



144 Sex ratio of *in vitro*-produced goat embryos

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Abstract

Goats are important livestock species because they produce meat, milk, and fibre and are also easily maintainable on small farms. Although goats provide many products and consumption of goat meat is increasing in the United States, the industry lags compared with many species with regard to IVF techniques to enhance goat production. It has been demonstrated in other species that male IVF embryos tend to develop faster than those of females. This may be due to increased tolerance of male embryos to inadequate conditions, particularly, glucose concentrations in culture media. However, the sex ratio of goat embryos produced utilising IVF remains unknown. The aim of this study was to determine the sex ratio of goat embryos utilising a commercially available media suite (IVF Biosciences, Falmouth, UK). Oocytes were harvested from ovaries obtained from 2 local abattoirs and matured *in vitro*. Frozen sperm from 1 of 2 billy goats were randomly assigned for each round of IVF. Embryos were evaluated daily from Days (D) 6 through 9 of *in vitro* culture. On the day an embryo reached the expanded blastocyst stage, it was removed from culture and placed into DNA extraction buffer (PicoPureTM DNA Extraction Kit, Applied Biosystems, Waltham, MA, USA) and stored at -20 for PCR analysis, typically within one month of collection. In all unknown samples, positive male (sperm) and female (uterus) controls, the amelogenin gene was amplified and products were evaluated on a 1.5% agarose gel with ethidium bromide. Embryos with 2 bands (202 and 262 bp) were classified as male, and those with 1 band (262 bp) were classified as female. Embryos with no bands were not included in analysis. Embryos reached the expanded blastocyst stage on D6 ($n = 29$), D7 ($n = 39$), and D8/9 ($n = 35$, combined for evaluation). A chi-squared analysis comparing the percentage of male and female embryos to the expected 50% was completed for each time point (D6, D7, D8/9), as well as overall ratios (D6-9). In total, 350 oocytes were utilised in 6 rounds of IVF resulting in a mean blastocyst rate of 32% (range 17-47%). There was no significant difference in the number of embryos that were male on D6 (55%) and D7 (46%). However, on D8/9 significantly fewer embryos were male (29% male; $P = 0.01$). Overall, there was no significant difference ($P = 0.14$) in the sex ratio, with 41% male and 59% female embryos. Our findings are somewhat consistent with other species, in that male goat embryos produced via IVF develop more quickly in culture conditions; however, female embryos were still able to tolerate culture conditions. Delayed blastocyst development may not necessarily be an indication of a reduced quality embryo but one that is slower to develop based on its sex. This could be due to expression of X-linked genes being unbalanced during pre-implantation embryo development stages and warrants further study. One influencer of sex ratio we are currently investigating is the impact of glucose during culture, to further understand metabolism in IVF embryos.