REPRODUCTIVE STRATEGIES FOR THE PRESERVATION OF THE ENDANGERED MARTINA FRANCA DONKEY

Contri Alberto DVM, PhD
Department of Veterinary Clinical Sciences
University of Teramo
Italy

acontri@unite.it
+39 0861 266975

STRATEGIES FOR PRESERVATION OF ENDANGERED SPECIES

The sustainability of a preservation program in some equine and donkey breeds pass by the development of an interest for them breeding. In Mediterranean countries, the donkey was for centuries an important form of transport and tool used in agriculture. However, as soon as the donkey was replaced by machines, this animal was essentially ignored until the interest of researchers was recently renewed for several reasons:

- Production of milk, because of the similarities between donkey and human milk composition.
- Pet therapy, because of slow movements and
- Heavy work (by the mule production) in restricted areas as National parks

These new uses have increased the interest on this breed and facilitated the breeding and the preservation.

Several documents from FAO (Food and Agriculture Organization) suggested the guidelines for the preservation of the biodiversity, and them were collected in the FAO programmes for the preservation of Animal Genetic Resources.

There are two main way to preserve an endangered specie:

1) the creation of a germoplasm banks, by semen, oocytes and embryo cryopreservation (ex-situ preservation).
2) The improvement of the reproductive performances, that allow:
    a. Increase the number of a limited population
    b. Manage the risk of an excessive inbreeding

SEMEN CRYOPRESERVATION IN MARTINA FRANCA DONKEY

Very few laboratories have studied the technology of donkey semen cryopreservation (Glatzel et al., 1981; Piao and Wang, 1988; Trimeche et al., 1996, 1998; Silva et al., 1997). The methods used for donkey semen are usually derived from those used for stallion semen. A method derived from the French stallion technique (Palmer, 1984) but using a higher amount of glycerol, the addition of glutamine and quail egg yolk instead of hen egg yolk was suggested by Trimeche et al. (1998). Semen cryopreservation is now successfully used in 80–85% of the stallions of light breeds in France (Vidament, 2005). The situation is very different for the donkey species, despite the close phylogeny with the stallion. Semen cryopreservation is not successful in the donkey species although donkeys of different breeds are often reported to produce large quantities of high quality semen (Santos et al., 1995; Gastal et al., 1997; Trimeche et al., 1998; Mirò et al., 2005).

In this study the effect of the concentration of semen during the cryopreservation in the donkey was evaluated. Seven adult Martina Franca jackasses (4-6 years, 300-350 kg in weight) of proven fertility were collected using a Missouri artificial vagina. After estimation of volume and concentration, raw semen was evaluated for motility using a computer-assisted sperm analyzer (CASA) IVOS 12.3 (Hamilton-Thorne Bioscience, Beverly, MA, USA). Six µl of diluted semen were loaded in a Makler chamber (Sefi Medical Instruments, Haifa, Israel) and analyzed. Total motility (TM, %), progressive motility (PM, %), average path velocity (VAP, µm/s), lateral head displacement (ALH, µm), beat frequency (BCF, Hz), straightness (STR, as (VSL/VAP)*100 - %),

and linearity (LIN, come (VSL/VCL)*100 - %) were considered and recorded. Progressive spermatozoa had VAP > 80 µm/s and STR > 75%. Based on the their VAP, spermatozoa were sub-classified as rapid (VAP > 80 µm/s), medium (VAP between 80 and 35 µm/s), slow (VAP < 35µm/s), static (VAP = 0). Then fresh semen was diluited 1:1 with INRAfreeze (IMV Technologies, L'Aigle, France), centrifuged (5 min at 1800 x g) and resuspended at 3 x 10^9 sperm/ml. Four aliquots were prepared and diluted at 100, 200, 500, and 1000 x 10^6 sperm/ml respectively. After cooling (75 min at 5°C) samples were loaded in French 0.5 ml straws and frozen. After 48 h, samples were thawed (1 min at 37°C) and analyzed for motility parameters. The analysis of the data showed similar parameters of fresh semen compared with those of centrifuged and cooled samples, suggesting a reduced effect of these manipulations on semen characteristics. Data reported in this study confirmed the low effect of some handling procedures such as centrifugation and cooling, independently to the semen concentration (Contri et al., 2010). As expected, the cryopreservation had a relevant impact on semen quality. A significant reduction in TM, PM and VAP was recorded after frozen, but the other parameters (ALH, BCF, STR, LIN) were marginally affected by this process, suggesting that cryopreservation act as "selector" on some spermatozoa, but these that survive had motility characteristics comparable with fresh and cooled semen. Despite a progressive decrease in the MT, PM and VAP when concentration increase, no significant differences were recorded (figure 1). These unexpected results should be due to the great variability of the cryoresistance to the freezing process between jackasses.

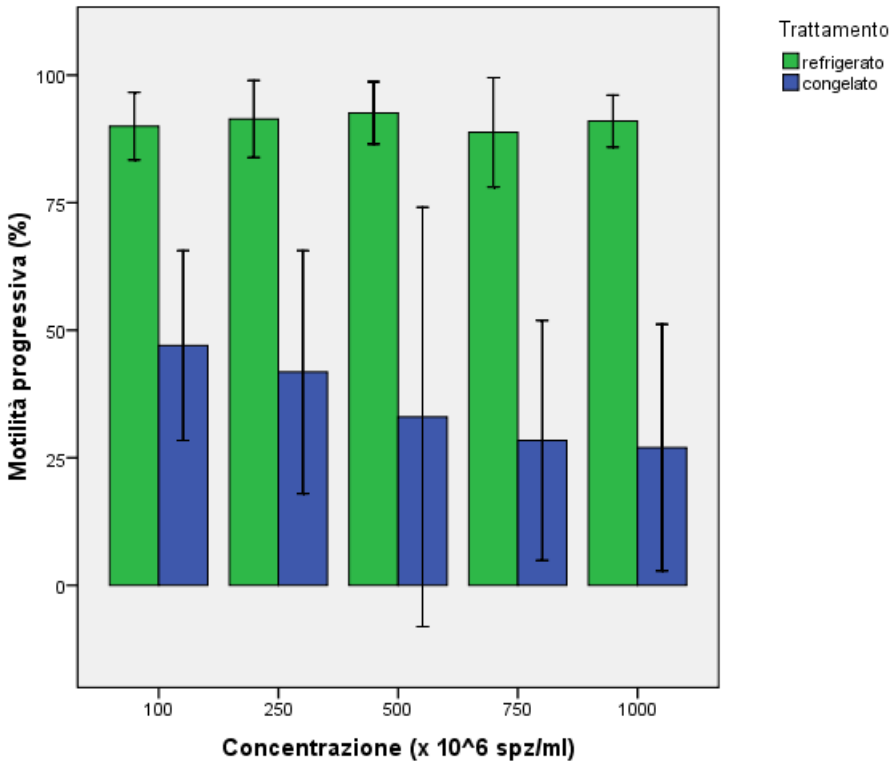

Figure 1. Progressive motility in cooled and frozen donkey sperm at different dose concentrations

In the horse, a different ability of spermatozoa from different stallion to survive after the freezing process were well documented. About 30% of the stallions tested for their freezability showed a unsatisfactory quality of frozen semen and were judged as "bad freezers" in France (Vidament et al., 1997). Probably this individual ability to resists after freezing cryoinjuries could be related to a different composition of the plasma membrane of spermatozoa, however this hypothesis is not still proved at this time.

Semen characteristics of fresh semen was in agreement with data reported in literature (Contri et al., 2010). As widely reported in the horse, there was a significant reduction of sperm characteristics in frozen-thawed semen, but a variability in the cryoresistance of semen between jackasses was found, suggesting the presence of good and poor freezers in the donkey. A progressive reduction, even if not significant, of sperm TM, PM and VAP was reported, in agreement with previous report in the horse (Heitland et al., 1996).

LIQUID SEMEN PRESERVATION AND SEASONALITY

The cryopreservation of equine semen is relatively expensive and frequently produces less than satisfactory fertility (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 hrs maintains a fertility rate similar to that of fresh semen (Jasko et al., 1992). In the horse, reproduction is influenced by the season and the time of insemination is limited by the presence of transitions and anestrus, so that cooled stallion semen is only available during the breeding season. Seasonal changes in sexual hormone profiles, seminal characteristics, and reproductive behaviours were reported in the stallion (Roser and Huges, 1992). Several studies have demonstrated that photoperiod affects reproductive activity by acting on the hypothalamic-pituitary-gonadal axis in seasonal breeders, including the stallion (Pickett et al., 1989). Unlike mares, fertile ovulatory cycles are present in most jennies throughout year, suggesting a low or different seasonal influence on the estrus cycle (Ginther et al., 1987; Blanchard et al., 1999). The absence of seasonal anoestrus 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. Since in donkey reproduction, the lack of both transitions and seasonal anestrus potentially extend 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 high daylight periods (May-June - MJ) and in lower ones (November-December - ND).

Eighteen ejaculates from 6 jackasses in both May-June and November-December periods were cooled with INRA96 or E-Z Mixin at a low cooling rate. Semen characteristics, such as viability, using propidium iodide-SYBR14 fluorescent stain and motility parameters (total motility - TMOT, progressive motility - PMOT, average path velocity - VAP, straight line velocity - VSL, curvilinear velocity - VCL, amplitude of lateral head displacement - ALH, beat cross frequency - BCF, straightness - STR, linearity - LIN) by computer assisted sperm motility were analyzed on fresh semen and every day over a 120 hour preservation period.

The results showed a significant extender influence on preservation time in both periods. Semen diluted with INRA96 maintained a progressive motility of 35.7 % and a straightness of 88.8 % at 120 hrs, while semen extended with E-Z Mixin had similar mean values at 48 hrs during the May-June period. Despite having the same initial characteristics, semen collected during low daylength periods showed a higher decline in semen quality during storage at 5°C, with slight better results for INRA96 (figure 2). The data reported in this study showed that the characteristics of raw jackass semen in both periods were similar. However in ND period the decline of semen characteristics was more pronounced, probably for a different sensitivity of spermatozoa to the cold shock phenomenon (Amann and Pickett, 1987). Seasonality in the mares only triggers a demand for cooled equine semen during the breeding season, with limited demand from April to September in the Northern Hemisphere (Nagy et al., 2000). For this reason, no data are available on the effectiveness of liquid storage at 5°C in the stallion during low daylength periods. However, a similar sensitivity of equine spermatozoa during non-breeding season to the low temperature was reported in the study by Blottner et al. (2001), in which a reduced quality of cryopreserved stallion semen was recorded during non-breeding season. These results demonstrated a strong seasonal influence on the preservability of extended semen during cooling but not on the characteristics of raw semen or on the resistance to centrifugation. Doubtless the season influences sperm resistance to cold shock, although the exact manner of action and target structures need to be further investigated.

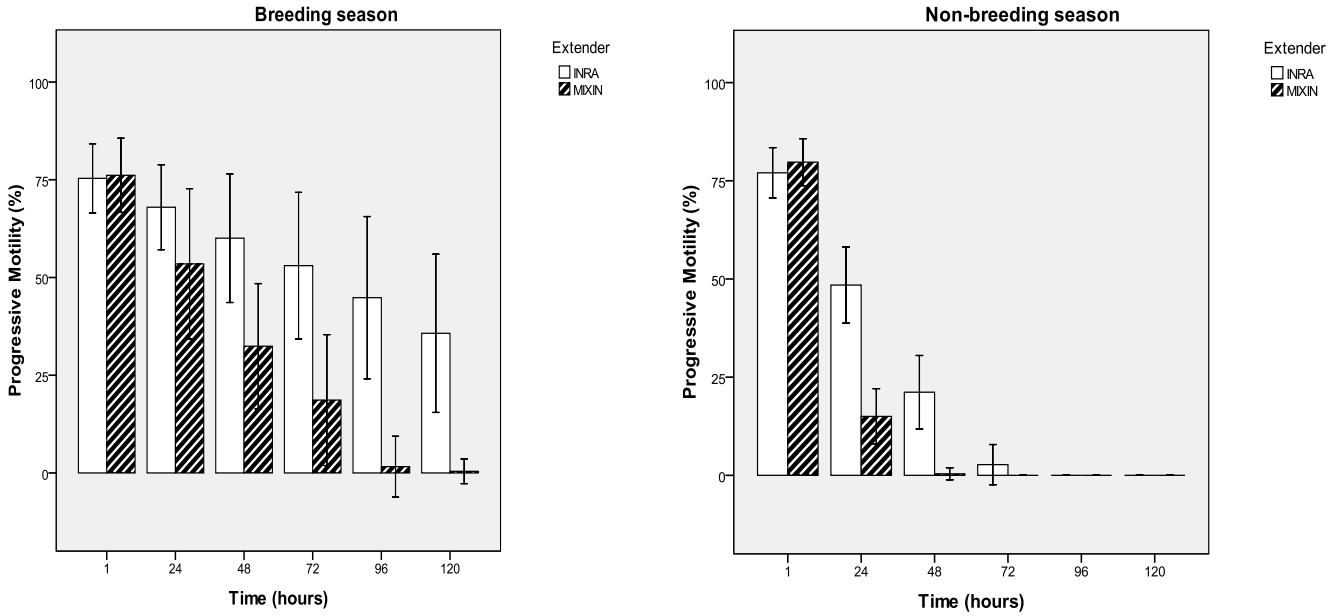

Figure 2. Trend of progressive motility of cooled semen during breeding season (left) and non-breeding season (right)

REFERENCES

Amann and Pickett, 1987. J. Eq. Vet. Sci. 7:145-173. – **Blanchard et al., 1999.** Theriogenology 52:827-834. – **Blottner et al., 2001.** Anim. Reprod. Sci. 65:75-88. – **Carluccio et al., 2003.** Veterinaria Pratica Equina 5:35-41. - **Contri A et al., 2010.** Anim. Reprod. Sci. 120:136-141. – **Gastal et al., 1997.** Theriogenology 47:627–638. – **Ginther et al., 1987.** Theriogenology 27:587-592. – **Glatzel et al., 1981.** Berl. Munch. Tierarztl. Wochenschr. 94:445–448. - **Heitland et al., 1996.** Equine Vet J 28:47-53. – Henry et al., 1987. J. Reprod. Fert., Suppl. 35:297-303. – **Jasko et al., 1992.** Theriogenology 37:1241–1252. – **Mirò et al., 2005.** Theriogenology 63:1706–1716. **Nagy et al., 2000.** Anim. Reprod. Sci. 60-61:245-262. - **Pagl et al., 2006.** Theriogenology 66:1115-1122. - **Palmer, E., 1984.** Proceedings 10th International Congress on Animal Reproduction and Artificial Insemination, 3, Urbana (Abstract 377). – **Piao and Wang, 1988.** Proceedings 11th International Congress on Animal Reproduction and Artificial Insemination, 3, Dublin (Abstract 286). – **Pickett et al., 1989.** Colorado State University Animal Reproduction Laboratory Bulletin No. 5., Fort Collins, CO. – **Roser and Hughes, 1992.** J. Androl. 13:214-223. – **Samper and Morris, 1998.** Theriogenology 49:895-903. – **Santos et al., 1995.** In: Equine Reproduction VI. Biol. Reprod. Mono 1:761–767. – **Silva et al., 1997.** Revista Brasileira de Reproducao Animal 21:140–146. – **Trimeche et al., 1996.** Theriogenology 45:1015–1027. – **Trimeche et al., 1998.** Theriogenology 50:793–806. – **Varner et al., 1989.** Theriogenology 32:515-525. - **Vidament et al., 1997.** Theriogenology 48:907-917. – **Vidament, 2005.** Anim. Reprod. Sci. 89:115–136.