

COMPARISON BETWEEN AN AUTOMATIC AND A MANUAL PROTOCOL FOR FREEZING CANINE SEMEN

Manuela PASCAL, Ruxandra COSTEA, Alin Ion BÎRȚOIU

Faculty of Veterinary Medicine of Bucharest

105, Splaiul Independentei, District 5, Postal code 050097, Bucharest, Romania

Corresponding author email: manuelastanescu@hotmail.com

Abstract

For the freezing of canine semen, slow to fast cooling rates have been used. Today, there are many ways of achieving this by using manual protocols, automatic protocols or ultrafreezers. Straws were divided in two groups: one batch (10 ejaculates) was automatically frozen, while the other batch was frozen manually. Motility (computer assisted sperm analyzer), morphology and acrosome status (Spermac[®] stain) were evaluated for fresh and frozen-thawed semen. The manual freezing protocol provided higher total (23.85%) and progressive motility values (20.41%) compared to the automatic protocol (13.26 % total motility, progressive motility 9.90%). The acrosome status was strongly influenced by the cryopreservation process, but there were no significant differences between the two protocols. When using the CaniPro Freeze[®] extender, a slower cooling rate (the manual protocol) gave better results than a fast one (the automatic protocol).

Keywords: canine semen, cryopreservation

INTRODUCTION

There are many factors that influence the quality of frozen-thawed canine semen. Among these factors, freezing rates are one of the most discussed and tested (Pena and Linde-Forsberg, 2000b; Schafer-Somi et al., 2006; Sirivaidyapong et al., 2000). For the freezing of canine semen a cooling rate of 10 to 50°C per minute for the critical interval between -15/-60°C is considered to be optimal (Olar et al., 1989; Pena and Linde-Forsberg, 2000a; Rota et al., 1998; Witte and Schafer-Somi, 2007). Today, there are many ways of achieving this by using manual protocols, automatic protocols or ultrafreezers (Alamo et al., 2005; Batista et al., 2006; Schafer-Somi et al., 2006; Yu et al., 2002).

The aim of our study was to compare two cooling rates used for the freezing of canine semen extended with CaniPRO Freeze[®] (Minitübe, Germany) and egg yolk.

MATERIALS AND METHODS

The research was developed between October 2010 and March 2012 in the Clinic of the Faculty of Veterinary Medicine of Bucharest. 20 ejaculates were collected manually from private owned stud dogs. Fresh semen was

evaluated: motility parameters (computer assisted sperm analyzer SpermVision[®], Minitübe, Germany), morphology and acrosome status (Spermac stain[®], Stain Enterprises, Onderstepoort, South Africa) were determined. Only good quality ejaculates were frozen: progressive motility >70%, morphology ical abnormalities < 20%. The extender (CaniPRO Freeze with 20% egg yolk) was prepared 30 minutes before the collection of semen and maintained at room temperature. The sperm rich fraction fraction was diluted in two steps: first – 1 ml of semen was diluted with 1 ml of CaniPRO Freeze A (with 20% egg yolk) and maintained 1 hour at 4°C; the second dilution was done with 1 ml of CaniPRO Freeze B (with 20% egg yolk) and the mixture was cooled at 4°C for 2 hours. Two batches were formed: batch AA and batch AM. Batch AA was frozen automatically (CryoCell[®], Minitübe, Germany) at a rate of -14°C for 6 minutes and maintained at -80°C for 6 minutes. Batch AM was frozen manually in a polystyrene box for 10 minutes at 6 cm above the liquid nitrogen. After thawing for 30 seconds at 37°C motility parameters (SpermVision[®], Minitübe, Germany), morphology and acrosome status

(Spermac stain®, Stain Enterprises, Onderstepoort, South Africa) were evaluated.

RESULTS AND DISCUSSIONS

For the automatic protocol, total motility of frozen-thawed semen was $13.26 \pm 2.19\%$ and progressive motility $9.90 \pm 1.87\%$.

For the manual protocol, total motility of frozen-thawed semen was $23.85 \pm 2.88\%$ and progressive motility $20.41 \pm 3.86\%$.

Regardless of the freezing protocol, it is noted that the cryopreservation process has a major effect on semen motility, reducing its values with more than 50% compared to fresh semen (fig. 1).

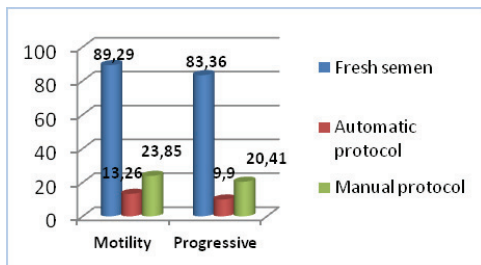


Figure 1. Comparison of mean motility values for fresh and frozen-thawed semen from batches AA and AM.

There is a statistically significant difference between the values of total and progressive motility determined for the two freezing protocols ($P < 0.05$), manual protocol providing clearly superior values (fig. 2).

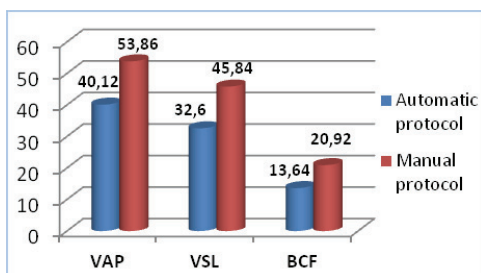


Figure 2. Comparison of motility parameters measured with SpermVision for the semen frozen-thawed by the automatic and the manual protocols.

Regarding the average velocity (VAP) and straight line velocity (VSL) of semen after

thawing, the manual protocol led to higher values of these parameters (VAP $53.86 \mu/\text{sec}$, VSL $45.84 \mu/\text{sec}$) compared to the automatic protocol (VAP $40.12 \mu/\text{sec}$, VSL $32.6 \mu/\text{sec}$). The beat cross frequency (BCF) of frozen-thawed semen was higher for the AM batch (20.92 hertz) compared to the AA batch (13.64 hertz) (fig. 3). Between the values of these motility parameters obtained for the AA and the AM batches there is a statistically significant difference ($P < 0.05$).

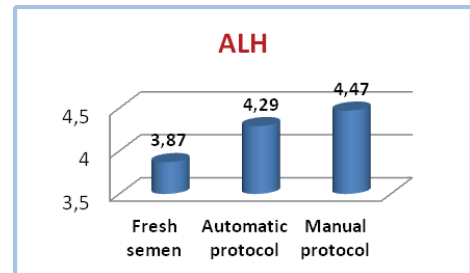


Figure 3. Evolution of mean value for amplitude of head lateral displacement (ALH) depending on the freezing protocol.

The amplitude of lateral head displacement (ALH) was $4.47 \pm 0.19 \mu$ for the semen frozen manually, and $4.29 \pm 0.24 \mu$ for the automatic protocol. ALH increases during capacitation due to sperm hyperactivation (Watson, 1995). Regardless of the cooling rate, ALH increases after freezing-thawing. Capacitation like changes are induced by the cryopreservation process and the external medium (Iguer-Ouada and Versteegen, 2001; Rota et al., 1999). Considering that ALH values do not differ significantly between the two freezing protocols ($P > 0.05$), we consider that the two freezing rates had a similar effect on sperm hyperactivation.

There are no significant differences between the percentage of normal spermatozoa in fresh semen (90.83%) and in the frozen-thawed semen (89.55% for the automatic protocol, 89.95% for the manual protocol) ($P > 0.05$). There are no differences between the morphological abnormalities for the two freezing protocols (manual and automatic). So, the freezing process did not affect semen morphology.

The percentage of acrosome reacted semen was 20.60% for the AA batch and 20.45% for

the AM batch compared to 2.75% in fresh semen. Acrosome status was strongly affected by the cryopreservation process regardless of the freezing rate, but there are no significant differences between the two protocols ($P > 0,05$).

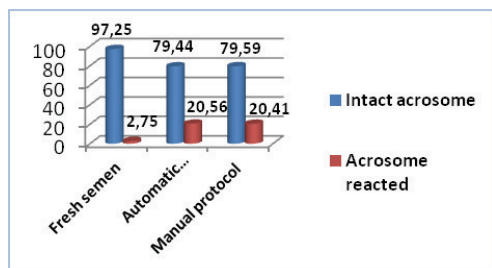


Figure 4. Comparison between acrosome intact spermatozoa for the different categories of semen.

Rota et al. (Rota et al., 2005) published a study concerning an automatic and a manual protocol for freezing canine semen. As in our study, the slower cooling rate proved to ensure superior values for motility and plasma membrane functions. Still, spermatozoa velocity (VAP, VSL, VCL) and the amplitude of lateral head displacement (ALH) were higher for the semen frozen at a fast cooling rate.

CONCLUSIONS

The manual freezing protocol provided higher total (23.85%) and progressive (20.41%) motility values compared to the automatic protocol (13.26% and 9.90%). For the amplitude of lateral head displacement (ALH), the manual freezing protocol led to a value of 4.47 Hz compared to 4.29 Hz for the automatic protocol, with no statistical significant differences between the two groups. The acrosome status was significantly influenced by the freezing process, but there were no significant differences between the two cooling rates.

ACKNOWLEDGEMENTS

This work was supported by the POSDRU project 88/1.5/S/52614 „Doctoral scholarships for high quality training for young researchers

in the field of agronomy and veterinary medicine”.

REFERENCES

- Alamo, D., Batista, M., Gonzalez, F., Rodriguez, N., Cruz, G., Cabrera, F., Gracia, A., 2005. Cryopreservation of semen in the dog: use of ultra-freezers of -152 degrees C as a viable alternative to liquid nitrogen. *Theriogenology* 63, 72-82.
- Batista, M., Alamo, D., Gonzalez, F., Cruz, M.G., Gracia, A., 2006. Influence of the freezing technique (nitrogen liquid vs ultrafreezer of -152 degrees C) and male-to-male variation over the semen quality in Canarian Mastiff breed dogs. *Reproduction in domestic animals = Zuchthygiene* 41, 423-428.
- Iguer-Ouada, M., Verstegen, J.P., 2001. Evaluation of the "Hamilton Thorn computer-based automated system" for dog semen analysis. *Theriogenology* 55, 733-749.
- Olar, T.T., Bowen, R.A., Pickett, B.W., 1989. Influence of extender, cryopreservative and seminal processing procedures on postthaw motility of canine spermatozoa frozen in straws. *Theriogenology* 31, 451-461.
- Pena, A., Linde-Forsberg, C., 2000a. Effects of Equex, one- or two-step dilution, and two freezing and thawing rates on post-thaw survival of dog spermatozoa. *Theriogenology* 54, 859-875.
- Pena, A., Linde-Forsberg, C.B., 2000b. Effects of spermatozoal concentration and post-thaw dilution rate on survival after thawing of dog spermatozoa. *Theriogenology* 54, 703-718.
- Rota, A., Linde Forsberg, C., Vanzozi, I., Romagnoli, S., Rodriguez-Martinez, H., 1998. Cryosurvival of dog spermatozoa at different glycerol concentrations and freezing/thawing rates. *Reprod Dom Anim* 33, 355-361.
- Rota, A., Martini, M., Milani, C., Romagnoli, S., 2005. Evaluation of dog semen quality after slow (biological freezer) or rapid (nitrogen vapours) freezing. *Reproduction, nutrition, development* 45, 29-37.
- Rota, A., Peña, A., Linde-Forsberg, C., Rodriguez-Martinez, H., 1999. In vitro capacitation of fresh, chilled and frozen-thawed dog spermatozoa assessed by the chlortetracycline assay and changes in motility patterns. *Animal reproduction science* 57, 199-215.
- Schafer-Somi, S., Kluger, S., Knapp, E., Klein, D., Aurich, C., 2006. Effects of semen extender and semen processing on motility and viability of frozen-thawed dog spermatozoa. *Theriogenology* 66, 173-182.
- Sirivaidyapong, S., Cheng, F.P., Marks, A., Voorhout, W.F., Bevers, M.M., Colenbrander, B., 2000. Effect of sperm diluents on the acrosome reaction in canine sperm. *Theriogenology* 53, 789-802.

- Watson, P.F., 1995. Recent developments and concepts in the cryopreservation of spermatozoa and assesment of their post-thawing function. *Reproduction, fertility, and development*, 871-891.
- Witte, T.S., Schafer-Somi, S., 2007. Involvement of cholesterol, calcium and progesterone in the induction of capacitation and acrosome reaction of mammalian spermatozoa. *Animal reproduction science* 102, 181-193.
- Yu, I., Songsasen, N., Godke, R.A., Leibo, S.P., 2002. Differences among dogs in response of their spermatozoa to cryopreservation using various cooling and warming rates. *Cryobiology* 44, 62-78.