CSIRO PUBLISHING

CORRIGENDUM
https://doi.org/10.1071/RDv36n2Ab65_CO

Reproduction, Fertility and Development

Corrigendum to: 65 Functional ablation of pregnancy-associated glycoprotein 7 affects attachment and growth of trophectoderm cell lines

E. Moreno, M. S. Ortega and K. G. Pohler

Reproduction, Fertility and Development 36, 183–184.
doi:10.1071/RDv36n2Ab65

The authors advise that the original list of authors appears in the incorrect order. The correct order of authors is as follows:

E. Moreno, K. G. Pohler and M. S. Ortega

Moreno E *et al.* (2024) *Reproduction, Fertility and Development*
doi:10.1071/RDv36n2Ab65_CO

RBPs in GCs cultured under HS (42°C) compared to thermoneutral (38.5°C) conditions using qRT–PCR. All tested RBP genes exhibited significantly higher expression levels (>2-fold change, $P < 0.05$) in the HS-GCs, except for *RBM42*. Notably, the expression of the *LIN28A* gene was found to be more than 8-fold higher in the HS-GCs. Certain RBPs, such as YBX and LIN28A, are already known for their involvement in miRNA sorting into EVs and the regulation of cellular stress. The identification and characterisation of these miRNA sequence motifs and their interaction with RBPs will present a unique opportunity to investigate the underlying mechanisms of miRNA-mediated intercellular communication during conditions of HS. Our future research will focus on a mechanistic analysis of the identified motifs and RBPs, exploring their roles in the packaging and release of specific miRNAs via EVs and assessing the subsequent impact of this interplay on cell survival and ovarian response under thermal stress conditions.

64 Is the proteome of the oviductal fluid in dairy cows affected by heat stress?

A. Assel[A], U. Besenfelder[A], K. Wagener[C], J. Allram[B], M. Tekin[C], C. Vogl[B], M. Drillich[D] and V. Havlicek[A]

[A]Reproduction Centre Wieselburg RCW, Institute for Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria
[B]Molecular Genetics, Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria
[C]Clinical Unit for Herd Health Management in Ruminants, University Clinic for Ruminants, University of Veterinary Medicine, Vienna, Austria
[D]Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Heat stress is one of the best-known manifestations of global warming in livestock, resulting in lower dry matter intake, milk yield, and fertility. Fertility problems associated with heat stress are mainly caused by the effect of increased body temperature on oocyte and embryonic development. In addition to the direct effect of heat stress, the early embryonic development might be affected by elevated temperature due to alterations of the maternal reproductive tract. The elevated metabolism of high-yielding dairy cows may further contribute to dysregulation of homeothermy in the oviduct. The aim of the study was to investigate the effect of short-term heat stress under Lower Austrian conditions on the maternal microenvironment in the bovine oviduct in cows with high or low milk yield. A total of 15 Simmental cows were divided into two groups according to their milk yield during Days 40 to 50 postpartum: low-yielding cows (296.66 l ± 35.65) and high-yielding cows (386.30 l ± 33.70). The oviducal fluid was collected both during the wintertime (temperature-humidity index (THI) < 72) and summertime (the animals were exposed to a THI ≥ 72 from the start of the synchronization to the collection of oviducal fluid). Only animals in a luteal phase were synchronised using $PGF_{2\alpha}$ and gonadotrophin-releasing hormone 48 h apart to induce ovulation. The oviducal fluid was collected by transvaginal endoscopy 48 h later: 0.5 mL of phosphate-buffered saline was flushed back and forth three times in the oviduct ipsilateral to the fresh corpus luteum (D1), the recovered fluid was centrifuged and prepared for further analysis. Three to four samples per group were digested with trypsin/lysC and injected in a nano-liquid chromatography (nLC) tandem mass spectrometry (MS/MS) system (Orbitrap Q Exactive Plus, ThermoFisher). Generated data were label-free quantified with the MaxQuant software (v2.2.0.0) and statistical analysis was performed in R (https://www.R-project.org/). In total, 2368 proteins were identified in all recovered oviducal fluids. The oviducal proteome of low-yielding cows differed significantly from that of high-yielding cows in summer and winter. The oviducal proteome of low-yielding cows differed between seasons, in contrast to that of high-yielding cows that showed negligible differences between both periods. In conclusion, it was shown that in addition to the metabolic load of high-yielding animals, the effect of heat stress on the proteome of the oviducal fluid is weak. Thus, the influence of elevated temperature was visible only in low-yielding cows with less metabolic activity. Further detailed analysis of proteome profiles is planned to better define the effect of elevated temperature on the composition of the oviducal fluid.

This study was funded by the Gesellschaft für Forschungsförderung NÖ, FTI-Call 2020 (FTI20–012).

65 Functional ablation of pregnancy-associated glycoprotein 7 affects attachment and growth of trophectoderm cell lines

E. Moreno[A], M. S. Ortega[A] and K. G. Pohler[B]

[A]University of Wisconsin–Madison, Madison, WI, USA
[B]Texas A&M University, College Station, TX, USA

Embryonic mortality between Days 8 and 30 of development represents ~20% of pregnancy losses in cattle. During this time, the embryo needs to grow into a conceptus and start the process of placentation. Pregnancy-associated glycoproteins (PAGs) are secreted by the trophectoderm (TE) mononucleated and trophoblast giant cells (TGCs) and have been used to monitor fetal viability and placentation in cattle. Previous research suggests that PAGs 7, 9, and 17 which are highly expressed by the TGCs between Days 20 and 45 of pregnancy, might have a role in extracellular matrix remodeling of the endometrium, which is essential for a successful placentation and therefore, pregnancy. However, the specific function or mechanism of action of PAGs is still unclear. The objective of this study was to elucidate how the functional ablation of PAG7 affects trophectoderm attachment and growth *in vitro*. To test this, a CRISPR/Cas9 system was used. Single RNA guides were designed to partially cut from exon 2 to 4 of *PAG7*, for a total edit of 1460 bp. Guides were individually annealed to a universal tracer RNA and then to CAS9mRNA. Abattoir-derived cumulus–oocyte complexes were matured, fertilized, and washed using our group standard protocol. Guide complex and CAS9 mRNA, or just CAS9 mRNA were electroporated into zygotes 15–17 h after insemination. Embryos were cultured until Day 8 of development. Embryos that reached the blastocyst stage were cultured for 2 additional days with 10% fetal bovine serum to promote hatching. On Day 10, hatched blastocysts were individually placed in gelatin-coated plates with an in-house TE culture medium. Daily pictures were taken to measure the area and the day of attachment was recorded for each embryo. On Day 25, cells were collected for endpoint polymerase chain reaction genotyping. A total of 37 PAG7 null TE cell lines and 24 CAS9 control

cell lines were produced in three *in vitro* embryo production rounds. The TE area measurement was done using ImageJ, v1.53 (National Institutes of Health, Bethesda, Maryland, USA). All data were analysed by ANOVA, with TE growth as response variable and replicate, genotype result (PAG7 null or not) and interaction as fixed effects, using the Statistical Analysis System SAS v9.4. The TE cell lines from PAG7 null embryos took 3 days longer ($P < 0.05$) to attach than CAS9 controls (Day 19 vs 16, respectively). The TE growth area was also affected in PAG7 null embryos compared to controls ($P < 0.05$). By Day 25 of culture, the TE area was 1.5 mm^2 for PAG7 null embryos and 4 mm^2 for the Cas9 controls. These preliminary results indicate that the ablation of PAG7 affects attachment and TE growth, supporting the proposed action of TGC PAGs on matrix remodeling; however, further research is required to elucidate if PAG7 knockout results in an inadequate response of the endometrium, which would not allow a successful placentation, leading to embryonic mortality.

This work was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021–67015–33675 from the USDA National Institute of Food and Agriculture.

66 Developmental potential of single blastomeres within individual embryos presenting bipolar or multipolar divisions

A. Fernández-Montoro[A], T. De Coster[A,B], D. Angel-Velez[A,C], Y. Zhao[B], K. Smits[A], J. R. Vermeesch[B] and A. Van Soom[A]

[A]Department of Internal Medicine, Reproduction, and Population Medicine, Ghent University, Merelbeke, Belgium
[B]Laboratory for Cytogenetics and Genome Research, Department of Human Genetics, KU Leuven, Leuven, Belgium
[C]Research Group in Animal Sciences, INCA-CES, Universidad CES, Medellin, Colombia

During multipolar zygotic divisions (direct cleavage into three or more blastomeres), entire parental genomes can segregate into distinct blastomeres, leading to mixoploid or chimeric embryos. Still, embryo arrest occurs frequently in those embryos, suggesting that blastomeres resulting from whole-genome segregation errors might have lower viability than normal, diploid blastomeres. To investigate this further, we compared the developmental potential of single blastomeres within an individual embryo after either bipolar (cleavage into two blastomeres) or multipolar division. To do so, bovine cumulus–oocyte complexes were collected from slaughterhouse-derived ovaries and matured separately per ovary ($n = 11$ cows, 7 replicates) in 500 μL maturation medium for 22 h. Since multipolar divisions are often triggered by polyspermic fertilization, oocytes were fertilized with semen of a bull with known high polyspermy rate. The presumed zygotes were denuded and transferred to 25 μL of culture medium droplets at 21 h postfertilization (hpf), and monitored from 26 to 36 hpf every 30 min to identify zygotes with bipolar and multipolar divisions. Immediately upon the first division, the zona pellucida was dissolved with pronase in 58 embryos and single blastomeres were isolated and cultured individually in well-of-the-well culture dishes. The development of the single blastomeres was checked at 72, 121, and 170 hpf. To evaluate the relation between the type of division and the developmental potential, a chi-squared test of independence was performed. At 72 hpf, the 2-cell stage was reached in a higher number of blastomeres from multipolar divisions (32.1%) than in blastomeres from bipolar divisions (0%; $P = 0.018$), while no difference was observed among groups in the percentage of blastomeres from embryos with multipolar or bipolar divisions that did not cleave any further (21.4% and 31.2%, respectively; $P = 0.469$). Interestingly, although there was no statistical difference, only 46.4% of blastomeres from multipolar divisions developed to the 3- to 5-cell stage compared to 68.8% ($P = 0.215$) in those from bipolar divisions. At 121 hpf, more blastomeres arrested at 1 cell or at the stage of 2–4 cells in the multipolar group (27.5% and 35%) than in the bipolar group (0%, $P = 0.023$; and 6.3%, $P = 0.036$), while more advanced development (5–10 cells) was reached in a higher proportion of blastomeres from bipolar divisions (87.5%) than from multipolar divisions (37.5%; $P < 0.01$). At 170 hpf, a similar number of blastomeres remained uncleaved in both groups (bipolar = 21.4%, multipolar = 41.1%; $P = 0.098$) or started the first divisions but arrested in 2–5 cells (bipolar = 7.1%, multipolar = 35.7%; $P = 0.122$), but blastomeres from bipolar divisions showed the highest developmental potential (71.4% blastocyst rate) compared to the multipolar group (26.8%; $P = 0.003$). To conclude, we showed that blastomeres resulting from bipolar divisions exhibit a higher developmental potential than those from multipolar divisions; and we developed a model to isolate and selectively grow cell lines with distinct parental contribution.

67 Deciphering the dialogue between the early bovine embryo and the oviduct: comparison of extracellular vesicle proteins from an *ex vivo* model and an *in vivo* environment

R. Mazzarella[A], J. M. Sánchez[A], B. Fernández-Fuertes[A], S. G. Egido[A], A. Álvarez-Barrientos[B], E. G. Jiménez[C], J. M. Falcón-Pérez[C], M. Azkargorta[D], F. Elortza[D], M. E. González Martinez[E], P. Lonergan[F] and D. Rizos[A]

[A]Department of Animal Reproduction, INIA-CSIC, Madrid, Madrid, Spain
[B]Servicio de Técnicas Aplicadas a la Biociencia, Universidad de Extremadura, Badajoz, Extremadura, Spain
[C]Exosomes Laboratory, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain
[D]Proteomics Platform, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain
[E]Department of Anatomy and Embryology, Veterinary Faculty, Complutense University of Madrid (UCM), Madrid, Madrid, Spain
[F]School of Agriculture and Food Science, University College Dublin (UCD), Belfield, Dublin, Ireland

Bovine oviducal explants provide an alternative *ex vivo* approach to study embryo-maternal communication, which is partly mediated by extracellular vesicles (EVs). We investigated the protein content of EVs from (1) oviducal explants cultured alone (EXP) with oviducal fluid (OF) of cyclic heifers (CY) and (2) oviducal explants co-cultured with embryos (EXP + EMB) with OF of pregnant (PG) heifers. Heifers