Effects of time of insemination relative to time of ovulation on embryonic sex ratio in mares
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Oocyte fertilization can occur in most mammalian species by sperm recently deposited in the female reproductive tract or several days after. It has been reported that the timing of artificial insemination (AI) in cattle, sheep, deer, and mice relative to the time of ovulation influences the sex ratio of embryos. The objective of this study was to determine the effects of timing of insemination relative to ovulation on the embryonic sex ratio in mares. One stallion and seventeen mares were used for preliminary study. The stallion was collected using an artificial vagina and the semen was evaluated for concentration, motility, and morphology. Semen was extended at one to one ratio using fresh semen extender (Animal Reproduction Systems, Chino, CA) and a minimum of five hundred million normal motile sperm cells were used to AI each mare within two hours of collection. Mares were examined by trans-rectal ultrasonography to determine follicular diameters and were divided into three groups. To increase the range of ovulation times, the first group was induced with 3000 IU of human chorionic gonadotrophin intravenously while the other two groups were not. Group 1: mares that had a 35 mm diameter follicle, were induced then AI 35 hours after injection. Group 2: mares with follicles that were 35 mm were AI. Group 3: mares that had any follicle greater than or equal to 35 mm were AI. All mares were examined by ultrasound every 24 hours until ovulation was confirmed. Mares were flushed between 8 and 10 days after ovulation and embryonic sex was determined by amplification of the ZFY and ZFX loci by PCR. Once an embryo was recovered from a mare, that mare was moved to an alternate treatment group. The sexed embryos were assigned to one of two categories: 1) those from ovulations occurring less than or equal to 48 hours after AI, and 2) those from ovulations occurring greater than 48 hours after AI. Of the 16 embryos in category one, nine were male (57%) and seven were female (43%). Of the 10 embryos in category two, three were male (30%) and seven were females (70%). The results of this preliminary study suggest that timing of insemination relative to ovulation may influence embryonic sex ratio in the mare. The study will need to be expanded to a minimum of 74 embryos before a statistically significant difference with a 0.8 power can be demonstrated in support of a 30 to 70 ratio, male to female due to ovulations occurring greater than 48 hours after AI.

Keywords: Semen, ovulation, embryo, sex, mares