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

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


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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 ± 35.65) and high-yielding cows (386.30 ± 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 FGF2a, 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.

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

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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 placentaion. 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 placentaion 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 placentaion 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. Abatior-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 inseminaion. 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
66 Developmental potential of single blastomeres within individual embryos presenting bipolar or multipolar divisions

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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


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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