Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

Seasonal variation of morphometrical characteristics of fresh and cryopreserved Saanen goat sperm

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Cryopreservation of sperm allows use of assisted reproductive technologies (ART) in animal husbandry more efficiently. Various studies have shown that the survival rate of sperm after cryopreservation depends on their initial morphofunctional characteristics. Cryopreservation of sperm with the best morphofunctional characteristics may allow them to be used for ART in goats throughout the year to effectively increase livestock. Therefore, the aim of the study was to evaluate the effect of seasonality on the morphometric characteristics of goat sperm before and after cryopreservation.

Ejaculates of 3 mature Saanen bucks were obtained by artificial vagina twice a month during the breeding season (September-December, n = 24) and non-breeding seasons (February-July, n = 36). Cryopreservation of sperm was performed in a HEPES buffer medium with 10% glycerol and 20% egg yolk. Sperm suspension with cryoprotectant was transferred into straws with a 0.25 ml volume, equilibrated for 30 min at room temperature (+20 °C), 2.5 h at +5 °C, 15 min in nitrogen vapors 4 cm from the liquid nitrogen and plunged into liquid nitrogen. The samples were thawed in a water bath (+37 °C) for 30 sec. Smears of fresh and cryopreserved sperm were fixed and stained using the Spermac Stain kit (FeriPro, Belgium) according to the manufacturer's protocol and visualized under a light microscope at a magnification of x1000. For morphometric measurements, sperm micrographs were taken and analyzed using ImageJ software (version 1.51j8, NIH, USA). Mann-Whitney U-test was used to compare the two samples, the difference was considered significant at $p \leq 0.05$.

Morphometric analysis of sperm showed that the size of the head and tail decreased after cryopreservation, compared with these measurements before cryopreservation. After cryopreservation, the length and width of the head decreased significantly ($p \leq 0.05$) compared to the corresponding sperm size before cryopreservation. Moreover, these changes were observed in both breeding and non-breeding seasons. When analyzing the morphological characteristics of the sperm tail, a reduction after cryopreservation was observed, probably due to the twisting, the formation of loops, and the separation of its part.

To conclude, the morphometric characteristics of Saanen goat sperm change significantly after cryopreservation, regardless of the season. After cryopreservation, the size of the head and tail of sperm is significantly reduced.

Keywords: Goat sperm, cryopreservation, morphometry, seasonality

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Placental vascularization in in vitro-derived pigs: a preliminary study.

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The placenta plays a critical role in maintaining and protecting the developing fetus. Placental vascularization abnormalities, including a decrease in arterial number, lumen size, and branching, have been extensively described in humans born from in vitro-produced (IVP) embryos (Riesche and Bartolomei, Seminars Reprod Med, 36:240-247, 2018) but studies on IVP pigs are very limited (Ao et al., Placenta 57:94-101, 2017). The objective of this study was to compare the placental vascularization in pigs born from in vitro- and in vivo-produced embryos (the latter born by artificial insemination; AI group). IVP embryos were produced after in-vitro fertilization (IVF) of in vitro matured oocytes and further culture (IVC) up to blastocyts stage in media supplemented with or without 1% porcine oviductal fluid and 1% uterine fluid (more details in Paris-Oller et al., J AnimSci and Biotech 12:32-44, 2021). Blastocysts produced with (RF-IVP group) and without (C-IVP group) reproductive fluids were surgically transferred at day 7 post-IVF. Both AI and IVP embryos were produced with spermatozoa from the same boar. After birth, placenta samples were collected at 3-5 cm from the insertion of umbilical cord, and fetal parameters were recorded. The placenta of 9 animals (3 per group) from different litters was selected following these criteria among animals: similar uterus position, birth weight, and crown-rump length; and a close male/female ratio among groups. Samples were fixed (10% formaldehyde solution) and paraffin embedded. Two complete placental sections (5 µm thickness) were stained (hematoxylin–eosin), photographed at 5x (ZEN 3.2, ZEN lite, Zeiss) and images processed (ImageJ) for a detailed study to record vessel number, area occupied by each vessel (µm^2), and total vascular area (%). Based on their size and histological characteristics, vessels were categorized by an expert operator as capillary (1-500 µm^2), arteriole/venule (501-1000 µm^2), small artery/vein (1001-3000 µm^2), medium-sized artery/vein (3001-30000 µm^2), and large artery/vein (>30000 µm^2). Data (mean±SEM) were analyzed by one-way ANOVA (Systat v13.1), and differences (P<0.05) were compared by Tukey's test. The total placental area observed, and total number of vessels analyzed was higher in AI (86.1±7.5 mm^2, 726 vessels) than C-IVP (45.9±6.8 mm^2, 544 vessels), and RF-IVP (52.8±5.1 mm^2, 637 vessels) (P<0.05). However, no differences were found in the total vascular area being 14.9±3.3% (AI), 19.9±2.7% (C-IVP), and 17.8±2.2 (RF-IVP) with similar pattern distribution in all groups: over 85% microvessels, 10-15% medium-size vessels and 5% macrovessels. However, the vascular area occupied by medium-sized vessels (arteries and veins) was significantly higher in the AI group (7.2±0.5%) than in IVP groups (2.1±0.3% and 1.8±0.2%) regardless of the addition of reproductive fluids (P<0.05). No differences in vascular areas of micro and macrovessels were observed. Preliminary results show that impaired placental vascularization in ART-derived pigs might occur due to a reduction of medium size vessels.

Keywords: placenta, vascularization, pig

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Transcriptional differences between cattle and buffalo growing follicles: possible effect over oocyte competence.

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Buffaloes are exceptional specie used to produce meat and milk under challenging conditions, especially in tropical areas. Since this is a relatively new production system, many practices used in cows have been applied to buffaloes, especially in the field of reproduction. Our team and others have been reported that the use of follicle-stimulating hormone (FSH) before aspiration increases the number of follicles and the number of oocytes recovered, and although FSH has its effect on the follicle, oocytes produced are no more competent than controls to form embryos producing similar blastocyst rate. To explore the different outcomes with a similar stimulation protocol, we studied the transcriptome of early dominant and subordinate follicles using granulosa cells as an indicator of follicular differentiation.

The somatic RNA was obtained from granulosa cells from growing follicles > 7mm obtained from ovaries of 10 buffaloes and 10 cattle recovered at the local slaughterhouse, sequenced with NovaSeq, and the differential expression was analyzed using EdgeR in Bioconductor. Files from differential expressed genes were cleaned, removing 9669 genes (low count) to be analyzed using Ingenuity Pathway Analysis.

Of 5393 genes that were retained for the analysis and with significant p-value, the most interesting canonical differences are related to eukaryotic initiation factor 2 (EiF2, growth), mammalian target of rapamycin (mTOR, energy), signal transducer and activator of transcription 3 (STAT3, differentiation), and protein kinase A (PKA, stimulation) on the upstream side. Progesterone and progesterone receptor (PGR) differences may indicate a difference in changes preparing for ovulation but transforming growth factor-beta (TGFB), p53, tumor necrosis factor (TNF), and phosphatase and tensin homolog (PTEN). These genes are all related to differentiation and apoptosis and may indicate a different timing in follicular dynamics between the two species. From a practical perspective, this result may indicate that coasting -FSH withdrawal prior to ovum picK-up- creates atresia faster in buffalos than in cows.

Keywords: Buffaloes, gene expression, oocyte competence, Cattle, Apoptosis

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Evaluation of two IVP bovine embryo sexing techniques according to their ability to preserve embryo viability after vitrification/warming

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Trophectoderm (TE) biopsies are frequently used for embryo genotyping, although they are invasive and harmful to further embryonic development. Cell-free DNA (cfDNA) found in blastocoele fluid (BF) can be considered as a non-invasive sexing method. The purpose of this study was to compare the accuracy of a non-invasive method using cfDNA present in BF, to the biopsy procedure in terms of determining the embryo's sex and its effect on embryo survival following vitrification/warming. Expanded Day 7 IVP embryos were randomly assigned to two groups: VIT-Collapsed (n=37), blastocysts artificially collapsed by aspiration of BF with an ICSI pipette; VIT-Biopsied (n=56): blastocysts biopsied by cutting off a small portion of the TE using a microblade. After sample collection, all embryos were vitrified/warmed by the Cryotop method and individually cultured *in vitro*. Intact embryos individually cultured (VIT-Single) (n=58) or cultured in group (VIT-Control) (n=56) after vitrification/warming were used as vitrification controls, whereas intact non-vitrified embryos were used as fresh controls (n=45). The survival of vitrified blastocysts was assessed as re-expansion and hatching rates at 24 h post-warming. Sex identification was performed in BF or biopsies as well as in the corresponding surviving embryos of VIT-Collapsed and VIT-Biopsied groups. BF samples underwent a whole genome amplification using REPLI-g single cell kit (Qiagen, Germantown, MD, USA), whereas biopsies and blastocysts were lysed by incubation with 100 µg/mL proteinase K at 55°C for 2h. Embryo sex was analyzed by PCR using two sets of primers: Y-chromosome specific primer (*BRY4a*) and bovine specific satellite sequence primer (*SAT1*). Products were visualized on a SafeView stained 2% agarose gel. Samples with *BRY4a/SAT* bands were considered male, while samples with only the *SAT1* band were assigned as female. Data were analyzed with a one-way ANOVA (P≤0.05). VIT-Collapsed blastocysts showed similar post-warming survival rates (87.55±16.1%) to those of fresh non-vitrified blastocysts (100%) and significantly higher than blastocysts from the VIT-Single and VIT-Control groups (79.0±9.1% and 72.0±14.3%, respectively). Blastocysts vitrified after biopsy showed the lowest (P≤0.05) survival rate (53.5±12.6%). No differences (P>0.05) between the two sources of DNA were observed either in their amplification efficiency (72.0% (18/25) in BF samples; 79.3% (23/29) biopsies) or in their accuracy in sex diagnosis (83.3% (15/18) in BF samples; 82.6% (19/23) in biopsies). In conclusion, the results of this study indicate that cell-free DNA analysis is an efficient and minimally invasive approach to sex IVP cattle embryos. Moreover, artificial collapse of blastocoel had a positive effect on embryo viability after vitrification/warming. Further studies to improve the efficiency of cell-free DNA collection and amplification are guaranteed.

Keywords: Cell-free DNA, Blastocoele collapse, trophectoderm biopsy

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