Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Antimicrobial resistance of Corynebacterium spp. in the vaginal flora of gilts and sows in Sweden

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Keywords: antimicrobial resistance, pigs, corynebacterium.

Semen extenders for fresh boar semen include antibiotics, according to official guidelines, to control the growth of bacteria contaminating the semen during collection. However, it is not known if this use of antibiotics leads to the development of antimicrobial resistance in the vaginal flora of inseminated pigs or whether this resistance could spread to other animals (including human beings) or the environment.

Objective: The antimicrobial resistance pattern of *bacteria* isolated from the vagina from non-inseminated gilts and from sows that had already had three litters of piglets following artificial insemination.

Methods: Vaginal swabs were taken from 30 sows and 30 gilts on three farms in the middle of Sweden during the autumn of 2018. The swabs were directly cultured on blood agar, lactose purple agar, mannitol salt agar, Colistin-Oxolinic Acid-Blood Agar (COBA) and Man, Rogosa and Sharpe agar (MRS-agar).

In total, 280 bacterial isolates were identified by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry: 73 % of them consisted of *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. Fifty-two isolates of *Corynebacterium* spp. were tested for susceptibility against 16 selected antimicrobial substances, assessed with VetMIC™ Lact-1 and VetMIC™ Lact-2 (SVA, Uppsala Sweden), by determining the antimicrobial minimum inhibitory concentrations (MIC). Epidemiological cut-off (ECOFF) values for determining susceptibility were obtained from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The difference in antibiotic resistance for *Corynebacterium* spp. between gilts and sows was analysed by Chi-squared or Fisher's exact test.

Results: The *Corynebacterium* isolated were identified as *C. aurimucosum, C. casei, C. confusum, C. freneyi, C. glucuronolyticum, C. glutamicum, C. stationis,* and *C. xerosis*. Most (>80%) *Corynebacterium* spp. were resistant to clindamycin, but the difference between gilts (85.71%) and sows (95.83%) was not significant ($p = 0.45$). A few (<20%) *Corynebacterium* spp. were resistant to gentamicin (3.57%:4.17%), penicillin (10.71%:12.5%), vancomycin (3.57%:4.17%), ciprofloxacin (3.57%:4.17%) and rifampicin (0%:4.17%), but no significant differences were found between the gilts and sows ($p > 0.05$). None of the *Corynebacterium* showed any resistance to linezolid. *Corynebacterium* isolated from gilts were more often resistant to tetracycline compared with *Corynebacterium* from sows (15%:4.17%) ($p = 0.04$). However, since only a few farms were included in the study, with few individuals per farm, this significance should be interpreted with caution.

Conclusion: *Corynebacterium* from both gilts and sows showed low resistance to most of the antibiotics tested, with the exception of clindamycin. More isolates from gilts were resistant to tetracycline compared with isolates from sows, which may be due to contact with this antibiotic during the early life of the gilts, followed by the waning of resistance with time. Unfortunately, it is not known which antibiotics were contained in the semen extender used for the inseminations since it was not obligatory at the time for the manufacturer of the extender to provide such information.

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Follicular size plays a critical role on durations of in-vitro maturation (IVM) in *Bos indicus* cattle oocytes

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Folliculogenesis involves a series of pivotal steps which lead to meiotic oocyte resumption. Oocytes harvested from various stages of follicular growth may vary accordingly. The research was designed to evaluate the effect of IVM period (21, 24, 27 h) for oocytes from different follicular sizes (small; ≤ 6 mm vs. medium; > 6 mm) on the nuclear maturation and early *in-vitro* embryo production. Ovaries were collected from a nearby abattoir, and follicles of small and medium size were aspired separately and poured into two separate 15 ml falcon tubes. After searching and washing of cumulus oocytes complexes (COCs), the good quality COCs (Grade I & II) were selected for further processing for IVM and were divided into three different groups: (1) 21 hours group, (2) 24 hours group and (3) 27 hours group.The COCs were incubated in the IVM media at 38.5^{o}C, 5% CO2 and 95% humidity. In Experiment 1, a total of 335 COCs, over six replicates, were processed for 21, 24, and 27h of IVM durations. The COCs were stripped off of the cumulus cell by gentle pipetting once the specific time window for each group was completed. The denuded oocytes were stained with Hoechst (Sigma 33342) and examined under inverted microscope equipped with fluorescence filter to estimate the nuclear maturation stages. Furthermore, Experiment 2 was performed to examine the effect of IVM period on the early embryonic development of oocytes. In Experiment 2, a total of 565 COCs, over 13 replicates, were processed for 21, 24, and 27h of IVM durations. The COCs were processed for IVF *after 24* h of incubation in the IVM media with the same bull's capacitated frozen thawed semen for each group. The presumptive zygotes were denuded and cultured for 7 days at 38.5^{o}C, 5% CO2, 5% O2 and 95% humidity after 16 hours of sperm-COCs incubation. On day 2 after IVF the cleavage rates were assessed. The data were analyzed using SPSS, using the Chi square method. For small-sized follicles, the maturation stage (MII) was highest at 27 h (48.1%) compared with 24 h (37.8%) and 21 h (32.1%) groups ($P > 0.05$), while the MII stage was the highest in 21 h (68.6%) group ($P > 0.05$) for medium-sized follicles. To conclude, oocytes harvested from small-sized follicles required 24-27h duration to achieve nuclear maturation while 21h duration is enough for oocytes from medium-sized follicles. Cleavage rates were highest at 21 h compared to 24 h and 27 h of IVM duration for oocytes aspirated either from small or medium-sized follicles ($P < 0.05$). 4-cell stage embryos were also significantly higher for oocytes aspired from small-sized follicles at 21 h (54.5%) compared to 27h (31.9%). The highest values for 4 cell stage embryos were observed at 24h (71.4%) of the IVM duration for oocytes aspired from medium sized follicles. It was concluded that the aging process begins after 24 h of IVM duration due to delayed nuclear maturation for oocytes aspired from either small or medium follicles, and that the developmental competence of these oocytes starts to decline after 24 h of IVM duration. Therefore, it is suggested that the oocytes should be processed for IVF between 21 to 24 h of IVM duration in *Bos indicus* cows for better developmental competence.

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Effect of season on follicular population, oocyte quality, in-vitro maturation and fertilization in Nili-Ravi buffalo

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Keywords*:* Season, Nuclear maturation, Nili-Ravi buffalo.

Unfavorable environmental conditions may influence the quality of COCs causing premature aging, and abnormal nuclear maturation leading to decrease in the reproductive efficiency of buffalo. The aim of the present study was to evaluate the effect of season on follicular population, COCs quality, nuclear maturation and *in-vitro* fertilization in *Nili-Ravi* buffalo under sub-tropical conditions. The season were classified as Autumn (Sep-Nov), Winter (Dec-Feb), Spring (Mar-May) and Summer (Jun-Aug). Ovaries (n=1836) were collected year-round from a local abattoir near Lahore and were categorized into cyclic and acyclic based on the presence or absence of the corpus luteum. To confirm the animals' cyclic or acyclic status, the animals were blood-sampled for plasma progesterone estimation. In the 1st Experiment, the cumulus oocyte complexes (COCs) were aspirated with an 18-G needle attached to a 10ml syringe. For further processing to IVM, the COCs with grade A and B (484 over six replicates) were selected and incubated for 24 hours at 38.5^{o}C, 5% CO2 and 95% humidity. The COCs were removed from the incubator after 24 hours of IVM incubation, and completely denuded by gentle pipetting and stained with fluorescent dye (Hoechst, Sigma 33343). The frozen semen from the same elite bull was thawed and utilized for IVF in the 2nd Experiment. A total of 904 COCs, over 8 replicates, were processed for IVF. The presumptive zygotes were processed for IVC at 38.5^{o}C, 5% CO2, 5% O2 and 95% humidity for 7 days after 6 hours of sperm-COCs co-incubation. The data were analyzed by One-way ANOVA and LSD test was used to test further differences between the groups. The proportional data were analyzed by Chi square test using SPSS. The meteorological data revealed that the temperature humidity index (THI) were (72 ± 4.0 vs. 56 ± 1.0 vs. 73 ± 4.1 vs. 81 ± 0.2) in Autumn, Winter, Spring and Summer, respectively.The results manifested that the follicle population per ovary were significantly higher for Autumn (3.35 ± 0.3) and Winter (2.94 ± 0.2) months compared to Spring (2.59 ± 0.3) and Summer (2.39 ± 0.2; $P< 0.05$) months while no significant difference was observed between Summer and Spring months. Moreover, it was observed that the follicular growth pattern started to improve after Summer months (medium and large-sized follicles improved in Autumn (0.29 ± 0.07, 0.13 ± 0.04) months compared with Summer (0.15 ± 0.03, 0.07 ± 0.03) months. Based on nuclear staining, the percentage of COCs, reaching the MII stage, improved during Autumn and Winter (69%, 73% Autumn and Winter seasons vs. 47%, 40% in Spring and Summer seasons respectively; $P < 0.05$). Furthermore, the Summer season also deteriorated the quality of COCs (only 25% A & B grade COCs during Summer months). However, the cleavage rate (54 vs. 48 vs. 52 vs. 39%) was not affected by season ($P > 0.05$). In conclusion, the ovarian dynamics, oocyte quality and maturation rate are affected by Summer season in buffalo under sub-tropical conditions as compared to other three seasons. It is therefore suggested that genetic harvesting for the production of embryos should be preferred in the Autumn and Winter when the heat stress is minimum.

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Embryonic disc formation in extended *in vitro* culture of ovine embryos

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Keywords*:* embryonic disc, *in vitro*, ovine.

The highest pregnancy losses in farm animals occur during the second week of pregnancy, when many critical events of embryo development take place*:* the inner cell mass differentiates into hypoblast, which covers the inner surface of the embryo, and epiblast, which will form the embryonic disc (ED). Unfortunately, the lack of an *in vitro* system able to support such events limits our understanding of those pregnancy losses. The aim of this study was to develop an *in vitro* culture system to achieve sheep embryo development after the blastocyst stage and ED formation. *In vitro*-produced blastocysts were cultured over agarose gels to prevent attachment from day (D)6/7 until D14. Blastocysts were randomly allocated to 5 different culture media*:* 1) SOF supplemented with 10% FBS (SOF-FBS n=16), 2) an *in vitro* culture medium (hIVC n=35) supporting ED formation in human embryos (Xiang et al., Nature, 2019), 3) chemically-defined N2B27 medium alone (N2B27 n=38), 4) supplemented with activin A (N2B27+A n=47) or 5) with activin A and Rho-associated protein kinase (ROCK) inhibitor (N2B27+A+R n=33). At E14, survival was recorded and embryo diameter, area and volume were measured with ImageJ. Cell apoptosis was analysed by TUNEL and development of specific lineages was assessed by immunostaining for SOX2 (epiblast), SOX17 (hypoblast) and CDX2 (trophectoderm). Embryo survival (Chi-square test; $p<0.01$) and size (One-way Anova; $p<0.05$) were significantly reduced in embryos cultured in SOF-FBS. The percentage of apoptotic cells was significantly higher in surviving embryos cultured in N2B27+A+R (7.96 ± 1.19) than in SOF-FBS (3.87 ± 1.05) or hIVC (2.10 ± 0.25) (One-way ANOVA; $p<0.01$). Complete hypoblast migration was observed in most of the surviving embryos cultured in N2B27 (20/26 ~77%), N2B27+A (21/22 ~95%) and N2B27+A+R (13/16 ~81%), but only in 3/6 (50%) embryos cultured in SOF-FBS and in 8/28 (~28%) in hIVC. No epiblast cells were detected in any embryo developed in SOF-FBS (0/6), and only 1/22 (~4%) in hIVC showed 3 SOX2+ cells. In contrast, 11/28 (~39%), 24/36 (~66%) and 9/17 (~53%) embryos developed in N2B27, N2B27+A and N2B27+A+R, respectively, exhibited SOX2+ cells. SOX2+ cell number was significantly higher in N2B27+A+R (222.44 ± 65.20) than in N2B27 (52.45 ± 13.5) or N2B27+A (57.75 ± 14.75) (One-way ANOVA; $p<0.01$). ED formation, evidenced by a round compact structure formed by more than 50 SOX2+ cells, was observed in 5/11 (~45%) embryos cultured in N2B27, 12/22 (~54%) in N2B27+A and 8/9 (~89%) in N2B27+A+R. In conclusion, neither SOF-FBS nor hIVC medium supported epiblast survival *in vitro* in ovine embryos. On the contrary, N2B27 medium, although inducing a higher percentage of apoptotic cells, supported complete hypoblast migration and epiblast development. Activin A supplementation enhanced epiblast survival and ROCK inhibitor promoted epiblast proliferation and embryonic disc formation *in vitro.* This system could provide a significant advance to understand early embryo mortality in livestock without the need of experimental animals.

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Role of granulosa cells as a monolayer on protecting *in vitro* buffalo embryo production under heat stress conditions

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Keywords: *in vitro* embryo production, heat stress, co-culture, buffalo.

Buffaloes experience low conception rate during hot months. Therefore, the impact of co-culture system using granulosa cells (GCs) to alleviate the adverse effect of physiological heat shock, at the most sensitive preimplantation stage (zygote), on *in vitro* embryo development and related molecular response was the aim of the present study. Cumulus-oocyte complexes (COCs) were collected from ovaries (*n*= 448) of cyclic slaughtered buffalo cows. Good quality immature oocytes (*n*= 1512) were subjected to *in vitro* maturation and fertilization. Nuclear maturation was detected for oocyte chromosomal configuration using 1% aceto-orcein stain after fixed in 3:1, methanol: glacial acetic acid for 24 hour. Post *in vitro* fertilization (18-22 h), presumptive zygotes were randomly assigned into four groups: (G1) No heat shock (38.5^{o}C), (G2) Heat shock (40.5°C) and (G3) Co-culture with GCs monolayer and heat shock and (G4) Co-culture with GCs monolayer and no heat shock. Heat-shocked groups were exposed to temperature of 40.5°C for the first two hours of culture (as well established and published protocol in this species) then continued *in vitro* culture at 38.5°C up to day 8 (day of fertilization = day zero). Embryo development (cleavage rate at D3 and blastocyst rate at D8) was monitored throughout pre-implantation period. Expression profile of 7 candidate genes (CPT 2, GLUT 1, SOD 2, HSF1, HSP 90, NANOG and NFE2L2) was analyzed in blastocysts of all experimental groups using quantitative Real-time PCR after RNA isolation and cDNA synthesis. The embryo development data were analyzed by General Linear Univariate model using SPSS while gene expression data was analyzed using SAS statistical analysis package.

The results indicated that COCs expansion rate was 90.8±1.1% and nuclear maturation rate (telophase + metaphase II) was 73.8%. Cleavage rate as recorded at day 3 was significantly higher ($p \leq 0.05$) for G1 (71.1±10.5%), G3 (80.2±7.0%) and G4 (70.5±7.9%) than G2 (43.7±7.0%). In addition, embryos of G3 showed approximately the same rate of developed embryos (Morula and blastocyst stages at D 8 of culture) as of G1 (50.9±5.3 and 51.7±7.9, respectively). The expression profile of genes regulating metabolic activity (CPT2 and GLUT1) was increased ($p \leq 0.05$) in G1 and G3 compared to G2 and G4 groups. In addition, relative abundance of antioxidant gene (SOD2) showed comparable results between G1 and G3 being however higher than G2 group. Two members of heat shock protein family (HSF1 and HSP90) were significantly up-regulated in G2 and G3 compared to G1 group. While no statistical differences were observed for pluripotent regulating gene, NANOG and stress resistance transcript NFE2L2 among the study groups. In conclusion, embryos cultured in the presence of GCs as a monolayer has a beneficial impact on alleviating heat stress through the regulatory mechanism of genes involved in metabolic activity, defense system and heat shock response highlighting crucial role of these mechanisms for embryo viability when buffaloes exposed to severe heat stress.

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APAF1-deficient bovine embryos develop normally through elongation

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Keywords*:* Elongation, haplotype, holstein, HH1, APAF1.

Genomics-assisted genetic selection in cattle has uncovered several deleterious haplotypes. These haplotypes are never found on homozygosity and thereby must induce pre-term mortality. Holstein Haplotype 1 (HH1) produces a non-sense mutation in the gene *APAF1*, truncating the protein to approximately one-third. However the developmental timing when HH1 double-carriers (i.e., *APAF1* knock-out, KO) die remains unknown. The objective of this study has been to determine whether *APAF1* KO embryos display developmental defects before maternal recognition of pregnancy. *In vivo* produced embryos were recovered at Days 9 (E9, expanded blastocysts), 11 (E11, ovoid conceptuses) or 14 (E14, elongated conceptuses) post-fertilization from superovulated cows heterozygous for HH1 mutation (Hz, 2 cows/stage) inseminated with semen from an Hz bull. Embryos were fixed in 4 % paraformaldehyde for 10 min and kept at 4 ºC until analysis. E9 embryos were subjected to immunostaining with anti-CDX2 to determine trophectoderm (CDX2+) and inner cell mass (CDX2-) cell number. Total and epiblast cell numbers were determined on E11 conceptuses by immunostaining for SOX2. Conceptus and embryonic disc length were measured on E11 and E14 conceptuses. Finally, Sanger sequencing was performed to determine the genotype of each embryo. E9 embryos showed Mendelian distribution of alleles (5*:*8*:*4 for wild-type(WT)*:*Hz*:*KO). At that stage, genotype did not determine blastocyst cell counts (TE*:* 98±7 vs. 106±4 vs. 105±5; ICM*:* 20±1 vs. 21±1 vs. 22±2; for WT, Hz and KO respectively, ANOVA p>0.05). E11 conceptuses also showed Mendelian distribution of alleles (4*:*12*:*5 for WT*:*Hz*:*KO). Conceptus or embryonic disc size was also similar across genotypes (conceptus length 535±84 vs. 546±89 vs. 446±68 µm, disc length 106±13 vs. 107±12 vs. 105±7 µm, for WT, Hz and KO, respectively, ANOVA p>0.05) and no differences were noted on total or SOX2+ (epiblast) cells (total cells 1262±209 vs. 1291±352 vs. 951±282; SOX2+ cells 50±4 vs. 54±10 vs. 50±7; for WT, Hz and KO, respectively, ANOVA p>0.05). Finally, Mendelian distribution was also unaltered in E14 conceptuses (2*:*3*:*3 for WT*:*Hz*:*KO) indicating that KO embryos are able to develop to elongated conceptuses. A significant cow effect was noted on conceptus and embryonic disc length at E14, but genotype did not influence any of these parameters (conceptus length 7.9±6.1 vs. 5.6±5 vs. 5.7±2.7 cm; disc length 0.52±0.11 vs. 0.44±0.16 vs. 0.57±0.13 cm, for WT, Hz and KO, respectively, two-way ANOVA p<0.05). In conclusion, APAF1 KO embryos develop normally to elongated conceptuses, suggesting that the developmental arrest induced by the causative mutation occur after maternal recognition of pregnancy.

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Mitochondrial DNA replicates during mouse preimplantation development

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Keywords*:* Mitochondria, mtDNA, CRISPR, POLG, oocyte quality, embryo quality.

Mitochondrial DNA (mtDNA) is believed to do not replicate during preimplantation development, as mtDNA copy number remains relatively stable up to the blastocyst stage. However, POLG, the nuclear-encoded polymerase responsible for mtDNA, is expressed in preimplantation embryos. The existence of mitochondrial replication during embryo development would imply that suboptimal culture conditions may alter mtDNA copy number contributing to embryonic damage. The objective of this study was to determine whether mtDNA replication occurs before reaching the blastocyst stage. For this aim, we have analyzed the mtDNA copy number in *Polg*-deficient mouse embryos (KO, generated by CRISPR/Cas9) compared with wild type (WT) embryos. C57CBAF1 female mice (7-8 weeks old) were superovulated by intraperitoneal injections of 5 IU of pregnant mare serum gonadotropin (PMSG) and an equivalent dose of human chorionic gonadotropin (hCG) at a 48-h interval. Superovulated female mice were mated with C57CBAF1 stud males. Mouse zygotes, collected ~20 h after hCG injection, were injected with mRNA encoding for Cas9 alone (WT) or combined with sgRNA against *Polg* (POLG group). Microinjected embryos developed *in vitro* to blastocyst in KSOM medium or to egg cylinder stage (sequential system, Bedzhov et al. Nature 2014). Clonal sequencing (10 clones/embryo) was performed to determine which embryos were KO within POLG group. Embryos were deemed KO when all alleles disrupted the open reading frame of the gene. Cell count was performed at the blastocyst stage by immunostaining for the trophectoderm marker CDX2, CDX2+ cells were deemed TE cells, whereas CDX2- DAPI+ cells were deemed ICM cells. Relative mtDNA content was analysed by qPCR. Development to blastocyst was similar (~75 %) between WT and POLG groups, and no differences were noted on total, TE or ICM cells between WT and KO blastocysts (total*:* 103±6 vs. 100±7; TE 77±7 vs. 84±6; ICM 25±5 vs. 15±3; WT vs. KO, ANOVA $p>0.05$). Relative mtDNA content at the blastocyst stage was significantly reduced following *Polg* ablation (2.6±0.3 vs. 1±0.1 for WT and KO, respectively, ANOVA $p<0.05$). Embryo development to egg cylinder was significantly lower for embryos of POLG group (37.5±3.5 vs. 14.7±2.5, for WT and POLG groups, ANOVA $p<0.05$) and the differences in mtDNA between KO and WT embryos were more evident than at the blastocyst stage (192.6±42.8 vs. 1±0.6, for WT and POLG groups, ANOVA $p<0.05$). In conclusion, *Polg* ablation does not alter blastocyst formation, but reduces mtDNA content, indicating that mtDNA replication occurs already before reaching the blastocyst stage in mouse embryos.

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Ovarian characteristics, and *in vitro* nuclear and cytoplasmic oocyte maturation in Duroc and Landrace pigs

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Keywords*:* porcine, breed differences, oocyte maturation.

Differences in reproduction traits are observed between pig breeds; on average 9.2 total number piglets born (TNB) per litter are reported in the Norwegian Duroc (ND) sire line compared to 13.8 TNB in the Norwegian Landrace (NL) dam line (Norsvin, 2019). Breed differences in this trait could be due to ovarian characteristics, which might also affect *in vitro* embryo production (IVP) outcomes. Therefore, the aim of this study was to assess ovarian characteristics and *in vitro* nuclear and cytoplasmic oocyte maturation in the ND and NL line. One day after weaning, follicular phase ovaries were collected from 37 ND and 20 NL sows. Ovary length and weight were measured, and the number of follicles (<3 mm and 3-8 mm) were counted. Cumulus oocyte complexes (COCs) were collected and cultured for 48 h in Porcine Oocyte Medium (POM) supplemented with 0.4% BSA (6% CO_2, 38.8°C). To assess individual COC area and to determine a cumulus expansion ratio per well, images were taken at 0 h and 20 h using a stereomicroscope. Oocytes were stained at 20 and 48 h with 8 µg/ml Hoechst-33342 and 100 µg/ml Lectin PNA-Alexa Fluor 568 to evaluate nuclear maturation and cortical granule (CG) distribution. Additionally, total glutathione (GSH) was measured at 48 h by a GSH/GSSG-Glo assay to further elucidate cytoplasmic maturation. Data was obtained from 3 replicates and mean values for ovarian characteristics, COC area, cumulus expansion and GSH content between the breeds were analysed by Student's t-test. Proportion of oocytes in the different nuclear stages and the six CG distribution classes were analysed between the breeds by Fisher's exact test. The data from parity one sows only (ND, n=11; NL, n=10) was used for analysis of ovarian characteristics as different parities were not equally represented across breeds. A larger average ovary length (3.0±0.3 cm vs. 3.2±0.3 cm, P=0.01) and a greater number of 3-8 mm follicles (13.6±5.4 vs. 21.6±7.9, P<0.001) were observed for NL ovaries compared to ND. For all sows (ND, n=37; NL, n=20), ND COCs had on average a significantly smaller area at 0 h (P<0.0001), but a higher cumulus expansion ratio was observed after 20 h compared to NL (364±46% vs. 278±27%, P=0.001). In addition, more ND oocytes exhibited advanced stages of nuclear maturation based on chromatin configuration at 20 h than NL oocytes. Significantly more ND oocytes were in the GV2 and MI stage compared to NL, while more NL oocytes were present in the GV1 stage. Contrary, the proportion of CG distribution groups of ≥ 4 showed more NL oocytes in the more advanced CG distribution groups compared to ND at the same timepoint (34% vs. 56%, P=0.0016). Maturation to MII stage at 48 h did not differ between ND and NL, 136/151 (90.1%) and 142/162 (87.7%), respectively. No differences were observed for GSH content or CG distribution at 48 h. In conclusion, differences with regard to ovarian characteristics as well as nuclear and cytoplasmic maturation at 20 h, but not at 48 h, were observed between breeds which could affect IVP outcomes. Further experiments are required to understand differences in fertilization and embryo development between the breeds.

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Alginate-based encapsulation of bovine cumulus-oocytes complexes during *in vitro* maturation

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Keywords*:* alginate, oocyte maturation, cattle.

Ovarian architecture prearranges oocyte quality. For example, the interaction between the stroma and the ovarian follicle has implications in polycystic ovary syndrome in humans (Fournier et al., 2017 Gynecol Obstet Fertil Senol 45) or culturing isolated preantral follicles in domestic species (Brito et al., 2016, Reprod Domest Anim 51). *In vitro* maturation (IVM) is a process in which cumulus-oocyte complexes (COCs) are removed from the ovary, and the final stages of oocyte development occur *in vitro*. Thus, IVM occurs in an altered physical environment, since cumulus-oocyte complexes (COCs) are deprived of the extracellular support that exists within the ovarian follicle. Full *in vitro* recapitulation of the events associated with successful oocyte maturation is not always achieved during IVM with only 30% of immature oocytes developing to the blastocyst stage (Lonergan and Fair, 2016 Annu Rev Anim Biosci 4). Here we tested the hypothesis that maintaining COCs within a three-dimensional structure during IVM for a better recapitulation of the physiological environment could improve nuclear and cytoplasmic maturation of the gamete. Inert biomaterials, such as alginate, have been used in numerous biomedical engineering applications due to its biocompatibility and ease of gelation (Shea et al., 2014 Annu Rev Biomed Eng 16). Thus, we examined the effect of encapsulating bovine COCs in alginate hydrogels on nuclear maturation and cortical granules (CG) migration. For IVM in alginate (ALG), 10 COCs were transferred to 0.5% ALG, aspirated with an automatic pipette in 10 µl volume, and transferred to the cross-linking solution (50 mM $CaCl_2$, 140 mM NaCl) to allow ALG gelification. ALG beads containing COCs were then washed and transferred to 500 µl BO-IVM medium (four beads/well). As a control, groups of 40 unencapsulated COCs were placed in IVM wells. Both groups were cultured for 22 h (38.5ºC, 5% CO_2 in air) and then COCs were released from the ALG beads following a brief treatment with 10 IU/ml alginate lyase. COCs in both treatment groups were denuded, and the oocytes were recovered. To assess CG distribution and nuclear status in the oocytes, the zona pellucidae were digested (0.1% pronase) and cells were fixed (4% paraformaldehyde) and stained (10 µg/ml *Lens Culinaris-Agglutinin*-FITC and DAPI-Vectashield). Confocal microscopy revealed no difference in the percentage of oocytes that reached metaphase II between groups (69.9±5.4%, N = 73 *vs.* 82.1±5.2% N = 56 for control and ALG, respectively). The proportion of oocytes that showed type III CG (CG fully migrated, arranged under oolemma and ready to be exocytosed) was 76.7±5.0% (control) and 82.1±5.2% (ALG). In conclusion, while there was a tendency of encapsulation to improve meiotic maturation and favorable CG distribution, these results were not significant. Additional fertilization and embryo development studies are warranted to examine whether encapsulation during IVM improves the developmental competence of the gamete.

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Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Embryonic disc formation following post-hatching bovine embryo development *in vitro*

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Keywords*:* bovine, embryonic disc, *in vitro* .

Embryonic mortality during the second week of gestation accounts for the greatest pregnancy losses in bovine, exerting a relevant economic impact on farming. By day 9, epiblast and hypoblast have emerged from the inner cell mass. The hypoblast then migrates to cover the entire inner surface of the embryo, and the epiblast forms a flat embryonic disc. These developmental events are poorly understood, mainly because they cannot be recapitulated *in vitro*. Previous studies have established an *in vitro* post-hatching development system that supports bovine embryo development beyond the blastocyst stage (Brand*ão* et al., Biol Reprod, 2004; Vajta et al., Theriogenology, 2004). This system, based on agarose gel tunnels and glucose-enriched (PHD) medium, achieves trophoblast expansion and proliferation of hypoblast cells, but embryonic disc formation is impaired. The aim of this study has been to develop an *in vitro* system able to support embryonic disc formation. *In vitro*-produced D9 blastocysts were allocated to SOF:PHD (1:1) or N2B27 and at D11 they were measured and cultured individually in PHD or N2B27, respectively, in different culture substrates*:* 1) inside agarose tunnels (n=69) or free-floating over an agarose-coated dish (n=48) in PHD medium (Synthetic Oviduct Fluid supplemented with 27.7 mM glucose and 10% FCS); 2) in PHD medium (n=62) or in a chemically-defined enriched medium (N2B27, n=47) over an agarose-coated dish and 3) with (n=32) or without (n=47) agarose coating in N2B27 medium. At D15, survival was recorded (dead embryos were clearly distinguishable as they collapsed and degenerated), embryo length, area and volume were calculated using Fiji, the abundance of transcripts encoding interferon Tau (*IFNT2*) and metabolic enzymes was analysed by RT-qPCR, and the development of specific lineages was assessed by immunostaining for SOX2 (epiblast), SOX17 (hypoblast), and CDX2 (trophectoderm). No differences were found on embryo survival until D15 and the main factor determining survival was the initial embryo size at D11 ($p<0.05$, Chi-square test). Culture inside agarose tunnels shaped embryo morphology by physical constriction, but it reduced embryo area and volume (1.92 ± 0.31 mm^2 and 1.52 ± 0.24 mm^3 inside tunnel *vs.* 3.98 ± 0.92 mm^2 and 7.50 ± 2.33 mm^3 free-floating, $p<0.05$, t-test) and did not provide any significant advantage in terms of development of hypoblast and epiblast lineages. *IFNT2* expression was higher in PHD medium and anaerobic glycolysis-related genes were upregulated in D15 vs. D9 embryos irrespective of the media used. In contrast to PHD, N2B27 medium supported complete hypoblast migration and epiblast survival *in vitro*, even in the absence of agarose coating*:* ~56 % of D15 embryos developed in N2B27 showed SOX2+ cells (6/11 over agarose and 9/16 without agarose) and ~22 % developed embryonic disc-like structures formed by SOX2+ cells (2/11 over agarose and 4/16 without agarose). In summary, we provide a culture system supporting trophectoderm proliferation, hypoblast migration and epiblast survival beyond the blastocyst stage.

Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Characterization and analysis of miRNA content of bovine oviduct and uterine extracellular vesicles across estrous cycle

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Keywords*:* oviduct, uterus, extracellular vesicles.

Recently, extracellular vesicles (EVs) found in various biological fluids and particularly in reproductive fluids, have gained a considerable attention for their possible role in cell- to- cell communication. Among, the different bioactive molecules cargos of EVs, MicroRNAs (miRNAs) are emerging as promising diagnostic biomarkers with high clinical potential. Therefore, aiming to understand the roles of EVs in bovine reproductive tract, we intended to characterize and profile the EVs of oviduct and uterine fluids (OF, UF) and their miRNA across the estrous cycle. For this, 5 reproductive tracts for each stage of estrous cycle (S1*:* days 1 to 4; S2*:* days 5-10; S3*:* days 11-17; S4*:* days 18-20) were selected according to their corpus luteum morphology from slaughtered heifers and transported to the laboratory on ice. EVs were isolated by size exclusion chromatography from a flushing of 1ml and 3ml of OF and UF, respectively, and concentrated by ultracentrifugation. The obtained EVs pellet was suspended in 100 µL of PBS. One part was used for EVs characterization by Nanotracking analysis (NTA), Transmission electron microscopy (TEM) and western blot. The other part was used for RNA extraction and miRNA expression profiling by primer based real-time PCR of 383 mature miRNA sequences. Statistical differences in miRNA level were assessed by ANOVA. Both NTA and TEM observations confirmed the existence of EVs in OF and UF at all stages, with a mean size ranging between 135-180nm. The NTA quantification showed an average concentration of 3.4 x 10^{10} EVs/ml and 6,0 x 10^{10} EVs/ml for OF and UF respectively, whatever the cycle stage. Moreover, western blot analysis evidenced the EVs expression of some classical markers described for exosomes*:* tetraspanin cell surface proteins (CD67 and CD9); heat shock protein 70 (HSP70). The miRNA analysis revealed the abundance of 232 and 332 miRNAs in OF and UF, respectively. 67% and 82% of these miRNAs are common to all stages of estrous cycle. 9 miRNAs were differentially abundant in OF between stages of cycle*:* 8 of them displayed a progressive increase from S1 to S4 ($P<0.05$) and one miRNA showed a reduction ($P <0.05$). In UF a total of 14 miRNA were differentially abundant between stages. Greater differences were observed between S1 and S3, with 11 miRNAs enriched in S3 compared to S1 ($P <0.05$). S2 showed enrichment of 4 miRNAs in relation to S1 ($P <0.05$). Reduction of 4 miRNAs was also observed in S4 compared to other stages ($P <0.05$). In conclusion, these preliminary results indicate a possible hormonal regulatory effect of the estrous cycle on miRNA content in EVs of bovine OF and UF. Ongoing bioinformatics analysis are aiming to predict the genes targeted by these miRNAs, their signaling pathways and functional annotation clusters associated with their biological processes.

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Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Porcine sperm attracted by chemoattractants, showed the highest curling tail when incubated in adverse osmolarity condition

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Keywords*:* Boar spermatozoa, chemotaxis, osmolarity condition

The hypo-osmotic swelling (HOS) test enables the identification of sperm with functionally intact membranes and is one of a range of tests commonly used to determine sperm viability. The osmotic stress caused by the hypo-osmotic medium is enough to induce an influx of water into the cell, which results in an increase in volume and hence curling of the tail. The aim of the study was to evaluate the reaction ability of porcine sperm in hypo-osmotic solution previously attracted by follicular fluid (FF), oviductal fluid (OF), conditioned medium (CM) and progesterone (P4). The chemotaxis system consisted of two wells (A and B) connected by a capillary. Five wells (A) were filled with fresh sperm washed (20x106/mL diluted in 500 µL of TALP) from different fertile boars. The opposite wells (B) were filled with TALP and TALP supplemented with corresponding chemoattractant*:* FF*:* 0.25%, OF*:* 0.25%, CM*:* 0.13%, and P4*:*10 pM. After 20 min of chemotaxis, the sperm (wells B) were incubated with a final hypo-osmotic solution of 100 mOsm/L for 20 min at 5% CO_2, 38.5 ºC, with saturated humidity. A total of 6000 sperms were evaluated in five replicates. ANOVA was used for the statistical analysis, and the means were separated using the Tukey test at $P < 0.05$. Results show that a higher curled tail concentration (%) was detected with FF (87.4±3.2a), OF (89.9±2.2a), CM (87.8±2.9a) P4 (88.6±2.1a) than control (84.3±3.2b) ($p<0.05$). In conclusion, our chemotaxis system, in combination with different chemoattracts, selects a pool of viable spermatozoa, as evidenced by the hypoosmotic swelling test. Further studies should be done to analyze both the phenotype and genotype of these spermatozoa, in the attempt to improve sperm selection prior to *in vitro* fertilization in the porcine species.

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Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Development of *in vitro* matured porcine oocytes after *in vitro* fertilization with ejaculated or epididymal spermatozoa

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Keywords: boar spermatozoa, *in vitro* fertilization, embryo.

In vitro production of pig embryos (IVP) is still the object of multiple studies, because a number of issues related to this method have not been resolved yet. Among problems connected with IVP are the quality of sperm after capacitation and the quality of *in vitro* obtained embryos. The aim of this study was to determine the quality and fertilization ability of ejaculated and epididymal boar spermatozoa. Ejaculated (n=7) and epididymal (n=4) sperm before and after capacitation were evaluated under a microscope and with the computer-assisted analysis method. The following parameters were evaluated: motility, progressive motility, curvilinear velocity (VCL), straight-line velocity (VSL) and hyperactivity. Ejaculated and epididymal spermatozoa underwent *in vitro* capacitation and *in vitro* fertilization (2 and 3 sessions, respectively) with the matured *in vitro* oocytes. Potential zygotes were cultured *in vitro* up to the expanding blastocyst for 6-8 days. The total cells number (mean) and number of apoptotic nuclei (mean) per blastocyst were assessed. Before and after capacitation, statistically significant differences ($p<0.05$, t- Student test) were found when comparing progressive motility of ejaculated (60.77% and 68.8%, respectively) and epididymal spermatozoa (30.0% and 55.7%, respectively). Moreover, after capacitation significant differences ($p<0.01$, t-Student test) between these two types of semen were recorded in the following motility parameters: VCL (49.2% and 83.3%, respectively) and hyperactivity (50.5% and 13.2%, respectively). Significant differences ($p<0.01$, t- Student test) in terms of hyperactivity before and after capacitation of ejaculated spermatozoa (14.81% and 50.5%, respectively) were also observed. We showed a similar *in vitro* fertilization ability of ejaculated and epididymal boar spermatozoa. The developmental potential of porcine embryos obtained as a result of fertilization with ejaculated and epididymal spermatozoa was also similar (53.7% of cleaved embryos and 25.9% of blastocysts, n=16; and 55.1% of cleaved embryos and 30.5% of blastocysts; n=4, respectively). A higher total number of cells per blastocyst was obtained as a result of *in vitro* fertilization with ejaculated spermatozoa (mean 34.2±8.3, n=70) when compared to epididymal spermatozoa (mean 28.5±6.45, n=13) ($p<0.05$, Chi^2 test). However, the number of apoptotic cells in blastocysts was similar in both groups (differences statistically nonsignificant). In conclusion, boar spermatozoa irrespective of type (ejaculated or epididymal), displayed a similar susceptibility to *in vitro* capacitation and *in vitro* fertilization. Developmental competence of porcine embryos obtained after *in vitro* fertilization with ejaculated and epididymal spermatozoa was also similar. Blastocysts obtained after *in vitro* fertilization with ejaculated spermatozoa showed a higher total number of cells in comparison to the same procedure carried out with epididymal spermatozoa.

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Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Seminal plasma downregulates the Signal Transducer and Activator of Transcription 5A gene (STAT5A), a modulator of immune response, in the preovulatory porcine endometrium

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Keywords: seminal plasma, transcriptomics, pig.

Seminal plasma (SP) is a complex secretory mix of proteins, cytokines, and hormones that acts as a carrier for spermatozoa as well as actively elicits molecular responses when females are inseminated. Thus, SP is playing a major role in male-female signaling and positively impacting early embryo development *(*Martinez CA et al., Front Vet Sci, 6:465, 2019*;* Alvarez-Rodriguez M et al., Int J Mol Sci, 20(3):E513, 2019*).* Alongside, glucocorticoid signaling, deemed essential for normal reproduction, seems to involve a wide range of molecules. Among them, there are the STAT-proteins, involved in JAK/STAT signaling, that modulate cytokine production during inflammatory processes, and heat shock 70kDa protein HSPA4L, implicated in osmotic stress adaptation and fertility. The present study evaluated the effects of infused boar SP on the expression of glucocorticoid related genes in the preovulatory reproductive tract of weaned fertile sows (n=8) on the 1st day of estrus. Samples of cervix, distal and proximal uterus, utero-tubal junction, isthmus, ampulla and infundibulum were surgically removed 24 h after cervical infusion with pooled sperm-free SP from the whole ejaculate (SP-Total; n=4) from mature fertile boars (n=5) or after infusion with the protein-free extender Beltsville Thawing Solution (control, n=4). RNA was isolated following a TRIzol-based protocol and analyzed for global transcripts using specific microarrays (PORGENE 1.0 ST GeneChip® array, Affymetrix) aiming to identify the specific expression for 22 glucocorticoid-related genes. The data was normalized (Robust Multiarray Average) and analyzed with the Transcriptome Analysis Console (RMA-method, -1>log fold change>1; p<0.05). Molecular functions and biological processes of all the analyzed genes were identified by PANTHER classification system. Functional pathways were described using the KEGG database. Significant changes in gene expression were triggered by SP in distal uterus (*HSPA4L* upregulation and *STAT5A* downregulation), and infundibulum (*PTGS2*, *STAT6*, and *NR3C1* downregulation) when compared to the control. The JAK-STAT (ssc04630), and also Th1, Th2 (ssc04658) and Th17 (ssc04659) cell differentiation pathways were enriched in the downregulated genes. Owing to the active role of *STATs* in inflammation, this SP-mediated *STAT5A* downregulation might modulate an increased uterine inflammatory to avoid a plausible harmful effect to spermatozoa during sperm transport towards the oviductal reservoir.

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Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

Natural mating represses pro-inflammatory responses in the pre-ovulatory porcine endometrium and endosalpinx (ampulla) by down-regulation of caspase-1 (CASP1) and caspase-12 (CASP12)

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Keywords: mating, pro-inflammatory response, pig.

Natural mating and artificial insemination (AI) elicit molecular responses in the sow genital tract upon the entrance of semen (Alvarez-Rodriguez M et al., Sci Rep, 10, 5061, 2020). Alongside, cysteine proteases i.e. caspases, play important functions in innate immunity, activating cytokines, and apoptosis. The inflammatory caspase-1 (*CASP1* gene) is activated in response to pathogen-derived and endogenous mediators, inducing secretion of the pro-inflammatory cytokines interleukin-1β and interleukin-18, both key modulatory factors in placenta development and attachment during early pregnancy in pigs. Besides, *CASP1* expression is upregulated by the presence of viable conceptuses in the porcine uterine lumen. Caspase-12 (*CASP12* gene), on the other hand, negatively regulates inflammatory processes, inhibiting the activation of caspase-1 and preventing the production of pro-inflammatory cytokines. This study investigated the effects of natural mating and AI on *CASP1* and *CASP12* gene expression along the sow reproductive tract during the pre-ovulatory stage. Samples of the reproductive tract (cervix, distal and proximal uterus, utero-tubal junction, isthmus, ampulla, and infundibulum) were surgically removed from sows 24 h after natural mating (NM, n=4) or cervical AI with 10 mL of the sperm-peak fraction (Semen-AI, n=4). Sows cervically infused with the protein-free extender Beltsville thawing solution were used as controls (n=4). RNA was isolated following a TRIzol-based protocol and analysed for global transcripts using specific microarrays (PORGENE 1.0 ST GeneChip® array, Affymetrix). Data was normalized (Robust Multiarray Average). Only *CASP1* and *CASP12* expression were analysed with the Transcriptome Analysis Console (-1>fold change>1, p<0.05). After NM the *CASP1* and *CASP12* expression were downregulated in uterus (distal: -1.71, -1.28; and proximal: -2.14, -1.73) and ampulla (-1.59, -1.32). Also, both NM and Semen-AI shared a downregulation of *CASP1* expression in the isthmus (-1.38, -1.42) and ampulla (-1.59, -1.53). The expression of *CASP12* in the ampulla was downregulated by NM (-1.32), whereas Semen-AI upregulated *CASP12* expression (1.44). Finally, Semen-AI downregulated *CASP1* expression in the utero-tubal junction (-1.47). Results indicate that both *CASP1* and *CASP12* were downregulated by the presence of spermatozoa in the female reproductive tract during the pre-ovulatory stage, although the verification of the results could be performed by PCR. Results confirm the presence of a mechanism of immune tolerance for spermatozoa and suggest that endometrial *CASP1* and *CASP12* expression is more strongly regulated by natural mating than for AI, probably induced by the act of mating itself regardless the presence of semen.

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Animal Reproduction

Embryology, developmental biology, and physiology of reproduction

In vitro bovine embryo production in high palmitic acid conditions decreases DNMT1 expression in bovine zygotes and lowers global DNA methylation in the produced morulas

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Keywords: bovine *in vitro* embryo, epigenetics, lipotoxicity.

Maternal metabolic disorders are associated with subfertility. Upregulation of lipolysis causes a rise in non-esterified fatty acids in the blood, which is reflected in the follicular and oviductal micro-environment. This has a lipotoxic effect on oocyte and embryo development, mainly due to elevated palmitic acid (PA) concentrations. Such disturbances in this micro-environment can cause persistent defects in later life. This is possibly mediated through epigenetic changes, like DNA methylation at cytosine residues (5-methylcytosine (5-mC)) in the oocyte or embryo. DNA methyltransferase 1 (DNMT1) is an important enzyme that maintains the DNA methylation pattern during mitosis. The dynamic nature of the epigenetic landscape during oocyte maturation and early embryo development makes this a vulnerable window for epigenetic alterations. We hypothesized that exposure of bovine oocytes to PA during *in vitro* maturation (IVM) and culture (IVC) alters expression of DNMT1 in the resulting zygotes and modifies global DNA methylation levels in the resulting morulas. In this study, bovine oocytes were exposed to a pathophysiological concentration of PA (150µM) or Solvent Control (SCONT) media during IVM (24h). Oocytes were *in vitro* fertilized (for 20h) and presumptive zygotes were cultured in either PA (230µM) or SCONT media, respectively. Cleavage rates were recorded at 48h post insemination (p.i.) and blastocyst rates at day 7 (D7) and 8 (D8) p.i. (n=538 oocytes, 4 replicates). Zygotes were collected at 20h p.i. and snap-frozen in pools of 5-10/treatment (4 replicates) to evaluate mRNA expression of DNMT1 by qPCR. This was measured in duplicates and normalized based on the expression of validated reference genes YWHAZ and GAPDH. Relative fold change was calculated with the delta-delta Ct method. Also, morulas (D5.7; n=7/treatment) were fixed in 4% paraformaldehyde for 5-mC immunostaining. Images were acquired with a Leica SP8 confocal microscope and quantified using Image-J to measure integrated density of the nuclei in different z-stacks (SCONT: mean cell number=36.3±7.5; PA: mean cell number=32.3±3.7). Developmental competence data were analysed using logistic regression, other numerical data using an independent samples t-test. Exposure of bovine oocytes and embryos to PA during IVM and IVC resulted in significant reduction of cleavage rates (68.3%) compared to the SCONT group (81.9%) (P<0.001). Blastocyst rates were significantly lower in the PA group (D7: 15.6%; D8: 25.2%) compared to the SCONT group (D7: 32.8%; D8: 39.1%) (D7: P<0.001; D8: P<0.001). DNMT1 expression in PA-exposed zygotes was significantly decreased (38-fold downregulation, P=0.02) compared to SCONT zygotes. Also, staining intensity of 5-mC was significantly decreased in morulas exposed to PA (34,7% reduction compared to SCONT, P=0.017). We conclude that exposure of bovine oocytes and embryos to PA during IVM and IVC hampers development, and alters global DNA methylation levels which is detected at the morula stage.

Animal Reproduction

ABSTRACTS: 36TH ANNUAL MEETING OF THE ASSOCIATION OF EMBRYO TECHNOLOGY IN EUROPE (AETE)

Embryology, developmental biology, and physiology of reproduction

Dietary caloric normalization or restriction as preconception care strategies: impact on oocyte developmental competence and blastocyst quality in high fat/high sugar-induced obese outbred mice

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Keywords: obesity, preconception care intervention, oocyte development.

Maternal metabolic disorders like obesity are linked to decreased oocyte and embryo quality, and reproductive failure. Obese patients are advised to lose weight before conception to increase pregnancy chances. However, as human studies show no univocal guidelines, more fundamental research might provide additional answers. Furthermore, it is unclear if oocyte quality can be restored over time after starting a preconception care intervention (PCCI). Therefore, we aimed to test the effect of diet normalization or caloric restriction (CR) as PCCI in high fat/high sugar (HF)-fed obese mice and examined the impact on oocyte development and embryo quality. Five week old female outbred Swiss mice were fed a control (CTRL; 10% fat) or a HF (60% fat in diet, 20% fructose in drinking water) diet for 7 weeks. Afterwards, HF-mice were put on different PCCIs for 2 or 4 weeks, resulting in four treatment groups: 1) CTRL diet for 9 or 11w (CTRL_CTRL), 2) HF diet for 9 or 11w (HF_HF), 3) switch from a HF (7w) to an *ad libitum* CTRL diet for 2 or 4w (HF_CTRL) and 4) switch to a 30% CR diet for 2 or 4w (HF_CR). Change in body weight (BW) was recorded twice a week (n=192 mice). *In vivo* matured oocytes were collected after superovulation, *in vitro* fertilized and cultured (n=6 mice/group/timepoint). Oocyte developmental competence (n=722 oocytes) was examined by recording cleavage (24h p.i.) and blastocyst rates (5 days p.i.). Blastocyst quality (n=183) was determined by caspase-3 immunostaining and DAPI. Total cell count (TCC) and apoptotic cell indices (ACI) were calculated. Categorical and numerical data were analysed using binary logistic regression and ANOVA, respectively, and were Bonferroni-corrected. In comparison with the CTRL group, HF diet increased BW after 7 weeks by 24.19% (*P=0.000*). After the start of the PCCI, both HF_CTRL and HF_CR mice progressively lost weight and reached values similar to control mice after two weeks. After 2 weeks of PCCI, oocytes from HF_HF mice displayed lower cleavage rates than those from CTRL_CTRL mice (36.26% *vs.* 64.52%, *P=0.002*) but blastocyst rates (26.37% *vs.* 35.48%, *P*>0.1) were not different. HF_CR, but not HF_CTRL, oocytes showed higher cleavage rates (68.48%, *P=0.000*) compared with HF_HF oocytes. Moreover, both HF_CTRL (44.64%, *P*=0.033) and HF_CR (59.78%, *P*=0.000) oocytes showed improved blastocyst rates when compared to the HF_HF group (26.37%). After 4 weeks of PCCI, HF_HF oocytes also displayed lower cleavage rates compared with CTRL_CTRL mice (42.17% *vs.* 62.11%, *P=0.040*) while blastocyst rate (34.94% *vs.* 45.26%, *P*>0.1) was not affected. HF_CR, but not HF_CTRL, oocytes showed higher cleavage (65.28%, *P=0.018*) but not blastocyst rates (52.78%, *P*>0.05) when compared to HF_HF. No significant differences in blastocyst TCC and ACI could be detected among relevant treatment groups at both time points (*P*>0.1). Based on this information, switching to a caloric restriction diet (HF_CR) seems to be more efficient in restoring oocyte quality in an obese mouse model than diet normalization (HF_CTRL).