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Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

Placental vascularization in in vitro-derived pigs: a preliminary study.

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The placenta plays a critical role in maintaining and protecting the developing fetus. Placental vascularization abnormalities, including a decrease in arterial number, lumen size, and branching, have been extensively described in humans born from in vitro-produced (IVP) embryos (Riesche and Bartolomei, Seminars Reprod Med, 36:240-247, 2018) but studies on IVP pigs are very limited (Ao et al., Placenta 57:94-101, 2017). The objective of this study was to compare the placental vascularization in pigs born from in vitro- and in vivo-produced embryos (the latter born by artificial insemination; AI group). IVP embryos were produced after in-vitro fertilization (IVF) of in vitro matured oocytes and further culture (IVC) up to blastocyts stage in media supplemented with or without 1% porcine oviductal fluid and 1% uterine fluid (more details in Paris-Oller et al., J AnimSci and Biotech 12:32-44, 2021). Blastocysts produced with (RF-IVP group) and without (C-IVP group) reproductive fluids were surgically transferred at day 7 post-IVF. Both AI and IVP embryos were produced with spermatozoa from the same boar. After birth, placenta samples were collected at 3-5 cm from the insertion of umbilical cord, and fetal parameters were recorded. The placenta of 9 animals (3 per group) from different litters was selected following these criteria among animals: similar uterus position, birth weight, and crown-rump length; and a close male/female ratio among groups. Samples were fixed (10% formaldehyde solution) and paraffin embedded. Two complete placental sections (5 µm thickness) were stained (hematoxylin–eosin), photographed at 5x (ZEN 3.2, ZEN lite, Zeiss) and images processed (ImageJ) for a detailed study to record vessel number, area occupied by each vessel (µm^2), and total vascular area (%). Based on their size and histological characteristics, vessels were categorized by an expert operator as capillary (1-500 µm^2), arteriole/venule (501-1000 µm^2), small artery/vein (1001-3000 µm^2), medium-sized artery/vein (3001-30000 µm^2), and large artery/vein (>30000 µm^2). Data (mean±SEM) were analyzed by one-way ANOVA (Systat v13.1), and differences (P<0.05) were compared by Tukey's test. The total placental area observed, and total number of vessels analyzed was higher in AI (86.1±7.5 mm^2, 726 vessels) than C-IVP (45.9±6.8 mm^2, 544 vessels), and RF-IVP (52.8±5.1 mm^2, 637 vessels) (P<0.05). However, no differences were found in the total vascular area being 14.9±3.3% (AI), 19.9±2.7% (C-IVP), and 17.8±2.2 (RF-IVP) with similar pattern distribution in all groups: over 85% microvessels, 10-15% medium-size vessels and 5% macrovessels. However, the vascular area occupied by medium-sized vessels (arteries and veins) was significantly higher in the AI group (7.2±0.5%) than in IVP groups (2.1±0.3% and 1.8±0.2%) regardless of the addition of reproductive fluids (P<0.05). No differences in vascular areas of micro and macrovessels were observed. Preliminary results show that impaired placental vascularization in ART-derived pigs might occur due to a reduction of medium size vessels.

Keywords: placenta, vascularization, pig

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