Seminal parameters and field fertility of donkey semen cryopreserved in two different extenders

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Cryopreservation of jackass semen has been successfully performed using extenders commonly used to preserve stallion semen. However, differences in sperm physiology and/or plasma membrane composition could potentially result in different media requirements for cryopreservation of jackass semen. Our hypothesis was that jackass semen could be successfully cryopreserved in a simplified egg yolk-extender (Nagase).1 The objective of the study was to compare seminal parameters and fertility of donkey sperm cryopreserved in Nagase versus lactose-EDTA extender.

Semen was obtained from five Pêga jackasses with histories of good fertility using fresh semen. In Experiment I, five ejaculates from each male were collected using an artificial vagina, diluted 1:1 (v/v) with skim milk-based extender and centrifuged at 600 x g for 15 minutes. After centrifugation, the supernatant was discarded and the sperm pellet was re-suspended to 200 x 10⁶ sperm/ml in Nagase or lactose-EDTA extender and loaded into 0.5 ml straws. Samples were cooled to 5 ºC for 1 h and then placed over LN₂ (4 cm) for an additional 20 min, before being plunged into LN₂. Subjective evaluations were performed immediately after semen collection, after cooling to 5 ºC and after freezing-thawing. Total motility (TM), progressive motility (PM) and plasma membrane integrity (HOST) were determined by a single person blinded to treatment groups. In Experiment II, semen from three males used in Experiment I and frozen in Nagase or lactose-EDTA extenders were used to inseminate 53 mares during a total of 60 cycles (n = 30 cycles per extender). Mares were inseminated within 6 h post-ovulation with > 300 x 10⁶ progressively motile sperm. Pregnancy diagnosis was performed by transrectal palpation and ultrasonography on days 15 and 25 post-ovulation. Seminal parameters were evaluated by ANOVA and individual means were compared by Tukey’s test. Pregnancy rates were compared by chi-square. Statistical significance was set at P<0.05.

Seminal parameters were similar between groups either after cooling or after thawing (Table 1). However, cryopreservation significantly decreased total and progressive motility compared to cooled samples. Similar pregnancy rates were obtained when mares were inseminated with semen cryopreserved in Nagase or lactose-EDTA on days 15 [53% (16/30) vs. 50% (15/30)] and 25 [43% (13/30) vs. 47% (14/30)], respectively.

Table 1. Seminal parameters of donkey semen diluted in Nagase or lactose-EDTA extender and evaluated after cooling to 5 ºC or after freezing-thawing (mean ± SD).

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<thead>
<tr>
<th></th>
<th>Cooled Samples</th>
<th>Frozen-Thawed Samples</th>
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<tbody>
<tr>
<td></td>
<td>TM (%)</td>
<td>PM (%)</td>
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<tr>
<td>Nagase</td>
<td>80 ± 6</td>
<td>71 ± 7</td>
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<tr>
<td>Lactose-EDTA</td>
<td>78 ± 6</td>
<td>70 ± 6</td>
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* Percentage of cells with intact plasma membrane (positive HOST).

In summary, cryopreservation of jackass semen using simplified egg yolk-base extender (Nagase) resulted in seminal parameters and fertility similar to those of lactose-EDTA. Our results provide a practical and less costly alternative to cryopreserve donkey semen.

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Reference