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Strategies to improve the fertility of fresh and frozen donkey semen

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ABSTRACT

Fertility rates of donkey semen in jennies are lower compared to mares. The aims of this study were to evaluate different sperm cryopreservation methods and insemination strategies to improve the fertility of donkey semen in jennies. Three experiments were performed: (1) the comparison of two freezing methods of donkey semen (conventional method and automated method); (2) the determination of a suitable insemination dose of fresh donkey semen for jennies and mares; and (3) the influence of the semen deposition site on fertility of jennies inseminated with frozen donkey semen. For experiment 1, no differences were observed in total motility, angular velocity, curvilinear velocity, straight-line velocity, and plasma membrane integrity between samples frozen with the conventional (Styrofoam box) and the automated method (TK 4000C). However, the automated method provided higher values of progressive motility and rapid cells in frozen-thawed samples in comparison with the conventional method ($P < 0.05$). For experiment 2, mares were bred using 500×10^6 fresh sperm (M); and jennies using 1×10^9 (J1) or 500×10^6 fresh sperm (J5). Pregnancy rates in M, J1, and J5 were 93% (14/15), 73% (11/15), and 40% (6/15), respectively. When using different insemination doses, 500×10^6 or 1×10^9 sperm, no significant difference was observed in pregnancy rates of mares (M, 14/15) and jennies (J1, 11/15). Furthermore, there was no significant difference between the two insemination doses in jennies. However, with an insemination dose of 500×10^6 fresh sperm, the pregnancy rates were significantly higher in mares (M, 14/15) than in jennies (J5, 6/15; $P < 0.05$). For experiment 3, the inseminations were carried out in the uterine body (UB) or in the uterine horn of jennies with frozen-thawed donkey semen. No pregnancies were achieved with inseminations performed in the UB (0/12). The pregnancy rate for uterine horn group was 28.26% (13/46) and thus significantly higher than the UB group (0%; 0/12; $P < 0.05$). In conclusion, the automated method showed higher values on progressive motility and rapid cells parameters compared to the conventional method and can be used as an alternative for freezing donkey semen. The increase in the number of sperm cells per insemination dose using fresh donkey semen improved the fertility rates in jennies. The deep horn inseminations using frozen-thawed donkey semen increased the pregnancy rate in jennies, and the multiple inseminations may be an option to improve the fertility rates of donkey semen in jennies.

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1. Introduction

Donkey species are bred around the world, and there is a particular interest for the production of hybrids in many countries [1–3]. The resulting hybrid of the mating of a horse (*Equus caballus*) with a donkey (*Equus asinus*) results in highly desirable products that combine the best features of the two species in a single animal [2], which has great physical strength and required strength for animal traction [1,3].

The use of frozen semen in artificial insemination (AI) represents the main tool for genetic improvement of most domestic animals. The advantages of frozen semen include lower transportation costs, continuous availability of semen, decrease in the risk of transmission of venereal diseases, and increase of the genetic pool [4]. In addition, the use of frozen semen could be an alternative for the preservation of endangered donkey breeds. There are approximately 185 donkey breeds worldwide [5], and the number of individuals in certain breeds is very low and demands an intervention to guarantee their survival [6,7].

However, the fertility rate of frozen semen in AI programs is lower than fresh or cooled semen [8–10]. The freezing process includes factors that are extremely harmful to sperm cells, such as changes of temperature, osmotic and toxic stress caused by exposure to cryoprotective agents, and the intracellular ice formation [10,11]. These conditions seriously affect sperm functionality and viability, leading to reduced sperm longevity in the female genital tract [12,13]. Moreover, the use of frozen semen induces a stronger inflammatory reaction in the uterus than fresh semen in mares [14].

The studies on the basis of the cryopreservation of stallion sperm have been reported for more than 50 years [15–19], however, the researches involving donkey semen are recent [20–24]. Despite the excellent semen quality, fertility rates of frozen donkey semen in jennies are still low (0%–36%) [20–22] compared to mares (33%–53%) [25,26]. According to the available literature, the highest result of pregnancy using frozen donkey semen was reported by Rota et al. 2012. The authors achieved a conception rate of 61.5% for jennies inseminated with donkey semen cryopreserved in INRA-96, using glycerol and postthaw re-extended with seminal plasma (SP).

The reproductive biotechnologies performed in donkeys correspond to the same applied in horses [21,27]. Transferring the biotechnologies from one species to another often results in unsatisfactory results, probably because of physiological differences between the studied species [28]. There are some important distinctions in the biophysical parameters of cells among animals, such as the cell surface and volume, amount of intracellular water, and membrane permeability to water, thus requiring the development of specific protocols for each species [29].

The low fertility rates achieved in studies using frozen donkey semen in jennies indicate the necessity of further research to improve the semen cryopreservation techniques and AI protocols. Therefore, the aims of this study were: (1) to evaluate two freezing methods for the cryopreservation of donkey semen; (2) to determine a suitable insemination dose of fresh donkey semen for jennies and mares; and (3) to evaluate the influence of the semen deposition site on fertility of jennies inseminated with frozen donkey semen.

2. Materials and methods

This study was approved by the Ethics Committee of School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP) and had ethical oversight from the same Institution.

2.1. Experiment 1: Comparative evaluation of freezing methods for the cryopreservation of donkey semen

2.1.1. Semen collection and handling procedures

A total of eight jacks (three Pega and five Brazilian Donkey jacks) aged between 8 and 15 years with proven fertility were used. Initially, the animals were collected for eight consecutive days to eliminate degenerated cells from the cauda epididymis and to stabilize the sperm parameters.

Two ejaculates were collected from each of eight donkeys using a Botucatu model artificial vagina (Botupharma, Brazil), with an interval of 3 days. After removal of the gel fraction, the semen was diluted in a skim milk based extender (BotuSemen; Botupharma) at a 1:1 ratio (vol:vol).

2.1.2. Freezing methods

After dilution, samples were centrifuged at $600 \times g$ for 10 minutes. The supernatants were discarded, and the sperm pellets were extended to a final concentration of 200×10^6 viable sperm/mL in an egg yolk freezing extender (BotuCrio, Botupharma) and packaged into 0.5 mL straws. Then, semen samples were transferred to a refrigerator (Minitub do Brasil, Brazil) and remained at a constant temperature of 5 °C for 20 minutes [30]. After cooling, the straws were allocated into two groups, as follows: manual and automated method.

For the conventional method, an isothermal box (Styrofoam box) of 42-L capacity filled with a depth of 3.5-cm liquid nitrogen (N_2) was used. The straws were placed horizontally at 6 cm above the level of N_2 for 20 minutes. Subsequently, straws were immersed into liquid nitrogen for storage [30]. The freezing method used in this study was the same described by Maziero et al. [31]. The authors reported that the freezing curve in this system was −10 °C/min between 5 °C and −60 °C and a velocity of −8 °C/min between −60 °C and −100 °C.

For the automated system, after cooling at 5 °C for 20 minutes, the straws were submitted to freezing process using a velocity curve of −15 °C/min from 5 °C to −10 °C and a velocity of −40 °C/min between −10 °C and −140 °C (TK 4000C, TK Tecnologia em Congelação, Brazil).

2.1.3. Semen evaluation

The straws were thawed at 46 °C for 20 seconds [32], and the motility parameters were evaluated by computer-assisted sperm analisys (CASA; HTM IVOS 12; Hamilton Thorne Research, USA). The computer-assisted sperm analisys setup was the same described by others [33]. The plasma membrane integrity (PMI) was evaluated