

Research Article/Artículo de investigación

EFFECT OF CRYOPRESERVATION ON ACROSOMAL INTEGRITY IN JERSEY BULLS

EFFECTO DE LA CRIOPRESERVACIÓN SOBRE LA INTEGRIDAD ACROSOMAL EN TOROS JERSEY

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ABSTRACT

A study was undertaken to evaluate the effect of cryopreservation on acrosomal abnormalities in Jersey bulls. Eight Jersey bull were maintained at Livestock Development Board, Sperm Station Palampur Himachal Pradesh, India. Total 64 ejaculates were evaluated using Giemsa stained semen smears at four stages of cryopreservation process: post-dilution, post-equilibration, post thaw, 1hr post-thaw incubation. Acrosomal abnormalities were assessed and classified into swollen, ruffled, abnormal contour and detached acrosome. Results revealed highly significant difference ($p < 0.001$) in the total acrosomal abnormalities among the four stages of semen processing and an increase of 30.51 points was observed from post-dilution stage to 1hr post-thaw. Ruffled acrosome (7.11 ± 0.5) and detached acrosome (10.55 ± 0.95) were the acrosomal abnormalities with highest proportions at post-dilution and post equilibration respectively, whereas detached acrosome was the abnormalities with highest proportions at post-thaw stage (14.99 ± 0.95) and 1 hour post-thaw incubation (22.35 ± 1.04). In conclusion, the high proportion of acrosomal abnormalities in the ejaculates of the Jersey bulls may be due to impaired spermiogenesis; cryopreservation affect acrosomal integrity, by which can compromise semen fertility. Further studies on new cryopreservation protocols that guarantee acrosomal integrity are necessary in Jersey bulls.

Keywords: acrosome, abnormalities, Jersey bulls, semen, cryopreservation.
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RESUMEN

Este estudio se desarrolló para evaluar el efecto de la criopreservación sobre las anomalías acrosomales en toros Jersey. Para ello se utilizaron ocho toros Jersey pertenecientes al Livestock Development Board, Sperm Station Palampur Himachal Pradesh, India. Un total de 64 eyaculados fueron evaluados usando la coloración de Giemsa en cuatro fases del proceso de criopreservación: post-dilución, post-equilibrio, post-descongelado, 1 hora post-descongelado. Las anomalías acrosomales se evaluaron y clasificaron dentro de acrosoma hinchado, acrosoma rizado, acrosoma con contorno anormal y acrosoma desprendido. Los resultados mostraron diferencias altamente significativas en el total de anomalías entre las cuatro fases de procesamiento y un incremento de 30.51 puntos fue observado desde la fase de post-dilución a 1 hora post-descongelado. Los acrosomas rizados (7.11 ± 0.5) y acrosomas desprendidos (10.55 ± 0.95) fueron la anomalías con mayores proporciones en la fase post-dilución y post-equilibrio, respectivamente, mientras que los acrosomas desprendidos fue la anomalía más frecuente en la fase post-descongelado (14.99 ± 0.95) y 1 hora post-descongelado (22.35 ± 1.04). En conclusión, la alta proporción de anomalías acrosomales en los eyaculados de toros Jersey puede ser debido a fallas en la espermiogénesis; la criopreservación afecta la integridad acrosomal, por lo cual puede comprometer la fertilidad del semen. Más estudios sobre protocolos de criopreservación que garanticen la integridad acrosomal son necesarios en toros de raza Jersey.

Palabras clave: acrosoma, anomalías, toros Jersey, semen, criopreservación.
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INTRODUCTION

Intactness of spermatozoal acrosomal cap is a prerequisite during the travel of sperm in the female reproductive tract so that the sperm is able to penetrate the zona-pellucida after acrosome reaction at appropriate time to release the acrosome enzymes. Therefore acrosome integrity is one of the important indicators of fertility (Neild et al., 2005 and Esteves et al., 2007). A small percentage of acrosomal abnormalities are commonly found in the semen of normal fertile bulls. Major abnormalities in acrosomal integrity is ruffling, swelling, rupture, missing part (incomplete acrosome) and loss of acrosomal membranes.

These abnormalities may be hereditary and may be involved in infertility (Saacke et al., 1968). Swollen acrosome can occur during epididymal aging (Sexual abstinence), with improper semen handling procedures (Blom, 1964) or due to injury during freeze-thaw whereas, acrosome with abnormal contour occurs due to defective spermatogenesis. In addition, the acrosome has a high sensitivity to the cryopreservation process (Rubio-Guillen et al., 2009). Cryopreservation reduced acrosome integrity and this was negatively relate to fertility (Ahmed et al., 2019) probably because damage of acrosome is relate to lower motility (Yániz et al., 2017). In view of this, a minimum of 65% of acrosomal integrity is desirable in frozen semen. Present communication describes the effect of cryopreservation on the proportion of different acrosomal abnormalities in semen of Jersey bulls.

MATERIALS AND METHODS

Jersey bulls reared in Livestock Development Board, Sperm Station Palampur Himachal Pradesh were used for this study. Semen collection was done bi-weekly using artificial vagina for four weeks at four stages of semen processing viz. post-dilution, post-equilibration, post-thaw and 1hr post-thaw incubation. Total 64 (8 per bull) ejaculates were collected. Sperm acrosome morphology and defects were examined in smears stained with Giemsa. At each preparation, 200 sperm cells were counted and the percentages of morphological defects were classified into four classes: swollen acrosome (Characterized by acrosome with swollen and ruffled apical ridge and neck); ruffled acrosome; acrosome with abnormal contour (Characterized by presence of acrosome with irregular shaped head); detached acrosome (Characterized by presence of acrosome with separated head, neck and midpiece). And proportion of acrosomal abnormalities was calculated at different stages of processing (Table 1) to access the effect of cryopreservation.

Statistical Analysis

Student's t-test using the Graph Pad Instat 3 was utilized for comparison of the means of acrosomal abnormalities at different stages of semen processing.

RESULTS

In the present study, highly significant difference ($p < 0.001$) was observed between total acrosomal abnormalities during four stages of semen processing and a subsequent increase in acrosomal abnormalities was observed as stages succeed, 30.51 points from post-dilution stage to 1hr post-thaw (Table 1). Ruffled and detached acrosome was the abnormalities with higher proportion. Ruffled acrosome (7.11 ± 0.5) and detached acrosome (10.55 ± 0.95) were the acrosomal abnormalities with highest proportion at post-dilution and post equilibration, respectively, whereas detached acrosome was the abnormalities with highest proportions at post-thaw stage (14.99 ± 0.95) and 1 hour post-thaw incubation (22.35 ± 1.04), respectively (Table 1). Different acrosomal abnormalities encountered during this study have been shown in Figures 1 and 2. Overall proportion of acrosomal abnormalities is given in Table 1.

Table 1. Proportion of different acrosomal abnormalities in the semen of Jersey Bulls

Stages	Swollen	Ruffled	Abnormal contour	Detached	Total abnormalities
Post-dilution	0.32±0.06 (0-2)	7.11±0.5 (2-19)	0.20±0.06 (0-2)	7.1±0.53 (1-16)	14.83±0.28^A (0-19)
Equilibration	0.58±0.10 (0-2)	10.03±0.64 (0-27)	1.66±0.43 (0-25)	10.55±0.95 (0-50)	22.83±0.4^B (0-50)
Post-thaw	1.06±1.66 (0-6)	14.68±1.1 (0-50)	0.94±0.38 (0-17)	14.99±0.95 (0-46)	32.80±0.588^C (0-50)
1 hour post-thaw incubation	0.92±0.21 (0-12)	21.34±1.35 (0-51)	0.71±0.44 (0-28)	22.35±1.04 (8-23)	45.34±0.79^D (0-51)

^{A, B, C, D} Values with different superscripts within the column for a particular parameter differ significantly ($p < 0.001$)

DISCUSSION

Acrosome is the acidic secretory organelle filled with hydrolytic enzymes. Assessment of the acrosomal status is a very important part of semen evaluation, in the view of the role of this structure in the maintenance of spermatozoa ability to penetrate the zona pellucida and the ability to fuse with the egg plasma membrane (Esteves et al., 2007).

Semen of bull also could had a high proportion of acrosome defects such as ruffled, incomplete and completely lost acrosome and this was observed in the present study. A proportion of these defects could be heritable (Chenoweth, 2005) and also strongly associated with fertility of the bull (Hough et al., 2002).

In contrast to the present study, Pant (2000) found the proportion of detached acrosomes and ruffled acrosomes to be 26-45% and 18-28%, respectively, which was higher than in the present study. Semen of Jersey bulls showed 75.1% of spermatozoa with defective acrosome. While 34.8% of sperm cells exhibited ruffled acrosome, the incomplete acrosome and spermatozoa with completely lost acrosome were 17.9% and 22.4% respectively (Sundararaman et al., 2014).

Acrosome integrity was reduced during the cryopreservation process and this was observed previously by Kumar et al., 2015, nevertheless impact of cryopreservation on acrosome integrity was higher in the present study (-30.51 points) in comparison with Kumar et al., 2015, (-17.11 points) who evaluated the effect of cryopreservation also in semen of Jersey bulls. Rubio-Guillén et al., 2009, observed an increases of 25.25 points in the percentage of damage acrosome after cryopreservation. A plausible explanation for increase in abnormalities at subsequent stages is cold shock. Fluctuation in temperature during subsequent steps of processing causes stress on plasma membranes, which is probably related to phase change in lipids and altered functional state of membranes (Watson, 1995).

CONCLUSIONS

To conclude, the higher proportion of acrosomal abnormalities in the ejaculates of the Jersey bulls may be due to impaired spermiogenesis, resulting possibly of genetic cause. Cryopreservation compromised acrosomal integrity and caused an increase in defects in this structure. More studies should be conducted to ensure cryopreservation protocols that preserve the integrity of the acrosome.

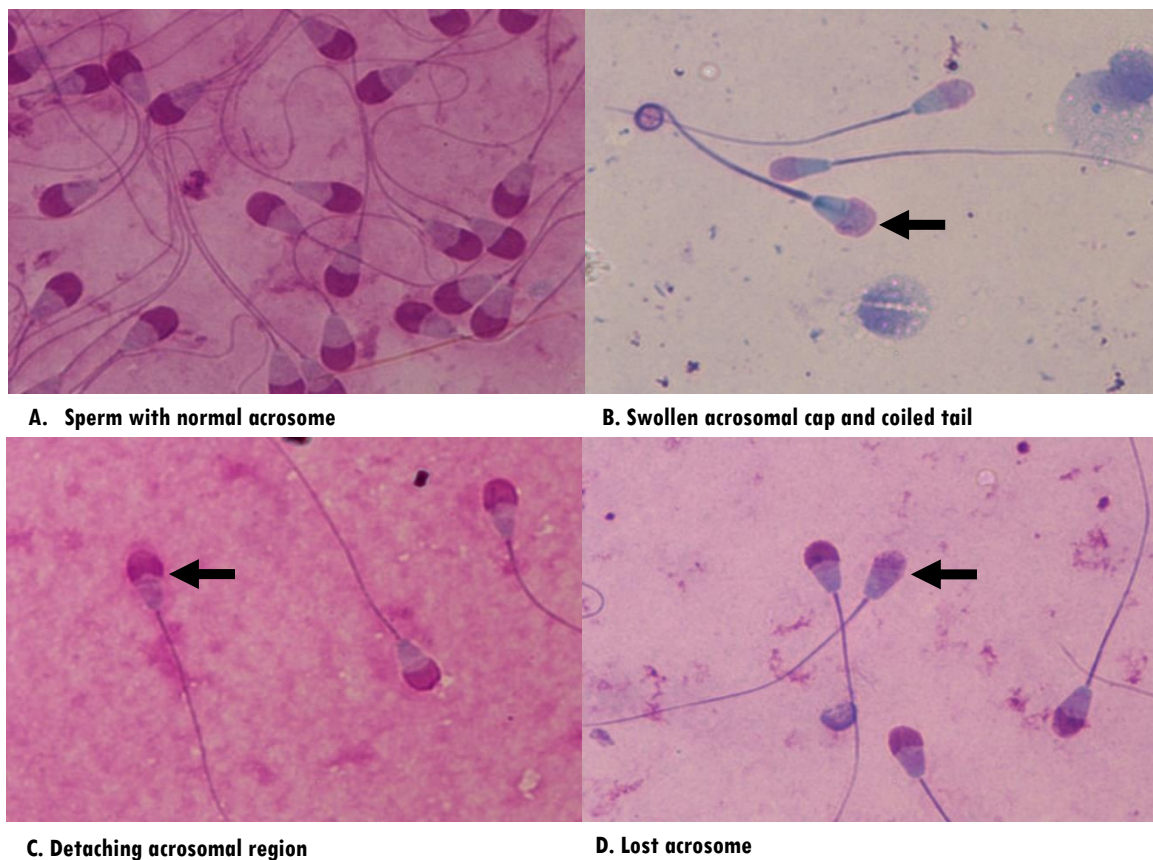


Figure 1. Different acrosomal abnormalities in Jersey Bulls

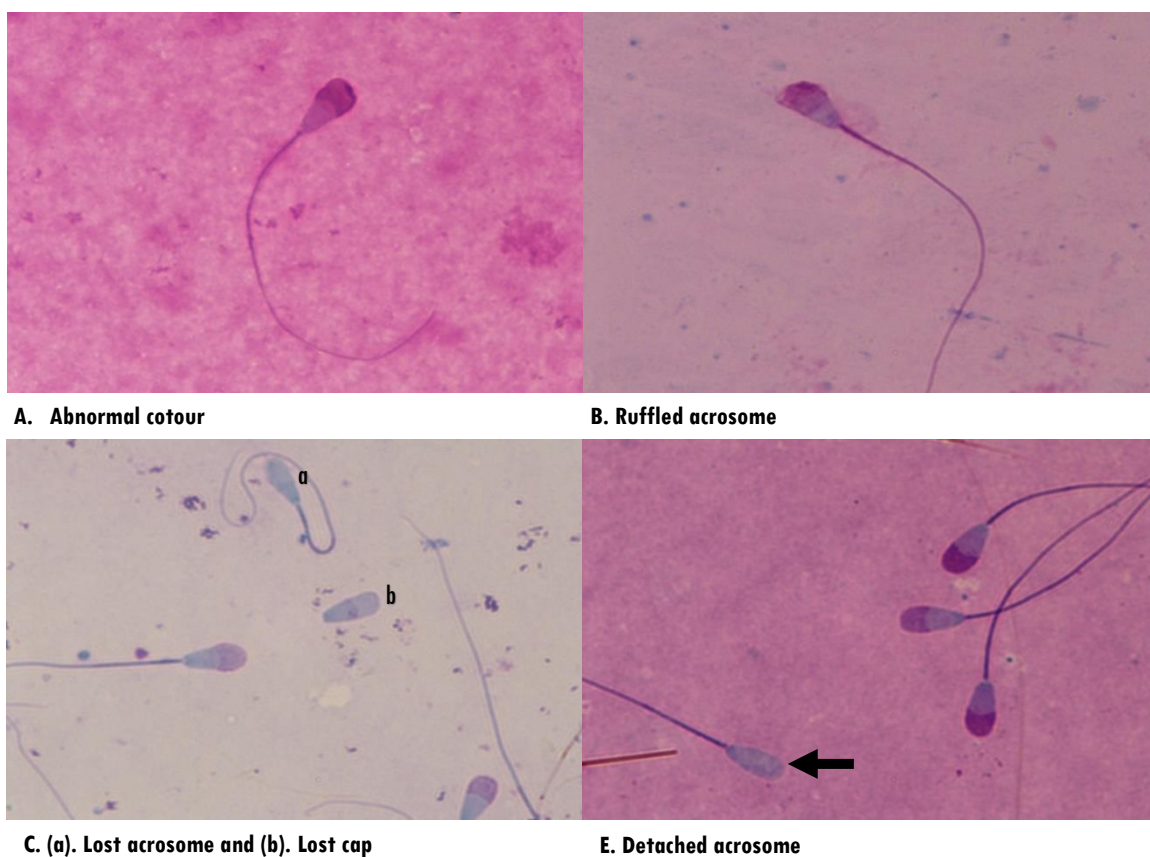


Figure 2. Different acrosomal abnormalities in Jersey Bulls

REFERENCES

- Ahmed H, Andrabi SMH, Shah SAH, Jahan S. 2019. Effect of cryopreservation on CASA characteristics, mitochondrial transmembrane potential, plasma and acrosome integrities, morphology and in vivo fertility of buffalo bull spermatozoa. **Cryo Letters** 40(3):173-180
- Blom E. 1964. The galea capitis as part of the acrosome cap in the bull sperm. **5th International Congress Animal Reproduction A.I. Trento**, pp. 656-660.
- Chenoweth PJ. 2005. Genetic sperm defects. **Theriogenology** 64: 457.
- Esteves SC., Sharma RK., Thomass AJ., Agarwal TA. 2007. Evaluation of acrosomal status and sperm viability in fresh and cryopreserved specimens by the use of fluorescent peanut agglutinin lectin in conjunction with hypo-osmotic swelling test. **International Brazilian Journal of Urology** 33: 364-376.
- Hough SR., Kaproth MT., Foote RH. 2002. Incidence of the acrosome reaction and zona free hamster oocyte penetration by a bull with complete tetraspermia versus a half-brother with normal sperm. **Journal of Andrology** 23: 98-106.
- Kumar U, Gawande AP, Sahatpure SK, Patil MS, Lakde CK, Bonde SW, Borkar PL, Poharkar AJ, Ramteke BR. 2015. Assessment of semen quality in pure and crossbred Jersey bulls. **Veterinary World** 8(10): 1266-1272.
- Neild DN., Gabela BM., Agüero A., Stout TAE., Colenbrander B. 2005. Capacitation acrosome function and chromatin structure in stallion sperm. **Animal Reproduction Science** 89(1-4): 47-56.
- Pant HC. 2000. In: **Morphological abnormalities in buffalo and cattle spermatozoa: relation to bull fertility**, National Dairy Development Board, Anand Press, Anand, India.
- Rubio-Guillén J., Quintero-Moreno, A., González-Villalobos, D. 2009. Efecto de la criopreservación sobre la integridad de la membrana plasmática y acrosomal de espermatozoides de toros. **Revista Científica FCV-LUZ**. XIX:382-389.
- Sacke RG., Amann RP., Marshall CE. 1968. Acrosomal cap abnormalities of sperm from sub-fertile bulls. **Journal of Animal Sciences** 27: 1391-1400.

Sundararaman MN., Gopinathan A., Subramanian A. 2014. Dag-like sperm defect and acrosome abnormalities indicating hereditary sperm defects in a Jersey bull. **Indian Journal of Animal Reproduction** 35: 27-30.

Watson PF. 1995. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. **Reproduction Fertility and Development** 7: 871-891.

Yániz JL, Soler C, Alquézar-Baeta C, Santolaria P. 2017. Toward an integrative and predictive sperm quality analysis in *Bos taurus*. **Animal Reproduction Science** 181:108-114. doi: 10.1016/j.anireprosci.2017.03.022.