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INFLUENCE OF THE CRYOPRESERVATION ON THE VITALITY OF THE SPERM OF THE DIFFERENT BREEDS OF RAMS

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ABSTRACT

The aim of the study was to determine the effect of cryopreservation on the vitality of sperm from different breeds of rams. For the purpose of the study were used 15 healthy rams from three breeds – Ile-de-France, Lacaune, Synthetic Population of Bulgarian Milk (SPBM), during their insemination campaign. Sperm viability was determined by a Computer-assisted sperm analysis (CASA) after staining with a kit containing nigrosin-eosin (NE) solution. After cryopreservation, the highest percentage of vital sperm were found in the Ile-de-France breed. A significance difference was found between the Ile-de-France and SPBM breeds ($P<0.001$) and between Ile-de-France and Lacaune ($P<0.01$), regarding the studied indicator.

Key words: cryopreservation, vitality, sperm, ram.

Introduction

The role of sperm cryopreservation for in vivo and in vitro production of human and animal embryos is evident (Byrne et al, 2000; Yildiz et al., 2007). Sperm cryopreservation is an effective way to manage and preserve male fertility in humans and pets (Sharma, 2011). Literature studies reveal that sudden temperature changes such as cold and warm shocks, as well as ice formation and dissolution during the freezing–thawing process, affects the integrity of cells at both the structural and substructural levels (Quinn et al., 1965; Nath, 1972; Watson, 1999). Assessment of sperm vitality is one of the main elements of sperm analysis and is particularly important in samples where there are many static sperm in order to distinguish between dead sperm and static living sperm (Björndahl et al., 2003).

The sperm cell membrane is a barrier to the infiltration of fluids, including various paints in the intracellular environment. Living sperm do not allow any type of coloring to pass into their intracellular environment while the dead sperm absorb them. This biophysical phenomenon has allowed the development of techniques aimed at distinguishing living sperm from the dead sperm (Fernández et al. 1998).

There are currently a number of techniques available to identify living and dead sperm. Multiple coloring have been described based on the selective permeability of the plasma membrane, such as eosin–nigrosin (Colas, 1975) and trypan blue (Suttiyotin and Thwaites, 1992), such that if the sperm are alive, the cell membrane acts as an impermeable barrier preventing the paint from passing through it, leaving the cell unpainted.

Eosin-nigrosin painting is a popular method for assessment sperm in both mammals and birds (Björndahl et al., 2004; Horst et al., 2009; Łącka et al., 2016). Eosin-Nigrosine is a coloring technique that estimates the viability of a sperm sample when the initial motility is less than 25%. Nigrosine increases the contrast between the background and the heads of the sperm, making sperm easier to visualize. Eosin paints only dead sperm, turning them dark pink while the live sperm look white (Agarwal, 2016).

The aim of the study was to determine the effect of cryopreservation on the vitality of sperm from different breeds of rams.

Materials and methods

Sperm samples were collected from 15 healthy rams (2–4 years), breeds – Ile–de–France, Synthetic Bulgarian Milk (SPBM) and Lacon. Two ejaculas were obtained from each ram – a total of 30 ejaculas were examined. After collection, the samples were transferred to the laboratory and kept in a 37 ° C water bath for examination. Ejaculates were collected from the rams using the artificial vagina. They are diluted with colloidal diluent 6AG (sodium citrate, lactose, sucrose, egg yolk and glycerol).

Vitality: BrightVit test, containing eosin and nigrosine was used to determine the number of living and dead sperm. In an Eppendorf tube was added 30 µl of BrightVit solution and a drop of 10 µl of the sperm sample and incubated at 37°C for 5–10min. As much as 10µl of the mixture was put onto and was spread on the glass, allowing the smear to dry at room temperature.

Microscopic analysis: The smear was examined under a microscope with 100x magnification and immersion oil and analyzed by Sperm Class Analyzer (SCA Microptic, Barcelona, Spain). Those cells that do not absorb the paint (not colored) were identified as living cells, while those that are (colored) are considered dead. As much as 100 sperm from several fields per slide were counted and the presence of live (non–colored head) and dead (head colored pink) sperm was noticed. Their total percentage was automatically calculated by the SCA. Sperm viability was determined before and after cryopreservation of the samples.

The freezing of semen was performed using Cassou's method (1964) and the thawing of sperm was performed in a 37°C water bath for 10s.

Statistical analysis. Data were analyzed with SPSS 23 to compare sperm characteristics using statistical tests of ANOVA and Paired T–test. The significance of the differences between groups was evaluated by t–criterion of Student. Findings were considered statistically significant if $P< 0.05$.

Results and discussion

Figures 1 and 2 show microscopic slides of alive and dead spermatozoa taken with the BrightVit test.

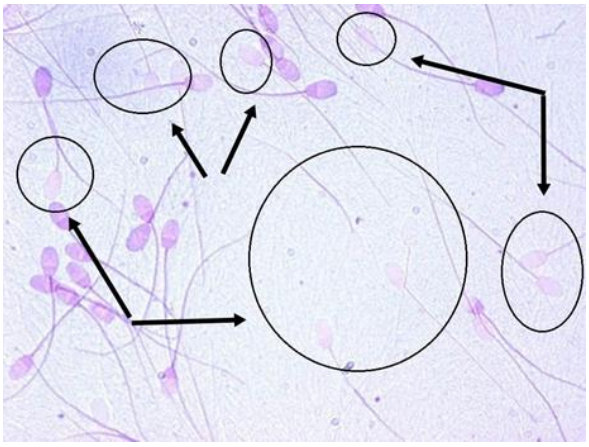


Figure 1: Alive spermatozoa.

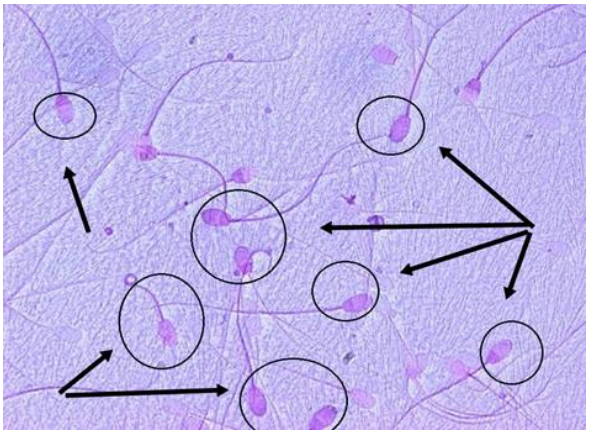


Figure 2: Dead spermatozoa.

The results of this study show a decrease in viability of freeze–thaw sperm. Table 1 presents the results of the effect of cryopreservation on the viability of sperm from the studied breeds.

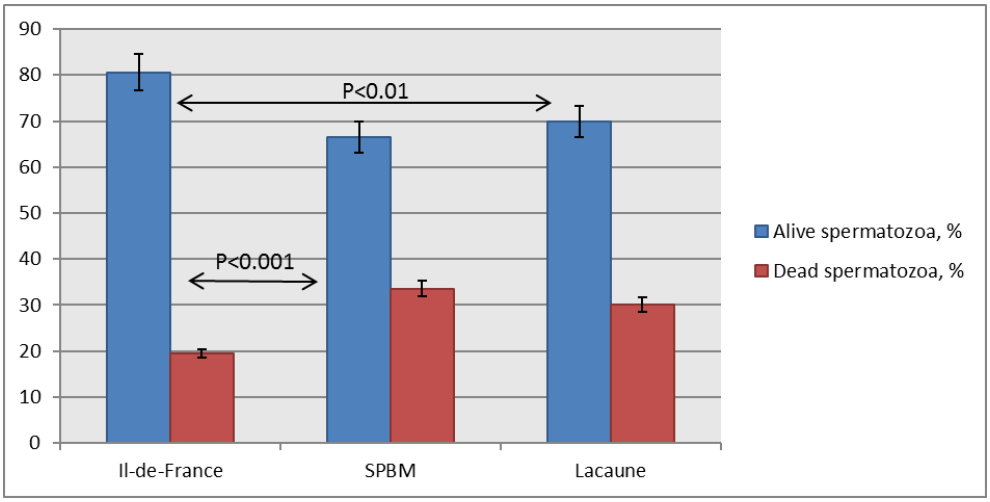
Table 1: Sperm vitality before freezing and after thawing

Breed	Alive before freezing, %	Alive after thawing, %	Dead before freezing, %	Dead after thawing, %
Il-de-France	85,90±7,69	80,60±7,16***	14,10±7,69	19,40±7,16***
SPBM	77,60±6,11	66,50±6,07***	22,40±6,11	33,50±6,07***
Lacaune	81,90±7,59	69,90±11,82**	18,10±7,59	30,10±11,82**

*Note: Results are presented as Mean ± SD, Significant differences ** P <0.01; *** P <0.001*

In the Ile-de-France breed, the difference between freezing and thawing is about 5%, while in the SPBM and Lacaune breeds the decrease in sperm viability is 10–12%. Regarding the average percentage of live sperm after thawing in the Ile-de-France breeds (80.6%), SPMB (66.50%) and Lacaune (69.90%), the results are lower than those obtained by Guerrero et al. (2009) – 90.2% and found by Hernández et al., (2012) – 91.4%, but higher than that reported by Hernandez et al. (2005) – 65. 8%. This is possible because in the first three studies the ejaculates were obtained by the artificial vagina (AV) method, while the ejaculates obtained by Hernandez et al. (2005) are by electro–ejaculation (EE). Decrease in viability of freeze-thaw sperm has been observed by other authors (Salmon and Maxwell, 1995). Freezing and the processes of thawing can cause irreversible damage to the sperm from the rams. According to Medeiros et al. (2002), a relatively high proportion (40–60%) of sheep sperm retain their motility after cryopreservation, but only about 20–30% remain biologically functional.

Regarding the sperm viability after thawing, a significance difference was found between the Ile-de-France and SPBM breeds (P<0.001) and between the Ile-de-France and Lacon (P<0.01) presented in Figure 3. Other authors in their studies also found breed significance differences (Aisen, 2004; Pelayo, 2019).



Note. Results are presented as Mean ± SE, %

Figure 3: Significance of breed differences between vitality after cryopreservation.

Conclusion

Regarding the Ile-de-France breed the ultra-low temperatures affect less the vitality of the sperm compared to the other two tested breeds. This is also a sign of better cryotolerance of the ejaculates from Ile-de-France breed.

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