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Corrigendum

43 Correlates of reproductive tract anatomy and uterine histomorphometrics with fertility in swine

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hypothalamus, with both tissues flash frozen and subsequently used for RNA sequencing. Differential expression of genes (DEGs) was determining using R Bioconductor package EdgeR (version 3.20.9). The resultant list of DEGs was then submitted to Ingenuity Pathway Analysis and Weighted Gene Co-expression Network Analysis (WGCNA). At slaughter, bodyweight was higher in HP calves (189.6 vs. 113.0 kg; $P < 0.001$). Compared with calves on MP, a high plane of nutrition altered the expression of 39 and 80 transcripts in the hypothalamus and ARC, respectively ($P < 0.05$). *COL15A1*, *CDH17*, and *IL20RA* were among the most upregulated transcripts in both hypothalamic tissue sections. The HP treatment induced downregulation of *AGRP* and *NPY* and upregulation of *POMC* in the ARC, which have previously been associated with early onset of puberty in heifers. Functional analysis highlighted the importance of DEGs in the hypothalamus and ARC related to diets, with stress-signalling pathways among the most highly dysregulated pathways in both tissues. Through pathway analysis of the ARC DEGs, a total of 7 networks were derived, including one involved in organ morphology, reproductive system development and function, and developmental disorder. Interestingly, *CGA*, the most upregulated DEG in the ARC and a potential upstream regulator in this brain region, was one of the most connected genes within this network. Furthermore, WGCNA revealed that expression of some hub genes in networks of co-expressed genes, previously associated with puberty in cattle, was affected by diet in the ARC (*POMC*, *CBLN2*, and *CHGA*) and the remainder of the hypothalamus (*PENK*). The results of this study indicate that an enhanced plane of nutrition during early calfhood alters the biochemical regulation of the hypothalamus consistent with advanced sexual development in the prepubertal heifer.

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42 Disruption of endogenous *SOX2* during porcine embryo development using the CRIPSR/Cas9 system

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The lineage specification of the pre-implantation embryo is important to understand the developmental process, but it remains unclear because the expression of lineage-specific genes is distinct among species. Pigs have genetic and physiological traits similar to humans; however, there are differences in gene expression during the pre-implantation stage. To select a candidate gene that affects the formation of the inner cell mass (ICM) in porcine embryo, we conducted preliminary experiments. First, we measured the expression level of candidate genes for lineage specification in parthenogenetic-activated embryos. The expression of pluripotent genes peaked on Day 3 and thereafter decreased gradually. Next, we conducted immunocytochemistry. OCT4 was expressed in all cells in morula and Day 5 blastocyst, but some Day 7 blastocysts expressed OCT4 in both ICM and trophectoderm (TE), whereas others expressed OCT4 only in ICM. NANOG was not observed in the morula stage, whereas SOX2 was located in a restricted area. To examine the effect of SOX2 in ICM formation, we injected plasmid expressing Cas9 and guide (g)RNA using Lipofectamine for efficient transgene expression at the 2-cell stage to increase viability by inducing mosaicism. The expression of enhanced green fluorescent protein (EGFP) contained in the plasmid confirmed that the plasmid was operating normally. In *SOX2*-knockout (KO) early blastocysts, the numbers of total cells and *SOX2*- and *NANOG*-positive cells were greatly decreased, while *OCT4* was expressed in most cells. As in early blastocysts, *SOX2*-KO late blastocysts had fewer cells expressing *SOX2*, *NANOG*, and *SOX17* than control. To identify the transcriptional consequences of *SOX2* reduction, we performed quantitative PCR analysis on non-injected and PX458-gRNA injected blastocysts. Injection of PX458-gRNA resulted in downregulation of *NANOG*, *SOX17*, and *SMAD7*, but not *SOX2* and *OCT4*. Furthermore, proliferation-associated genes were downregulated in injected blastocysts. In conclusion, *SOX2*-targeted porcine embryos showed blastocoel formation, the inner cell mass formed poorly, and embryos have inefficient cells. Also, the depletion of *SOX2* in porcine blastocysts downregulated pluripotent genes and proliferation genes.

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43 Correlates of reproductive tract anatomy and uterine histomorphometrics with fertility in swine

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Economic potential of the swine industry hinges upon the reproductive performance of sows, which may be enhanced by improving uterine capacity, a component trait of litter size and piglet productivity. Previous attempts at characterising morphological traits indicative of high uterine volume have not been completely successful, resulting in the continued need for a reliable method of predicting reproductive value to improve production efficiency of the sow. Hence, the main objective of this study was to scrutinize macro- and micro-morphology of the sow's reproductive tract for quantitative correlations with fertility indices. Reproductive records from Polish Landrace × Polish Large White sows (mean ± s.e.m.

parity: 4.3 ± 0.6, range: 2–8) were used to examine the associations between fertility and ovarian/uterine morphology ($n = 34$) or uterine histomorphometry ($n = 10$) posthumously. Simple linear regression was performed to determine the relationship between anatomical or histological parameters and various measures of reproductive performance. Several measures related to the ovary, including right and left ovarian weight ($r = 0.50$, $P = 0.005$, and $r = 0.49$, $P = 0.006$, respectively), were positively correlated with the litter size, whereas left ovarian number of corpora lutea ($r = -0.38$, $P = 0.04$) was negatively correlated with the mean litter size. Analysis of histomorphological characteristics of the uterine wall collected during the luteal phase of the oestrous cycle revealed correlations between mean litter size and myometrial vascular content ($r = 0.75$, $P = 0.03$), the proportion of myometrial stroma ($r = -0.68$, $P = 0.03$), and the variability of endometrial thickness ($r = -0.72$, $P = 0.02$) in sows. Eight ovarian, vaginal, and uterine characteristics were significantly correlated with mean lifetime numbers of live born and stillborn piglets/litter or the last litter size before slaughter. In conclusion, several anatomical and histomorphological metrics that relate to reproductive performance of swine may be used to inform production protocols and as a tool for selection of elite breeding sows, warranting future research into noninvasive or minimally invasive techniques for obtaining such measures.

44 Exploring the use of silver and diamond nanoparticles on sperm cell *in vitro* and chicken embryo *in ovo*

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In poultry industry, chick viability is a crucial factor determining profitability from fertilized egg to placement at the farm. However, decreases in fertility and hatchability have been observed. Recently, there has been renewed interest in the use of silver nanoparticles (Ag-NPs) due to their antimicrobial properties and growth-promoting ability, and diamond nanoparticles (D-NPs) due to their biocompatibility properties. The aim of the study was to evaluate the effect of silver and diamond nanoparticles on chicken embryo oxidative status, biochemical indices, and expression of immune-related genes and on sperm cell viability. The experiment was conducted in Ross 308 chicken embryos and Ross 308 cockerels. One hundred and fifty fertilized eggs were divided randomly into 5 groups (5 × 30). Fertilized eggs were injected with 50 mg/L Ag-NPs at volumes of 100 μL (group 1), 200 μL (group 2) or 50 mg/L D-NPs at volumes of 100 μL (group 3) or 200 μL (group 4), or received no nanoparticles (control; group 5) and incubated at 37°C and 55% humidity for 20 days. Then, chicken blood was collected and centrifuged to evaluate alkaline phosphatase (ALP), alanine transaminase (ALT), lactate dehydrogenase (LDH), glucose, urea, and free haemoglobin. Chicken embryo liver was used to evaluate antioxidant capacity (TAC) and chicken embryo spleen was used to evaluate expression of the immune-related genes interleukin-1β (IL-1β), toll-like receptor (TLR)4, TLR2, and TLR15. Semen was randomly divided into 1 control and 8 treatment groups and treated with 50 mg/L Ag-NPs: group A (0.1 ppm), group B (1 ppm), group C (5 ppm), group D (10 ppm) or 50 mg/L D-NPs: group E (1 ppm), group F (5 ppm), group G (10 ppm), and group H (20 ppm). Sperm viability was analysed using prestoblue metabolic assay. Data were analysed using PROC in GLM procedure of SAS 2014 (SAS Institute Inc.). Decrease in sperm cell viability was recorded in a dose-dependent manner. Sperm cell viability decreased ($P < 0.005$) as the concentration of Ag-NP or D-NP increased. Addition of 100 μL of Ag-NPs increased the growth rate of chicken embryo but not 200 μL of Ag-NPs or addition of D-NPs. Increases in ALP, ALT, LDH, glucose and urea enzyme were observed in a dose-dependent manner in both Ag-NPs and D-NPs. Addition of 50 mg/L Ag-NPs or 50 mg/L D-NPs increased ($P < 0.001$) TAC of chicken embryo as the volume increased. Additions of 200 μL of Ag-NPs, 100 μL of D-NPs, and 200 μL of D-NPs were haemolytic ($P < 0.001$) but addition of 100 μL of Ag-NPs was not. Additions of 100 or 200 μL of Ag-NPs or 100 μL of D-NPs downregulated IL-1β and 200 μL of D-NPs upregulated IL-1β compared with the untreated control group. Additions of 100 or 200 μL of Ag-NPs or 200 μL of D-NPs induced expression of TLR4 and TLR15. Furthermore, addition of Ag-NPs did not result in expression of TLR2. We concluded that administration of 50 mg/L Ag-NPs and 50 mg/L D-NPs *in ovo* improve immune status and administration of 100 μL of Ag-NPs improved the growth rate of chicken embryo. However, toxicity associated with 50 mg/L Ag-NPs and 50 mg/L D-NPs remains a concern and need to be addressed before use.

45 Embryonic disc development *in vitro* in ovine embryos

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Embryonic mortality during the second week of pregnancy has an important economic impact on farming. At this time, the embryo undergoes critical developmental events that cannot be recapitulated *in vitro*, limiting our understanding of these pregnancy losses. After the blastocyst stage, the hypoblast migrates to cover the inner surface of the embryo and the epiblast forms a flat embryonic disc (ED) that initiates gastrulation. The aim of this study was to develop an *in vitro* culture system to support sheep embryo development after the blastocyst stage. Day 6/7 *in vitro*-produced blastocysts were cultured over agarose gels to prevent attachment and allocated to different media: synthetic oviductal fluid with 10% fetal bovine serum (SOF-FBS, $n = 52$), an *in vitro* culture medium (hIVC, $n = 35$) supporting ED formation in human embryos (Deglincerti *et al.* 2016 Nature **533**, 251-254; https://doi.org/10.1038/nature17948), and chemically defined N2B27 medium ($n = 38$) supporting ED formation in bovine embryos (Ramos-Ibeas *et al.* 2020 Reproduction **160**, 579-589, https://doi.org/10.1530/REP-20-0243). At Day 14, survival and embryo area were recorded, the abundance of transcripts encoding interferon Tau (*TP1*) and metabolic enzymes was analysed by RT-qPCR, and the development of epiblast and hypoblast was assessed by immunostaining for SOX2 and SOX17. Embryo survival and size and the percentage of embryos achieving complete hypoblast migration were significantly reduced in SOF-FBS (Chi-squared test and one-way ANOVA; $P < 0.05$). Only N2B27 medium supported epiblast survival in 11/28 embryos. *TP1* expression increased at Day 14 in all culture conditions and metabolism-related genes revealed a shift from anaerobic glycolysis