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ELUCIDATION OF IMPRINTED GENES RESPONSIBLE FOR HYPERPLASIA IN SOMATIC CELL NUCLEAR TRANSFERRED PLACENTAS

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BACKGROUND-AIM
Somatic cell nuclear transfer (SCNT) is the sole reproductive technique to produce live animals from differentiated somatic cells. However, their birth rate is very low (usually less than 5% for transferred embryos) and many abnormalities are observed. We recently reported that one of the placenta-specific imprinted genes, Sfmbt2 microRNA cluster, was overexpressed in SCNT placentas by loss of imprint, resulting in placental hyperplasia in mice. We found that normalization of their expression level ameliorated their placental morphologies; however, their placental weights could not be corrected entirely to the level comparable to those of IVF placentas (0.10±0.004 g vs. 0.20±0.01 g). In this study, we investigated relationships between other placenta-specific imprinted genes and SCNT placental hyperplasia.
METHODS
We focused on three placenta-specific imprinted genes (Jade1/Phf17, Smoc1, Platr20) overexpressed in SCNT placentas. Their knockout (KO) mice lines were produced by the CRISPR/Cas9 system. Cumulus cells were collected from (C57BL x DBA/2)F1 female mice with a maternal KO allele and transferred into enucleated oocytes. Reconstructed oocytes were cultured in KSOM for 1 h and activated with Ca2+-free KSOM containing 2.5 mM SrCl2, 50 nM trichostatin A and 5 μM latrunculin A. 2-cell stage embryos were transferred into oviducts of pseudopregnant ICR female mice on the next day and SCNT fetuses were retrieved on day 19.5.
RESULTS
Two imprint genes, Jade1/Phf17 and Smoc1, revealed sublethal in homozygous KO fetuses. On the other hand, the homozygous KO of Platr20 did not show any lethality. When SCNT fetuses were produced with maternal KO cumulus cells from three KO lines, their birth rates were comparable to that of wild-type SCNT. In Smoc1 and Platr20 maternal KO, SCNT placental weights were not changed (wild-type: 0.32±0.02 g, Smoc1: 0.29±0.02 g, Platr20: 0.30±0.03 g), while those of Jade1/Phf17 maternal KO showed decreased placental weights (0.23±0.03 g).
CONCLUSIONS
Together with our previous study, we identified that loss of imprint of two imprinted genes, Sfmbt2 miRNA and Jade1/Phf17, could be the causes of placental hyperplasia in mouse SCNT. The details of placental morphologies are under-investigated at present.

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COMPARISON OF COMBINED THICKNESS OF THE UTERUS AND THE PLACENTA BETWEEN 2 BREEDS OF MARES AND THEIR POTENTIAL RELATIONSHIP WITH ESTROGENS DURING NORMAL PREGNANCY.

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BACKGROUND-AIM
To the best of our knowledge, combined thickness of the uterus and the placenta (CTUP) has never been compared between different breeds of horses, using the same settings. This study compares the CTUP in 4 to 11 months pregnant Spanish Pure-Breed (SPB) and showjumping Belgian Saddle-Breeds (SJ) mares. The potential relationships between CTUP and estradiol (E2), estrone (E1) or estrone sulfate (E1S) concentrations in maternal sera will also be investigated.
METHODS
Once a month, CTUP was measured and blood was collected in 15 SPB and 11 SJ mares. Mares presenting clinical signs of placentitis during the pregnancy or after foaling were excluded of this study. The CTUP was measured in 3 different places of the cervical area by transrectal ultrasonography using the previously described technique (Renaudin et al., 1997). The mean of the 3 measures was recorded and assigned to the month of pregnancy. Estrogens were assayed in serum using the previously validated Liquid Chromatography coupled to Mass Spectrometry technique (Dufour et al., 2021).
RESULTS
For the same month of pregnancy, no difference in CTUP was observed between breeds of mares. The CTUP gradually increased during the pregnancy and was significantly larger ($p<0.01$) at 11 months (mean: 8.39 ±2.02mm), showing a decreased and heterogenous echogenicity. No significant difference in CTUP was observed between 9th and 10th month (respective means: 6.29 ±1.23mm and 6.57 ±1.14mm), but they both tended to be higher ($p<0.1$) than those observed at 4, 5 and 6 months of pregnancy (respective means: 4.34 ±0.56mm, 5.45 ±0.65mm, 5.41 ±0.82mm). No correlation was observed between CTUP and estrogens concentrations.
CONCLUSIONS
Gradual increase of CTUP was observed from 4 to 10 months of pregnancy. During the last month, fœto-maternal unit thickened more quickly and it was associated with an heterogenous echogenicity. Some previous reports leaded to think that there were differences in CTUP between breeds of mares. However, with this design, such differences were not observed. The CTUP was not related to E2, E1, E1S concentrations, showing that morphologic and endocrinologic evolutions of placenta are not associated: maximal CTUP was observed at 11 months whereas estrogens peak is described between 5 and 6 months.