A252E Embryology, Developmental Biology and Physiology of Reproduction

A novel 3-D culture system to study bovine oviduct physiology, gamete interaction and early embryo development

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Keywords: 3-D culture system, cattle, embryo development, gamete interaction, oviduct physiology.

Successful fertilization depends on processes that take place in the oviduct. Due to its intra-abdominal location, it is difficult to study intra-oviductal processes *in vivo* in mammals. Instead, *in vitro* models that retain essential cell morphological and functional characteristics are being developed. In culture, bovine oviduct epithelial cells (BOECs) rapidly lose differentiated cell properties (e.g. secretory activity and cilia), while suspended cells have a limited lifespan. Progress with insert culture models and 3-D printing technologies prompted us to develop two independent BOEC culture systems, in which *in vivo*-like differentiation and function is re-established, to study oviduct physiology: (i) 3-D printed U-shaped inserts mounted with PET membranes with 0.4 μm pores (3D U-shaped culture) and (ii) hanging inserts (polycarbonate with 0.4 μm pores) containing 150 μL of Matrigel (3D culture). BOECs were harvested by scraping, and cultured for 24h to agglomerate into floating vesicles with outwardly oriented cilia. The vesicles were plated and, 7 days later, the resulting monolayers were scraped, washed and seeded onto the 2 systems described above and cultured at an air-liquid interface. For comparison, BOECs were also seeded onto coverslips as monolayers (2D culture). After 28 days, the apical side of all BOEC monolayers was washed to harvest secreted proteins, and the inserts were fixed for immunocytochemistry. Proteins (20 μg) were separated by SDS-PAGE and visualized by silver staining, or blotted onto nitrocellulose and immunostained for oviduct specific glycoprotein (OVGP1). Epithelial cell differentiation was indicated by immunodetection of laminin and the presence of primary cilia. Ciliated cell presence (acetylated α-tubulin) and secretory activity (OVGP1) characteristics of BOECs in 3D cultures were comparable to freshly harvested BOECs. The 3D culture yielded 46 silver-stainable protein bands versus 30 in 2D cultures (n=3 per system). In 3D U-shaped cultures, the polarized state (laminin and primary cilia) and their amenability to direct fluorescence microscopy (allowing live cell imaging) are currently determined. In conclusion, 3D culture methods promote polarization and differentiation of BOECs. The extent to which physiological function is maintained is under investigation. Studies in progress to assess the BOEC differentiation using the 3D U-shaped cultures include basolateral co-culture of stromal cells. Ultimately, we aim to develop an oviduct-like environment to study gamete activation, fertilization and early embryo development *in situ*.

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Detection of pregnancy-associated glycoproteins (PAGs) in prolific and non prolific ewes from early to late gestation and postpartum

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Keywords: ELISA, PAGs, non prolific, prolific, RIA, sheep.

Pregnancy-associated glycoproteins (PAGs) are placental antigens that were initially characterized as pregnancy markers in the maternal circulation of bovine species (Zoli et al., 1992, Biol. Reprod. 46: 83-92). After that, the measurement of such molecules in maternal blood as a method for pregnancy diagnosis in other ruminants has been demonstrated in several species. It can give useful information to develop appropriate feeding strategies for pregnant females and to assure requirements of the mother and the growing of fetuses and to avoid metabolic disorder associated to pregnancy. The aim of the present study was to investigate the use of a PAG ELISA-Sandwich kit (Ref. code E.G.7. CER. Marloie, Belgium) *vs* two homologous radio-immunoassay described in El Amiri et al. (2007; Reprod. Domest. Anim. 42:257-62) to detect PAGs in blood samples collected from Boujaâd (non prolific, n=8) and Boujaâd x D'man (prolific, n=20) sheep from early to late gestation and postpartum. Ewes were assumed to be pregnant when PAG concentrations were higher than 0.8 ng/ml in ELISA and 0.3 in RIAs. In addition the samples were also explored by the double immunodiffusion radial (El Amiri et al., 2003, Theriogenology. 59:1291-301) after PAG extractions. The results show that in both systems (ELISA *vs* RIAs), the PAG concentrations were significantly lower in Boujaâd a non prolific sheep than in Boujaâd x D'man a prolific sheep. Furtemore, the concentrations in RIAs were 3 folds higher than those in ELISA. In all systems, the concentrations decreased rapidly after lambing (21 weeks) reaching basal values at fourth week postpartum in ELISA *vs* RIAs. In ELISA all pregnant females showed PAGs level above 1.4 ng/ml from day 24. The double radial immunodiffusion showed positive reactions in ewes carrying more dead fetus. In conclusion, the plasma PAG investigated in the present study showed that the ELISA technique is proved to be a convenient and reliable means for early pregnancy diagnosis as well as for pregnancy fellow up in sheep. From 24 days of gestation, its reliability achieved 100% and, therefore, matches conventional approaches of pregnancy detection. The PAGs could also be detected after extraction from plasma of pregnant ewes using the double radial immunodiffusion. However, for using this technique in routine, further studies are necessary.

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Effect of asynchronous embryo transfer on glucose transporter expression in equine endometrium

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Keywords: asynchronous embryo transfer, endometrium, expression of the glucose transporter, horse.

Equine pregnancy is characterized by an unusually long pre-implantation period (40 days) during which the conceptus is entirely dependent on uterine secretions for nutrient provision. Moreover, horse embryos tolerate a wide range of uterine asynchrony following embryo transfer (ET); however negative asynchrony (recipient behind the donor) of more than 5 days markedly retards conceptus growth and development, and thereby offers a unique tool for studying the effect of the uterine environment on early development. Glucose is an important nutrient during pre-implantation development, however little is known about its transport from the endometrium into the uterine lumen. The aim of the current study was to evaluate the effect of uterine asynchrony on glucose transporter expression in the equine endometrium. Day 8 horse embryos were transferred to recipient mares that ovulated on the same day (synchronous; n=10), or 5 days after (asynchronous; n=10) the donor mare. The resulting conceptuses and matched endometrial biopsies were collected 6 or 11 days after ET (14 or 19 days of embryo development: n=5 per group). Endometrial expression of mRNA for glucose transporters was evaluated by qRT-PCR, and the effects of asynchronous ET and stage of pregnancy were analyzed by two-way ANOVA followed by independent-samples t-tests. Gene expression for SLC2A3, 4, 5, 8, 10 and SLC5A1 was stable over time and treatment, whereas endometrial SLC2A1 mRNA expression was down-regulated in the asynchronous group at day 14 of embryonic development ($p<0.05$), but did not show differences between the two treatment groups at day 19. In summary, the expression of SLC2A1, one of the main glucose transporters in the endometrium, is negatively affected by asynchronous ET and, although its expression seems to be restored by day 19 of conceptus development, this might be a contributor to the delayed development observed in asynchronous pregnancies.

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The effects of hypo- and hyperglycemia during lipolysis-like conditions on bovine oocyte maturation, subsequent embryo developmental and glucose metabolism

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Keywords: glucose, metabolic disorders, NEFA, oocyte.

Elevated follicular NEFA concentrations, commonly present in cattle in NEB or women suffering obesity or type 2 diabetes, are known to disrupt oocyte and embryo development and alter subsequent embryo metabolism. However, NEB cows exhibit systemic hypoglycemia whereas humans suffering metabolic disorders have hyperglycemic insults. Both metabolic features may affect oocyte development. Little is known about whether elevated NEFA concentrations in combination with hyper- or hypoglycemic conditions influences oocyte viability. In this study, we hypothesized that glucose interacts with high NEFA levels during *in vitro* oocyte maturation to affect developmental capacity and metabolism of the resulting blastocysts. Thus, 647 bovine grade I COCs were matured (3 repeats) under 4 conditions: 1) physiological NEFA (72µM; palmitic, stearic and oleic acid) and routine IVM glucose (GLUC) concentrations (5.50mM) (CNTRL), 2) pathophysiological NEFA (420µM) and routine GLUC (HI NEFA), 3) HI NEFA and high GLUC (10mM) (HI NEFA+HI GLUC) and 4) HI NEFA and low GLUC (2.75mM) (HI NEFA+LO GLUC). Subsequently, matured oocytes were routinely fertilized and cultured for 7 days. At day (D) 7 post insemination (pi) all blastocysts were individually cultured for 24 hours in 4µl drops of modified SOF medium under oil after which droplets were analyzed on GLUC concentrations as described by Guerif *et al.* (PLOSone, 8, e67834, 2013). Cleavage (48h pi), blastocyst rates (D8 pi) and the rates of D8 blastocysts from cleaved zygotes were recorded. Developmental competence and GLUC consumption data were compared between 4 treatments using a binary logistic regression model and mixed model ANOVA, respectively. Replicate, treatment and the interaction of both factors were taken into account (IBM SPSS Statistics 20). Significant lower cleavage rates were observed for HI NEFA+LO GLUC (56%) compared with CNTRL (73%; P=0.006) and HI NEFA+HI GLUC conditions (70%; P=0.048). At D8 pi, blastocyst rates of HI NEFA+LO GLUC exposed oocytes (18%) were significantly lower compared with CNTRL (38%, P<0.001), whereas development of HI NEFA+HI GLUC D8 blastocysts (25%) tended to be reduced compared with CNTRL (P=0.066). The capacity of cleaved zygotes to develop to blastocyst stage by D8 showed a similar profile: HI NEFA+LO GLUC (32%) significantly reduced and HI NEFA+HI GLUC (35%) tended to reduce development compared with CNTRL (53%; P=0.024 and P=0.066, respectively). Interestingly, with no significant difference in developmental stage at D7, these HI NEFA+LO GLUC blastocysts consumed significantly less GLUC from D7 to D8 (12.14 ± 4.10 pmol/embryo/h) compared with CNTRL (25.53 ± 2.96 pmol/embryo/h; P=0.020). In conclusion, low GLUC concentrations seem to be more deleterious than high GLUC concentrations in the presence of elevated NEFAs in terms of embryo development and the lower ability of the surviving D7 embryo to consume GLUC as an energy source for its further development.

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Integreated andrological evaluation in Angora goat

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Keywords: integrated andrological evaluation, sperm parameters, testicular parameters.

Integrated andrological evaluation (IAE) is a practice to identify Satisfactory (S), Questionable (Q) and Unsatisfactory (US) males. IAE procedure mainly involves classical and modern methods. The routine evaluation system contains physical and reproductive examination, while the innovative approach is more based on ultrasound detection of testicular and accessory glands, scrotal surface thermography, GnRH challenge test, CASA semen analysis (Computer Assisted Semen Analyzer), fluorescent staining, seminal plasma biochemistry, testicular fine needle aspiration cytology (TFNAC). The aim of this trial was to conduct a pilot study with an IAE based evaluation system (except of the TFNAC) in four healthy adult (4/6 years) Angora goat in Kazan-Turkey. Physical traits such as age, BSC, hereditable defects on: eyes, mouth, legs/feet, prepuce, penis, scrotum and its components were recorded and scrotal circumference, testicular ultrasound (ESAOTE MyLab5, Genoa, Italy with convex probe 2.2 – 6.6 MHz) and scrotal thermography (Flir, E60 during GnRH challenge test with 8.4 μg Busereline IV) were performed. Semen parameters such as color, volume, concentration, sperm motility (SCA Microptics), viability and morphology (Eosin-Nigrosin stain), acrosome integrity (FITC-PNA) were measured in fresh and frozen-thawed semen samples. Correlation indices and mathematical tendencies were calculated using Sigma Stat Software 2.05 and Microsoft Excel version 14.4.9. Three males were evaluated as Q, and one as US because of the presence of feet and mouth defects. One buck has not been evaluated by reasons of higher delta Testosteronemia during GnRH Challenge Test and echotexture testicular classification (Lower Mineralization Index). All mature bucks showed similar scrotal thermal pattern. Seminal plasma mean values of cholesterol, glucose, LDH, triglycerides, total protein, GGT and magnesium were 30.5 mg/dl, 77.8 mg/dl, 470.1 u/L, 8.8 mg/dl, 82.5 g/l, 46.8 u/L and 2.03 mg/dl, respectively. Bucks with higher testicular functionality, according to the physical examination, had the best freezability (Delta Viability and Intact Acrosome) and higher levels of cholesterol (34.5 mg/dl) glucose (87.4 mg/dl), LDH (585.4 u/L), triglycerides (11.15 mg/dl), total protein (89.0 g/l), GGT (53.4 u/L) as well as the lower levels of magnesium (1.88 mg/dl) in the seminal plasma. A correlation between testicular functionality and frozen-thawed semen parameters was also confirmed by sperm kinetic parameters, viability and morphology results. Application of IAE in Angora goat may indicate the buck selection for specific purposes such as breeding, cryopreservation or exclusion from any application.

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Effect of epidermal growth factor on nuclear and cytoplasmic *in vitro* maturation of guinea pig oocytes

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Keywords: apoptosis, cortical granule, EGF, guinea pig, mitochondria, oocyte maturation.

The guinea pig may represent an animal model for research on ovarian infertility and improvement of the in vitro maturation (IVM) conditions is needed in this species. The aim of the present work was to immunolocalize the Epidermal Growth Factor (EGF)-Receptor in the guinea pig ovaries and to study the effect of EGF on meiotic and cytoplasmic maturation, and apoptotic rate in cumulus-oocyte-complexes (COCs). Immunohistochemistry was performed in paraffined ovaries using a rabbit polyclonal antibody EGF-R (1:100; Santa Cruz Biotechnology) and the ABC Vector Elite kit (Vector Laboratories). For the IVM, COCs were collected by aspiration of follicles >700µm under a stereoscopic microscope. They were cultured at 37°C in 5% CO2 during 17 h with TCM-199 supplemented with glutamine, pyruvate, BSA, and different concentrations of EGF (Sigma) [0 (control), 10, 50 or 100 ng/mL] or 10% Fetal Calf Serum (FCS). After IVM, 564 oocytes were fixed and stained with 10 µg/mL Hoechst to assess nuclear configuration in terms of Metaphase II (MII) rate. A total of 143 oocytes were treated progressively with 0.5% pronase, 4% paraformaldehyde, 0.02% Triton X-100, 7.5% BSA and 100 µg/mL FITC-LCA for cortical granule (CG) staining. Also, 78 oocytes were stained with 180 nm MitoTracker RedCMXRos (Molecular Probes Inc) for active mitochondria visualization. CG and mitochondria patterns were analyzed with laser scanning confocal microscopy (Leica TCS SP2). Apoptosis rate in cumulus cells (n=58 COCs) were visualized with TUNEL (In Situ Cell Death Detection Kit, Roche) and analyzed with Image J software. Chi-square test was used to compare nuclear maturation, CG and mitochondria migration rates. The apoptotic index was analyzed by a one-way ANOVA using Duncan post-hoc test. Positive immunostaining for EGF-R was found in granulosa and theca cells and oocytes in all follicular stages. MII were significantly higher in oocytes supplemented with 50 ng/mL EGF group (75.9%) compared to other experimental groups (43.5, 51.8, 53.7 and 59.5% for 0, 10, 100 ng/mL EGF and 10% FCS, respectively, $P<0.05$). Group matured with 50 ng/mL EGF showed higher rate of oocytes with peripheral migration pattern of CG (compatible with cytoplasmic maturation) compared to control group (71.9 vs. 32.4%; $P<0.05$) and migrated mitochondrial pattern compared to the control group and the group supplemented with 100 ng/mL EGF (80.0% vs. 27.8% and 31.3%, respectively; $P<0.05$). Apoptotic rate was lower in 50 ng/mL EGF (17.2±0.9%) and 10% FCS (16.0±1.2%) groups related to the control one (28.7±1.4%) ($P<0.05$). In conclusion, the presence of EGF-R in guinea pig ovaries, suggests that EGF may exert a direct effect on ovarian function. A dose of 50 ng/mL EGF seems to be the most appropriate concentration for IVM of guinea pig oocytes, since it improves nuclear and cytoplasmic oocyte maturation and reduces apoptosis in the cumulus cells.

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Immunoradiometric assay (IRMA) of Pregnancy-Associated Glycoproteins (PAG) in bovine milk: determination of profiles in ongoing and failed pregnancies

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Keywords: cattle, milk, pregnancy, pregnancy-associated glycoproteins,

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Pregnancy-Associated Glycoproteins (PAGs) are used since early eighties as pregnancy markers in cattle and other ruminant species. Until now, they are mainly assayed in plasma or serum samples by using radioimmunoassay or ELISA systems. In cattle, concentrations of PAG are detectable in maternal blood from Day 28 to Day 30 after fertilization. Milk concentrations are 20-30 times lower than in blood samples and cannot be quantified by existing immunoassay systems. Recently, a new sensitive and robust immunoradiometric assay (IRMA) was developed allowing PAG quantification in bovine milk. Purified bovine PAG 67kDa was used as standard at concentrations ranging from 100 to 50,000 pg/mL. Highly purified immunoglobulins (hp-Ig) were obtained from two distinct rabbit polyclonal antisera by using a specific affinity chromatography (anti-PAG 4B-Sepharose gel). The hp-Ig708 (purified from polyclonal antiserum raised against caprine PAG 55kDa+59kDa) was used as capture antibody (0.01 µg/tube). The hp-Ig727 (purified from polyclonal antiserum raised against purified boPAG67kDa) was used as detection antibody (1:8,000). Radiolabeled streptavidin (125I-Strep; 50,000 cpm/100 µL) was used to reveal the Ab-Ag-Ab-Biot complexes. The aim of this study was to quantify PAG concentrations in bovine milk for pregnancy follow-up in cattle. Milk was collected from pregnant cows (n=20) during the whole duration of lactation until dry-off. Samples were frozen until assay. Before analysis, milk samples were thawed at 37°C, centrifuged (2,500 x g) and fat was removed. Samples giving high PAG concentrations were serially diluted in order to fit with standard curve range. In non-pregnant cows, concentrations remain lower than 40-50 pg/mL at all time points. In pregnant cows, milk PAG concentrations increased from Week 10 (56.9 ± 13.1 pg/mL) to Week 11 (93.5 ± 20.4 pg/mL) and Week 12 (135.2 ± 27.7 pg/mL). Thereafter, PAG concentrations increased regularly until Week 32 (2,177.6 ± 496.2 pg/mL) and slightly decreased until dry-off at Week 35 (1,615.9 ± 663.9 pg/mL). Immediately after parturition, PAG concentrations reached 5,615.3 ± 615.7 pg/mL and decreased continuously until Week 11 postpartum (36.6 ± 2.1 pg/mL). In this experiment, we could also follow three cows with pregnancy failure (2330, 7722 and 7725). Two of these cows (7722 and 7725) showed very low levels of PAG before pregnancy failure. In Cow 2330, PAG concentrations clearly decreased around the time of pregnancy failure. In conclusion, in the present study we describe the use of a sensitive and quantitative IRMA allowing pregnancy follow-up in dairy cows. This approach offers the possibility (in time or in retrospective studies) of an individual follow-up without any additional manipulation of female neither any stress induced by the investigator.

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Cumulus cells protect the oocyte against free fatty acids

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Keyword: bovine cumulus cell, Carnitine-PalmitoylTransferase-1A, DiGlyceride-AcylTransferase, free fatty acids, Stearoyl-CoA-Desaturase.

Cumulus cells have an intimate contact with, and provide metabolites to, the oocyte. The importance of cumulus cells for the oocyte extends into potential protection of the oocyte against free fatty acids (FFA)[1]. Exposure of cumulus-oocyte-complexes (COCs) to elevated FFA levels results in massive lipid accumulation in cumulus cells and normal developmental competence of oocytes (Aardema et al., BoR, 2013; 88, 164). Two potential mechanistic routes by which cells are protected against saturated FFA are lipid storage and β-oxidation (Henique et al., JBC, 2010; 285, 36818-827). To further unravel the presumed protection against FFA by cumulus cells, oocytes with and without cumulus cells were exposed to FFA. To investigate the potential mechanism by which cumulus cells may protect the oocyte, gene expression of cumulus cells from COCs matured in the presence or absence of FFA was analysed for DiGlyceride-AcylTransferase (*DGAT*; lipid storage), Carnitine-PalmitoylTransferase-1A (*CPT-1A*; β-oxidation) and Stearoyl-CoA-Desaturase *(SCD)*, the enzyme that converts saturated FFA into unsaturated. COCs were collected from bovine slaughterhouse ovaries and during 23h matured with or without 250μM saturated stearic acid followed by standard fertilization and culture. After 8h of maturation, cumulus cells were removed from part of the COCs and oocytes were placed back in maturation medium. Gene expression of cumulus cells from COCs was analysed by QPCR for *DGAT, CPT-1A* and *SCD* before and after 23h culture with or without FFA, and from cumulus cells without an oocyte for *CPT-1A* and *SCD*. Statistical analysis was performed by a paired sample t-test (gene expression) and general linear model (culture data). Materials and methods according to Aardema et al. (BoR, 2013; 88, 164) Removal of cumulus cells after 8h maturation resulted in oocytes with normal developmental competence (27±2.8%; 24±1.1% for COCs). Exposure to stearic acid resulted in strongly reduced developmental competence of oocytes cultured without cumulus during the last 15h (1±1.0%; $P<0.01$) compared to oocytes matured as COC (18±4.2%). Expression of *CPT-1A* ($P<0.01$) and *SCD* ($P<0.01$) in cumulus cells increased during maturation of COCs, independent of the presence of FFA. *DGAT* expression was not different among groups. The presence of an oocyte during culture resulted in higher *SCD* expression levels in cumulus cells after 23h of culture ($P<0.05$). These data indicate that cumulus cells are essential to protect the oocyte against saturated stearic acid. The increase in *CPT-1A* expression was independent of the condition and is in line with the necessity of β-oxidation during COC maturation. *SCD* expression has to our knowledge, not been investigated before and showed a marked, oocyte dependent, increase during maturation. We suggest that conversion of saturated FFA into harmless unsaturated FFA by cumulus cells protects the developmental competence of the oocyte.

A260E Embryology, Developmental Biology and Physiology of Reproduction

Mobilization of intracellular lipids by supplementation of IVM and IVC media with L-carnitine improves bovine embryo quality

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Keywords: gene expression, *in vitro*, mitochondria.

Mobilization of embryo lipid by supplementing culture media with metabolic activator is one of the promising tools to improve quality of in vitro produced bovine embryos. Therefore, the present study investigated the effect of L-carnitine supplementation during in vitro maturation (2.5 mM) and embryo culture (1.5 mM) on embryo developmental rates, quality and gene expression profiles. Cumulus-oocyte complexes recovered from slaughter house ovaries were morphologically evaluated and only grades 1and 2 were used in this study. Treatment groups were: T1=IVM+LC, T2=IVC+LC, T3=(IVM and IVC)+LC and control. In vivo produced embryos were included in all analyses. Development rate was calculated based on the number of embryos reached blastocyst stage at day 8 of culture. Total cell count as well as number of apoptotic cells was evaluated using Tunnel-Hoechst assay. The activity of mitochondria and intensity of lipid was measured using fluorescent probes. Expression of embryo selected candidate genes was profiled using quantitative real-time PCR. Our results showed no differences ($P < 0.05$) in cleavage rate between L-carnitine treated groups and control. Although there was an increase in blastocyst rate in T2 (44.4%) and T3 (42.1%) groups compared to T1 (39.2%) and control (38.2%), it was not statistically significant. Embryos cultured with L-carnitine and in vivo group had greater total cell number (T1: n=140.2, T2: n=164.8, T3: n=155.9 and in vivo: n=160) than the control (n=129.4). On the other hand, the percentage of apoptotic cells from total number of cells was greater ($P < 0.05$) in control (11.2%) than L-carnitine treated (T1: 4.2, T2: 3.8 and T3: 2.9%) and in vivo derived blastocysts (0.3%). Cytoplasmic lipid content was reduced by 1.8, 2.7, 2.4 and 5.1 times in T1, T2, T3 and in vivo produced blastocysts compared to their control counterparts. Whereas, intracellular mitochondria density was increased by 2.0, 4.8, 4.5 and 6.3 folds in embryos cultured with L-carnitine and in vivo group. Genes regulating lipid oxidation (CPT2 and CPT1B), fatty acid transport (SLC27A1) and mitochondria transcription (TFAM) were up-regulated while a lipid storage marker transcript (PLIN2) was down-regulated in embryos cultured in presence of L-carnitine and in vivo ones compared to control. Collectively, the lipolytic effect of L-carnitine was linked with increased mitochondrial activity, reducing apoptotic cells and modulating gene expression of in vitro produced embryos which will most likely enhance their survival after cryopreservation and transfer to recipients.

A261E Embryology, Developmental Biology and Physiology of Reproduction

Intrauterine expression of insulin-like-growth factor family members during early equine pregnancy

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Keywords: conceptus, horse, IGF-family members, pre-implantation period.

Insulin-like growth factor (IGF) family members are known to regulate fetal and placental growth and development. Insulin (INS), IGF1 and IGF2 stimulate cell proliferation and differentiation via their receptors INSR, IGF1R and IGF2R. The actions of IGF are further regulated by the IGF-binding proteins. The horse is unique with regard to an unusually long pre-implantation period (40 days) offering a unique tool to study the dialogue between conceptus and endometrium. We evaluated the expression of IGF system components in equine conceptus membranes, and endometrium during the cycle and early pregnancy. Endometrial biopsies were harvested on days 7, 14, 21 and 28 from pregnant mares, following conceptus collection, and days 7, 14 and 21 from cycling mares (n=4 per group). Bilaminar trophoblast was isolated from day 14 and 21 conceptuses, and the yolk-sac and allantochorion from 28 day conceptuses were separated. Expression of mRNA for IGF system components (INS, INSR, IGF1, IGF1R, IGF2, IGF2R) were investigated by qRT-PCR. The effect of conceptus developmental stage was analyzed by one-way ANOVA, and the effects of pregnancy and days after ovulation on endometrium by two-way ANOVA followed by independent-sample T-tests. INS mRNA was not detected in endometrium or conceptus membranes. IGF1 and IGF2R mRNA levels were uniform in cycling and pregnant mare endometrium. INSR gene expression increased in the endometrium of pregnant mares only from day 7 to 14 ($p<0.05$) and showed a higher expression than in cyclic mares on day 21 of pregnancy ($p<0.05$). IGF2 mRNA increased sequentially from day 7 to 14 to 21 of pregnancy ($p<0.05$). IGF1R expression was elevated on day 14 in both cyclic and pregnant mares ($p<0.05$). In the conceptus membranes, mRNA expression for INSR, IGF1, IGF1R, IGF2 and IGF2R was low on days 7 and 14 but showed up-regulation from day 21 ($p<0.05$). In summary, IGF family members are expressed uniformly in endometrium from cycling mares whereas endometrial expression increases during early pregnancy. Conceptus membrane expressions of IGF family genes increases from day 21, when the blastocyst capsule would start to disintegrate. We propose that the INS/IGF system plays an important role in early equine embryonic growth and the preparation for placentation.

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A262E Embryology, Developmental Biology and Physiology of Reproduction

Bovine oviduct epithelial cells: an *in vitro* model to study early embryo-maternal communication

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Keywords: bovine oviduct epithelial cells, embryo-maternal communication, IVP.

We aimed in this study to: (1) assess the expression of oviduct epithelial cells markers on bovine oviduct epithelial cells (BOECs) cultured *in vitro* under two different systems (suspension or monolayer) and (2) determine the BOECs response to the presence of early bovine embryos. BOECs were mechanically extracted by squeezing the isthmus regions of oviducts collected from slaughtered heifers during the early luteal phase, determined by the appearance of the corpus luteum. Part of the oviduct extract was frozen in liquid nitrogen and stored at -80^{o}C for gene expression analysis (fresh BOEC), while the rest was cultured in SOF+10% FCS for either 24 h (suspension) or 7 days (monolayer). Suspension or monolayer BOECs were co-incubated for 24 h with Day 2 (2- to 4-cell) or 3 (8- to 16-cell) bovine embryos produced *in vitro* to determine the embryonic effect on BOECs. A control group without embryos was included for each BOEC culture. RNA extraction from BOECs was carried out by Trisure™ (Bioline, Madrid, Spain) and Dynabeads (Dynal Biotech, Oslo, Norway) and gene expression was analyzed by qPCR, using *ACTG1* as housekeeping gene. Statistical differences were assessed by ANOVA. *OVGP1*, *GPX4* and *FOXJ1* were chosen as markers for oviductal epithelial cells and based on their function to support early embryo development, protect gametes against oxidative stress, and cilia formation, respectively. *KERA* and *PRELP* are genes implicated in extracellular matrix and *ROCK2* and *SOCS3* are genes involved in cytokinesis, all of which were found to display a response to the early embryo *in vivo* (Maillo et al., Biol Reprod 2015, DOI:10.1095/biolreprod.115.127969). Among BOECs markers, *OVGP1* and *FOXJ1* were significantly downregulated in suspension cells compared with fresh BOECs, losing their expression in a monolayer; however, *GPX4* was significantly higher in monolayer than fresh and suspension BOECs, suggesting that although monolayer BOECs lost some of their functional characteristics, they still conserved others like protection against oxidative stress. Regarding the effect of the embryos on *in vitro* cultured BOECs, only suspension BOECs showed an embryonic effect on gene expression: *ROCK2* and *SOCS3* were significantly upregulated in cells co-cultured with Day 2 compared with Day 3 embryos. In conclusion, based on the markers studied, BOECs cultured *in vitro* lost some of their functional characteristics, with suspension cells being closer to *in vivo* controls than monolayer. In addition, under our experimental conditions, suspension cells were more adequate to detect possible embryo signals than monolayer.

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Effect of high hydrostatic pressure (HHP) stress on intercellular ATP content in pig embryo

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Keyword: embryo development, hydrostatic pressure, pig.

Embryos exposed to high hydrostatic pressure (HHP) have a greater resistance to further stress and a higher survival rate in cryopreservation or nuclear transfer processes (Pribenszky C., Biology of Reproduction 83; 690-697, 2010). It is known that efficient metabolism is one of the main factors response for a proper development of pig embryos (Romek M., Reproduction in Domestic Animals 46; 471-80, 2011). In addition, preliminary measurements of the inner mitochondrial membrane potential (ΔΨm) has shown lower ΔΨm in HHP embryos compared to untreated embryos. If the HHP directly affects on metabolism of embryos reducing the ΔΨm, perhaps the total amount of adenosine triphosphate (ATP) content is changing. The aim of this study was to examine the effect of HHP treatment of porcine zygote, on ATP level in embryos at various stages of development. Pig zygotes (number of 217 embryos) used in the experiment were collected surgically from superovulated gilts breed polskiej zwisłouchej. Gilts were superovulated by an intramuscular injection of 1500 IU of PMSG (pregnant mare serum gonadotrophin, Serogonadotropin, Biowet) followed 72 h later by 1000 IU of hCG (human chorionic gonadotropin, Chorulon, Biowet). Embryos were treated by HHP in HHP device (Cryo-Innovation Ltd, Hungary) for 1 h in 39°C at a pressure of 20 MPa. Afterwards cultured in vitro in medium NCSU-23 in 39°C and 5% CO2. Before ATP analysis, embryos from experimental and control groups were frozen in 5µl Gibco® HEPES buffer (Thermo Fisher Scientific Inc., MA USA) in 1.5ml eppendorf (4-8 embryos in each tube). Analysis of ATP content was performed using Adenosine 5'-triphosphate (ATP) bioluminescent somatic cell assay kit (Sigma Chemical Company, USA) and the luminometer Lumat^3 LB 9508 (Berthold Technologies, USA). In order to examine the statistical differences a one-way ANOVA were used. The intracellular ATP content in HHP treated group (A) and control group (B) at zygote stage (a), 8-16 cells (b), morula (c) and blastocyst (d), looks like this: Aa 1.63 ± 0.26 pmol/embryo, Ab 1.55 ± 0.25 pmol/embryo, Ac 0.97 ± 0.09 pmol/embryo, Ad 0.88 ± 0.23 pmol/embryo, Ba 1.51 ± 0.40 pmol/embryo, Bb 1.40 ± 0.12 pmol/embryo, Bc 1.01 ± 0.35 pmol/embryo, Bd 0.62 ± 0.15 pmol/embryo. Pig embryos treated by HHP at zygote stage show not significant differences in intercellular ATP content compared to control group. Significant differences in ATP content between zygote, 8-16 cells and morula, blastocyst stages in both groups of HHP treated and untreated embryos were observed. It means that ATP content in pig embryo is changing during development.

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Lipid profile analysis of bovine *in vitro* blastocysts deriving from insulin treated oocytes by desorption electrospray ionization – mass spectrometry (DESI-MS)

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Keywords: blastocyst, cattle, insulin, IVP, lipid profile analysis.

The aim of this study was to characterize the lipid profile of bovine blastocysts produced from oocytes exposed to different insulin concentrations during maturation by DESI-MS. Insulin is a key metabolic hormone and its concentration in blood and follicular fluid changes in situations of metabolic imbalance as obesity, diabetes or negative energy balance (NEB). The impact of insulin on the lipid profile of blastocysts can provide important insights on the metabolic changes induced by this hormone on early development. Blastocysts were produced from abattoir derived oocytes according to standardized IVP-protocols in our laboratory. Insulin treatment was performed during 22 h of maturation using 0 (INS0); 0.1 (INS0.1) or 10 (INS10) µg/ml bovine insulin. After maturation, all treatment groups were submitted to equal conditions during fertilization and culture. On day 8, blastocysts were separately frozen at -80ºC in PBS with 0.1% PVA and individually transferred to glass sides in randomized order. A total of 63 blastocysts were used for DESI-MS lipid profile analysis. Lipids such as diacylglycerols (DAG), triacylglycerols (TAG) cholesteryl esters (CE), squalene and ubiquinone were detected in positive ion mode as silver adducts. Average full scan mass spectra of the three different treatment groups indicated few changes in the lipid profiles. Multivariate statistics by PCA (Principal Component Analysis) was used to comprehensively explore the chemical information of the full mass spectral dataset and visualize the grouping of samples resulting from chemical similarity. PCA showed some extent of discrimination between INS0 and INS10 whereas the discrimination between INS0 and INS0.1 was less evident. Data suggests down-regulated mitochondrial metabolism (indicated by ubiquinone abundance) in INS10 as well as few changes in TAG- and cholesterol metabolism comparing the treated groups (INS10 and INS0.1) with the control (INS0). Overall, the low extension of changes observed in the DESI-MS lipid profiles indicates minimal impact of insulin exposure during oocyte maturation on lipid content during preimplantation embryo development. The results of the lipid profile analysis shows that the lipid profile was not significantly different in the day 8 blastocyst after exposure of insulin during maturation. Possible explanations could be that the insulin exposure during the IVM period is not sufficient to promote extensive end-point metabolism changes in the lipids detected during preimplantation development, or that the early embryo strongly compensates for the impact of a metabolic stressor as insulin during oocyte maturation by a subsequent change in gene expression, leading to compensating mechanisms to obtain balance in the chemical profile and permitting a viable phenotype.

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Laparoendoscopic single site surgery (LESS) approach to the porcine oviduct for *in vivo* evaluation of physicochemical parameter

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Keywords: approach, laparoscopy, monoport, oviduct, pig, single-site surgery.

We aimed to define a surgical approach to the porcine oviduct capable of combining minimally invasive techniques with a time effective and accurate insertion of biosensors of physico-chemical parameters. Gilts (n= 14) and sows (n=6), of a range weight of 85 to 280 Kg were used. Animals were anaesthetized and placed in lateral right recumbent position. Then, a 5-6 cm incision in the skin followed by layer-by-layer surgical approach to the abdominal cavity was done so as to place the single-site monoport device GelPOINT Advanced (Applied Medical®, Rancho Santa Margarita, California, USA). Under laparoscopy conditions -CO2 pneumoperitoneum (8-10 mmHg)- the left uterine horn was grasped with non-traumatic forceps. Pneumoperitoneum and the single port cap were then removed, and the reproductive organs pulled up towards the incision so as to allow a direct manipulation of the oviduct. The rapid identification of the abdominal opening allowed a rapid and effective insertion of biosensors within the lumen, thus allowing the evaluation of the oviduct microambient, i.e. pH, O2 or temperature. After settling and stabilizing the probes within the oviduct lumen, the organs were put back into the abdominal cavity and in vivo recording of physiological parameters started. The laparoendoscopic single-site surgery (LESS) approach was successful in all the animals, independently of weight and reproductive maturity. Manipulation of reproductive organs was always minimal, although in 3 cases (2 gilts and 1 sow), small and slight hyperaemic areas caused by the forceps were observed in the uterine horn. During the approach no damage to the ovary, oviduct or any other abdominal organ such as intestine was produced. The average duration of the whole procedure since the beginning of the incision in the skin till the insertion of the biosensor within the oviduct was approximately 19.5 min (12-27 range), with a current duration of pneumoperitoneum conditions of 5.5 min (4-7 range). Independently of the LESS approach occasional bleeding of the mesosalpingeal vessels was observed during the manipulation required for stabilizing the probes within the oviduct. The laparoendoscopic single-site (LESS) approach described here proved very efficient in terms of allowing a rapid, minimally invasive and hardly manipulative approach to the reproductive organs, and particularly to the oviduct lumen. This approach benefits from the advantages of both laparoendoscopy (minimal trauma) and traditional laparotomy (by-hand manipulation of organs). The LESS approach is been successfully used to evaluate pH, CO2, O2 and temperature within the oviduct.

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Periovulatory pH within the porcine oviduct and uterus obtained by laparoendoscopic single-site surgery

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Keywords: laparoscopy, oviduct, pH, pig, uterus.

To determine *in vivo* pH values within the oviduct (ampulla and isthmus) and uterus in the porcine species with a minimally invasive approach. Eight pre-pubertal gilts (G) and 7 sows (S) were used. G were treated with intramuscular 1500 IU of eCG and 750 IU of hCG to induce ovulation. 36-44 hours after hCG injection (G) or the onset of oestrus external signs (S) pigs were anaesthetized and placed in lateral right recumbent position. A left lateral paralumbar laparoendoscopy single-site surgical approach (GelPOINT Advanced, SingleMedical®) was carried out under CO2 pneumoperitoneum (8-10 mmHg). Laparoscopy non-traumatic forceps were used to pull up the ovary towards the incision and upon visual inspection pigs were assorted into preovulatory (PreO) or postovulatory (PostO) stages. A flexible 1.6 mm diameter pH probe (MI508, Microelectrodes®, New Hampshire, USA) was sequentially inserted into the ampulla (Amp), isthmus (Isth) and uterus (Ut) for a time period of 10-12 min after signal stabilization. A reference electrode (MI401, Microelectrodes®, New Hampshire, USA) was also required for measurements. To simulate the physiological ambient registers were obtained after replacing back the organs -with the pH probe inserted- into the abdominal cavity and the surgical port closed. Anova of repeated measures was carried out with SPSS 19 (IBM®) to evaluate for a significance level of $p<0.05$. pH values (mean ± SD) within the Amp and Isth were significantly different (7.41 ± 0.17 and 7.10 ± 0,21 respectively, $p<0.001$). pH within the uterus (7.55 ± 0.16) was within the range of the Amp ($p>0.05$) and significantly higher than in the Isth ($p<0.001$). Regarding the PreO and PostO stages pH differences were found in the oviduct ($p=0.02$) for either the Amp (7.45 ± 0.15 vs 7.34 ± 0.12) or the Isth (7.15 ± 0.24 vs 7.04 ± 0.12), but not for the Ut (7.57 ± 0.15 vs 7.52 ± 0.07). While no differences between G and S were observed (7.35 ± 0.24 vs 7.33 ± 0.25) a significant interaction between sex maturity (G vs S) and the phase of the estrous cycle (PreO vs PostO) was found ($p=0.02$). The recorded pH values in the oviduct were lower than those of Nichol (Can. J. Physiol. Pharmacol. 75:1069, 1997), which could be related with the use of a different pH probe and surgical approach. The pronounced pH contrast between the Amp and Isth, and between the Isth and Ut is a relevant result that should be considered to better understand the microambient experienced by the porcine gametes and early embryos.

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Omega-3 fatty acids enhance developmental competence of bovine oocytes under metabolic stress conditions *in vitro*

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Keywords: blastocysts, bovine oocytes, developmental competence, IVP, Omega-3 fatty acids.

Metabolic stress conditions such as negative energy balance in dairy cows are associated with fat mobilization and elevated saturated (stearic; SA, palmetic; PA) and monounsaturated (oleic; OA) fatty acids (FAs) in serum and follicular fluid. We have shown that these FAs have direct detrimental effects on oocyte quality (Van Hoeck et al., ARS, 149:19-29, 2014). In contrast, we demonstrated that polyunsaturated α-linolenic acid (*n-3* 18:3; ALA) can enhance oocyte competence (Marei et al., BOR, 81:1064-1072, 2009). Here, we examined the effects of ALA supplementation (at physiological follicular fluid concentration; 50 μM) during *in vitro* oocyte maturation on subsequent embryo development in the presence of high follicular fluid concentrations of SA, PA and OA (HNEFA, 425μM). Cumulus cell expansion was scored at the end of oocyte maturation (0-3: 0; not expanded, 3; fully expanded). The proportions of cleaved and fragmented embryos were recorded on day 2 post-fertilization. Blastocyst rates were recorded on day 7 and 8. Day 8 blastocysts were categorized as Normal (not expanded), Expanded, or Hatched, and were fixed and immunostained with anti-cleaved-caspase-3 antibody and Hoechst. Total cell counts and apoptotic cell indices were calculated. Data were obtained from 5 independent repeats using 1529 oocytes derived from slaughter house material. A total of 179 blastocysts were stained. Categorical data were analyzed by binary logistic regression using SPSS, and numerical data were analyzed using ANOVA. Pairwise comparisons were performed using Bonferroni correction. *P* values <0.05 were considered significant. Compared with FA-free solvent controls, supplementation with HNEFA resulted in: inhibition of cumulus cell expansion (score: 1.7±0.2 vs. 2.8±0.04, $P<0.05$); higher fragmentation rates (16.8% vs. 9.5%, $P<0.05$); and lower blastocyst rates on day 7 ($P<0.05$), either expressed as a proportion from the total number of fertilized oocytes (15.6% vs. 22.8%) or from the total number of cleaved embryos (20.4% vs. 30.6%). Hatched and expanded blastocysts produced from HNEFA-exposed oocytes had higher apoptotic cell indices. In contrast, these negative effects were alleviated by ALA supplementation. In the HNEFA+ALA group, cumulus expansion score (2.4±0.16), fragmentation (6.9%), blastocyst rate on day 7 (21.4% from total fertilized oocytes and 28.7% from cleaved embryos), and apoptotic cell index were similar to the controls. In addition, HNEFA+ALA group had significantly higher total cell numbers in expanded and normal blastocysts compared with those from HNEFA group. In conclusion, ALA supplementation enhanced oocyte developmental capacity during maturation under metabolic stress conditions. The underlying mechanisms of action are currently under investigation. These results may have clinical implications to improve fertility through dietary interventions in animals and humans suffering from metabolic disorders associated with lipolysis.

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Addition of omega-3 DHA during *in vitro* maturation affected embryo development

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Keyword: bovine oocytes, *in vitro* maturation, omega-3 DHA.

Several studies have suggested a positive effect of n-3 poly-unsaturated fatty acids (PUFA) on bovine reproduction. Indeed, n-3 PUFA reduced prostaglandin secretion in uterine environment, thus providing more favorable conditions for embryo development. Other studies suggested a direct effect of n-3 PUFA on the oocyte that could enhance fertility. In the present study, we aimed at investigating *in vitro* the effect of docosahexaenoic acid (DHA, C22:6 n-3, Sigma, Saint-Quentin Fallavier, France) on bovine oocyte maturation and developmental competence. Oocyte cumulus complexes (OCC) were collected from slaughtered cows. In first experiment, *in vitro* maturation (IVM) with DHA 1, 10 and 100 µM was performed (n=3 replicates, 50-60 OCC per condition). After IVM, oocyte viability was assessed using Live/DEAD staining and then meiotic stages were determined by using Hoechst staining after oocyte fixation. Neither difference in viability nor in maturation rate was observed after IVM between control and treated oocytes whatever the DHA concentration. 83.1% of mature oocytes in control IVM and 78.9%; 84.0%; and 84.0% in presence of DHA at 1, 10, 100 µM, respectively, were observed. In second experiment (n=5 replicates, 50-60 OCC per condition), after 26h IVM with or without DHA 1, 10 and 100 µM, oocytes were subjected to parthenogenetic activation (ionomycin 5 µM, 5 min and 6DMAP 2 mM, 4h). Oocytes were then *in vitro* developed in modified synthetic oviduct fluid supplemented with 1% estrus cow serum for 7 days. Cleavage rate and a number of blastomers were assessed in resulting embryos at day 2 post activation. Cleavage rate significantly increased after IVM with DHA 1µM (84.3%) but significantly decreased with 100µM DHA (66.2%) as compared to control (76.0%) embryos (Chi-square test p=0.02). Moreover, the percentage of embryos that progressed further than 4 cells at day 2 was significantly higher (p=0.02) in the presence of 1 and 10 µM DHA (40.8% and 40.4%, respectively) than in control (31.2%) and with DHA 100 µM (22.2%). At day 7, embryos from DHA 1 µM-treated oocytes encountered more cells than those from control and other DHA groups (10 and 100 µM). Altogether these data suggest that a low dose of DHA (1µM) during IVM might improve oocyte developmental competence through possible effect on cytoplasm but not nuclear maturation. Also, we confirmed that a high dose of DHA (100µM) is deleterious for oocyte developmental potential.

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Periconceptional body condition induces placental adaptations but does not affect foal growth and metabolism in horses

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Keywords: body condition, foal, glycemia, horse, periconception, placenta, pregnancy,

Objectives: It has been shown in several species that the periconceptional environment can affect offspring long-term phenotype. This study aims to investigate the effects of periconceptional body condition on feto-placental biometry, post-natal foal growth and glucose metabolism. Materials and methods: 32 saddlebred mares of similar size were allocated to one of two groups depending on their body condition score (BCS, 1-5 French scale) at the time of artificial insemination (AI). Group High (H, n=18) had a median BCS of 3.9 (range: 3–4.25) whereas group Low (L, n=14) had a significantly lower BCS (median: 2.5, range: 2–3.75, p=0.01). Both groups were kept in pasture until the 7th month of gestation when they were housed indoors and fed forage and concentrate (barley). Food intake was not different between groups. Mares were weighed every 2 weeks and their BCS was monitored monthly. Placentas and foals were weighed and measured at birth. Foals were measured and their fasting glucose assessed regularly until 12 months of age. A frequently sampled intravenous glucose tolerance test (FSIGT) was performed at 3 days and 4 months of age. Results were analyzed using a Mann-Whitney test. Results: H mares maintained a significantly higher BCS (median≥3.75) than L mares from AI until foaling (median at foaling: 3.75, p<0.0001). L mares reached a peak BCS of 3.75 at the 7th and 8th month and thereafter lost BCS until foaling (median BCS at foaling: 2.75). Mares' body weight was not different between groups at any time. Gestation length did not differ between groups. H placentas tended to be 15% lighter with a 10% reduced surface compared to L placentas (p=0.071). Foals' weight and measurements at birth were not different but the placental efficiency (foal/placental weight) tended to be 12% higher in H mares (p=0.078). There was no difference in foals' growth until 12 months. H foals' fasting glucose tended to be higher at 3 days (p=0.063) but there was no difference in the glucose response to the FSIGT. Plasma insulin concentrations are pending. Conclusion: H mares tended to have a lighter placenta and with a reduced surface area that was more efficient than L mares. Their foals tended to have greater fasting plasma glucose than L foals at 3 days. The fact that the BCS of H and L mares throughout gestation matched their BCS at AI highlight the importance of periconceptional BCS. This study follows a previous one showing that feeding mares in the 2nd part of gestation with two different energy sources does not affect feto-placental biometry and foal development until the age of 6 months (Peugnet et al. 2015, Plos One 10, e0122596). Nevertheless, periconceptional BCS appears to induce placental adaptations that are currently being characterized.

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Involvement of phosphodiesterase 5 (PDE5) on lipid accumulation in bovine oocytes and embryos produced *in vitro*

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Keywords: cryotolerance, IVP, lipid metabolism, melatonin, nucleotide.

The aim of this study was to investigate the involvement of PDE5 on lipid metabolism in bovine oocytes by assessing the effects of PDE5 inhibition during in vitro culture on lipid contents in oocytes and resulting in vitro produced (IVP) embryos, and their cryotolerance. In Experiment 1, cumulus-oocye complexes (COCs) from slaughterhouse ovaries were submitted to IVM in TCM199 supplemented with 0.4% BSA or 10% FCS associated or not with a PDE5 inhibitor (10^{-5}M sildenafil- Sigma-Aldrich) and after 22h oocytes were denuded and stained with Nile Red (1µg/ml, 30 min) to assess cytoplasmic lipid levels measured by fluorescence intensity. In Experiment 2, 10^{-5}M sildenafil (SDF) was included during IVM and/or IVC (SOFaa) during embryo development after IVF (TALP medium using frozen sperm from the same bull prepared by Percoll gradient). Controls were cultured without SDF and all groups were cultured with 10% FCS. After 22h IVM, 20h IVF and seven days IVC, embryos were assessed for cleavage (Day 4) and blastocyst development rates. Day 7 blastocysts (BL) were fixed and stained with Nile Red to evaluate lipids. In Experiment 3, the same groups were assessed plus two others including melatonin (10^{-7}M) as an antioxidant during IVC in SDF treated groups. Cleavage and BL rates were determined and embryos were vitrified. After thawing, BLs were cultured for 24h to assess reexpansion and 48-72 h for hatching. Cultures were at 38.5ºC under 5%CO_2 in air. Statistical analyses were performed by ANOVA followed by Tukey test using SAS and significance level was 5%. In Experiment 1, SDF reduced ($P<0.05$) lipid content in oocytes matured with BSA (13.1) or FCS (16.3) when compared to controls matured only with BSA (17.6). SDF groups were similar ($P>0.05$). Reduction in lipids was only observed in BLs produced with SDF in both IVM and IVC (30.2; $P<0.05$). Oocytes matured only with FCS had highest lipid content (20.1, $P<0.05$). In Experiment 2, there was no effect of SDF or melatonin on cleavage or BL rates (79 and 31%, respectively, $P>0.05$) or reexpansion and hatching (89 and 64%, respectively, $P>0.05$). In conclusion, PDE5 inhibition during IVM reduces lipid content in oocytes, but in embryos, inhibition is necessary during both IVM and IVC. Lipid reduction, however, did not translate into improved cryotolerance, neither did the addition of the antioxidant melatonin. PDE5 appears to be involved in lipolysis in bovine oocytes and embryos possibly related to cGMP levels and PKG activity and may be an interesting target for studies to understand lipid metabolism in oocytes and IVP embryos. To our knowledge, this is the first study to show the possible relationship between this pathway and lipid metabolism in bovine oocytes and embryos.

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In vitro production of bovine embryos as a toxicological model: impact of polychlorinated biphenyl (PCB) 126 during maturation

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Many of the experimental animals used in toxicological studies are for assays involving reproductive toxicity and the vast majority use the small rodents as models for the human. There are many factors making the human and cow much more similar than humans and rodents. The aim of this study was to explore the bovine IVP system for the impact of PCB 126 during oocyte maturation. All PCB congeners are lipophilic persistent environmental pollutants, of which PCB 126 is the most dioxin-like (activates the aryl hydrocarbon receptor) and therefore considered to be the most toxic congener. For maturation, 254 abattoir derived oocytes were used (in three replicates). The oocytes were randomly divided into two groups for maturation and the treated group contained an addition of 100.6 pg/ml of PCB 126, a concentration previously found to affect cleavage and blastocyst development (Krogenæs et al., Reprod Toxicol 12:575-80 1998). Apart from the addition of PCB 126, the maturation, fertilization and culture were done according to standardized protocols (Abraham et al., Acta Vet Scand 54:36 2012). The embryo development was assessed through cleavage at 44 h after fertilization and blastocyst development (stage and grade) at day 7 and 8 after fertilization. At day 8 after fertilization the blastocysts were stained for number of nuclei (DraQ-5, Bionordica, Stockholm, Sweden) and neutral lipid (HCS LipidTOX, Invitrogen, Paisley, UK). The embryos (n = 63) were examined for number of nuclei and for neutral lipid staining intensity with fluorescent microscopy and ImageJ 1.48v (http://imagej.nih.gov/ij). Statistical analysis of the effect of PCB 126 on cleavage rate and blastocyst rate, stage and grade was done by logistic regression (logistic procedure of SAS, Milltown, USA). Continuous variables were analysed in the GLM procedure. Replicate was considered as an influencing factor and was included in all models. The mean cleavage rate for the control group was 76.3% ±0.12 (mean ±SD) and in the PCB 126 treated group 70.0% ±0.09. Blastocyst rate (calculated from number of oocytes to maturation) on day 7 was higher in the control group (19.5% ±0.1) than the PCB 126 group (10.4% ±0.04). On day 8 the corresponding figures were 28.5% ±0.06 (control) and 21.4% ±0.04 (PCB 126 group). The difference in blastocyst rate between the control and the PCB 126 group was significant (p=0.04) on day 7, but not on day 8. There was no effect of PCB 126 on blastocyst stages, grades or number of nuclei. The mean pixel intensity of the LipidTOX stain was lower in the control group (334 ±139) compared to the PCB 126 group (454 ±212) but this was not statistically significant (p=0.18). In conclusion, addition of PCB 126 during maturation seemed to affect early embryo development in this small study, and could possibly be related to lipid metabolism. Bovine IVP should be further explored as a model for toxicity on oocytes.