A258E Embryology, Developmental Biology and Physiology of Reproduction

Cumulus cells protect the bovine oocyte against lipotoxicity by converting saturated into unsaturated fatty acids using stearoyl-CoA-desaturase during in vitro maturation

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Keywords: cumulus, oocyte, free fatty acid.

Elevated levels of free fatty acid (FFA) in blood are a metabolic indication for either obesity or a sign for a negative energy balance and adversely influence cell functioning, including that of oocytes. In particular, saturated palmitic (C16:0) and stearic (C18:0) acid can induce lipotoxic events in cumulus-oocyte-complexes (COCs) and result in reduced developmental competence of the oocyte (Leroy et al., Reproduction; 130: 485-495, 2005). In contrast, elevation in mono-unsaturated oleic acid (C18:1) levels prevents the loss in oocyte developmental competence even in presence of relatively high levels of saturated FFA (Aardema et al., Biol Reprod; 85: 62-69, 2011). We have recently reported on the role of cumulus cells in protecting the oocyte against lipotoxic effects associated to elevated levels of FFA (Aardema et al., Biol Reprod; 88: 164, 2013; Lolicato et al., Biol Reprod; 92: 16, 2015). Here we extend that study and investigated whether stearoyl-CoA-desaturase (SCD-1 which converts C18:0 into C18:1) is functional in cumulus cells as a protecting enzyme.

COCs were retrieved from bovine slaughterhouse ovaries, matured for 23h, were fertilized and the presumed zygotes were cultured until day 8 according to our standard protocol. The SCD-1 gene- and protein expression in cumulus cells were detected by quantitative RT-PCR and immunoblotting respectively. Results are presented as means ± SD. Statistical analyses was performed in SPSS version 22.0 by the use of an univariate general linear model for blastocyst rates and an univariate analysis of variance on the log transformed lipid data to achieve normally distributed data. $P < 0.05$ was considered significant. Inhibition of SCD-1 activity (1μM, Biovision) in the presence of C18:0 (250 μM) during maturation of COCs resulted in a reduction in the blastocyst rate when compared to the not inhibited control group in the presence of C18:0 (10 ± 4.7% and 25 ± 7.5%, respectively; $P < 0.001$, n =~280 in 3 runs per group). C18:0 and C18:1 levels were determined by HPLC mass spectrometry after total lipid extraction and hydrolysis. A decrease in the C18:1/C18:0 ratio (0.5 ± 0.03) was identified in cumulus cells after inhibition of SCD activity in the presence of 250μM C18:0 versus the group without SCD inhibition (1.3 ± 0.46; $P < 0.01$), suggesting active conversion of C18:0 into C18:1 by SCD.

Combined, the data indicate that in cumulus cells SCD-1 converts the potentially toxic saturated C18:0 into less harmful mono-unsaturated C18:1. These data unravel the mechanism of how cumulus cells are competent to protect maturing oocytes against saturated FFA.

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A comparative analysis of the protein composition of the oviductal and uterine fluids in cattle during the periovulatory phase by 2D fluorescence difference gel electrophoresis (DiGE)

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Keywords: oviductal fluid, uterine fluid, protein composition.

During the periovulatory phase of the estrous cycle processes important for fertilization are going on in the female genital tract. These processes include, e.g., the transport and maturation of gametes. The oviduct and uterus lumen contain complex fluids, which interact with gametes; however, the composition is not entirely known. The oviductal fluid (OF) and uterine fluid (UF) consist of secreted components derived from epithelial tissue as well as transudate from blood serum. The identification of the components of these fluids involved in transport and interaction of gametes during fertilization can be very useful for improvement of culture media used in IVF. The aim of this study was to identify, through a comparative study, differences in the protein composition of OF and UF. Six Simmental heifers were synchronized using gonadotropin-releasing hormone 500 µg IM (Receptal®, MSD) in conjunction with two intramuscular injections of PGF2alpha (10 µg , Estrumate®, MSD). A rectal examination was performed confirming the presence of corpora lutea and the heifers were slaughtered three days after the second PGF injection. OF and UF from slaughterhoused animals classified according to the ovarian morphology (ovarian follicle of approximately 15mm diameter) were pooled. The fluids (150 µg of OF and 150 µg of UF) were labeled with different cyanine fluorescent probes (Amersham) and separated according to the isoelectric point using immobilized pH gradient strips (3-10 pH, 17 cm, Protean® IEF cell system, Bio Rad). The second dimension was performed in a polyacrylamide gel (12%) in the presence of SDS using a Protean II XL system (Bio Rad). The images were obtained with a Typhoon 9410 scanner (Amersham). Image analysis was performed with the Progenesis SameSpots software v 4.0. Approximatively 1000 spots were identified in both, oviductal and uterine fluids. The image analysis showed that 23 spots were different between the two samples ($P < 0.01$ and fold difference >5). Thus, a number of 16 spots were more abundant in UF and 7 more abundant in OF. Future studies will identify these different protein spots and this information would provide more detailed information about their roles during the fertilization.

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Proteomic characterization of oviductal extracellular vesicles along the estrous cycle in cattle

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Keywords: Extracellular vesicles, oviduct, estrous cycle.

Exosomes along with microvesicles, named globally as extracellular vesicles (EVs) (Raposo and Stoorvogel, 2013) have been suggested as mediators of the interactions between gametes and embryos and the maternal tract. EVs contain proteins, lipids, and nucleic acids that can be transferred to recipient cells as a new way of cell-to-cell communication. The delivery of the molecular cargo from the maternal tract to sperm and embryos may have a functional impact, promoting sperm capacitation and fertilization ability or supporting early embryo development. Since the reproductive tract undergoes many physiological and hormonal changes during the estrus cycle regulating the environment surrounding gamete and embryos, these changes may also modulate EVs cargo. Thus, the objective of our study was to determine the influence of the stage of the oestrous cycle on the oviductal EVs content at protein level. To perform this study, a bovine *in vivo* model was used. Pairs of oviducts with their attached ovaries were collected from cyclic (corpus luteum (CL) present), non-pregnant bovine reproductive tracts at the slaughterhouse. To estimate the stage of the oestrous cycle, the CL morphology and follicle populations were assessed according to Ireland et al. (1980): Stage 1 (d 1-4) recently ovulated follicle; Stage 2: (d 5-11) early CL development; Stage III: (d 11-17) yellow or orange CL and Stage 4: (d 18-20) regressing CL with little vasculature and a large preovulatory follicle present. Subsequently, oviducts were flushed to collect their fluid. EVs were isolated by ultracentrifugation. Protein composition from EVs samples was analyzed by high resolution tandem mass spectrometry using GeLC-MS/MS strategy combined to label free quantitative method (samples from 5 replicates were pooled for each stage, n = 20 samples). Our first preliminary quantitative proteomic results based on spectral counting showed differential protein abundance of EVs along the bovine oestrous cycle. Among the 336 clusters of proteins identified, 170 were differential among different stages of oestrous cycle (p-value <0.05, ratio <0.5 or ratio>2). From the 498 individual proteins identified from all stages, only a small number of proteins were specific of each stages (2 proteins at Stage 1, 1 at Stage 2, 1 at Stage 3 and 2 at Stage 4), while 454 proteins were common to all stages but with significant differences in abundance between stages. Among the common proteins, we found significant differences in proteins involved in gamete interaction and embryo development such as OVGP: P = 0.00010 S1 <S2; HSPA8: P = 0.00010 S1 <S2; Myosin-9 (P = 0.0028) S2 <S1; HSP90: P = 0.00010 S2 <S1). The characterization of oviduct-derived EVs under different regulation by oestrogen and progesterone will extend our understanding of gamete/embryo-maternal communication with potential impacts on infertility.

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Alpha-tocopherol affects gene expression patterns of rabbit cumulus-oocyte complexes and reduces apoptosis rate during in vitro maturation

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Keywords: a-tocopherol, *in vitro* oocyte maturation, rabbit.

Oxidative stress compromises oocyte developmental competence during *in vitro* maturation (IVM). Antioxidants such as vitamin E may avoid this imbalance. The aim of this study was to investigate the effect of a-Tocopherol (α-TocOH) on the relative mRNA abundance of genes involved in cumulus expansion (*GJA1, PTGS2*), cell cycle and viability (*AKT1*), cell cycle regulation and apoptosis (*Tp53, CASP3*) and antioxidant response (*SOD2, GPX1, CAT*) in rabbit cumulus oocyte complexes (COCs) *in vitro* matured. The apoptosis index in cumulus cells (CCs) and the hydrogen peroxide (H_2O_2) released by the COCs in maturation media were also assessed. For these purposes, COCs from follicles ≥1mm were recovered, selected and *in vitro*-matured for 16h (38ºC, 5% CO_2) in a medium containing TCM-199 (Sigma, Madrid, Spain) with 0.3% bovine serum albumin (Sigma, Madrid, Spain) and 10 ng/mL Epidermal Growth Factor (EGF) (Sigma, Madrid, Spain) supplemented with 0, 100, 200 or 400 μM α-TocOH (Sigma, Madrid, Spain), named as 0E, 100E, 200E and 400E groups, respectively. After IVM, maturation media without cells was collected and stored at -32ºC and H_2O_2 concentrations were measured by the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Life Techonologies, NY, USA). The mRNA transcripts were quantified in 203 oocytes and their respective CCs (n = 51, n = 50, n = 50, n = 52 for 0E, 100E, 200E and 400E groups, respectively) by qRT-PCR to contrast relative levels of histone *H2AZ* and genes described above. Apoptotic index was studied in 43 COCs by TUNEL technique (Roche Diagnostics, SL, Barcelona, Spain) (n = 10, n = 10, n = 10, n = 12 for 0E, 100E, 200E and 400E groups, respectively). Data were analysed using one way ANOVA and Bonferroni test to compare means. In oocytes, *SOD2*, *CAT* and *Tp53* poly (A) mRNA contents were down regulated with 100 μM α-TocOH supplementation compared to the control group without this antioxidant ($P < 0.05$). In CCs, *CASP3* mRNA transcripts were lower in groups with intermediate concentrations of antioxidants (100E and 200E) compared to 0E and 400E groups ($P < 0.005$), in spite of the apoptosis rate was significantly reduced in all groups supplemented with α-TocOH (100E: 9.12 ± 1.81%, 200E: 10.26 ± 2.75%, 400E: 8.50 ± 2.63% vs 0E: 22.50 ± 3.40%, $P < 0.05$). However, the amount of H_2O_2 released by the COCs to the maturation media was similar in all the experimental groups (7.62 ± 0.60, 10.93 ± 1.23, 7.76 ± 0.00 and 7.75 ± 0.45 μM in 0E, 100E, 200E and 400E groups, respectively). This study has demonstrated that supplementation of α-TocOH in IVM medium induced significant changes in the molecular machinery of oocytes. Thus, α-TocOH reduced the apoptosis rate in CCs despite non-differences in H_2O_2 concentrations were found among groups. We acknowledge UCM, CM and MICINN for funding.

We acknowledge UCM, CM and MICINN for funding.

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Liquid preservation of bovine embryos as an alternative to cryopreservation

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Keywords: biopsy of bovine embryos, liquid preservation, hypothermic storage.

One of the most promising challenges in modern cattle breeding since the introduction of genomic breeding value estimation (GBVE) employing SNP genotyping is the GBVE of early embryos. To determine the genomic breeding value a biopsy of the embryo needs to be taken. In total it lasts approximately seven days until the result is announced. During this time span the embryo has to be preserved. At present, the possibilities to preserve biopsied embryos are either fresh transfer into recipients or cryopreservation. Due to lower pregnancy rates after transfer of biopsied and cryopreserved embryos especially when they are generated via in vitro production (IVP), an alternative preservation method needs to be developed. Recently published data show similar pregnancy rates of non-biopsied in vivo generated embryos which were stored for seven days at temperatures around 4°C in comparison to their fresh-transferred embryos (75% vs 77%; Ideta et al. Scientific Reports, 2013). The aim of this study is to develop an efficient medium that can be used to preserve the viability of biopsied, in vitro produced bovine embryos for up to seven days under hypothermic conditions. Therefore, day 6 embryos were generated employing a standard IVP protocol and assessed according to the IETS standard. Only morulae were used. With the aspiration technique the zona pellucida was perforated and some blastomeres were aspirated. After 24 hours of culture the embryos were examined a second time. Then the embryos were preserved in TCM supplemented with either FBS (25% and 50%) or BSA (1 mg/ml and 10 mg/ml) for seven days at 0-4°C. A control group of non-biopsied embryos was handled and stored under the same conditions. After liquid preservation, embryo quality was determined at the morphological level. Furthermore, live-dead staining was performed. First results show that embryos stemming from all groups have similar total cell numbers (25% FBS non-biopsied and biopsied: 118.4 ± 28.5 vs 106.6 ± 23.4; 50% FBS non-biopsied and biopsied: 98.8 ± 29.5 vs 103.8 ± 19.3; 1mg/ml BSA non-biopsied and biopsied: 100.0 ± 29.0 vs 103.4 ± 27.0; 10 mg/ml BSA non-biopsied and biopsied: 110.5 ± 30.5 vs 111.9 ± 28.5). The live/dead ratio was affected by the protein source, irrespective of the concentration (25% FBS non-biopsied and biopsied: 14.8 ± 14.4 vs 15.9 ± 8.6; 50% FBS non-biopsied and biopsied: 10.2 ± 6.1 vs 14.3 ± 11.9; 1 mg/ml BSA non-biopsied and biopsied: 9.0 ± 6.1 vs 8.1 ± 4.8; 10 mg/ml BSA non-biopsied and biopsied: 7.8 ± 3.9 vs 6.5 ± 3.2) suggesting that the biopsy procedure itself does not harm further embryo quality. These results indicate that liquid preservation employing FBS might be an alternative preservation method for early embryos. Further analyses employing antifreeze protein (AFP Type III) as preservation protein are underway.

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A263E Embryology, Developmental Biology and Physiology of Reproduction

In vitro maturation of guinea pig oocytes supplemented with Epidermal Growth Factor and Insulin-Like Growth Factor I

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Keywords: Oocyte maturation, growth factors, Guinea pig.

Insights in oocyte maturation process in guinea pigs are essential for the development of *in vitro* culture systems in this species, since it represents an interesting animal model in reproduction field (Suzuki et al. Mol Reprod Dev 2003; 64, 219–25). The goal of this study was to elucidate the influence of both Epidermal Growth Factor (EGF) and Insulin-like Growth Factor I (IGF-I) on *in vitro* oocyte maturation (IVM) medium of guinea pig. We assessed meiotic and cytoplasmic oocyte maturation, in terms of cortical granules (CG) and mitochondrial distribution, apoptotic rate and steroidogenic response of cumulus-oocyte-complexes (COCs) after IVM. A pool of 500 COCs from adult guinea pigs were cultured in groups of 40 COCs in four replicates in TCM-199 with 2 mM/mL glutamine, 0.1 mg/mL sodium pyruvate and 0.003% BSA for 17h (38ºC, 5%CO_2) (Sigma Chemical Company). Oocytes were distributed in different combination doses of growth factors as follows: group 0 (without growth factors); group EI (50 ng/mL EGF + 100 ng/mL IGF-I); group EI-FCS [50 ng/mL EGF + 100 ng/mL IGF-I + 10% Fetal Calf Serum (FCS)] and group FCS (10% FCS). After IVM, 456 oocytes were randomly selected, fixed and stained with 10 µg/mL Hoechst 33342 to assess nuclear configuration [Metaphase II (MII)]. Among them, a total of 152 oocytes were denuded and stained with 100 µg/mL FITC-LCA for CG visualization (n = 93) or with 180 nm MitoTrackerRedCMXRos (Molecular Probes Inc) (n = 59) for mitochondria assessment. CG and mitochondria patterns were analyzed with laser- scanning confocal microscopy (Leica). Estradiol (E_2) and Progesterone (P_4) production by COCs was measured by ELISA assay (DEMEDITEC Diagnostics GmbH) in the maturation medium. In the rest of COCs (n = 44) apoptosis rate was visualized with TUNEL technique (Roche Diagnostics, SL) and analyzed with Image J software. Chi-square test and one-way ANOVA with Duncan *post-hoc* test were used. MII rate significantly increased in oocytes from EI and EI-FCS groups compared to 0 and FCS groups (78.3 and 83.7% *vs* 38.4 and 55.8%, respectively; $P < 0.05$). EI-FCS group showed higher rate of oocytes with peripheral migration of CG (76.9%) (compatible with cytoplasmic maturation) compared with 0 group (23.8%) ($P < 0.05$) whereas EI and FCS groups showed intermediate results (59.1 and 50.0%, respectively). There were no significant differences between groups in the mitochondrial distributions EI-FCS COCs´ showed the lowest apoptosis rate (6.6 ± 0.7%) and the highest E_2 (0.3 ± 0.01ng/mL) and P_4 (1.9 ± 0.05 pg/mL) production compared to the remaining experimental groups ($P < 0.05$). In our conditions, combination of 50 ng/mL EGF, 100 ng/mL IGF-I and 10% FCS seems to be a suitable medium for IVM system in guinea pig oocytes since it offers superior results of oocyte maturation and quality of CCs compared to the other groups studied included when FCS was added alone. Future studies using these oocytes for IVF and IVC are needed to assess the potential of such COCs. Funded by UTPL and UCM.

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A264E Embryology, Developmental Biology and Physiology of Reproduction

In vitro embryo production of lamb oocytes after IVF, ICSI and Parthenogenetic Activation in Autumn and Winter

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Keywords: Season, prepubertal sheep, IVEP.

Previous reports in our laboratory (Catalá 2014. Rep. fert. & dev.; 27(1):216-7) showed significant differences in blastocyst production of prepubertal goat oocytes after IVF in autumn (7%) and winter (33%). In the present study we have compared the effect of autumn and winter on cleavage rate and blastocyst production of lamb oocytes after IVF, ICSI and parthenogenetic activation (PA).

For this purpose, prepubertal sheep (4 month) ovaries were collected in autumn (22 september to 21 december) and winter (22 december to 21 march) from a local slaughterhouse and transported to the laboratory in sterile dulbecco's (PBS) held at 34-37ºC. COC's were obtained by slicing the ovarian surface in Hepes-TCM199 medium. Afterwards, 30 COC's were selected and cultured in 100 µL drop of maturation medium (TCM 199 supplemented with hormones and 10% FBS (fetal bovine serum)) for 24 h at 38.5ºC in 100% humidified atmosphere and 5% CO_2.Matured oocytes were subjected to the different IVEP techniques. Briefly, IVF with thawed semen selected by density gradient (Nidacon®INT. AB. Sweden) was done during 20 h in SOF (2% FBS and 1% w/v BSA) medium supplemented with 10% of estrous sheep serum. ICSI was performed by injecting thawed selected spermatozoa (Nidacon® density gradient) into matured oocytes. Immediately after ICSI, injected oocytes were cultured in 1µM Ionomicyn solution for 4 min, and subsequently rinsed carefully. Finally, PA was achieved by culturing matured oocytes in 1 µM Ionomicyn solution during 4 min followed by 3 h in 5 µM 6-DMAP (6-Dimethylaminopurine). Presumptive zygotes produced by the different IVEP techniques were cultured (6 zygotes/10 µL drop) during 8 d in SOF medium at 38.5ºC with 5% CO_2, 5% O_2 and 90% N_2 in humidified atmosphere. At day 5 of IVC, medium was refreshed. Cleavage rate was evaluated at 48 h post-insemination (*pi)*, and blastocyst rate was recorded at day 8 *pi*.

Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., USA). In each experimental group, oocytes were randomly distributed. Differences in embryo development (cleavage and blastocyst rates) among the experimental groups (IVF, ICSI and PA) in autumn and winter were analysed by one-way ANOVA, using the generalized linear model procedure and LSMEANS statement of the SAS and Tukey post-hoc test.

Results did not showed significant differences among seasons (autumn and winter) in cleavage rate for IVF (64.6 ± 9.8% vs 69.4 ± 8.2 %), ICSI (88.9 ± 7.9vs76.6 ± 4.6%) and PA (78.9 ± 1.9 vs 83.0 ± 3.7%) and blastocyst production (IVF: 13.7 ± 2.8% vs 12.3 ± 5.2%; ICSI: 15.5 ± 1.9% vs 14.5 ± 2.3%; and PA: 11.1 ± 3.0% vs 15.6 ± 3.4%, respectively).

In conclusion, under our conditions, no season effect was identified on *in vitro* embryo production between Autumn and Winter in prepubertal sheep oocytes.

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A265E Embryology, Developmental Biology and Physiology of Reproduction

A polarized oviduct epithelial cell culture model supports murine early embryo development without additional medium supply

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Keywords: mouse, oviduct epithelial cells, embryo development.

The oviduct hosts fertilization and early embryo development. It provides the only optimal micro milieu for zygotes and preimplantation embryos. During IVP procedures efforts are made to mimic the oviductal environment, however in some species with suboptimal results. Recently, oviduct epithelial cells of human, pig, and cattle have been cultured on porous membranes at the air-liquid interphase (ALI), which closely recapitulated the phenotype of native oviduct tissue. However, to our knowledge, no attempt has been made to apply this approach in IVP procedures.

In this study we aimed to establish a culture model of mouse oviduct epithelial cells (MOEC) using the ALI approach. We tested whether MOEC are capable to support in vitro embryo development without additional IVC medium.

Mice included in this study originated from the FBN mice strain FztDU. After isolation MOEC were seeded on porous PET inserts. Initial proliferation for 7d was conducted at the liquid-liquid interface, followed by 14d of differentiation at the air-liquid interface (medium only from basolateral side). Apical fluid generated by MOEC was removed with each medium change. Trans-epithelial electrical resistance (TEER) was measured and samples were processed for histology on d3, 7 and 21 (n = 5 mice each). For the embryo co-culture experiments, apical fluid was removed from the insert on d21. 3 days later (d24) 30-50µl of fluid was re-generated on the apical cell side. In total 83 potential zygotes were collected from naturally mated female mice approx.12h post conception, and transferred to the apical side of MOEC (d24). In experiment 1 (n = 64) embryos were harvested on d2 of co-culture and assessed for cleavage based on the Theiler staging criteria. In experiment 2 (n = 19), cleavage rate was also assessed on d2, but the co-culture period was subsequently extended until d4.5.

Using the ALI approach MOEC achieved full differentiation: they were polarized and composed of secretory and ciliated subpopulations (confirmed by immunofluorescence for acetylated tubulin). From d3 onwards cells possessed moderate TEER with mean values ranging from 282 to 619 $\Omega*cm^2$. After 2d of co-culture, uncleaved zygotes/COCs developed to 2-4 cell stage with cleavage rates of 73% (exp. 1) and 95% (exp. 2), respectively. When extending trial 2 to 4.5d, 32% of the embryos developed to morulae and 53% reached blastocyst stage. The timing of embryonic development in co-culture was in line with reports on embryonic development in vivo.

In conclusion, we established the first ALI culture model for MOEC. This model successfully supported mouse embryos in passing the 2-cell block and developing to the blastocyst stage without addition of any IVC medium. However, further experiments including in vivo embryo transfer have to be conducted to assess the quality of ALI-produced blastocysts.

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A266E Embryology, Developmental Biology and Physiology of Reproduction

Progesterone and Estradiol concentrations of follicular fluid according to the follicular size from Prepuberal and Adult ewes

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Keywords: Follicle size, Follicular fluid, Prepuberal and Adults ewes.

The aim of this study was compare the hormonal status (Estradiol and Progesterone) from follicles of different sizes (diameter) from prepuberal and adult ewes. Eleven ovaries from adults (n = 7) and prepuberal ewes (n = 4; from two to three months old), were obtained from abattoir and transported to the laboratory in PBS at 34 to 37^{o}C. These follicles were dissected and grouped as: 1) smaller (S: from 2.0 to 2.9 mm) and; 2) large (L: ≥ 3mm) for prepuberal (P) and adults (A) ewes/group. The dissected follicles were carefully cut with microscissor and follicular fluid was recovered into vial of 200 mL and centrifugated at 10000 x g for 15 minutes. The supernatant was stored and analysed by radioimmunoassay to detect Estradiol (E2) and Progesterone (P4) concentrations. Steroids were analysed by radioimmunoassay using ImmuChem Double Antibody P4 and E2 125I RIA kit (MP Biomedicals™, Santa Ana, California, USA). The intra-assay and inter-assay coefficient of variation and the limit of sensitivity were 7.0 and 7.1%, 14.2 and 7.9% and 0.2 and 1.7 ng/mL, for E2 and P4, respectively. Outlier and samples without both steroids analyzed were excluded from analyses. Data of E2 and P4 were transformed to logarithms and analysed using Two-Way ANOVA where size, age of the ewe and their interactions were included in the model. Comparisons were performed using Tukey's test. Estradiol concentrations for S-P (n = 20), L-P (n = 15), S-A (n = 19) and L-A (n = 23) follicles were 1.8 ± 0.6, 18.7 ± 5.9, 3.1 ± 1.4 and 21.2 ± 5.8 ng/mL, respectively. Progesterone concentrations for S-P, L-P, S-A and L-A groups were 5.1 ± 1.9, 8.1 ± 1.3, 66.4 ± 28.0 and 73.8 ± 17.4 ng/mL, respectively. Follicular fluid from S-P and S-A follicles showed less E2 than L-P and L-A ($P < 0.01$). Progesterone concentration was minor in follicular fluid from prepuberal than adult ewes ($P < 0.05$). In conclusion, E2 concentration is related to the size of the follicle while P4 is related to age of the donor ewe.

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The effects of hypo- and hyperglycemia during lipolysis-like conditions on bovine oocyte physiology

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Keywords: NEFA, glucose, oocyte.

Next to elevated non-esterified fatty acid (NEFA) concentrations, lipolytic metabolic conditions can be associated with hypo- and hyperglycemia. Previous research has shown that in the presence of high NEFAs, hypoglycemia (LO GLUC) during IVM hampers embryo development to a greater extend as compared to hyper- (HI GLUC) and normoglycemic conditions. Blastocyst metabolism and carbohydrate- and oxidative stress related gene expression were not affected, but blastocysts from LO- and HI GLUC exposed oocytes showed a higher degree of apoptosis. As a consequence we aimed to study the effects of hypo- and hyperglycemia in presence of elevated NEFAs on oocyte metabolism, apoptosis and reactive oxygen species generation (ROS). Hereto bovine cumulus oocyte complexes (COCs) were routinely matured (1 COC/10µL medium) during 24h under different NEFA and GLUC levels: 1) physiological NEFA (72µM palmitic, stearic, oleic acid) and routine IVM GLUC (5.5mM) (= CONT), 2) pathophysiological NEFA (420µM) and routine GLUC (= HI NEFA), 3) HI NEFA+HI GLUC (10mM) and 4) HI NEFA+LO GLUC (2.8mM). Initial and conditioned medium was sampled (4 repeats) and analyzed for glucose and lactate concentrations. After IVM, all COCs were fixed and stained with caspase-3 and HOECHST to determine apoptosis (n = 182, 3 repeats) or denuded and stained for intracellular ROS during 30min using H_2DCFDA (n = 79, 3 repeats). H_2DCFDA fluorescence intensity was quantified using ImageJ and COC apoptosis was classified as: <25%, 25-75% and >75% cumulus cell apoptosis. All data were compared between 4 treatments using a mixed model ANOVA and Bonferroni post-hoc (IBM SPSS Statistics 20). Means ± SEM are presented. COCs exposed to HI NEFA+HI GLUC consume significantly less glucose (485 ± 63 pmol/COC/h) compared with the other treatments (mean of 894 ± 35 pmol/COC/h). HI NEFA+HI GLUC and HI NEFA+LO GLUC exposed COCs produced significantly less lactate (1738 ± 192 and 1848 ± 51 pmol/COC/h, respectively) than CONT and HI NEFA exposed COCs (3573 ± 212 and 3494 ± 289 pmol/COC/h). In addition, LACT/GLUC ratio was significantly lower in all treated COCs compared with CONT, with the lowest LACT/GLUC ratio in HI NEFA+LO GLUC exposed COCs indicating a shift of glucose towards pathways other than glycolysis. No differences were observed in COC apoptosis between treatments. Oocyte ROS was significantly higher in HI NEFA+HI GLUC exposed oocytes (15.85 ± 1.87) compared with HI NEFA+LO GLUC (13.06 ± 1.28) and HI NEFA oocytes (12.06 ± 1.11). In conclusion, lipolytic conditions with or without glycemic perturbations influence the oocyte's glucose and lactate metabolism. Whereas hypoglycemia in the presence of elevated NEFAs hampers embryo development the most, high GLUC exposed oocytes suffer from increased intracellular ROS. This could not be substantiated by increased cumulus cell apoptosis.

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Reproductive performance and milk production of Damascus goats raised under the intensive system in southeastern Anatolia

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Keywords: Damascus, intensive system, milk yield.

The Damascus goat, also known as the Shami, is a native breed of Syria and other Near East countries. It has been practiced in a commercial goat enterprise (Pan Hayvancilik) since 2011 with 4500 heads under intensive system. Pregnancy and kidding rates in different months of mating; lactation periods and milk yield of in does of different parity were reported.

A total 1427 lactating goats were used in this study. Estrus of lactating does was synchronized with CIDR (EAZI-BREED™ CIDR®) for 14d in three different months such as August, September, October. Does were placed with sexually experienced Shami bucks in the ratio 1 buck to 10 does following CIDR removal. Thirty days after mating, pregnancy status was determined by trans-abdominal ultrasonography using a Real-time B-mode.

Pregnancy rates of does were found 88%, 94% and 86% ($P < 0.05$) in August, September and October mating groups, respectively. Kidding rates of does mated in different months were found similar (99%). Lactation period and milk yield of first, second, third and fourth parities of does were found 231 d and 419 l, 293 d and 617 l, 283 d and 637 l, 299d and 680 l, respectively.

It was concluded that Damascus goat under intensive system in southeastern Anatolia performed well in terms of reproductive performance and lactation yield. We found that Damascus goat subjected to selection in a commercial farms showed higher reproductive performance and milk yield than those reported by various researchers (Khazaal, 2009; Monem et al., 2005; Khoury, 1996).

A269E Embryology, Developmental Biology and Physiology of Reproduction

Effect of non-esterified fatty acids during sperm capacitation or IVF on developmental competence of bovine oocytes

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Keywords: bovine embryo, IVF, development.

Deviating metabolic conditions, present in dairy cows suffering negative energy balance (NEB), are reflected in the follicular and oviductal fluid (Leroy *et al.* (2015), RFD 27: 693-703). Elevated non-esterified fatty acid (NEFA) concentrations, associated with NEB, during *in vitro* maturation and culture have significant carry over effects on embryo quality and physiology (Van Hoeck *et al.* (2014), ARS 149: 19-29). Moreover, the oviduct plays an important role in sperm storage and selection, regulation of sperm motility and capacitation (Holt *et al.* (2010), MRD 77:934-943). This implicates that fertilization can be influenced by alterations in oviductal fluid composition. Therefore, we hypothesized that exposure of sperm cells to elevated (NEB-like) NEFA concentrations shortly before and during IVF can affect fertilization and further embryonic development. To differentiate between possible effects on both spermatozoa and oocytes, two experiments were conducted. Bovine oocytes were matured following standard procedures. In experiment 1, mature oocytes were fertilized under 4 conditions: 1) standard lab conditions (CONT), 2) solvent control (SOLV), 3) physiological NEFA conditions (mixture of 23 µM palmitic acid (PA), 28 µM stearic acid (SA) and 21 µM oleic acid (OA)) (BAS-NEFAs) or 4) lipolytic NEFA conditions (mixture of 230 µM PA, 280 µM SA and 210 µM OA) (HIGH-NEFAs). In experiment 2, spermatozoa were pre-exposed for 4h under conditions CONT, SOLV, BAS-NEFAs or HIGH-NEFAs, then washed and used for IVF of mature oocytes in FA-free media. After 24h, presumptive zygotes were cultivated in serum-free medium until day 8 and developmental competence was assessed. Development was analyzed using binary logistic regression.
In experiment 1, cleavage rate was not significantly different between all treatment groups. A significantly higher proportion of HIGH-NEFAs zygotes showed 2-cell block (24.8%) compared to CONT (6.9%; P = 0.001), SOLV (11.5%; P = 0.016) and BAS-NEFAs (13.1%; P = 0.057) zygotes. Blastocyst rate was significantly decreased in the BAS-NEFAs (36.7%; *P* = 0.007) and HIGH-NEFAs (36.6%; P = 0.024) compared to the CONT group (54.3%). In experiment 2, no differences in developmental competence were observed among treatments.
In conclusion, exposure to elevated NEFA concentrations during IVF has no obvious effect on the fertilization process itself since cleavage rate is not significantly affected. However, further embryonic development is hampered due to NEFA exposure during fertilization. NEFAs have no influence on the fertilizing capacity of pre-exposed sperm suggesting that NEFA-induced reduction in developmental competence is through alterations in oocyte quality but not through affecting sperm quality. More research is ongoing to investigate underlying mechanisms.

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Detection of Brucellosis in seropositive superovulated sheep embryo flushing media

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Keywords: ovine, brucellosis, embryo.

The efforts have been made to analyses the risks associated with importing in vivo derived ovine embryos. Few studies have been made of the interaction between embryos and pathogens in small ruminants in comparison with those conducted on bovine embryos. As a consequence, few disease agents affecting sheep and goats have been categorized by the International Embryo Transfer Society Import/Export Committee (IETS) Research subcommittee for their capacity to be transmitted via ET. The characteristics of embryos and their interactions with pathogens cannot be generalized. Embryos of different species differ in the glycoprotein composition of the ZP. This structure in sheep and goats differs from that in cattle (Chen and Wrathall 1989; Dunbar et al 1991). It has been suggested that ovine ZP is 'stickier' than that of bovine embryos, and less likely to resist penetration and adherence of pathogens (Singh et al 1997). This may explain the higher probability of binding between the ZP and various pathogens in these species. In the absence of relevant information, infection patterns for sheep and goat embryos are based on studies of infection of bovine genetic material. The risk estimate for B ovis in sheep without risk management measures is negligible for embryos however in the case of B melitensis without risk management measures is high for embryos. To the best of our knowledge, no work has yet been done to investigate the possible carriage of Brucella melitensis by embryos despite the fact that this is an important cause of disease in small ruminants particularly in the peri-Mediterranean regions.

The experiment was conducted to test for the recovery of Brucella oganisms from uterine flushings of sero positive embryo donor females. We used 14 donor ewes with history of being chronically seropositive to the plate serum agglutination acidified plate antigen card (B. abortus, B. melitensis). Donors were superovulated with single shot FSH (9 cc Folltropin) combined with and eCG (500 I.U.) injection 24h prior to sponge withdrawal and artificially inseminated at 24 hours following the onset of estrus with Brucella free semen. Samples of recovered flushing medium were placed into a validated in vitro culture system to detect the presence of brucella bacteria. Uterine flushings from donor females were free from B mellitensis, ovis and abortus contamination. It was concluded that the superovulatory treatment is not likely to reactivate the release of Brucella into the uterine lumen during the period when embryos are normally collected.

A271E Embryology, Developmental Biology and Physiology of Reproduction

Bovine embryo production is very sensitive to toxins released from 3-D printed acrylate chambers

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Keywords: oviduct, embryo, 3-D printing.

The oviduct provides an ideal microenvironment for gamete interaction and early embryo development. However, in conventional monolayer culture bovine oviduct epithelial cells (BOEC) undergo rapid loss of typical differentiated BOEC properties (e.g. cilia and secretory activity). Recently, we developed a BOEC culture chamber using 3D-printing with acrylate-based resins (3-D BOEC). The printed chambers were successfully detoxified to allow colonization with BOEC monolayers that formed cilia, secreted OVGP1 and supported sperm activation and fertilization of oocytes. However, embryo development was blocked at the 4-cell stage. In fact, when the chambers were used without BOECs no fertilization was observed at all, whereas in the absence of the 3-D chamber the same medium allowed >80% fertilization. We therefore tested whether the BOEC monolayer protects the apical compartment from toxic components leaking out of the 3-D chambers. Primary BOECs (originating from 4 different cows) were seeded onto Transwell® inserts (Polycarbonate, 0.4 μm pore), grown to confluence and cultured at an air-liquid interface for up to 58 days (Transwell® culture). *In vitro* fertilization (IVF) and culture (IVC) media were conditioned for 24 h with routinely detoxified 3-D chambers, and compared to identical media that had not been exposed to 3-D chambers. Transwell® culture supported the formation of differentiated cuboidal to columnar BOECs, and assessment of trans-epithelial electrical resistance at day 28 indicated that they established a functional barrier (mean value: 578 ohm/ cm^2). Transwells with or without BOECs were used for IVF and IVC using a routine IVP protocol. A mixed model ANOVA was used to evaluate statistically significant differences ($P < 0.05$) between groups and a total of 4 replicates, using 100 oocytes per experimental group each (400 oocytes per group) were performed. Using non-conditioned media, cleavage rates were similar in Transwells with or without BOEC (77.45 ± 3.54% and 79 ± 0.92% respectively $P > 0.05$), and considerably better ($P < 0.05$) than when 3-D chamber-conditioned media were used in the Transwells (56.07 ± 12.4% and 11.21 ± 4.18%; with or without BOEC, respectively). Notably, the presence of BOEC significantly reduced the toxic effect ($P < 0.05$). Likewise, in non-conditioned media blastocyst rates were similar (29.5 ± 1.3% and 27 ± 1.8% of matured oocytes; with and without BOEC), whereas they were lower ($P < 0.05$) or completely blocked ($P < 0.05$) when conditioned media were used (19 ± 1.8% with BOEC and 0 ± 0% without BOEC). Again the BOEC had a protective effect ($P < 0.05$). Clearly, the acrylate-based 3-D chambers release a toxic component to which fertilized oocytes, but not BOECs, are extremely sensitive. Moreover, a functional BOEC barrier partially protects the early embryo from this toxic effect. Future studies should identify the component(s) responsible for embryo toxicity, while the data emphasize that care is needed in selecting and testing materials for 3-D printing technologies before applying them to *in vitro* embryo production.

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Response of bovine oviduct epithelial cells to early embryos in vitro

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Keywords: BOEC, Embryo, Transcriptome

In vitro culture of bovine oviduct epithelial cells (BOEC) has been widely used for its beneficial effect on early embryo development; however, limited evidence exists describing alterations in the transcriptome of these cells in response to the presence of an embryo(s). In order to elucidate that response on *in vitro* conditions, we established a primary cell culture of BOEC which was co-cultured with 4-cell stage bovine embryos. Cells were mechanically harvested from a pool of three oviductal isthmus sections collected from heifers slaughtered during the early luteal phase. BOECs were cultured in 500µl of Tissue Culture Medium-199 supplemented with 10% fetal calf serum (FCS) in four-well culture plates (NUNC, Roskilde, Denmark) in a humidified atmosphere at 5% CO_2 in air at 38.5ºC. Half of the medium was renewed every 48 h. Six days later, the, medium was replaced with synthetic oviductal fluid (SOF) supplemented with 10% FCS. One day later, 4-cell stage *in vitro* produced embryos selected at 52 h post IVF were added to the BOEC for 24 h. In order to limit the area of contact bewteen the embryos and the BOEC, embryos were cultured in a nontoxic woven polyester mesh (Sefar Petex; Sefar, Bury, Lancashire, UK) in a 7x7 grid (i.e., 49 embryos /well). After 24 h co-culture, the BOEC directly beneath the embryos (Group 1) were recovered as well as cells in the same well but outside this area (i.e., not in direct contact with the embryos (Group 2, control+) and cells from a different well without embryos (control-). Cells were snap frozen in liquid nitrogen and stored at -80ºC. mRNA extraction was carried out with Dynabeads (Dynal Biotech, Oslo, Norway). The relative abundance of genes previously shown to display alteration in the presence of embryos *in vivo* [*SLC26A3* (ion transport), *MCTP1* (calcium ion binding), *BMP5, SMAD6* (BMP signaling pathway) *ROCK1, ROCK2, SOCS3* (Cytokinesis)] or *in vitro* [*GPX4, NFE2L2* (oxidative stress), *SCN9A* (Sodium ion binding), *EPSTI1* (Tissue remodeling), IGFBP3 (Insulin-like growth factor binding)] were analyzed by qPCR using *H2A.Z* and *ACTG1* as housekeeping genes. Statistical differences were assessed by ANOVA. The expression of *EPSTI1* was significantly decreased in BOEC in direct contact with embryos compared with cells not in contact with embryos, either from the same or a different well. Increased expression of *EPSTI1* has been implicated in endometrial remodeling prior to embryo implantation in cattle. All other genes studied either were not different between groups or, in the case of *SLC26A3, MCTP1, BMP5* were not detectable. In conclusion, based on the relatively small number of genes analyzed, this study provides limited evidence for an embryo-induced transcriptomic response in the cells of the oviduct.

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Porcine sperm bind to beads conjugated to ZP2 protein under in vitro conditions

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Keywords: ZP proteins, beads, sperm-binding

The oocyte is encapsulated by a filamentous structure composed of several glycoproteins termed zona pellucida (ZP),that acts as a matrix mediating interaction with sperm. It has been shown that processing of ZP2 at N terminal position (LADEN) mediates the recognition between gametes (Avella M, J Cell Biol. 205(6):801-9, 2014). The study of these molecular mechanisms is very limited due to the ethical problems, the difficulty of obtaining mature oocytes in many mammalian species, the high cost of genetically modified mice and the inability to transfer this knowledge to other species such as swine. Therefore, we propose an *in vitro* model that mimics the oocyte shape, allowing the research on the interaction of gametes, identification and characterization of the ZP proteins activity and the conditions for sperm recognition. In this work, the proposed model is developed by combining magnetic beads (His Mag Sepharose™ Excel) conjugated with porcine ZP2 and ZP4 recombinant proteins. This novel model besides increasing our knowledge of the oocyte-sperm interaction, could also be industry-wide implemented as an evaluator of mammalian sperm quality. For this study ZP2 and ZP4 recombinant proteins were marked with a Flag and V5 tag recognition site, respectively, and with a histidine tag for easy identification and adhesion to the beads. The proteins were expressed in mammalian cells (CHO) and once secreted, identified by electrophoresis and western blot. Then, the secreted recombinant proteins were conjugated with the magnetic beads. Groups of 40-45 ZP proteins conjugated-beads and beads raised with growth CHO-cell medium (Control group) were coincubated for 2hr with boar spermatozoa (heterospermic dose) in TALP medium at a final concentration of 200.000 spermatozoa/ml. After 2h coincubation, the beads were washed twice in PBS, fixed and stained with Hoechst. Bound sperm in each bead was scored by fluorescence microscopy and the obtained results analysed by one-way ANOVA. Three replicates in a blind analysis were done and *P*-value <0.05 was taken to denote statistical significance. Secreted proteins ZP2 and ZP4 were identified by electrophoresis and western blot with anti-Flag and and Anti-V5 antibodies, respectively. The ZP2 showed a molecular weight of 100 kDa and ZP4 a molecular weight of 65 kDa. Adhesion of secreted proteins to the beads was confirmed by western blot. Finally, number of sperms bound to ZP2-beads (8.56 ± 0.64, n = 230) was significantly higher (P < 0.001) than sperm bound to ZP4-beads (3.00 ± 0.27, n = 233) and control (4.00 ± 0.36, n = 207). In conclusion, a novel in vitro model combining magnetic beads with ZP proteins (ZP2 and ZP4) was developed in order to study the role of ZP2 protein on sperm-oocyte interaction. Future studies could increase our knowledge of the oocyte-sperm interaction and could be also implemented as an in vitroselection and quality evaluation technique of potentially fertile mammalian spermatozoa.

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The presence of L-carnitine during maturation improves bovine embryo production

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Keywords: bovine oocytes, L-carnitine, IVF outcome.

Supplementation of IVM media with mitochondrial stimulators can improve oocyte cytoplasmic maturation and embryo production in farm animals. Although the positive effect of L-carnitine on oocytes and embryos was described (Takahashi et al., Rep Fert Dev 25, 589–599), there is no information about the specific impact of L-carnitine on oocytes with different developmental competence.
This study was designed to define the effect of L-carnitine during maturation on bovine oocytes with different developmental competence in terms of IVF outcomes. Ovaries from the growth to stagnation phase were used for oocyte collection. Meiotically more competent (MMC) and meiotically less competent (MLC) oocytes were isolated regarding the follicles size (medium or small). The oocytes maturing with or without 2.5 mM L-carnitine (Sigma-Aldrich Co., Prague, Czech Republic) were fertilized and cultured into blastocysts, using standard protocols (Machatkova et al., Zygote 16: 203–209, 2008). The effect of L-carnitine treatment on mitochondrial cluster formation, lipid consumption, fertilization, cleavage and blastocyst differentiation was assessed. The results were statistically analysed by the ANOVA procedure using Chi-square and Fisher's least difference tests, significant level was set at $P < 0.05$. No significant differences were found in the proportions of MII oocytes among MMC and MLC oocytes matured with or without L-carnitine. However, a significantly higher proportion of MII oocytes with mitochondrial clusters was observed in MLC oocytes matured with L-carnitine than with those matured without L-carnitine (67.2% vs 49.2%). A significantly lower mean lipid content was also detected in MLC oocytes matured with L-carnitine in comparison with those matured without L-carnitine. A significantly higher fertilization (91.0% vs 85.9%) and syngamy rates (55.0% vs 46.7%) in MLC oocytes but similar fertilization and significantly lower syngamy rates (36.4% vs 52.9%) were found in MMC oocytes when they matured with L-carnitine compared with those matured without L-carnitine. Although no significant difference in cleavage rates was found among oocytes matured with or without L-carnitine, significantly more MLC oocytes matured with L-carnitine developed into D7 early blastocysts and D8 expanded blastocysts compared with the controls (31.7% vs 23.1% and 33.3% vs 25.8%, respectively). On the other hand, a significantly higher proportion of D8 expanded blastocysts was obtained in MMC oocytes matured with L-carnitine compared with the controls (72.7% vs 59.3%). It can be concluded that L-carnitine treatment during maturation enhances production of bovine embryos from meiotically less competent oocytes and accelerates differentiation of blastocysts developed from more competent oocytes.

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A275E Embryology, Developmental Biology and Physiology of Reproduction

In vitro monolayer barrier function of bovine oviduct epithelial cells is modified due to high concentrations of non-esterified fatty acids

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Keywords: Bovine oviduct, Maternal metabolism.

Early post-partum negative energy balance in high yielding dairy cows has considerable repercussions on reproductive ability and economic merit of these animals. Typically, lipolysis is upregulated and the associated rise of non-esterified fatty acids (NEFAs) has been proposed as a key factor in the decline of oocyte and embryo quality. However, the effects on the oviductal micro-environment remain largely unknown. In this study, we hypothesized that elevated NEFAs may modify *in vitro* bovine oviduct epithelial cell (BOEC)-physiology by altering the BOEC-barrier function, and thus may potentially affect overall fertility. Hereto, fatty acid (FA)-transfer was evaluated, monolayer permeability was linked to transepithelial electric resistance (TER), and BOEC *TJP1*-expression and lipid droplet (LD) formation were analyzed.

In 4 repeats, early luteal BOECs were seeded in a polarized cell culture (PCC)-system. After reaching 100% confluency (D9), monolayers were NEFA-exposed (PA+SA+OA) for 24h in 4 groups: 1) CONTROL (0µM NEFA + 0%EtOH), 2) SOLVENT CONTROL (0µM NEFA + 0.45%EtOH), 3) BASAL NEFA (720µM NEFA + 0.45%EtOH in the basal compartment), 4) APICAL NEFA (720µM NEFA + 0.45%EtOH in the apical compartment). Next, spent medium was photometrically assessed for total FA-concentration and subjected to gas chromatography for FA-profiling. Also, a 3h permeability assay using FITC-albumin was performed, and related to pre- and postexposure TER-measurements. BOEC-mRNA was retrieved for qRT-PCR of *TJP1* to assess expression levels of tight junction protein 1. LD-formation was studied using Bodipy® 493/503 and confocal imaging. All data were analyzed with one way ANOVA.

Spent medium analyses showed a 19.5% NEFA-decrease in the supplemented compartment of BASAL NEFA, with paracellular passage to the non-supplemented, apical compartment of PA (56.0%↑), SA (60.0%↑), OA (33.5%↑) as free FAs. However, in APICAL NEFA 53.4% of FA-decrease was observed in the supplemented compartment, while no FA-increase was apparent at the non-supplemented side, suggesting intracellular FA-uptake, which was positive for LD-formation in APICAL NEFA. FITC-albumin flux increased significantly (27.59%) in APICAL NEFA, associated with a reduced relative TER-increase (46.85%) during the NEFA-exposure. *TJP1*-expression was not affected by the treatments.

In conclusion, elevated NEFAs in the apical, 'oviductal lumen' compartment may decrease the tightness of cell-cell interactions. BOEC-barrier function was thereby compromised in APICAL NEFA. Also, the PCC allows to observe FA-transfer across BOEC-monolayers and the resulting response strongly depends on cell polarity. These data substantiate the concept of the oviduct as a possible gatekeeper that shields its micro-environment from detrimental metabolites, such as high NEFAs.

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The P4 and E2 treatment and protein expression of PGR and PGRMC1 in porcine endometrial cells

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Keywords: progesterone receptors, luminal epithelial cells, pig.

The endometrium consists of a layer of columnar epithelium and connective tissue. In the endometrium can be distinguish two zones based on their involvement in the changes during the oestrus cycle. The functional layer contain the luminal part of the endometrium and it is the site of cyclic changes in the endometrium.
The proper functionality of endometrial tissue is regulated by paracrine and endocrine pathways that activate several mediators or metabolic pathways and gene cascades. This study was aimed to investigate the influence of estradiol 17-beta (E2) and progesterone (P4) on progesterone receptor (PGR) and progesterone receptor membrane component 1 (PGRMC1) protein expression in porcine luminal epithelial cells and their influence on the proliferation of these cells in real-time.
Surface uterine luminal epithelial cells were removed using sterile surgical blades from uterine horns of ten crossbred anestrus gilts. Following treatment with collagenase I, cells were separated and transferred into 48-well E-Plates for use in a real-time cell analyzer (RTCA, Roche-Applied Science, GmbH, Penzberg, Germany). The luminal epithelial cells were cultured *in vitro* (IVC) in standard Dulbecco's Modified Eagle's Medium (DMEM; Sigma Aldrich, Madison, USA) and incubated with E2 (10 pg/ml, 40 pg/ml, 500 pg/ml) and P4 (10 ng/ml, 40 ng/ml, 500 ng/ml). The cell proliferation index was analyzed after 0-240h, 0-120h, 120-240h. The analysis was carried out using RTCA and confocal microscopic observations. Results were performed using analysis of variance (ANOVA) and Tukey test, and by using Imaris 7.2 software (BitPlane, Zurich, Switzerland).
Using RTCA analysis we found an increased proliferation of luminal epithelial cells after treatment of low doses of P4 (10 and 40 ng/ml), ($P < 0.001$). Higher doses of P4 leads to decrease of proliferation ($P < 0.001$). Conversely, higher doses of E2 (500 pg/ml) increased proliferation index as compared to low doses (10 pg/ml) and control ($P < 0.001$).
Confocal microscopic observations revealed that higher concentrations of E2 up-regulate the expression of both PGR and PGRMC1. Additionally, P4 used in lower concentrations stimulated the expression of these receptors.
Our study demonstrated that E2 and P4 treatment significantly regulated the expression of PGR and PGRMC1, which is accompanied by real-time proliferation of porcine luminal epithelial cells. The relationship between PGR or PGRMC1 expression and the proliferation of luminal epithelial cells may be influenced by E2 or P4 in a steroid type- and dose-dependent manner.

A277E Embryology, Developmental Biology and Physiology of Reproduction

Developmental competence of bovine oocytes that have not finished growth phase in vivo

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Keywords: oocyte, prolactin, IVF.

In our previous studies we demonstrated that the addition of bovine prolactin (PRL) to culture media enhanced the proportion of blastocysts obtained from bovine oocytes selected by brilliant cresyl blue (BCB, Heleil B. et al., J. Reprod. & Infertility 1 (1):01-07, 2010). Process of nuclear maturation of BCB^- oocytes (oocytes that have not finished growth phase in vivo) are slowly compared to the BCB^+ oocytes (oocytes that have finished growth phase in vivo). Most of the BCB $^+$oocytes (81%) reached the metaphase-II after 24 hours and only 52% BCB^- oocytes completed nuclear maturation (Kuzmina T. et al. Tsitologiya. 55 (9): 664-665, 2014).

The aim of the present study was to evaluate the developmental competence of BCB^- oocytes matured in medium supplemented by PRL at prolongation of the time of cultivation to 30 h.

Ovaries were collected at slaughterhouse. Compact cumulus oocyte complexes (COC) were aspirated from follicles 3-8 mm diameter. Before IVM COC were incubated in 26 μM BCB (B-5388, Sigma) solution for 90 minutes. Oocytes were divided into BCB^- (colorless cytoplasm) and BCB+ (colored cytoplasm). COC were cultured 15 h in TCM 199 + 10% (v/v) FCS + 50 ng/ml PRL with 106 /ml granulosa cells. Then medium were supplemented by 10 IU/ml hCG. The time of cultivation for BCB^+ and BCB^- oocytes were 24 h in control and 30 h in experimental groups. After IVM oocytes were fertilized and embryos were cultured by standard protocols up to Day 8. All chemicals used in this study were purchased from Sigma - Aldrich (Moscow, Russia). Data were analyzed by chi-square.

Chromatin of 193 BCB $^+$ and 176 BCB $^-$ oocytes (total 369 oocytes, in 5 replicates, 17-20 oocytes/group) was evaluated after 24 and 30 hours of cultivation. 81 % (77/95) and 85 % (83/98) of BCB $^+$ oocytes reached metaphase II after 24 and 30 h of cultivation, respectively. 52% (46/89) of BCB^- oocytes reached metaphase II after 24 h, but after 30 h of cultivation percentage of oocytes significantly increased [71%(62/87), $P < 0.01$]. These results suggest the possibility of BCB^- to complete the nuclear maturation in vitro with the prolongation of culture time. In the control group the cleavage rates were significantly higher in the BCB^+ oocytes in comparison to the BCB^- oocytes [87% (107/123) vs 63% (87/139), $P < 0.01$]. Prolongation of the time of cultivation significantly increased percentage of cleavage after IVF of BCB $^-$ oocytes [63% (87/139) vs 78% (118/151), $P < 0.05$]. We did not find significant differences between the percentage of late morulae and blastocysts that developed from BCB^+ oocytes independently of prolongation of maturation time [41% (50/123) and 38% (56/147)]. The BCB^- oocytes yielded a higher proportion of late morulae and blastocysts by the prolongation of the time of cultivation to 30 h [12 % (17/139) vs 30 % (45/151), $P < 0.01$].The prolongation of maturation time to 30 h improved the developmental competence of BCB^- oocytes. Further investigation is needed to evaluate the potential of obtained embryos to development (including evaluation level of apoptosis, gene expression, ET).

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Progesterone is involved in anti-aging effects of prolactin on bovine cumulus-enclosed oocytes matured in vitro

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Keywords: prolactin, progesterone, oocyte aging.

In matured oocytes, aging processes are accelerated that reduces the ovum fitness. However, little is known about physiological factors responsible for the oocyte protection from precocious senescence. We have found previously that prolactin (PRL) can maintain the developmental capacity and apoptosis resistance of *in vitro* matured bovine oocytes aging *in vitro* (Singina et al., Reprod Fert Dev, 27:204, 2015; Singina et al., Reprod Domest Anim, 50(S3):77, 2015). The goal of this study was to test a hypothesis that these anti-aging effects of PRL might be achieved through stimulation of progesterone production by cumulus cells (CCs). Bovine cumulus-enclosed oocytes (CEOs) were cultured for 20 h in the IVM medium (TCM 199 supplemented with 10% fetal calf serum, 10 μg/ml FSH, and 10 μg/ml LH). Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). A part of in vitro matured oocytes was set free from their CCs. Then CEOs and denuded oocytes (DOs) were cultured for additional 12 or 24 h in the aging medium (TCM 199 containing 10% fetal calf serum) without (Control group) or with bovine PRL (50 ng/ml; Research Center for Endocrinology, Moscow, Russia) and/or trilostane (an inhibitor of progesterone synthesis). Apoptosis was detected in oocytes aged for 24 h using the In Situ Cell Death Detection Kit (Fluorescein, Roche, Indianapolis, USA). Oocytes aged for 12 h were subjected to the IVF procedure described previously (Singina et al., Reprod Fert Dev, 26:154, 2014). The embryo development was evaluated at Days 2 and 8 for cleavage and blastocyst formation. The data for apoptosis (n = 4, 80-90 oocytes per treatment) and IVF/IVC (n = 5-6, 135-175 oocytes per treatment) were analyzed by ANOVA. After 24 h aging, the rate of apoptotic CEOs in the PRL-treated group was reduced as compared to the Control group (8.2 ± 3.3 vs. 24.5 ± 3.3%, $P < 0.001$), but did not differ from that prior to aging (3.5 ± 1.8%). Meanwhile, PRL did not affect this rate in the medium containing 1 or 10 μM trilostane (1 μM: 23.8 ± 3.4 vs. 29.0 ± 1.6%; 10 μM: 30.7 ± 3.4 vs. 36.2 ± 2.0%). Furthermore, the effect of PRL on oocyte apoptosis disappeared when removing CCs. After IVM for 20 h, the blastocyst rate in our IVF/IVP system was 25.9 ± 3.0%. Following the prolonged culture of CEOs for 12 h, the blastocyst yield in the Control group decreased to 9.9 ± 0.9% and was lower than in the PRL group (18.5 ± 2.8, $P < 0.01$). The addition of 1 μM trilostane to the aging medium containing PRL (but not to the Control medium) caused the blastocyst yield to decline up to 11.7 ± 2.4% ($P < 0.05$). At the same time the developmental capacity of cultured DOs was unaffected by PRL. Thus, the supporting effects of PRL on the developmental capacity and apoptosis resistance of aging oocytes are mediated through CCs and related to stimulation of progesterone synthesis. Therefore, the anti-aging effects of PRL may be achieved, at least in part, due to a pro-survival action of progesterone on mature oocytes.

The study was supported by the Russian Science Foundation (project No. 16-16-10069).

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Developing a responsive mouse *in vitro* fertilization model with focus on sperm concentration

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Keywords: *in vitro* fertilization, mouse, sperm concentration.

Pesticides and other xenobiotics are assessed for reprotoxicity prior to approval. For this, a model based on rodents is often used, with natural mating and litter size as the end-point. The use of an *in vitro* embryo production (IVP) system would provide us with more details about the potential effects of the chemicals, as this includes the fertilization process (IVF) itself followed by the embryo development (PLoS One, 2013:e70112).
When assessing sperm fertilizing capacity, however, the concentration used for IVF is important. The fraction of fertilized oocytes in an IVF system will increase with increasing sperm concentration until reaching a maximum attainable plateau – we call this "the responsive range" of sperm concentrations. Adding an overload of sperm, i.e. more sperm than necessary to reach the maximum attainable plateau, will not increase the level of this plateau. Furthermore, an overload of sperm in an IVF system could mask compromised sperm fertilizing capacity, dependent on the type of sperm defect, resulting in a compromised assessment of sperm fertilizing capacity. In mouse IVF, the commonly used sperm concentration in relation to toxicity tests of pesticides is $1x10^6$ sperm/ml, which could result in an overload of sperm. Therefore, we studied the effect of using different sperm concentrations for IVF in a complete mouse IVP system on fertilization rates and subsequent embryo development, to develop a sensitive assessment test for sperm fertilizing capacity.
Sperm from 20 NMRI males (9-15 weeks old) and 3,416 oocytes from 195 C57BL/6J females (3-4 weeks old) were used for IVF (Theriogenology 65:1716). A total of nine sperm concentrations were used in the range of $1x10^4$ to $2x10^6$/ml, using three to four concentrations from each male. To get representative sperm samples, the sperm was mixed in capacitation drops before use for IVF. Fertilization capacity was expressed as percentage of oocytes developing to the 2-cell stage (2-cell rate). The mean values of 2-cell rates at concentration of $1x10^4$, $2.5x10^4$, $4x10^4$, $5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$, $1.5x10^6$ and $2x10^6$ sperm/ml were 23%, 42%, 47%, 55%, 56%, 45%, 36%, 50% and 41%, respectively. The data of 2-cell rates were used for a breakpoint analysis based on segmented negative binomial regressions with R software. The breakpoint analysis was made to achieve a model of the evolvement over the whole range of sperm concentrations rather than focusing on the specific sperm concentrations. The breakpoint analysis revealed a maximum 2-cell rate (51%, 95% CI: 38-69%) at 35,892 sperm/ml (95% CI: 20,999 – 61,348).
Based on these results, we will use a sperm concentration of $2.5x10^4$ sperm/ml, i.e. somewhat lower than the estimated breakpoint, in order to be within the "the responsive range" in our ongoing work with evaluating pesticide reprotoxicity. We conclude that a relatively low sperm concentration is a precondition in a mouse IVF system in order to detect reprotoxic effect on sperm cell quality.

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Effects of recombinant porcine OVGP1 protein on bovine embryo gene expression

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Keywords: Oviductin, early development embryo, recombinant protein

Oviductin is the most abundant non-serum protein present in the oviductal fluid. It has been detected bound to the zona pellucida (ZP) of gametes and embryos indicating a potential biological role during fertilization and development. We have detected porcine oviductin (pOVGP1) bound to the ZP and inside of multivesicular bodies of *in vitro* matured pig oocytes with a positive effect on *in vitro* fertilization (IVF). Previously, we tested the effect of two concentrations of pOVGP1 (10 and 50µg/mL) in a heterologous system using bovine cumulus-oocyte complexes *in vitro*. Whereas the supplementation during fertilization or culture (IVC) did not show any statistical effect on the cleavage rate nor the blastocyst yield, there was a positive tendency in the blastocyst rate when 50µg/mL of pOVGP1 was used during IVC. Therefore, the aim of this study was to evaluate if pOVGP1 had any effect on bovine embryo gene expression. Purified recombinant pOVGP1 tagged with histidine tail was obtained from HEK 293T cells. Embryos were produced by *in vitro* maturation and fertilization of oocytes derived from ovaries from slaughtered heifers. The effect of 10 or 50µg/mL of pOVGP1 was tested during IVF (day 0-1), IVC (day 1-3.5) or both IVF+IVC (day 0-3.5). Media used for IVC until day 3.5 was SOF supplemented with pOVGP1 or not (control). Then all groups were changed into SOF supplemented with 3mg/ml BSA. At day 8 a representative number of embryos from each group were fixed and the presence of pOVGP1 was analysed using anti-his antibody by confocal microscopy. Gene expression was analysed in three pools of 10 expanded blastocysts recovered from day 7 and 8 for each experimental group using RT-qPCR. Relative mRNA abundance was analyzed using one-way ANOVA. Day 8 embryos cultured in 50µg/mL of pOVGP1 during IVC were the only ones which showed fluorescent signal bound to ZP. Supplementation with 50µg/mL of pOVGP1 during IVF increased the expression of *ATF4,* gene related with endoplasmic reticulum homeostasis, and both concentrations of pOVGP1 up-regulated the expression of aquaporin 3 (*AQP3*), a gene positively correlated with survival after vitrification ($P < 0.05$). In addition to those genes, supplementation with 10 and 50µg/mL of pOVGP1 during IVC also increased the expression of desmocollin 2 (*DSC2),* a gene involved in cell to cell communication ($P < 0.05$). Finally, supplementation during IVF+IVC up-regulated the same genes in both concentrations (*ATF4, AQP3* and *DSC2*) and only when 50µg/mL concentration was used *DNMT3A,* gene related with epigenetics, was up-regulated ($P < 0.05$). In conclusion, pOVGP1supplementation during *in vitro* fertilization and culture has a positive effect on developmental related genes, indicating an improvement on bovine embryo quality.

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Dynamic changes of telomere length during bovine preimplantation development

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Keywords: Telomere bovine embryo.

Telomeres cap linear chromosomes and provide protection against nucleases, aberrant chromosomal end-to-end fusions and progressive chromosomal shortening due to the end replication problem. Telomere length (TL) has been associated with lifespan, cancer development, age and pluripotency, and it has been suggested to be established during preimplantation development. TL dynamics during embryogenesis have been thoroughly studied in the mouse model. However, laboratory mouse present extraordinarily long telomeres compared with other mammals, including bovine and humans, which makes them a poor model to understand telomere biology in humans. The objective of this study has been to determine telomere length across bovine preimplantation embryo development. Bovine embryos were produced *in vitro* and telomere length was analyzed in 20 samples per stage: matured oocytes (8 oocytes per sample), zygotes (18 hours post insemination –hpi-, 8 embryos per sample), 2-cell embryos (32-34 hpi, 4 embryos per sample), morulae (125 hpi, 1 embryo per sample) and blastocysts (200 hpi, 1 embryo sample). Zona pellucida was removed by incubating the embryos in a 0.5 % pronase solution in order to improve embryo digestion. Immediately after zona removal, embryos were stored in PCR tubes and frozen at -80 ºC until sample analysis. Samples were digested in 8 µl of a 100 µl/ml proteinase K buffered solution for 1 h at 65 ºC and proteinase K was inactivated by incubation at 95 ºC for 10 min. Relative TL was determined by quantitative PCR by contrasting the amplification of the telomeric sequence to the genomic sequence 18S, which served as an internal control to relativize the amplification of the telomeres to the total DNA amount present in the lysate. Relative TL did not vary significantly from oocytes to 2-cell embryos (oocytes 1 ± 0.15, zygotes 1.4 ± 0.17, 2-cell embryos 1.13 ± 0.11; mean ± standard error of the mean –s.e.m.-), but experienced a significant increase at the morula stage (2.31 ± 0.33, ANOVA $P < 0.05$). Beyond the morula stage, TL experienced a sharp increase in the morula to blastocyst transition (10.37 ± 1.37, ANOVA $P < 0.05$), in agreement with previous findings using Q-FISH (Schaetzlein S. et al. PNAS 2004). In conclusion, telomere lengthening during bovine preimplantation development starts before the morula stage experiencing a sharp increase in the morula-to-blastocyst transition.

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USP9Y is necessary for male development in early bovine embryo

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Keywords: DDX3Y, siRNA, sex ratio.

A third of transcripts present in the early bovine embryos have sex-specific expression patterns, which might contribute to a sexual dimorphic development of the early embryo, such as faster development, higher total cell number and greater total glucose metabolism in male embryos. Expression of Y-linked genes, such as *DDX3Y, EIF1AY, TSPY, USP9Y, ZFY*, and *ZRSR2Y,* has been identified in bovine blastocysts (Hamilton *et al*. Theriogenology 8:1587. 2012). The ubiquitin specific peptidase 9, Y-Linked (*USP9Y*) gene is involved in spermatogenesis and shortening or deletions cause azoospermia or oligozoospermia, but its role in early embryo development is not known. This study examined the role of *USP9Y* in pre-implantation bovine embryo development by investigating the effects of *USP9Y* knock-down by siRNA injection on development to the blastocyst stage. In vitro embryos produced according to Ashkar *et al*. (Hum Reprod, 252: 334. 2010) were microinjected at the 1-cell-stage at 16 h post-fertilization in 3 treatment groups: Non-injected (NI), scrambled siRNA injected (SI), and *USP9Y* knockdown siRNA injected (KD). *USP9Y*-specific siRNA was designed (Invitrogen custom primer software) assuring no homology to the X counterpart *USP9X*. After microinjection, lysed zygotes were removed and the remaining zygotes were cultured to the blastocyst stage. Cleavage and blastocyst rates were assessed at 48 and 168h post-fertilization, respectively. Relative transcript levels of *USP9Y* and the male specific gene *DDX3Y* were quantified in blastocysts by quantitative PCR (qPCR) with *GAPDH* and *PPIA* as reference genes. One-way ANOVA analysis showed that zygotes microinjected with either scrambled or *USP9Y* siRNA resulted in a significant increase in the number of lysed zygotes compared to non-injected controls (SI: 8.4% n = 538, KD: 28% n = 698 vs NI: 0% n = 487, respectively; $P < 0.05$). Scrambled and *USP9Y* siRNA microinjection significantly decreased cleavage rates (NI: 69.0% n = 487, SI: 42.8% n = 493 and KD: 43.9% n = 503; $P < 0.05$), while only *USP9Y* siRNA injection significantly decreased blastocyst rate (NI: 17.7%, SI: 12.9% and KD: 5.5%; $P < 0.05$) when compared to non-injected. qPCR relative quantification of *DDX3Y* to determine blastocyst sex in the three groups and showed only 27% of male blastocysts in KD group, compared to 44% in both NI and SI groups ($P < 0.05$). When the extent of the knock-down was assessed by qPCR, KD blastocysts showed approximately 25% less *USP9Y* expression compared to NI and SI groups. In conclusion, approximately 25% effective knock-down of *USP9Y* was reflected in significantly less male embryos reaching the blastocyst stage, allowing us to speculate that a baseline level of *USP9Y* might be necessary for development of males. Further experiments achieving a higher *USP9Y* knock-down efficiency will confirm our findings.

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The effect of L-carnitine supplementation during IVM and/or IVC on sex ratio of transferable bovine embryos depends on the combination of the IVM/IVC media

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Keywords: cattle, sexual dimorphism.

Mobilization of embryo lipids by supplementing culture media with metabolic activator is one of the promising tools to improve bovine embryo quality (Ghanem et al., Theriogenology 82(2):238-50, 2014; Ghanem et al., the Proceedings of the 31st Scientific meeting of AETE, Ghent, Belgium 11th - 12th of September 2015, p. 126). The effect of such metabolic modulator on sex ratio of transferable bovine embryos, which is of interest in breeding schemes, has not been studied. The present study investigated the effect of L-carnitine supplementation during in vitro maturation of oocytes (2.5 mM, Jeseta et al., 2014; COST action FA1201, Epiconcept, Proceedings of workshop 2014, p. 41) and/or embryo culture (1.5 mM; Ghanem et al., 2014) on the sex ratio of transferable bovine embryos. Unless stated otherwise all the chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Slaughterhouse-derived oocytes were matured 24 h in TCM199 with glutamax-I (Gibco™; Invitrogen Corporation, Paisley, UK) supplemented with 0.25 mM Na-pyruvate, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 ng/ml FSH (Puregon, Organon, Oss, Netherlands), 1 µg/ml β-estradiol (E-2257) and 10% heat inactivated FBS (Gibco™, New Zealand) at 38.5°C in maximal humidity in 5% CO_2 in air. Following 20-h fertilization with washed sperm of IVF-proven bull the zygotes were cultured in G1/G2 media (Vitrolife, Göteborg, Sweden) or modified SOFaaci (Holm et al., Theriogenology 52(4):683-700, 1999 supplemented with 3.4 mM of glucose from day 5) supplemented with FAFBSA (4 mg/ml) at 38.5°C in maximal humidity in 5% O_2, 5% CO_2 and 90% N_2. The treatment groups were: C/C = control (no L-carnitine), C/LC = 1.5 mM L-carnitine in IVC, LC/C = 2.5 mM L-carnitine in IVM, LC/LC= L-carnitine in IVM (2.5 mM) and IVC (1.5 mM). All day 7 and 8 (IVF=day 0) morulae and blastocysts (G1/G2: n = 451, mSOFaaci: n = 458) were collected from the eight IVP replicates for diagnosis of sex by PCR. The data were analyzed by comparing the observed sex ratios of embryos to the expected 1:1 ratio within each treatment group using chi-square test. Following culture in G1/G2 the sex ratios (% males) of day 7-8 embryos were 52.3%, 65.8% ($P < 0.05$), 61.5% ($P < 0.05$) and 58.4% for the C/C, C/LC, LC/C and LC/LC groups, respectively. Following culture in mSOFaaci the corresponding sex ratios were 59.3%, 45.1%, 53.3% and 55.1%, respectively. Taken together, L-carnitine supplementation during IVM or IVC skewed the sex ratio of transferable bovine embryos in favor of males when G1/G2 IVC media were used.

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Royal jelly improves embryonic developmental competence and affects transcript levels of apoptosis-related genes in goat cumulus-oocyte complexes

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Keywords: Royal jelly, goat oocyte, development.

Royal jelly (RJ) has been described with its vital biological property an antioxidant. This antioxidative capacity of RJ has been linked with protecting female/male gametes and improving post-thaw sperm motility. Considering such beneficial effects, here we have performed experiments to evaluate whether addition of RJ to *in vitro* maturation medium can affect oocye developmental competence and transcription of apoptosis-related *Bax*, *Bcl-2*, and *p53* genes in goat.

Good quality cumulus-oocyte complexes were recovered from slaughterhouse ovaries. Capsulated pure RJ (Natural Life; Brookvale, NSW, Australia) was supplemented in maturation medium during the 24 h of *in vitro* maturation at three different concentrations (2.5, 5 and 10 mg/mL). A cohort of oocytes without any RJ treatment was assigned as control group. Embryo cleavage as well as blastocyst rate were recorded at days 3 and 8 postinsemination, respectively for all groups. Gene expression of apoptosis related transcripts (*Bax*, *Bcl-2* and *p53*) was profiled using Real-time PCR. Differences in mean values were tested using ANOVA followed by a multiple pair wise comparison using t-test. The relative expression data were analyzed using the General Linear Model of SAS. A *P-value* of less than 0.05 was considered significant.

The percentage of cleaved embryos and Day 8 blastocysts was higher ($P < 0.05$) in the RJ-treated groups at concentration of 5 (70.2 ± 3.2 and 33.1 ± 2.2) and 10 mg/mL (69.8 ± 2.1 and 26.4 ± 3.5) than in 2.5 mg/mL (59.2 ± 3.3 and 21.2 ± 4.1%) and the control groups (54.5 ± 3.6 and 22.3 ± 3.7%). The expression profile of apoptotic induced (*Bax* and *p53*) was down-regulated ($P < 0.05$) in oocytes treated was RJ at 5 and 10 mg/mL compared with control counterparts. On the other hand, the transcript abundance of antiapoptotatic gene (Bcl-2) was up-regulated ($P < 0.05$) in all oocytes treated with RJ at concentration of 5 and 10 mg/mL while at 2.5 mg/mL expressed similar ($P \geq 0.05$) profile of other all groups.

Overall, the addition of RJ at concentrations of 5 mg/mL has enhanced embryo development through reducing expression of genes inducing apoptosis.

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A proteomic approach to monitor interactions between oviductal fluid and spermatozoa across the estrous cycle

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Keywords: spermatozoa, oviduct, tubal fluid.

In the bovine during estrus (pre-ovulatory period), both oviducts are able to maintain sperm viability in the so called "sperm reservoir" with the assumed aim of preserving sperm viability up to the time of ovulation. However, very few molecules interacting with bull spermatozoa (spz) and playing a role in the maintenance of bull sperm viability in vivo were identified. Furthermore, the effect of the stage of the estrous cycle on sperm-oviduct interactions is barely known. The aim of this study was to identify proteins present in the oviductal fluid (OF) that interact with spz and to determine how stage of cycle affects these interactions.

Bovine oviductal fluids (OF) were collected at a local slaughterhouse at 3 stages of the estrous cycle based on ovarian morphology: before ovulation (Pre-ov; preovulatory follicle, *corpus albicans*), after ovulation (Post-ov; *corpus hemorragicum*) and during the luteal phase (Lut; *corpus luteum*), and stored at −80°C until used. Frozen-thawed spermatozoa (spz) from one bull were washed then incubated at a final concentration of 330.10^6 spz/ml in phosphate-buffered saline supplemented with 14% OF (7 mg proteins/ml, based on a positive effect of OF at this concentration on sperm viability) at one of the three stages or a protein-free medium (Synthetic oviductal fluid or SOF, control group) for 1h at 37°C, then washed again. Total proteins from spz were migrated on a 10% SDS-PAGE and each lane was divided in 3 bands for in-gel digestion before proteomic analysis by nano LC-MS/MS. Proteins were considered to originate from the OF and to interact with spz when detected in at least one treatment group but not in the control group. Normalized spectral counts of interacting proteins were compared between stages with T-tests and considered differential when $P < 0.05$ and ratios >2 or <0.5.

A total of 270 protein clusters were identified, among which 53 oviductal clusters (that included 55 proteins) interacted with spz, including several myosins (MYH), heat shock proteins (HSP), annexins (ANX) and protein disulfide isomerases (PDI). The proportion of proteins interacting with spz increased from Pre-ov (60%, 33/55) to Post-ov (78%, 43/55) and was maximal at Lut stage (98%, 54/55). Furthermore, interacting proteins shared between Lut and Pre-ov or Post-ov were always more abundant at Lut than at periovulatory stages. When comparing Post-ov and Pre-ov, 20 interacting proteins (including MYH9, HSPB1 and ANXA2) were found more abundant at Post-ov and 4 (OVGP1, GRP78, PDIA3, PDIA6) at Pre-ov.

In conclusion, a new approach was proposed to identify and quantify proteins interacting with spz in the oviductal fluid. These results need to be confirmed with more animals. Further studies are also required to decipher the roles played around the time of ovulation by interacting proteins on sperm function.

A286E Embryology, Developmental Biology and Physiology of Reproduction

Cotyledon Efficiency: A Novel Parameter to Assess Placental Efficacy in Small Ruminants

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Keywords: placental efficiency, cotyledon efficiency, small ruminants.

Function of placenta has vital importance providing the needs of fetus.Until recently researchers thought that placental exchange in the ovine/caprine placenta is dependent on the number and size of the placentomes, which may be influenced by both maternal and fetal factors. Whilst these studies examined efficacy factors for each genus, comparisons between the ovine and the caprine were not profoundly assessed.We aimed to cross compare the differences in placental components between goat and sheep and if there is any particular element that could be used in order to assess the efficacy of placental and fetal development.Thus objective of this study was to investigate relationships between placental components (placental weight, birth weight, parity, cotyledonary characteristics and total surface area of cotyledons) within species and differentiate weather there is any similarity between those traits based on extensively comparison of cotyledonary traits to ascertain the main influential factors in placental efficiency.A total of 120 sheep and 150 goat placentas were used to determine the efficacy factors.Measurement of each cotyledon surface area was carried out on a 1 cm grid matrix whiteboard and was recorded; corresponding to the doe/ewe and after measuring each cotyledon's surface area they were individually dissected from the chorioallantois and weighed in digital scales according to their size.Placental efficiency (PE) was calculated for each ewe/doe, as the ratio of litter weight (LW) to placental weight (PW), according to Molteni et al. (1978). The proposed new parameter for measuring cotyledon efficiency (CE) was defined as the ratio of litter weight (LW) to the total cotyledon surface area (TCSA).Species were statistically compared by t-test and the variables were assessed with multi variate analyses. The Pearson Correlation coefficient was used to test the possible relationships between placental features. Placental traits were not affected by fetal sex ($P < 0.05$) birth type significantly affected ($P < 0.001$) birth weight, LW, PW and total cotyledon surface area. High positive correlation was observed between CE and PE ($r = 0.85$), sex did not have a significant effect on cotyledon number (CN). PE for sheep and goats were determined as 7.8 and 8.2 respectively. Both species had similar placental and cotyledon efficiency values. Strong positive correlation was recorded between birth type (BT) and LW ($r = 0.92$) and PW and LW ($r = 0.76$). Cotyledon number in goats ($n = 121$) were found far more higher than in sheep (55) as well as for TCSA (715 vs 301). PW in sheep and goats were found 515g and 577g, respectively and were significant between species ($P < 0.05$). Placentas with less TCSA give heavier offspring indicate that TCSA had significant effect on CE efficiency. Based on the recent outcomes cotyledon efficiency based on total cotyledon surface area seems to be a more reliable parameter to assess placental efficiency rather than CN. The marked quantitative differences in placental features between two species were determined as PW, CN and TCSA.

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Cell ultrastructure in bovine preimplantation embryos in relation to cow´s body condition score

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Keywords: embryo, BCS, ultrastructure.

The aim of the study was to examine ultrastructure of several embryo cell organelles from cows with different body condition score (BCS). Embryos (first numeral in brackets) were recovered on 7th day after the insemination by a standard non-surgical flushing of the uterine horns from superovulated Holstein-Friesian cows (second numeral in brackets) with BCS2 (71/17), BCS3 (141/31), BCS4 (48/11) and BCS5 (9/4). Thereafter, the good quality embryos (only those at blastocyst stage) were processed for transmission electron microscopy, and electronograms were evaluated by stereological analysis determining relative volumes of important organelles (mitochondria, lipid droplets, vacuoles, inclusion bodies and apoptotic bodies). Statistical analysis of cellular components was done using one-way ANOVA and Mann-Whitney U test. No differences were observed in the embryo recovery per cow among BCS2 (4.2 ± 0.8), BCS3 (4.6 ± 0.9) and BCS4 (4.4 ± 1.3) groups, whilst BCS5 group showed lesser embryo recovery rate (2.3 ± 0.6). Blastocysts from BCS3 cow with relative volumes (%) of 4.9 ± 0.32, 5.5 ± 0.5, 4.6 ± 0.5, 6.9 ± 1.05 and 1.6 ± 0.5 for mitochondria, lipid droplets, vacuoles, inclusion bodies and apoptotic bodies, respectively were served as a control. Ultrastructure of blastocyst cells in the BCS2 group was similar to those in the BCS3 group: relative volumes of lipid droplets was 7.9%, vacuoles and inclusion bodies - 9.2%. The relative volume of lipid droplets in BCS4 and BCS5 embryos increased significantly (18.5 and 22.6%) when compared to BCS3 embryos. The volume of apoptotic bodies did not significantly differ among the embryos of BCS2-BCS5 cows. In the embryos from the BCS4 or BCS5 cows we observed different morphological patterns of mitochondria: oval, round-shaped or mitochondria with vacuoles inside. Cell nuclei from BCS4 and BCS5 embryos showed the signs typical for low transcription activity (none or very few reticular nucleoli). In conclusion, differences in the ultrastructural morphology of embryos between over-conditioned (BCS4 and BCS5) and average-conditioned (BCS3) cows, in particular the higher lipid content in the cytoplasm, may indicate their low quality, and this fact can be a contributing factor to subfertility in over-conditioned cows.

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A288E Embryology, Developmental Biology and Physiology of Reproduction

FFAR4 is involved in docosahexaenoic acid effects on oocyte developmental potential during in vitro maturation

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Keywords: PUFA, oocyte maturation.

Besides affecting uterine environment, a direct effect of n-3 poly-unsaturated fatty acids (PUFA) on the oocyte could enhance fertility. We previously showed that docosahexaenoic acid (DHA, C22:6 n-3, Sigma), when provided during *in vitro* maturation (IVM), improved oocyte developmental competence through possible effects on cytoplasm but not nuclear maturation and without affecting lipid metabolism gene expression in cumulus cells (CC) (Oseikria et al *Theriogenology* 85:1625-1634. 2016). DHA could act through several mechanisms of action: i.e. via surface fatty acid receptors (free fatty acid receptor 1 or 4, FFAR1 and 4) or sensors involving PPAR or NFkB pathways; via changes in composition of cell membrane phospholipids; via production of eicosanoids… The aim of the present work was to investigate whether the FFAR4 was involved in the DHA effects previously reported on oocyte quality. We therefore investigated the effect of a specific agonist of the FFAR4, TUG-891, on embryo development after IVF. The response of surrounding CC to DHA or TUG treatment was also studied by gene expression analyses.

Oocyte cumulus complexes were collected from slaughtered cows. The protein FFAR4 was first localized by immunohistochemistry, by using a customized antibody produced specifically against the bovine protein. FFAR4 is expressed in CC and localized close to the cellular membrane, as expected.

After 22h IVM with or without DHA 1 µM or TUG 1 and 5 µM oocytes were subjected to *in vitro* fertilization (IVF) and *in vitro* development in modified synthetic oviduct fluid supplemented with 10% fetal calf serum for 7 days. At day 7, both blastocyst and expanded blastocyst rates were significantly increased with either DHA 1µM or TUG 1 or 5 µM (logistic regression, $P < 0.05$).

In order to decipher the DHA mechanisms linked to oocyte developmental competence, we then investigated the common pathways of DHA and TUG actions. Microarray hybridization of CC after 4h IVM in the presence or absence of 1µM DHA was performed (n = 4 samples per condition). A customized 60K bovine microarray (Agilent technology) including 97.4% of Ensembl *Bos taurus* transcripts was used (GEO accession: GPL21724). Only 14 differentially expressed genes varied more than two-fold and were enriched in gene ontologies related to regulation of translation, RNA splicing and spliceosome formation, oxidation/reduction, actin cytoskeleton organization and vesicle-mediated transport.

The kinetic of expression of these genes is currently characterized by qRT-PCR analysis on CC samples at 0, 4, 10 and 24h IVM with or without DHA 1 µM, TUG 1 or 5 µM.

Altogether the IVF data suggest that DHA exert its effect partly through FFAR4 on oocyte developmental competence. Also, we are studying the common transcriptomic modulation between DHA and TUG to provide insights on its detailed mechanism of action.

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A289E Embryology, Developmental Biology and Physiology of Reproduction

Reactive oxygen species level in pig embryos cultured in hypoxic conditions

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Keywords: pig embryo, hypoxic conditions, ROS.

It has been previously shown that low oxygen tension (5%) during culture of porcine embryos improves the development of zygotes to the blastocyst stage. Such produced blastocysts possess lower apoptotic index and higher total cell number in comparison with those cultured in ambient oxygen tension (21%). Alternatively reactive oxygen species (ROS) generated during mitochondrial electron transport are detrimental to embryo development. Therefore, considering the positive effect of hypoxic condition on *in vitro* embryo development we examined whether oxygen tension may alter ROS level in pig embryos during culture. Pig zygotes were collected surgically after flushing oviducts of superovulated and inseminated gilts and then were cultured up to the blastocyst stage in NCSU-23 (North Carolina State University-23) medium, at 39°C in an atmosphere containing 5% CO_2 and 5% O_2 for experimental group (A) as well as 5% CO_2 and 21% O_2 for control group (B). To estimate ROS level embryos at the zygote, 2- to 4-cell, 8- to 16 cell, morula and expanded blastocyst were labelled with 5 mM CM-H_2DCFDA dye (Molecular Probes Inc., OR, USA) for 30 min. at 39°C and then examined under a Nikon Eclipse fluorescence microscope with a CCD camera. We measured ROS level in embryos as the amount of fluorescence emitted from each labelled embryo in arbitrary unit. The data was analyzed using one-way analysis of variance and post-hoc Tukey test. ROS level (mean ± standard deviation) in group (A) was:1.32 ± 0.31 (n = 15, zygote), 1.85 ± 0.53 (n = 18, 2to 4 cell), 1.67 ± 0.38 (n = 20, 8-16 cell), 1.51 ± 0.44 (n = 22, morula) and 4.61 ± 1.23 (n = 27, blastocyst), whereas in group (B) ROS level was: 1.27 ± 0.30 (n = 18, 2 to 4 cell), 1.84 ± 0.35 (n = 19, 8 to 16 cell), 2.34 ± 0.49 (n = 21, morula) and 5.62 ± 1.41 (n = 18, blastocyst). ROS levels remained low and unchanged up to the 8 to 16 cell and morula stage in (A) and (B) group, respectively ($P < 0.01$). Then this parameter significantly increased at the morula and blastocyst stages for (B) group and at the blastocyst stage for (A) group ($P < 0.01$). Additionally, ROS level in morula and blastocysts cultured in atmosphere containing 5% O_2 were significantly lower ($P < 0.01$) then those cultured in ambient oxygen tension (21% O_2). Simultaneously, we observed similar developmental timing of pig embryos cultured in 5% O_2 and 21% O_2. In conclusion, oxygen tension during embryo culture reduces ROS level in porcine embryos, especially during morula compaction and blastocoel formation, i.e. when embryo aerobic metabolism significantly increases. Our findings suggest that the beneficial effect of hypoxia condition on pig embryo development and blastocyst quality may be related to ROS level decrease.

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Prolactin supports the developmental competence and apoptosis resistance of aging bovine oocytes through the same signaling pathway

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Keywords: prolactin, oocyte aging, developmental capacity.

The time-dependent senescence of mammalian oocytes attained the M-II stage results in a decline of their quality *in vivo* and *in vitro*. We have previously shown that the decelerating effect of prolactin (PRL) on age-associated alterations in M-II chromosomes in bovine cumulus-enclosed oocytes is related to activation of Src-family tyrosine kinases, Akt, and protein kinase C (Lebedeva et al., Front Genet, 6:274, 2015). The aim of the present research was to study mechanisms of PRL actions on the developmental competence and apoptosis resistance of bovine oocytes aging *in vitro*. Bovine cumulus-oocyte complexes (COCs) were cultured for 20 h in TCM 199 containing 10% fetal calf serum, 10 μg/ml porcine FSH, and 10 μg/ml ovine LH at 38.5°C and 5% CO_2. Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). After IVM, COCs were transferred to the aging medium (TCM 199 supplemented with 10% fetal calf serum) and cultured for 12 or 24 h in the absence (Control) or in the presence of 50 ng/ml bovine PRL (Research Center for Endocrinology, Moscow, Russia) and/or protein kinase inhibitors. The following inhibitors were applied: (1) PP2 (an inhibitor of Src-family tyrosine kinases), (2) triciribine (an inhibitor of Akt kinase), and (3) calphostin C (a protein kinase C inhibitor; Calbiochem, Darmstadt, Germany). After the prolonged culture for 12 h, oocytes underwent IVF and IVC as described previously (Singina et al., Reprod Fert Dev, 26:154, 2014). The cleavage and blastocyst rates were assessed at Days 2 and 8, respectively. Apoptosis was detected in oocytes following 24 h aging using the TUNEL kit (Roche, Indianapolis, USA). The data for apoptosis (52-57 oocytes per treatment) and IVF/IVC (177-212 oocytes per treatment) were analyzed by ANOVA. For oocytes fertilized just after IVM, the cleavage and blastocyst rates were 67.9 ± 4.2% and 22.1 ± 1.6%, respectively. After 12 h aging, the blastocyst yield declined to 7.7 ± 1.2% (Control), whereas PRL raised the yield to 14.9 ± 2.6% ($P < 0.01$). Calphostin C (0.5 μM) eliminated ($P < 0.01$) this effect of PRL on aging oocytes, although it did not affect the blastocyst rate in the control medium. Triciribine (25 μM) reduced the yield of blastocysts both in the control and PRL-treated groups (to 3.3 ± 1.2 and 7.9 ± 2.8%, respectively, $P < 0.05$), whereas PP2 (10 μM) did not. Furthermore, PRL decreased the apoptosis frequency in aging oocytes from 23.3 ± 3.4% (Control) to 9.0 ± 2.4% ($P < 0.01$), while this frequency was 4.1 ± 2.3% before aging. The hormonal action on apoptosis was abolished by calphostin C (1 μM) but not by triciribine (50 μM) or PP2 (20 μM). Our findings indicate that PRL can maintain the developmental competence and apoptosis resistance of bovine cumulus-enclosed oocytes aging *in vitro* by activating protein kinase C. Thus, the supporting action of PRL on the oocyte developmental capacity is likely to be related to its pro-survival action.

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A291E Embryology, Developmental Biology and Physiology of Reproduction

Dimethyl sulfoxide supplementation affects bovine in vitro embryo development

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Keywords: IVP, bovine Embryo.

Many techniques for IVP of embryos make use of dimethyl sulfoxide (DMSO) as solvent or cryoprotectant. Based on its high glass forming characteristics it is essential for vitrification. It is known that high concentrations could be embryo-toxic. Only little attention has been paid to small concentrations present in culture media when used as vehicle. Earlier studies deemed concentrations up to 0.4% in in vitro maturation (Avery and Greve 2000, MolReprod Dev 55 (4):438-445) and 0.1% in in vitro culture (IVC) as safe with regards to morphological criteria (Stinshoff et al. 2013, ReprodFertil Dev 26 (4):502-510).
Recently it has been shown that the live/dead ratio, the apoptotic index and fat accumulation were affected in bovine IVP blastocysts employing DMSO concentrations of 0.10% (0.20%) and 0.15% (Stöhr et al. 2016, ReprodFertil Dev 28 (1-2):157-158).
In the present study, cumulus-oocyte-complexes (COC) isolated from abattoir derived ovaries were matured and fertilized in vitro. Thirty zygotes were randomly allocated per group and cultured in vitro without oil-overlay in SOFaa + BSA supplemented with the following DMSO concentrations during IVC: 0% (control), 0.05%, 0.10%, 0.15%, 0.20% and 0.25%. The chemicals which were used for IVM, IVF and IVC were purchased by Sigma Aldrich (Steinheim, Germany).
Blastocysts at day 8 were analyzed via RT-qPCR to support these morphological results at the molecular level.
Data were analyzed for normal distribution using a Kolmogorov-Smirnov test followed by an ANOVA and a Tukey test employing SigmaStat 3.5 software (Systat Software GmbH, Germany). The level of significance was set at $P \leq 0.05$.
The following gene transcripts were assessed: ACAA1, FASN, CPT2, SCD1,SCL2A3, G6PD, BCL2L1, BAX,HSP1A1. Transcripts related to fat metabolism show an increased relative abundance (RA) of ACAA1 and FASN in blastocysts of the 0.10% DMSO group in comparison to the embryos out of other groups ($P \leq 0.05$). The RA of CPT2 was significantly lower in embryos of groups with 0.05%, 0.15% and 0.25% DMSO than in embryos out of control group ($P \leq 0.05$). Transcripts related to apoptosis demonstrated that the relative amount of BCL2L1 was significantly lower in embryos out of group supplemented with 0.15% DMSO than in the ones out of control group and 0.10% DMSO ($P \leq 0.05$). The RA of BAX was significantly decreased in embryos out of the DMSO groups in comparison to control ones with exception of embryos out of group with 0.10% DMSO supplementation($P \leq 0.05$). The RA of G6PD was significantly higher in embryos of groups with 0.05%, 0.15% and 0.25% DMSO than in embryos out of control group ($p \leq 0.05$).
In conclusion, it seems possible that the supplementation of 0.10% DMSO is suitable for working with bovine embryos in vitro.

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Effect of maternal genotype on embryo and foetal survival using rabbit as a model

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Keywords: Maternal effect; Implantation; Offspring.

Actually, the influence of maternal and embryonic genotype in prenatal survival continues being unclear. However, it is know that maternal genotype partially determines the uterine environment, leading to the possibility of induced helpful or detrimental long term effects in the developing fetus. *Whilst several studies have* linked prenatal survival with maternal genotype, others suggest that embryonic genotype could modify uterine secretions, so that prenatal survival no longer depended exclusively on maternal factors. Specifically, in rabbit has been demostrated that both embryonic and maternal genotype can affected embryo survival. In the current study, we aimed to evaluate the maternal effect on embryo and foetal survival using superovulation treatment and embryo transfer technology to discard the embryonic effect. Maternal effect was determined by comparison among the three different genotype-phenotype: two lines selected for reproductive traits but with different genetic selection processes, named line A and line V and one line selected by individual selection on daily gain from weaning to slaughter age (28 and 63 days), named line R. Nulliparous donors from line R were superovulation with one subcutaneous injection of corifollitropin alfa (3 µg, Elonva®). Sixty hour after, does were inseminated (AI) and the embryos were collected 72 h after AI. Embryos from each donor were distributed equally (7-10 embryos) among maternal genotypes to discard the embryonic genotype influence, and transferred into multiparous does using laparoscopic technique. A total of 453 embryos from 13 donors were transferred (151 in each line). The embryo survival rates were assessed by laparoscopy at day 14 and at birthday noting implantation rates and birth rates, respectively. A GLM was used (SPSS 21.0 software package). The error was designated as having a binomial distribution using probit link function. A P value of less than 0.05 was considered to indicate a statistically significant difference. The data presented as least square mean ± standard error mean. Our results indicate a clearly influence of maternal genotype on embryo and foetal survival. Specifically, lines selected for reproductive traits showed a significantly higher implantation rate and development to term (63 ± 4.0% and 36 ± 4.0% and 59 ± 4.0% and 32 ± 4.0%, for line A and V, respectively) than that line selected by daily gain (47 ± 4.3% and 24 ± 3.6%, line R). Foetal losses were similar between groups (21 ± 4.8%, 20 ± 4.8% and 18 ± 4.8%, for line A, V and R respectively). Results showed that after transplantation one peak of loss occurs before implantation, but after implantation till the end of gestation all maternal genotypes followed similar paths. In conclusion, our observation appears to be explained by the importance of the intrauterine environmental differences.

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A293E Embryology, Developmental Biology and Physiology of Reproduction

Maternal impact of metabolic diseases: effect of nutrient-sensing pathways on developmental and differentiation programs in the bovine embryo

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Keywords: nutrient-sensing, embryo, differentiation.

Maternal metabolic disorders affect reproductive physiology, leading to a disappointing fertility. A correct proliferation and differentiation process of the embryo inner cell mass (ICM) versus trophectoderm (TE) cells is a prerequisite for successful embryo-endometrial cell interactions upon arrival in the uterus. Recent cancer cell studies revealed that regulation of cell differentiation occurs via nutrient-sensing mechanisms. In this context, glucose and amino acids are upstream factors regulating the mTOR driven nutrient-sensing pathways, coupling metabolite availability to cell growth and differentiation.

Here, the earliest preimplantation stages of bovine *in vitro* embryo development were studied as 'window' for nutrient-sensitive manipulations. Embryos were cultured during 4 days under distinct nutrient conditions: [C1] CONTROL based on the SOF medium; [C2] HIGH GLUCOSE using 3.5mM glucose; [C3] LOW AMINO ACID containing only 10% of amino acid concentrations as presented in C1. At morula stage, i.e. 120h post insemination, embryos were transferred to a routine IVC medium (SOF with 5% serum; without oil overlay; 5 morulae per well). In D8 blastocysts from 4 replicates, cell differentiation (ICM/TE ratio) and apoptotic cell index (ACI) were evaluated using CDX2, Casp3 and Hoechst immuno-staining techniques. Embryo development was analyzed using binary logistic regression and other parameters with mixed model ANOVA.

Cleavage and blastocyst rates were similar for all groups ($P > 0.05$). However, the capacity of cleaved zygotes to reach blastocyst stage tended to drop after embryo culture till morula stage under C2 and C3 conditions (29.2% and 30.8%, respectively) compared to the C1 group (37.2%) ($P < 0.1$). No differences in total cell numbers were observed when comparing treatment groups. Nevertheless, a significant shift in cell lineage commitment was noticed; C2 and C3 (0.41 ± 0.02 and 0.49 ± 0.02, respectively) displayed dropped ICM/TE ratios compared to C1 (0.65 ± 0.04) blastocysts ($P < 0.02$). Furthermore, the overall ACI was twice as high in blastocysts from C2 (0.30 ± 0.04) and C3 (0.35 ± 0.03) compared to C1 (0.15 ± 0.02) ($P < 0.001$). More specifically, the ACI of the ICM fraction was drastically increased in C2 and C3 (0.58 ± 0.09 and 0.55 ± 0.06; respectively) compared to C1 (0.11 ± 0.01) blastocysts ($P < 0.001$). The latter can contribute to the observed drop in ICM/TE ratios in C2 and C3 blastocysts. However, also an increased TE ACI was noticed in C2 (0.14 ± 0.02) and C3 (0.23 ± 0.03) compared to C1 (0.10 ± 0.01) blastocysts ($P < 0.05$).

In conclusion, a bovine preimplantation embryo responds to nutrient availability in its microenvironment, resulting in changed blastomere cell fates. The latter might jeopardize first maternal-embryonic interactions and thereby establishment of pregnancy can be threatened in females suffering metabolic disorders.

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miR-21 expression in ovine oocytes: Implications for developmental competence

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Keywords: MicroRNA-21, oocyte, developmental competence.

It is known that microRNAs (miRNAs) have important roles in diverse cellular processes, but not much is known about their identity and functions during oocyte growth and development. One of these, miR-21, has been shown to have proproliferative and antiapoptotic cellular activity, which directly regulate *Bcl-2*/*Bax* expression. Taking into account that apoptosis could directly affect oocytes quality, we aimed to evaluate the expression of miR-21 and its downstream genes in oocytes with different developmental competencies. Visualization of cytoplasmic lipid content was performed for selecting the oocytes with high and low competence for the expression study. To re-confirm our oocyte quality selection criteria, a preliminary test was made to evaluate the developmental potantial of oocytes with high , middle, and low cytoplasmic lipid content (HCLC, MCLC, and LCLC, respectively).
Visible follicles were aspirated from abattoir-derived ewes' ovaries to obtain cumulus–oocyte complexes (COCs). COCs were then classified as HCLC, MCLC, and LCLC under stereomicroscope. For maturation, COCs were cultured for 24 h at 39°C and 5% CO2 in a humidified atmosphere. After maturation, a cohort of oocytes from HCLC, MCLC, and LCLC groups were evaluated for their nuclear maturation rates. Second cohort of oocytes from aforementioned groups was then activated parthenogenetically for assessing subsequent embryonic developmental potential followed by *in vitro* embryo culture. Third cohort of oocytes from HCLC and LCLC groups were analyzed for relative expression of miR-21 and its downstream *Bcl-2*/*Bax* genes using Real-time PCR. Differences in mean values were tested using ANOVA followed by a multiple pair wise comparison using t-test. The relative expression data of each gene were analyzed using the General Linear Model of SAS. A *P-value* of less than 0.05 was considered significant.
As previously demonstrated by our group HCLC oocytes (80.75 ± 6.09) revealed higher ($P < 0.05$) maturation rate than LCLC oocytes (67.25 ± 6.62) while the differences between MCLC oocytes (75.89 ± 6.29) and these two groups were not significant ($P > 0.05$). HCLC oocytes also showed a higher ($P < 0.05$) cleavage (89.85 ± 4.37) and blastosyst (39.4 ± 6.73) rates as compared to the LCLC oocytes (74.9 ± 5.80 and 27.18 ± 3.21, respectively). However, cleavage (82.49 ± 5.60) and blastocyst (35.61 ± 7.39) rates of MCLC oocytes showed no differences ($P > 0.05$) with the other two groups. The transcript abundance of miR-21 and its downstream *Bcl-2* gene decreased ($P < 0.05$) in HCLC oocytes as compared to LCLC oocytes. However, *Bax* gene expression did not showed any change between the groups upon application of this screening methodology.
It is postulated that miR-21 and its downstream target gene (*Bcl-2*) has lower transcript abundance in competent (HCLC) vs incompetent (LCLC) oocytes. More studies are needed to fully delineate the antiapoptotic role of miR-21 in oocyte growth and development in ovine.

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A295E Embryology, Developmental Biology and Physiology of Reproduction

Effects of BOEC and VERO co-culture systems on bovine blastocyst transcriptome

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Keywords: Bovine Blastocyst, BOEC cells, VERO cells.

Early embryo development is known to be impacted by its environment and especially by oviductal secretions *in vivo*. In cattle, embryo co-culture with bovine oviduct epithelial cells (BOEC) has thus been developed to mimic the *in vivo* oviduct/embryo crosstalk. Nevertheless, to the best of our knowledge, whether BOEC had a specific impact on embryo transcriptome hasn't been investigated yet.

To answer this question, we compared bovine blastocysts obtained by co-culture with BOEC to blastocysts obtained with another co-culture system: VERO cells (an epithelial cell line derived from monkey kidney). Control blastocysts were obtained in standard conditions, i.e. at 5% O_2 in SOF medium (Minitüb, Tiefenbach, Germany) + 5% Fetal Calf Serum (FCS). Because co-culture systems require 20% O_2 to maintain feeder cells alive, embryos cultured at 20% O_2 in SOF + 5% FCS were included as an additional control.

Cleavage rates and timing of blastocyst appearance were similar in the four culture conditions. A significant decrease in blastocyst rate was observed at 20% O_2 without feeder cells. Day 8 blastocysts transcriptome was analyzed on a new customized bovine microarray including more than 26 700 transcripts and 250 retroviral ESTs (GEO platform GPL21734). Hierarchical clustering of the samples revealed very weak differences between culture conditions but a clear clustering of samples depending on the presence or absence of feeder cells. Considering an adjusted P value <0.05 and a fold change >2 (Limma test), 36 transcripts were found differentially expressed between blastocysts obtained in SOF medium in 5% or 20% O_2. Comparing the two co-culture conditions revealed only 10 differentially expressed transcripts suggesting almost no difference induced by the origin of cells used in co-culture systems on bovine blastocyst transcriptome. Nevertheless, the presence of BOEC or VERO cells induced differential expression of 192 and 229 transcripts respectively when compared to 5% O_2 and 542 and 881 transcripts respectively when compared to 20% O_2. A large proportion of the transcripts affected by co-culture with BOEC were also impacted by VERO cells. Several biofunctions relative to cell cycle regulation, free radical scavenging and glucose and lipid metabolism were impacted by both cell types when compared to culture in SOF without feeder cells. Collectively, co-culture systems, using BOEC or VERO cells, do not improve cleavage and blastocyst rates and induce weak and closely related modifications of blastocyst transcriptome when compared to 5% O_2 culture condition in SOF medium.

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A preliminary study focused on the comparison of meiotic maturation effectiveness between canine and porcine oocytes undergoing two-step *in vitro* culture under analogous biochemical and biophysical conditions

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The efficiency of *in vitro* oocyte maturation (IVM) in domestic dog (*Canis lupus familiaris*) persists at the disappointingly low levels that range from 0% to approximately 25%. For this reason, either development of the effective strategies used for IVM in this member of the *Canidae* family or optimisation of the procedures adapted from IVM in other mammalian species seem to be inevitable to successfully generate canine embryos by applying such assisted reproductive technologies (ARTs) as standard *in vitro* fertilization (IVF), microsurgical IVF by intracytoplasmic sperm injection (ICSI) and intra- or inter-species somatic cell nuclear transfer (SCNT). The aim of the present study was to compare the frequencies of canine (Group I) and porcine (Group II) oocytes reaching the metaphase II (MII) stage under similar biochemical and biophysical conditions of sequential (two-step) IVM. The medium intended for the first step of IVM was comprised of TCM 199 and enriched with 10% foetal bovine serum, 10% porcine follicular fluid, 5 ng/mL recombinant human basic fibroblast growth factor, 10 ng/mL recombinant human epidermal growth factor, 1.2 mM *L*-cysteine, 0.1 IU/mL human menopausal gonadotropin (hMG), 5 mIU/mL porcine follicle-stimulating hormone (pFSH) and 1 mM dibutyryl cyclic adenosine monophosphate (db-cAMP). In the second step of IVM, cumulus-oocyte complexes (COCs) were cultured in the medium depleted of hMG, pFSH and db-cAMP. In Group I, canine COCs were matured *in vitro* for 22 h in the hMG-, pFSH-, and db-cAMP-supplemented medium. They were subsequently incubated for a further 50 h in the medium deprived of hMG, pFSH and db-cAMP. In Group II, porcine COCs that had been selected for IVM were cultured for 22 h in the hMG-, pFSH-, and db-cAMP-enriched medium, followed by 22-h incubation in the medium lacking hMG, pFSH, and db-cAMP. The meiotic (nuclear) maturity status of canine and porcine cumulus-denuded oocytes was determined on the basis of morphological evaluation (the presence of fully extruded the first polar bodies) and Hoechst 33342-mediated fluorocytochemical analysis. Sequential IVM resulted in achieving the complete meiotic maturity at the MII stage by 0/177 (0%)^{A} bitch oocytes as compared to 163/189 (86.2%)^{B} gilt/sow oocytes (^{A,B} $P < 0.001$; χ^2 test). Moreover, after 22 h and 72 h or 44 h of sequential *in vitro* culture, the degeneration rates of canine or porcine oocytes were maintained at the levels of 75.1% (133/177)^{C} and 100% (177/177)^{E} or 2.6% (5/189)^{D} and 4.2% (8/189)^{F}, respectively ($^{C,D;\ E,F}$ $P < 0.001$; χ^2 test). Summing up, canine oocytes failed to acquire the meiotic competence and to attain the nuclear maturity status under analogous conditions of two-step IVM that were used to culture porcine oocytes. Further investigations are indispensable to develop and adapt efficient and more cytobiochemically or cytophysiologically relevant approaches to extracorporeal meiotic maturation of oocytes derived from domestic dog bitches.