, we demonstrated that intramuscular application of Vitamin E reduces ROS concentration in the dominant follicle. In conclusion, the four-day fasting resulted in an increase in NEFA concentrations in serum. Among the NEFA, only the concentration of oleic acid was higher in fasting groups in FF. In addition, vitamin E has beneficial effects in preventing oxidative stress in pre-ovulatory follicles of cows in NEB.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Importance of CTGF and other classic YAP-TEAD target genes regulation by FSH in granulosa cells during the establishment of follicle dominance in cattle

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Abstract

Despite years of research, the molecular mechanisms that drive the GC differentiation into a more estrogenic profile during follicular divergence have not been completely elucidated (1). The Hippo signaling has, however, emerged as a potential key player to explain such complex process. The core Hippo pathway consists of a kinase cascade that ultimately regulates the activity of the transcriptional activator yes-associated protein 1 (YAP) (2). When Hippo signaling is inactive, YAP accumulate in the nucleus and form complexes with numerous transcription factors, notably those of the TEAD family of transcription factors, resulting in the modulation of the transcriptional activity of several target genes as the classic tissue growth factor (*CTGF*) (3). Conversely, when Hippo signaling is activated, YAP is phosphorylated resulting on its nuclear export to the cytoplasm and therefore compromising YAP-TEAD dependent transcriptional activity (4). A study employing rat GC cultures demonstrated that *Ctgf* mRNA downregulation coincides with FSH-induced GC differentiation (5). In addition, a study employing a bovine *in vitro* GC model, clearly showed that when *CTGF* mRNA levels are increased in this cell type, there is a decrease in *CYP19A1* expression and, consequently, a significant reduction in E2 secretion levels (6). Taken together, these findings led us to hypothesize that, during ovarian follicular divergence in cattle, FSH regulates CTGF expression in granulosa cells to allow the future dominant follicle to launch its augmented estrogenic capacity. To address this, we first employed a bovine GC culture model in which cells were cultured in the presence of graded doses of FSH. The results showed that FSH downregulated in a concentration-dependent manner ($P < 0.05$) the mRNA levels not only for *CTGF* but also for ankyrin repeat domain 1 (*ANKRD1*) and cysteine-rich protein 61 (*CYR61*), both considered classic YAP-TEAD target genes along with CTGF (7). These findings were explained by the fact that FSH treatments did not alter total YAP protein levels ($P > 0.05$) but significantly promoted YAP phosphorylation in a concentration-dependent manner ($P < 0.05$). To better elucidate the functional importance of such FSH-induced downregulation of YAP-TEAD target genes, we then cultured GC in the presence of distinct concentrations of verteporfin (VP) or peptide 17 (P17), two pharmacological inhibitors known to interfere with YAP binding to TEADs. The results showed that both VP and P17 increased *CYP19A1* basal mRNA levels in a concentration-dependent manner ($P < 0.05$). To complement our *in vitro* findings, we decided to perform an *in vivo* experiment to obtain GC samples from dominant (F1) vs. subordinate (F2) follicles isolated from ovaries collected at days 2 (D2), 3 (D3), and 4 (D4) of the first follicular wave (collection time points correspond to the day before, during and after ovarian follicular follicular deviation in bovine, respectively). Although mRNA levels for *CTGF*, *CYR61* and *ANKRD1* were not significantly higher in GC of subordinate follicles collected at D2 and D3 ($P > 0.05$), mRNA levels for those 3 genes were significantly higher is GC from subordinate follicles collected at D4 ($P < 0.05$). Taken together, our results demonstrate that Yes-associated protein 1 transcriptional activity must be altered in bovine granulosa cells to allow the increased estrogenic capacity of the future dominant follicle.

References

1. Hsueh AJWW, Kawamura K, Cheng Y, Fauser BCJMJM. Intraovarian Control of Early Folliculogenesis. Endocr Rev [Internet]. 2015 Feb 1;36(1):1–24. Available from: https://academic.oup.com/edrv/article/36/1/1/2354672

2. Zhao B, Li L, Lei Q, Guan KL. The Hippo-YAP pathway in organ size control and tumorigenesis: An updated version. Genes and Development 2010 p. 862–74.

3. Boopathy GTK, Hong W. Role of Hippo Pathway-YAP/TAZ signaling in angiogenesis. Front Cell Dev Biol. 2019;7(APR):1–12.

4. Cairns L, Tran T, Kavran JM. Structural Insights into the Regulation of Hippo Signaling. ACS Chem Biol. 2017;12(3):601–10.

5. Harlow CR, Davidson L, Burns KH, Changning Y, Matzuk MM, Hillier SG. FSH and TGF-β superfamily members regulate granulosa cell connective tissue growth factor gene expression in Vitro and in Vivo. Endocrinology. 2002;143(9):3316–25.

6. Han P, Relav L, Price CA. Regulation of the early growth response-1 binding protein NAB2 in bovine granulosa cells and effect on connective tissue growth factor expression. Mol Cell Endocrinol [Internet]. 2020;518(September):111041. Available from: https://doi.org/10.1016/j.mce.2020.111041

7. Wang K, Yeh Y, Nguyen P, Limqueco E, Lopez J, Thorossian S, et al. Flow-dependent YAP/TAZ activities regulate endothelial phenotypes and atherosclerosis. Proc Natl Acad Sci [Internet]. 2016 Oct 11;113(41):11525–30. Available from: https://pnas.org/doi/full/10.1073/pnas.1613121113

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Strategies to prolong the luteal phase in gilts without the use of progestagens

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Abstract

Gilts are a key category for genetic improvement in pig herds and numerous farms use estrus synchronization protocols to optimize management. However, some protocols have unsatisfactory results or use components that are banned in some countries, such as pseudopregnancy protocols that use estradiol (1). The most widespread synchronization protocol for gilts involves daily administration of altrenogest (2) by oral route on top of feed or directly in the mouth for 14 to 18 days (3). In this case, the adequate ingestion of the dose and the duration of treatment could be considered as limitations of this protocol. Gonadotropins, in turn, are mostly used to overcome anestrus in gilts by injecting a commercial product that contains 400 IU of eCG and 200 IU of hCG in a single dose (4). In the present study, different protocols using gonadotropins injection for estrus synchronization in cyclic gilts were evaluated and subsequent reproductive performance was assessed. A total of 36 gilts from a gilt development unit that exhibited estrus (heat no-service) in the same day (day 0; D0) were randomly assigned into three treatment groups: Control (n=12), no hormonal treatment; eCG+hCG group (n=12), that received 400 IU eCG on D10 and 500 IU hCG on D12; and hCG2 (n=12), which received two doses of 1500 IU hCG I.M., on D12 and D15. On D30, the gilts that did not express estrus received two doses of 250 µL cloprostenol, I.M, within a 6-h interval. Blood samples were collected from all gilts on D10, D20, D25, D30 and D35 to assays progesterone levels by chemiluminescence method. At the estrus the females were inseminated (AI) up to three times (depending on estrus duration) through intracervical AI with 2.0×10^9 spermatozoa, at 24-h intervals. Progesterone concentrations highly differed among groups, with group hCG2 exhibiting high levels of serum progesterone for a longer period. All gilts expressed the second estrus after treatments and were subsequently inseminated. In the control, most gilts expressed estrus within D20 and D23. Within D24 and D33, most estrus expressions were from gilts in the eCG+hCG treatment, whereas gilts from the hCG2 treatment showed estrus signs from D33 to D36. The inter-estrus interval was longer for gilts treated with hCG2 (36.2 ± 1.6 d; $P < 0.05$) than for those in the other treatments. Compared to the control (22.2 ± 1.6 d), gilts in the eCG+hCG treatment (29.7 ± 1.6 d) presented longer inter-estrus interval ($P < 0.05$). The farrowing rates did not differ among groups and were the same for the three (91.7%). In the first parity, the litter size was higher ($P < 0.05$) in the control group (15.3 ± 1.3) compared to hCG2 (11.3 ± 1.3) but did not differ from eCG+hCG group (13.1 ± 1.3). The gonadotropin-treated groups did not differ regarding litter size. We hypothesized that the differences in the number of total piglets born and born alive was due to the higher P4 serum concentrations observed in hCG2 group, which could have interfered with the follicular dynamics of the gilts in this group, resulting either in the ovulation of less oocytes or possibly oocytes of poor quality. These data indicate that hCG2 treatment provided an efficient way to prolong luteal phase in gilts, with luteolytic response to PGF2α analogous, though it impacted litter size in first parity.

Acknowledgments

Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina – FAPESC, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul – FAPERGS, that financed the study

References

1 - Noguchi M, Yoshioka K, Suzuki C, Arai S, Itoh S, Wada Y. Estrus synchronization with pseudopregnant gilts induced by a single treatment of estradiol dipropionate. J Reprod Dev. 2010;56(4):421-427. http://dx.doi.org/10.1262/jrd.10-006t.

2 - Estill, C. Current concepts in estrus synchronization in swine. J Anim Sci. 2000;77 (E-Suppl):1-9. Doi: h10.2527/jas2000.77E-Suppl1m.

3 - Wang Z, Liu BS, Wang XY, Wei QH, Tian H, Wang LQ. Effects of altrenogest on reproductive performance of gilts and sows: A meta-analysis. Anim Reprod Sci. 2018;197:10-21. http://dx.doi.org/10.1016/j.anireprosci.2018.08.035.

4 - Kraeling RR, Webel SK. Current strategies for reproductive management of gilts and sows in North America. J Anim Sci Biotechnol. 2015;6(1):3. http://dx.doi.org/10.1186/2049-1891-6-3. PMid:PMC4382856.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Cell proliferation in aorta-gonad-mesonephros region from bovine embryos occurs only during early development

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Abstract

The first progenitors of definitive hematopoiesis (1) arise from the differentiation of the endothelium of the dorsal aorta in the aorta-gonad-mesonephros (AGM) region (2) and subsequently migrate to the embryonic yolk sac (3), to the fetal liver, and finally to the bone marrow (4), in which it remains throughout life. The use of reproductive biotechnologies in cattle, especially nuclear transfer, can bring about significant changes in this system, including changes in the formation of blood vessels in the yolk sac, gene expression (VEGF and its receptors), and the development of vascular changes in different organs. On the other hand, mapping the regions that present niches of proliferating, undifferentiated, or progenitor cells have shown a growing interest in recent years due to its applicability in reproduction and Regenerative Medicine. Thus, this work's objective was to characterize the AGM region in bovine embryos and identify the periods of higher proliferative activity throughout early embryonic development (5). Therefore, embryos (n = 10) with gestational ages of 20 to 40 days from pregnant cows (natural breeding) of mixed breeds were collected in slaughterhouses at São João da Boa Vista, São Paulo, Brazil. The age of the embryos was estimated according to the measurement of the crown-rump, a methodology established by Evans and Sack (1973). After fixation in 4% paraformaldehyde, the samples were processed for histological and immunohistochemical analysis using the primary antibody proliferating cell nuclear antigen (PCNA) at a concentration of 1:100 (6). Proliferating cells (PCNA+) were identified in the regions of the aorta, tubules, and undifferentiated gonads in embryos with an estimated age of 20 to 25 days of gestation. From the 26th day, the AGM region of bovine embryos reduces its proliferative capacity, ceasing the expression of PCNA in embryos at 30 days of gestation. Thus, our results indicate that the AGM region presents higher proliferative rates at embryonic ages below 25 days, favoring the isolation of hematopoietic progenitor cells.

References

1. Nishikawa et al. (2001). Role of the microenvironment of the embryonic aorta-gonad-mesonephros region in hematopoiesis. Annals of the New York Academy of Sciences, 938(1), 109-116.

2. Ivanovset al. (2011). Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. Journal of Experimental Medicine, 208(12), 2417-2427.

3. Mançanares et al. (2019). Morphological and molecular analysis of in vitro tubular structures from bovine yolk sac-derived MSCs. Stem Cells International, 2019.

4. Godin et al. (1999). Stem cell emergence and hemopoietic activity are incompatible in mouse intraembryonic sites. The Journal of experimental medicine, 190(1), 43-52.

5. Kumaravelu et al. (2002). Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver.

6. Moldovan et al. (2007). PCNA, the maestro of the replication fork. Cell, 129(4), 665-679.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

First characterization of feline oviduct epithelial spheroids and their extracellular vesicles

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Financial support: CAPES, CNPq, and FAPERJ

Abstract

Oviduct epithelial cells (OEC) are involved in sperm capacitation, fertilization, and early embryo development. These cells produce extracellular vesicles (EV), which are involved in intercellular communication among gametes, embryos, and maternal organism. During *in vitro* culture, OEC form spheroids clusters (OES) maintaining their differentiation and function over a long period when compared to monolayer cultures. Although the great importance of OEC for reproductive events has already been described for bovine and swine, there is no evidence about production and characterization of feline oviduct epithelial spheroids (fOES) and their EV. Thus, the aim of the present study was to describe and characterize, for the first time, the production of fOES and analysis of the EV they produce, in view of their future use in reproductive technologies related to the preservation of endangered feline species. After elective ovarian hysterectomy, eight domestic cat oviduct pairs were dissected, washed quickly in alcohol 70% and subsequently in PBS bath. The oviduct lumen was exposed and scraped using a sterile slide in Petri dish. The recovered cells were diluted in TCM 199 HEPES supplemented with 10 mg/mL gentamycin and 0.4 g/L BSA (TCM-H), and then vortexed for 30 s. The cell pellet was recovered by gravity sedimentation, washed twice in TCM-H, and diluted (1:100) in TCM 199 bicarbonate supplemented with gentamycin (10 mg/mL) and BSA (0.1%; T1) or FCS (10%; T2), transferred to a 4-well dish, and cultured for 7 days in 5% CO_2 in humidified atmosphere at 38.5° C. On D4, fOES were selected and transferred to another well. Half of the culture medium was changed every 48 h. The fOES were evaluated regarding their quantity, size, and cell number on D4 and D7. The fOES-EV were isolated by ultracentrifugation. For that, culture supernatant from T1 was centrifuged twice at 300 x *g* and 12,000 x *g* for 15 min at 4 °C. The resulting supernatant was filtered (0.20 µm) and ultracentrifuged twice at 100,000 x *g* for 70 min at 4 °C. The final EV pellet was resuspended in 50 µL Ca^{2+}/Mg^{2+}-free PBS and evaluated by nanoparticle tracking analysis. The data are presented as mean ± standard error. On D4, T1 generated 19 fOES and T2 produced 64 fOES, with 58% and 66% of them presenting a circular morphology, whilst 42% and 34% displayed a lobular shape, respectively. On D4, T1-derived fOES showed a mean size of 51.2±11.8 µm with a mean of 283±51.4 cells/spheroid, whilst T2-derived fOES presented a mean size of 54.7±8.4 with 254±83.7 cells/spheroid. On D7, T1-derived fOES mean size was 38.6±6.8 µm with only 99±32.6 cells/spheroid, while T2-derived fOES mean size was 51.5±7.5 µm with 283±51.4 cells/spheroid. The EV size distribution showed a homogeneous population ranging from 60 to 400 nm, presenting a mean of 234.9±3.0 nm, mode of 157.7±7.0 nm, and a concentration of 1.48±0.06x10^9 particles/mL. In conclusion, this is the first report to demonstrate that it is possible to produce feline OES *in vitro*, which are able to release EV in the medium. Moreover, apparently, supplementation with FCS is preferable for fOES culture, generating a higher number and better maintenance of the spheroids throughout the culture than BSA. This is the first attempt to study the use of fOES and their EV on the feline early reproductive events and opens the possibility of their use as a tool to improve different *in vitro* reproductive biotechnologies.

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

THE EFFECT OF AI SHEATHS ON THE PREGNANCY RATE OF DAIRY CATTLE

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Abstract

The profitability of cattle farming depends on the success of reproductive performance among the animals, when an adequate reproductive performance is not achieved, it can result in great economic losses to the producers. The search for tools is constant to increase pregnancy rates in cattle, one of them is the use of sanitary Sheaths in the Artificial Insemination (AI), to reduce the probability to bring to the uterus external contamination that might compromising the conception rate. The objective of this study was to evaluate the pregnancy rate of dairy cows inseminated with and without the use of AI Sheaths. For that, we used 823 multiparous cows distributed in five farms located in the state of RS/Brazil from April/August of 2022. The animals were randomly separated into two experimental groups. G1 - Insemination without the use of AI Sheaths (N= 449); and G2 inseminated with the use of AI Sheaths (N= 374), all the animals were inseminated by the same veterinarian. Both groups underwent the FTAI protocol with the use of intravaginal implant, and pregnancy detection was conducted on day 30, using the ultrasound (EASISCAN GO - IMV Imaging Scothland); Statistical analyzes were performed using the X^2 test in the R statistical environment. The pregnancy rate in G1 was 34% (N= 152/449) while the animals from G2, the pregnancy rate was 44% (N= 127/374). No statistical difference was observed among the treatments at 30 days of pregnancy at the first AI ($P>0.05$). However, even not observing an statistical difference among treatments, an increase of 10% of pregnancies were achieved, representing 30 additional calves being born as a result in use of the sanitary Sheaths, which alone justifying its use. It will be interesting to evaluate the use of sanitary Sheaths efficacy on different housing systems of dairy cows, to assess the effect of this tool.

Acknowledgements

INOVAVET, IMV – Technologies do Brasil, University of São Paulo, University of Passo Fundo

Animal Reproduction

Thematic Section: IX International Symposium on Animal Biology of Reproduction (ISABR 2022)

Effect of exogenous melatonin on the reproductive performance in ewes with different body condition scores during the anestrous season

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Abstract

Reproductive seasonality and nutritional condition are the main factors that influence reproductive performance in ewes, particularly in the central region of Argentina, where the availability of food is highly seasonal. Inadequate nutrition can compromise luteal function and embryo development. On the other hand, melatonin treatment is an effective method for inducing estrous cycles, increasing ovulation, and improving embryo viability during anestrus. Therefore, the effect of exogenous melatonin on reproductive efficiency was investigated in ewes with different initial body condition scores (BCSi) during the anestrous season. The work was carried out at the facilities of the Animal Reproduction Department (UNRC), in Río Cuarto, Argentina (30º07´S, 64º14´W). The present protocol was approved by the Ethics Animal Research Committee of UNRC, Argentina. During seasonal anestrus (October to December), thirty-one adult, non-cycling, non-pregnant, crossed-breed ewes were separated into 2 groups according to their BCSi: fat (F, BCSi: 3.8±0.10) or thin (T, BCSi: 2.75±0.08) (scale 1-5, $P<0.05$). In turn, each of them was separated into 2 groups, considering treated (+ MEL) or not (-MEL) with a melatonin implant. Thus, the design included 4 groups: F-MEL (n=7), F+MEL (n=7), T-MEL (n=8) and T+MEL (n=9). All animals received a diet based on alfalfa hay and corn grain to cover maintenance requirements and *ad-libitum* water. After 40 days from the insertion of melatonin implants, all females received a treatment to induce ovulation and were inseminated via cervical, with fresh semen, at 48 h after the removal of the intravaginal sponges (AIFT). Fourteen days after AIFT, estrus was detected, once per day, using a vasectomized ram, during a period of 21 d. The females that returned to estrus were mated with an adult male. Ovarian ultrasound was performed at 7 days post-AIFT to determine the presence and area of the corpora lutea (CL). The pregnancy diagnosis was made by ultrasound at 30 and 50 days post-AIFT. Statistical comparisons of proportional values were based on the Chi-squared Test. The level of statistical significance was set to $P<0.05$. The BCS presented differences between groups F and T, during the entire experimental period ($P<0.05$). There was an effect of exogenous melatonin on ovulation in T ewes (T-MEL: 50%, T+MEL: 88.9%; $P<0.05$) but no effect on F groups was observed (100% of ewes had ovulation in each group). A luteotropic effect of melatonin was observed in F+MEL ewes, due to the presence of a larger CL area (cm2) compared to the rest of the treatments (F+MEL: 0.16±0.02 versus F-MEL: 0.11±0.01, T-MEL: 0.07±0.03 and T+MEL:0.09±0.01; $P<0.05$). There were no differences in the number of T ewes that returned to estrus, although the % of general pregnancy was better in this group ($P<0.05$). On the other hand, melatonin improved fertility in F+MEL sheep compared to F-MEL (28.6 and 14.3%, respectively; $P<0.05$), however, this effect was not observed among the T groups (T-MEL: 37.5, T+MEL: 33.3%; $P<0.05$). In conclusion, under the conditions of this experiment, the use of exogenous melatonin increased ovulation rate and the resumption of ovarian function in ewes with low BCS during seasonal anestrus, although the effect on fertility was only observed in F ewes. A better understanding of modulating effects in the interaction of melatonin and nutritional status on ovarian function is essential to develop management systems looking to enhance reproductive performance in anestrous ewes. This study was supported by grant PIP2014-2018 from CONICET.