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Gene silencing during development of *in vitro*-produced female bovine embryos

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ABSTRACT. In early development, female embryos (XX) produce twice the transcripts of X-linked genes compared with male embryos (XY). During the course of development, inactivation of the X chromosome equilibrates gene dosage, making the development of female embryos viable. Moreover, the biotechnologies used for producing embryos *in vitro* seem to work better with male embryos, making it easier for them to reach the blastocyst stage and allow for complete gestation. We investigated the expression of three X-linked genes that are involved in development, XIST, G6PD, and HPRT, and of the transcript interferon-τ, in male and female bovine blastocysts produced by nuclear transfer (NT) and by *in vitro* fertilization (IVF). Oocytes that had been matured *in vitro* were enucleated and reconstructed with somatic cells from adult animals at 18 h post-maturation. After fusion (two pulses of 2.25 kv/cm) and chemical activation (5.0 μM ionomycin for 5 min and 2.0 mM 6-DMAP for 3 h), the oocyte-somatic cell units were cultivated in CR2 with a monolayer of granulosa cells at 38.8°C, in a humidified 5% CO_2 atmosphere. IVF embryos were inseminated, after centrifugation in a Percoll gradient, with 2 x 10^6 sperm/mL TALP medium supplemented with BSA and PHE and cultivated under the same conditions as the cloned embryos. We used real-time PCR to

analyze the gene expression of individual blastocysts compared to expression of the housekeeping gene, GAPDH. The gene XIST was expressed in female embryos and not in male embryos produced by IVF, though it was expressed at low levels in male embryos produced by NT. Unlike previous reports, we found lower levels of the transcript of G6PD in females than in males, suggesting double silencing or other mechanisms of control of this gene. Female embryos produced by IVF expressed the HPRT gene at a higher level than female embryos produced by NT, suggesting that gene silencing proceeds faster in NT-produced female embryos due to "inactivation memory" from the nucleus donor. In conclusion, male and female embryos express different levels of X-chromosome genes and failures of these genes that are essential for development could reduce the viability of females. Nuclear transfer can modify this relation, possibly due to epigenetic memory, leading to frequent failures in nuclear reprogramming.

Key words: X-chromosome inactivation; Nuclear reprogramming; Bovine embryos; Nuclear transfer and sex

