Animal Reproduction

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

Folliculogenesis, oogenesis, and superovulation

# Long-term changes in granulosa cells and oocytes following bacterial infection of the uterus in Holstein dairy cattle

[1]Martin Sheldon[1], Mackenzie J Dickson[2], Rachel L Piersanti[2], E B de Oliveira[2], Fahong Yu[2], Zhengxin Ma[2], Kwangcheol C Jeong[2], Jose E P Santos[2], Jeremy Block[3], John J Bromfield[2]

[1]Swansea University, United Kingdom; [2]University of Florida, Gainesville, USA; [3]Ovatech, Gainesville, USA.

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**Keywords**: oocyte, endometritis, cattle.

Postpartum uterine disease reduces fertility in dairy cows, even after the resolution of the bacterial infection. However, it is not clear how fertility continues to be impaired after the bacterial infection has resolved. We hypothesised that bacterial infection in the uterus compromises oocyte competence. To test our hypothesis, without the potential confounding effects of lactation or negative energy balance, we induced endometritis in virgin Holstein heifers (n = 4) and non-lactating Holstein dairy cows (n = 12). Animals were infused intrauterine with endometrial pathogenic bacteria, *Escherichia coli* and *Trueperella pyogenes* in 30 ml Luria-Bertani broth (Sigma, USA), and the animals developed endometritis; control heifers and cows (n = 6 and 11, respectively) received an intrauterine infusion of 30 ml sterile Luria-Bertani broth. In the heifers, we collected oocytes on day 60 after infusion by ovum pick-up, using an oocyte pick-up instrument with an 18-guage needle and a 7.5 MHz convex ultrasound probe, as described in detail previously (Biology of Reproduction, https://doi.org/10.1093/biolre/ioaa069), and granulosa cells were collected by aspiration from dominant follicles at the time of slaughter, as described in detail previously (Reproduction, https://doi.org/10.1530/REP-19-0564). In the cows we collected a total of 933 oocytes by ovum pick-up on days 2, 24, 45 and 66 after intrauterine infusion. In the heifers, we used RNAseq profiling and Ingenuity Pathway Analysis to compare the transcriptomes of oocytes and granulosa cells between bacteria-infused and control animals. We found that amongst the > 11,700 expressed genes, uterine bacterial infusion led to 539 differentially expressed genes (log2 fold change > 2) in oocytes collected 60 days after infusion, and 89 differentially expressed genes in the granulosa cells collected 94 days after infusion. Predicted upstream regulators of differentially expressed genes in the oocytes and granulosa cells included innate immunity (LPS, TLR4) and cytokines (IL-1, IL-6 and TNF). Oocytes collected from the cows were subjected to *in vitro* fertilization in BO-IVF media overlaid with mineral oil, and then embryo culture in BO-IVC embryo culture medium (all IVF Bioscience), as described in detail previously (Biology of Reproduction, https://doi.org/10.1093/biolre/ioaa069). Uterine bacterial infusion reduced the proportion of cleaved oocytes developing to morula compared with control (30.7% vs 45.0% for all oocytes collected 2, 24, 45 and 66 days after intrauterine infusion), with the greatest reduction for oocytes collected 24 days after intrauterine infusion (21.4% vs 45.6%). In conclusion, independent of lactation and negative energy balance, bacterial infection of the uterus in dairy cattle altered the transcriptome of oocytes and granulosa cells months later, and compromised the developmental capacity of oocytes. Our findings imply that bacterial infections of the uterus have long-term effects on oocyte competence, and that cows that have a history of postpartum uterine disease may not be optimal oocyte donors.
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# Plasma extracellular vesicle miRNAs as potential biomarkers for ovarian superstimulatory response in cattle

**Ahmed Gad[1,2], José María Sánchez[3], John A. Browne[3], Lucie Nemcova[1], Jozef Laurincik[1,4], Radek Prochazka[1], Pat Lonergan[3]**

[1]Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Liběchov, Czech Republic; [2]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt; [3]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland; [4]Constantine the Philosopher University in Nitra, Nitra, Slovakia.

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**Keywords***:* EV-miRNAs, superovulation, bovine.

Ovarian superstimulation (OS) in cattle is utilized to stimulate the growth and ovulation of multiple ovarian follicles to o btain a high number of viable embryos from elite donor cows. However, the high individual variability in response to OS is one of the disadvantages of this technology. Prediction of superstimulatory response could be a beneficial tool in assisted reproduction. The objective of this study was to analyse the extracellular vesicle microRNA (EV-miRNA) expression profiles in the blood plasma of heifers with variable response to OS. Oestrous cycles of crossbred beef heifers (n=25) were synchronized using an 8-d intravaginal progesterone device with GnRH at insertion and PGF2α 24 h before removal. On D10 after standing oestrus (=D0), OS was induced by the administration of decreasing doses of FSH twice a day for 4 d with PGF2α administered with the $6^{th}$ FSH injection followed by AI 24 and 36 h after the last FSH injection. Blood samples were collected on D7 of the unstimulated (U) and superstimulated (S) cycle from each heifer. All heifers were slaughtered on D7 of the S cycle. Corpus luteum (CL) measurements, as well as the total number of recovered/transferable embryos, were recorded for each heifer. A subset of High (H, n=3) and Low (L, n=3) responders was selected depending on their response to OS and EV-miRNAs profiles were analysed in each. Total weight of luteal tissue (33.3±13.9 *vs*. 107±13.9 g) and the total number of recovered (6±6.2 *vs*. 16.3±4) and transferable (2.6±2.5 *vs.* 11.3±1.5) embryos were lower in SL *vs.* SH heifers, respectively. Total vesicular RNA, was isolated from blood plasma using exoRNeasy Serum/Plasma Kit (Qiagen). MiRNA expression profile was analysed in individual plasma samples using small RNA-seq technology (NextSeq500; Illumina). Approximately 200 known miRNAs were detected in each sample with 144 commonly detected in all samples. MiR-16, miR-125, miR-126, and members of let7 family were among the most highly abundant miRNAs in all samples. Differential expression (DE) analysis revealed that 12 miRNAs (including miR-1, miR-133a, miR-206, and miR-6517) and 14 (including miR-17-5p, miR-181a, miR-199c, miR-206, and miR-6517) were dysregulated in UH *vs.* UL and in SH *vs.* SL heifers, respectively. Interestingly, miR-206 and miR-6517 exhibited the same expression pattern in H compared to L heifers both before and after OS. KEGG pathway analysis for the DE miRNA-target genes revealed that estrogen, MAPK, and Wnt signaling were among the top pathways targeted by the downregulated miRNAs while FOXO, PI3K-Akt and RAP1 signaling pathways were targeted by the upregulated miRNAs in H compared to L heifers. In conclusion, heifers with divergent ovarian responses exhibited differential expression of plasma EV-miRNAs which may be used as a potential biomarker to predict individual animal response to OS.
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## Different ovarian stimulation protocols used prior laparoscopic ovum pick-up in Saanen goats: preliminary results

**Anastasiia Bogdaniuk[1], Oleg Berestovoyy[1], Vasyl Tsvyk[1,2], Volodymyr Garkavii[2], Narhiz Yusifli[1], Oleh Sigunov[1], Olga Kramareva[3]**

[1]Institute of Contemporary Veterinary Technologies, Cherevky, Ukraine; [2]Tetyana 2011, Cherevky, Ukraine; [3]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine.

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**Keywords***:* goat, ovarian stimulation, laparoscopy, oocyte.

Laparoscopic ovum pick-up (LOPU) is a convenient way to retrieve oocytes for *in vitro* embryo production from healthy efficient goats. This process requires ovarian hyperstimulation to obtain many big follicles (≥ 5 mm) that can be easily visualized by laparoscopic optics and conveniently aspirated with a high recovery rate. The objective of this study was to compare the effect of different ovarian stimulation protocols on the number of aspirated follicles and oocyte recovery rate. The study was conducted using 9 sexually mature Saanen goats with an average age 2.8 ± 0.2 years and average weight 75.6 ± 4.6 kg that were divided into 3 groups. Estrus synchronization was performed using intravaginal sponges with 45 mg flugestone acetate (Chronogest CR®, MSD, Walton, UK) for 14 days. Ovarian stimulation was induced by injecting PMSG (Sergon 500® Bioveta, Ivanovice na Hane, Czech Republic) intramuscularly with treatment regimens, as follows: group 1 - three doses (375, 250, and 125 IU) at 24 h intervals; group 2 - five doses (300, 300, 300, 250 and 1000 IU) at 24 h intervals; group 3 - six doses (500, 500, 500, 500, 250 and 1000) at 24 h intervals. The last dose in all protocols was applied 36 h before LOPU that was performed once for each goat. Although group 2 received almost 3 times more total dosage of hormone than group 1, there was no significant difference ($P > 0.05$, Mann–Whitney U test) between the two groups in the number of aspirated follicles (14.3 ± 2.5, 13.0 ± 2.0; mean ± SD), retrieved oocytes (9.3 ± 0.6, 8.7 ± 0.6) and recovery rate (66.0% ± 8.2%, 67.3% ± 6.6%). Group 3 showed significantly more aspirated follicles (22.0 ± 3.6; $P \leq 0.05$) and retrieved oocytes (16.0 ± 2.0; $P \leq 0.05$) than groups 1 and 2, however, the recovery rate was not influenced by the protocol (73.1% ± 4.2%; $P > 0.05$). To conclude, these preliminary results showed that ovarian stimulation protocol with six doses of PMSG provided higher number of follicles and retrieved oocytes in Saanen goats.

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## Changes in acetyl-CoA metabolism alter histone acetylation profile and global gene expression in bovine cumulus cells

**João Vitor Alcantara da Silva[1,2], Jessica Ispada[2], Érika Cristina dos Santos[2], Aldcejam Martins da Fonseca Junior[2], Camila Bruna De Lima[3], Marcella Pecora Milazzotto[2]**

[1]Universidade Federal do ABC, Brazil; [2]Universidade de Mogi das Cruzes, Brazil; [3]Laval University, Canada.

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**Keywords**: oocyte maturation; acetylation; metabolic pathways.

The cumulus cells (CC) are somatic cells that are closely attached to the oocytes. Among other functions, CC support maturation by allowing the bi-directional transfer of essential molecules involved with cell signaling and metabolism. Besides, in mammalian cells, most of the cytosolic acetyl-CoA, the major source of acetyl groups for histone acetylation, is derived from citrate produced in the tricarboxylic acid cycle (TCA). Thus, alterations in mitochondrial function could impact histone acetylation, with consequences to chromatin permissiveness and gene expression, possibly impacting the maturation process and the oocyte quality. In this work, we hypothesized that the modulation of mitochondrial function in bovine CC correlates with changes in histone acetylation profile and global gene expression. Bovine COCs (grade I from 3-5mm follicles) were collected from slaughterhouse ovaries and *in vitro* matured in the presence or absence (control) of 1.5 mM of dicholoracetate (DCA), an inhibitor of pyruvate dehydrogenase kinases (25 per group in 3 replicates – culture conditions: 90ul drops of tissue culture medium 199 (TCM-199), 10% fetal bovine serum (FBS), 0.2 mM pyruvate, 0.5 mg/mL FSH, 100 IU/mL human chorionic gonadotrophin and 1.25 mg/mL gentamicin 38°C, 5% $CO_2$ and high humidity). COCs were collected at different time points (immature, 4, 8, 16 and 24 hours) and assessed for H3K9 acetylation levels (immunostaining) and global synthesis of new transcripts (Click-iT® RNA imaging kit). At 24h, mitochondrial activity was also assessed (MitotrackerTM Red CMXRos). At each time point, images were acquired by fluorescence microscopy (LAS X Life Science Software) under the same conditions and parameters. Fluorescence intensity of CCs was calculated considering a round area including the oocyte and approximately 10 CC layers (Image J software). Then, oocyte area was subtracted, and the resulting values were submitted to statistical analysis. Results were compared by t-student (treatment vs. control) with $p<0.05$. CC from DCA group had an increase in mitochondrial activity suggesting, albeit indirectly, the greater activity of the TCA cycle. Associated with that, CC from treated group showed higher H3K9 acetylation levels at all analyzed timepoints. Moreover, at 8 and 16h after the onset of IVM, we also observed a significant increase in the synthesis of new transcripts compared to control group. At 24h, however, levels of new transcripts did not differ between groups, suggesting the action of additional epigenetic regulation at the end of maturation. In conclusion, results corroborate our hypothesis and clearly demonstrate the close relationship between energy metabolism and epigenetic control in bovine CC, suggesting that a higher mitochondrial activity modulates the generation of substrates for histone acetylation, and leads to changes in the global transcription levels of CC.

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## Pharmacokinetics of a long-acting progesterone formulation in female camels

**Hamid Chhaibi[1], Ahmed Tibary[2], Alexis Campbell[2]**

[1]Institut agronomique et veterinaire hassan II, Morocco; [2]Department of clinical sciences,college of veterinary medicine, Washington state University, USA.

**Keywords**: camelids, embryo, progesterone.

Progesterone administration is used extensively in camel embryo transfer programs for synchronization of recipients and donors. Daily intramuscular administration (IM) of 100 to 150 mg of progesterone in oil for 14 days is recommended in order to achieve the desired effect. Daily IM injections to a large group of animals present several difficulties associated with frequent animal handling and compliance with timing and dose of injection. In addition, frequent injections may render some animals less tractable. Other techniques of delivery of progesterone for several days include the use of CIDRs. However, these devices are not always well tolerated by camels, may be lost, and are associated with development of vaginitis. Similar problems have been encountered in other species such as horses. Studies in mares have shown that administration of Biorelease progesterone formulations results in serum progesterone levels comparable to those observed with normal luteal function, for a period of 10 to 12 days.

The present experiment was designed to evaluate progesterone pharmacodynamics following a single standard dose administration of compounded proprietary long-acting progesterone that was formulated for mares. Fourteen (n=14) nulliparous female camels of 3.5 years of age and of similar weights were included in the study. Each female was given an intramuscular injection of 5 mL of a proprietary progesterone formulation (BioRelease P4 LA300, 300 mg of progesterone per mL). All females were examined by transrectal ultrasonography and only females with no corpora lutea present on the ovaries were included in the study. Blood samples were collected daily starting one day prior (Day 0) and continuing for 14 days after injection. Serum was isolated and stored at -20°C until assayed for progesterone using radio-immunoassay. Change in daily progesterone level following treatment was examined using a repeated measurement ANOVA.

As expected progesterone level was low (Mean ± SEM = 0.2 ± 0.07 ng/mL) prior to injection and increased significantly (36.76 ± 3.8 ng/mL, $P<0.05$) within 24 hours of treatment. Serum progesterone level remained above 2 ng/mL in all animals for 10 days. By 12 days after injection only 50% of the females had progesterone levels below 2 ng/ml. By 14 days after treatment, five females (36%) had serum progesterone between 1 and 2 ng/mL while all the other has less than 1 ng/ml.

In conclusion, this study demonstrated that administration of 5 ml of BioRelease P4 LA300 to female camels provides elevated serum progesterone levels that are comparable to those expected during the luteal phase for at least 10 days. This treatment may be useful to eliminate the need for repeated daily administration for at least that period of time. Studies are underway to determine the effect of this compounded long-acting progesterone on ovarian function. Sources of variation in individual response need further examination.