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Efficiency of different extenders on cooled semen collected during long and short day length seasons in Martina Franca donkey

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ABSTRACT

Artificial insemination with cooled semen is routine in equids because of its good fertility rates and relatively low costs. In several donkey breeds, especially in restricted populations, the use of cooled semen could be seen as the best way of improving reproductive performance and avoiding excessive inbreeding. Furthermore, most jennies have ovulatory estrous throughout the year, and thus, cooled semen could also be used during short day length season. The aims of this study were to evaluate the effects of different extenders on sperm quality during cooling in the Martina Franca breed, and to verify the preservation of cooled semen collected during long day length (May–June) and short day length (November–December) seasons. Three ejaculates were collected at 10-day intervals from each of six jackasses during both May–June and again in November–December time periods. Each ejaculate was cooled in INRA96 or E-Z Mixin at a low cooling rate and evaluated daily over a 120-h preservation time. The results showed a significant extender influence on preservation time in both periods. Semen diluted with INRA96 maintained a progressive motility of 36% and a straightness of 89% at 120 h, whereas semen extended with E-Z Mixin had a mean progressive motility of 32% and a straightness of 81% at 48 h during the May–June period. Despite having the same initial characteristics, semen collected during the short day length season had a higher rate of decline in semen quality during storage at 5 °C with E-Z Mixin.

1. Introduction

Interest in donkey reproduction has been increasing over the last few years, in particular, with regard to its use in the production of hypo-allergenic milk, in pet therapy, and in the production of mules for agricultural work in those areas in which the use of machines is banned. Moreover, several donkey breeds are considered by the FAO as being endangered because of their small population size. Despite common belief, the transfer of knowledge and procedures from horse to donkeys often achieves poor results (Trimeche et al., 1998; Vidament et al., 2005), as reported in connection with other endangered species (Wildt et al., 1995).

One of the strategies for increasing reproductive performance, especially in small-size populations, is the optimization of both male and female reproduction. Other authors have pointed out the need to increase jackass efficiency through the use of cooled semen in artificial insemination programs as a tool for improving gene distribution and for reducing the risk of excessive inbreeding (Rota et al., 2008). Because cryopreservation of equine semen is relatively expensive and frequently produces less than satisfactory fertility (Amann and Pickett, 1987;

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Weitze and Petzold, 1992; Samper and Morris, 1998), cooled semen is routinely used in the equine industry (Varner et al., 1989) because semen stored at 5 °C for roughly 24 h maintains a fertility rate similar to that of fresh semen (Jasko et al., 1992). Although the use of cooled semen in horse reproduction has been investigated in depth, much less information is available with regard to donkeys (Santos et al., 1995; Cottorello et al., 2002; Contri et al., 2005; Rota et al., 2008). Furthermore, there are restricted populations of several donkey breeds and the use of artificial insemination with cooled semen could optimize breeding management, reducing the risks of excessive inbreeding (Rota et al., 2008). In the horse, reproduction is influenced by the season (Ginther, 1992), and the time of insemination is limited by the presence of transitions and anestrous (for a review, see Sharp and Davis, 1993), so that cooled stallion semen is only available during the breeding season. Seasonal changes in sexual hormone profiles, seminal characteristics, and reproductive behaviors were reported in the stallion (Berndtson et al., 1983; Harris et al., 1983; Johnson and Thompson, 1983; Clay and Squires, 1987; Clay et al., 1988; Roser and Hughes, 1992) and several studies have demonstrated that photoperiod affects reproductive activity by acting on the hypothalamic–pituitary–gonadal axis in seasonal breeders, including the stallion (Lincoln and Short, 1980; Karsch et al., 1984; Pickett et al., 1989; Hoffmann and Landeck, 1999).

Unlike mares, fertile ovulatory cycles are present in most jennies throughout year, suggesting a low or different seasonal influence on the estrous cycle (Ginther et al., 1987; Blanchard et al., 1999). The absence of seasonal anestrous in jennies was reported in 40% (Henry et al., 1987) and 100% (Carluccio et al., 2003) of jennies. Thus inseminations and, consequently, pregnancies can be continued throughout the year, leading to a possible year-round demand for and employment of cooled jackass semen.

Because, in donkey reproduction, as the lack of both transitions and seasonal anestrous potentially extends the breeding season to the entire year, the aim of this study was to verify semen characteristics in Martina Franca jackasses after preservation at 5 °C in both long (May–June, MJ) and short (November–December, ND) day length periods. The second aim of this study was to evaluate the efficiency of two different commercial equine extenders for cooling donkey semen at 5 °C during these periods.

2. Materials and methods

2.1. Animals

In this study, 6 adult and reproductively mature Martina Franca jackasses, 4–9 years of age and weighing 380–450 kg, were used. The males were routinely used for reproduction programs and had a normal fertility. The jackasses were housed in the veterinary teaching farm of the University of Teramo in individual 5 m × 5 m boxes with access to an outdoor paddock. They were fed with 5 kg of hay supplemented with 1.5 kg of commercial balanced stallion fodder twice daily, while water was freely available.

2.2. Semen collection

Semen was collected from the jackasses using a Missouri artificial vagina in the presence of a natural estrous jenny. The reaction time (RT), being the time between jackass presentation to an estrous jenny and ejaculation, was recorded for each collection. In order to evaluate the photoperiod influence on semen characteristics and 5 °C preservation, semen was collected in May–June (MJ period) and in November–December (ND period).

In order to reduce the variability due to the time of last ejaculation, 7 days before the experiment, one ejaculation for each stallion was collected but not evaluated. Thereafter, semen collections were performed every 10 days for a total of three collections in both MJ and ND periods. Each batch of collected semen was evaluated within 15 min.

2.3. Semen evaluation

After collection, the total volume of the ejaculate was recorded; the semen was then filtered through sterile gauze to remove the gel fraction after which the gel-free volume was estimated. Sperm concentration was evaluated by hemocytometer (Bürker chamber; Merck, Leuven, Belgium), and pH was measured within 5 min using a pH-meter (PH210, Hanna Instruments Ltd., Leighton Buzzard, Luton, UK).

Sperm membrane integrity was used to assess sperm viability, which was evaluated using a propidium iodide (PI) and SYBR-14 fluorescent staining (LIVE/DEAD sperm viability kit, Molecular Probes Inc., Eugene, OR, USA) as previously described by Garner and Johnson (1995) with some modifications. Briefly, an aliquot (200 μl) of diluted semen was incubated with 2.4 μM of PI and 20 nM of SYBR-14 at 37 °C under light-proof conditions. After 10 min, spermatozoa were fixed with 1 μl of 3% glutaraldehyde and 6 μl of this solution placed on a slide. A coverslip was applied and the stained spermatozoa were examined under an Olympus BX 51 epifluorescent microscope. Spermatozoa with bright green fluorescence (SYBR–14) were considered viable, whereas those partially or totally red (PI) were considered dead. At least 200 spermatozoa were examined.

2.4. Sperm motility analysis

Donkey semen motility parameters were assessed using CASA system IVOS 12.3 (Hamilton-Thorne Bioscience, Beverly, MA, USA). This device can reconstruct the trajectories of spermatozoa by determining the head position in frame sequences. Given the lack of validated studies each setting parameter was calibrated to track donkey spermatozoa and was optimized to analyze all sperm and exclude debris using the playback function. For CASA analysis, a 2-μl aliquot of semen was loaded into a 4-chamber 20-μm slide (Leja, Nieuw-Vennep, the Netherlands) and 12 non-consecutive microscopic fields were evaluated. In this study, the following setting was used: 60 frames per second (Hz), 45 frames per field. Motility parameters considered were total motility (TMOT, %), progressive motility (PMOT, %), average path velocity (VAP, μm/s), straight line velocity