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The X-Chromosome and Its Expression in Embryos Produced *In Vitro* and by Cloning

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In domestic mammals females have two X-chromosomes while males have one X- and one Y-chromosome. The X chromosome makes up about 5% of the total genome and contains approximately 1000 genes involved with a wide range of functions including housekeeping, stress buffering, embryo, fetal and placental growth and development, reproduction, fertility, ovarian development, and intelligence. The Y chromosome is considerably smaller consisting of approximately 150 coding sequences mainly involved in sex determination and fertility. Embryos produced by *in vitro* fertilization and nuclear transfer (cloning) has yielded several unexpected observations that involve the sex chromosomes or sex chromosome related development. For example, male embryos develop to blastocyst stage faster than females when cultured in glucose rich media. Also, significantly more male than female calves are born when embryos produced by *in vitro* fertilization are transferred to recipient cows. Thus, these effects appear to be related to the production method and/or the *in vitro* culture environment. Since these effects may be controllable, they are of significant biological and commercial interest and we have begun to systematically evaluate the role of sex chromosome compliment of embryo development in *in vitro* produced (IVP) and nuclear transfer (NT). We have investigated timing of cleavage in male and female IVP embryos, X and Y-chromosome make-up in IVP and NT embryos and the expression of X-chromosome linked genes in IVP and NT embryos in cattle and sheep.

When the duration of sperm-oocyte interaction *in vitro* was reduced from 18 h to 6 h fertilization decreased but significantly more male

than female 4-cell, 8-cell and blastocyst stages resulted. The sex ratio among embryos resulting from longer co-incubation (9, 12, 18 and 24 h) did not differ from the expected 1:1 ratio. Similar results have been obtained among sheep IVP embryos. These observations suggest a preferential fertilizing advantage for Y-chromosome bearing spermatozoa during the initial phases of sperm-oocyte co-incubation. Cytogenetic analysis of IVP and NT embryos has revealed a higher incidence of chromosome abnormalities than among embryos of comparable stage produced *in vivo*. When we specifically examined the sex chromosome make up of IVP and NT embryos in cattle and sheep using fluorescent *in situ* hybridization we observed that NT embryos had significantly more abnormalities involving the sex chromosomes. We speculate that the sex chromosome, particularly the X-chromosome is susceptible to errors in cell division in cloned embryos.

To accommodate the difference between males and females in the number of copies of genes on the X chromosome, one of the two X-chromosome inactivates in female embryos beginning with the trophoblast. This process, known as X-inactivation, is a histone-mediated epigenetic modification of all but one of the X-chromosomes. To investigate the timing of X-chromosome inactivation in bovine *in vivo* produced, IVP and NT embryos we have examined global gene expression levels using real-time PCR to verify the level of mRNA at specific stages of development. Preliminary results have shown major differences in transcription of 12 X-chromosome linked genes between IVP and *in vivo* derived embryos, with some transcripts reaching 800 times higher levels in the IVP group. Initial observations in sexed IVP blastocysts analyzed by real-time PCR showed higher levels of all X-linked transcripts in females. Some genes were not detected consistently while others are detected only in females. The process of X-inactivation begins gradually in bovine embryos with the first recognizable feature detectable at the 8- cell stage. By blastocyst stage, male and female embryos have similar levels of mRNA for X-linked genes such as G6PD indicating that dosage compensation has been initiated.

It was concluded that the X-chromosome and its complement of

genes is susceptible to alteration in embryos produced *in vitro* or by nuclear transfer. These alterations may partly explain the unexpected skewing of sex ratio and high rate of embryo loss among *in vitro* produced embryos.