

Fertility trials with frozen-thawed jack semen

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Pregnancy rates following artificial insemination with frozen-thawed jack spermatozoa are relatively low compared to those attained in other species. Glycerol and cholesterol are known to interfere with post-thaw fertility of jack and stallion semen (1, 2). Altering the amount of cholesterol and the cryoprotectant in the freezing extender may improve the fertility of frozen-thawed jack semen.

Semen was collected from a single jack and extended in EZ Mixin[®] and slowly cooled to 5°C. Samples were then centrifuged at 400G for 10 minutes and the supernatant was removed. The spermatozoa were then resuspended in the appropriate freezing medium to a final concentration of 500×10^6 cells/mL and frozen in liquid nitrogen vapor.

A total of twenty-six mares have been utilized for the fertility trials over 4 breeding seasons. Mares 2 to 18 years in age, were mostly client owned and some mares were inseminated more than once. Follicular assessment was made by rectal palpation and transrectal ultrasonography. Human chorionic gonadotropin (hCG) was utilized to induce ovulation once the dominant follicle obtained a size of 35 mm or greater. Mares were inseminated with approximately 500×10^6 viable cells using standard insemination techniques within 6 hours pre-ovulation and again within 6 hours post-ovulation. Pregnancy diagnosis was performed at 14 to 18 days post-ovulation by transrectal ultrasonography.

Pregnancy rates in 1999 were <15% (1 pregnancy out of 8 matings) when 4% glycerol (GLYC) and 20% egg yolk (EG) were used in the standard freezing medium. Because of the poor results of the first year, the following treatments were tested: 1) 20% EY and 2% ethylene glycol (EG), 2) 10% EY and 2% EG, 3) 5% EY and 2% EG, 4) 5%EY and 5% GLYC, and 5) 20% EY, 2%EG and 60mM hydroxypropyl- β -cyclodextrin (β -CD). Hydroxypropyl- β -cyclodextrin binds cholesterol in its hydrophobic pore.

Pregnancy rates for each treatment were as follows: 1) 0% (7 matings), 2) 0% (4 matings), 3) 56% (14 pregnancies, 25 matings), 4) 30.7% (4 pregnancies, 13 matings), and 5) 63.6% (7 pregnancies, 11 matings). These data support the theory of cholesterol and glycerol interfering with post-thaw fertility of jack semen (1, 2). By adding β -CD to the freezing medium we were able to bind the cholesterol and prevent it from interfering with post-thaw fertility. Additional studies to understand the function of β -cyclodextrins in the improvement of post-thaw fertility are on-going in our laboratory.

- 1) Trimeche,A; Renard,P; Tainturier,D (1998): A procedure for Poitou Jackass sperm cryopreservation. *Theriogenology* 50, 793-806.
- 2) Zahn, FS; Papa, FO; Dell'Aqua Jr., JA (2002): Cholesterol incorporation on equine sperm membrane: effects on post-thaw sperm parameters and fertility. *Theriogenology* 58, 237-240.

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