Genetics & Biodiversity Journal
Journal homepage: https://journals.univ tlemcen.dz/GABJ/index.php/GABJ
ISSN: 2588-185X

Original Research Paper

Effect of post-mortem testicular storage on epididymal sperm motility and viability in the Berber breed in eastern Algeria

OUENNES Houria[1.2], SAHI Sameh[2.3], AFRI-BOUZEBDA Farida [1.2], BOUZEBDA Zoubir[2]

[1]*Institute of Agro-Veterinary Sciences, Department of Veterinary Sciences. University Mohamed Cherif Messaadia Souk Ahras, 41.000.Algérie.*

[2] *Laboratory of Animal Production, Biotechnology and Health. Institute of Veterinary Sciences. University Mohamed Cherif Messaadia ,Souk Ahras, 41.000.Algérie.*

[3] *Chadli Bendjedid University of El Tarf. BP: 73, El Tarf 36000, Algeria.*

***Corresponding Author**: Ouennes Houria. Institute of Agro-Veterinary Sciences, Department of Veterinary Sciences. University Mohamed Cherif Messaadia Souk Ahras, 41.000.Algérie; Email: h.ouanes@univ-soukahras.dz

Article history: Received: November 23th 2022, revised: February 27th 2024 Accepted: March 7th 2024.

Abstract

The use of epididymal sperm is a new technique that can be used for artificial insemination or in vitro fertilisation in sheep in order to save breeds in decline. The objective of our work is to study the effect of storage after slaughter for 2h and 24h at a refrigeration temperature (5°c) on the mobility and viability of epididymal spermatozoa collected from 60 rams with an average age of 8-12 months, slaughtered a few hours ago, recovered from the slaughterhouse of Annaba. The results of the spermatic characteristics after preservation of the testicles 2 h and 24 h post-slaughter, do not show significant differences ($p > 0.05$). Without alteration of sperm quality. In rams, the refrigeration temperature (5°C) of the spermatozoa allows a very good quality of epididymal sperm to be maintained for up to 24 hours after death before freezing.

Key words: Algeria, Berber ram, Preservation, Epididymal sperm

الملخص

يعد استخدام الحيوانات المنوية البربخية تقنية جديدة يمكن استخدامها للتلقيح الاصطناعي أو الإخصاب في المختبر في الأغنام من أجل إنقاذ السلالات في طريق الانقراض. الهدف من عملنا هو دراسة تأثير التخزين بعد الذبح لمدة ساعتين و 24 ساعة عند درجة حرارة التبريد (5 درجات مئوية) على حركة وصلاحية الحيوانات المنوية البربخية التي تم جمعها من 60 كباش بمتوسط عمر 8-12 شهرًا ، مذبوحة قبل ساعات قليلة شفيت من مسلخ عنابة. نتائج خصائص الحيوانات المنوية بعد الحفاظ على الخصيتين بعد ساعتين و 24 ساعة بعد الذبح لم تظهر فروق نتائج الخصائص المنوية بعد حفظ الخصيتين بعد ساعتين و 24 ساعة بعد الذبح، لا تظهر فروقاً كبيرة .($p > 0.05$) دون تغيير في جودة الحيوانات المنوية. في الكباش، تسمح درجة حرارة التبريد (5 درجات مئوية) للحيوانات المنوية بالحفاظ على نوعية جيدة جداً من الحيوانات المنوية البربخية لمدة تصل إلى 24 ساعة بعد الوفاة قبل التجميد.

الكلمات المفتاحية: الجزائر، البربر الحمل، الحفظ ، الحيوانات المنوية البربخية

Introduction

In Algeria, sheep farming is considered the most important sub-sector of animal production (MADR, 2017). It is characterised by very high potential, particularly due to the diversity of breeds (Djaout *et al.*, 2017; Abdelkader *et al.*, 2018), and the importance of its qualities of adaptation to the modes of management of farms (Benyoucef *et al.*, 2000). With regard to reproduction, one of the most important aspects remains, to date, very underdeveloped (Smith *et al.*, 2018), as breeders generally use the natural control method (Deghnouche *et al.*, 2017). Epididymal sperm harvesting is one of the most current methods and consists of collecting semen directly from the epididymis in order to obtain fertile spermatozoa for the various reproductive techniques (artificial insemination, In vitro fertilization and Intracytoplasmic injection).

Multiple observations have shown that the epididymal medium is an excellent storage medium (Guérin *et al*., 2003; Rizal, 2009; Martınez-Pastor *et al*., 2005; Abdel-Khalek *et al*., 2009; Khalil, 2009; Karja *et al.*, 2010). Its composition is complex and results from the reabsorptive and secretory activity of the epithelium of this organ. Many proteins present in this medium are probably linked to the protection of gametes, particularly against peroxidation (Guérin *et al.*, 2003). Epididymal sperm in

rams is extremely resistant to various cryobiological stress conditions than ejaculated sperm, which is more sensitive to stress factors such as cold cooling.

This work aims to propose an easy to implement method of using semen from sheep which are difficult to collect from injured or sacrificed animals, by studying the effect of preservation on the fertilizing power of epididymal spermatozoa harvested after 2h of slaughter with that harvested after preservation in situ at 5° C for 24 h with the aim of their potential use in artificial insemination or in vitro fertilization.

Materials and Methods

The study involved 60 Berber rams with an average age of 8-12 months; the gonads were collected after slaughter from the slaughterhouse in Annaba (eastern Algeria), transported in preservation bags in a cooler at 18°C, and taken to the laboratory according to the method of Khalil Rezk (2009). The gonads of the same ram were examined 2 h after collection, for one of the gonads and the other was treated after 24 h preservation at 5°C.

One of the two testes is dissected out of its tunica vaginalis and washed with tap water (Abdel-Khalek *et al.,* 2009), the other is preserved for later observation in 5° storage bags. The technique used consists of isolating the epididymis, wiping it, and carefully drying the surface of the tail (Martınez-Pastor *et al.,* 2005; Tamayo-Canul *et al.,* 2011).

The epididymis is placed in a 60 *mm* petri dish and the tail of the epididymis is incised under aseptic conditions (Barati *et al.,* 2009).

Several longitudinal incisions were made on the distal end of the tail, to extract the sperm into the external environment and sometimes apply slight pressure (Garcia-Alvarez *et al.,* 2009). The epididymis tail is rinsed by spraying with 3 *ml* of saline solution (0.9%), using a *5 ml* syringe. The incubation at 37°C was performed in the oven over the entire sample for 15 *minutes*. The tissue is removed and the remaining medium is centrifuged at 3000 rpm for 8 minutes. The supernatant is removed and the pellet analysed (Julienne Posière, 2002).

A drop of sperm was observed by light microscopy at low magnification (×10) to assess motility (scale 0-5). Ranked from 0 to 5 (0 = no movement, 5 = mass motility). The percentage of live sperm was assessed after staining with eosin (1%) and nigrosin (10%).

Statistical analysis

Statistical analysis was carried out using the equality of variance study (95% confidence intervals for standard deviations), to compare the different sperm parameters, 2h and 24h (storage at 5°C) after slaughter.

Results and Discussion

Sperm motility and viability are considered important factors in determining the quality of sperm ejaculate and therefore fertility of a ram (Martí *et al.,* 2011; Martí *et al.,* 2012; David *et al.,* 2015).

Motility

The motility varied between 3.34± 0.12 (2 h) and 2.87 ± 1.34 (24 h); this parameter tended to decrease with time (Table 1). The comparison between the values obtained at the different periods does not reveal a significant difference ($p > 0.05$). This means that the ejaculates produced by the rams were of high quality (Benhenia et al., 2016). This explains the good protection of spermatozoa by the epididymal fluid. Namely, epididymal fluid is composed of organic (proteins, glucose and triglycerides) and non-organic (mainly minerals: K, Na, Mg, Ca and), which allows sperm survival (Hajirezaee *et al.*, 2009).

Table 1. Variation in mass motility of epididymal sperm stored for 24 h at 5°C.

Storage time (h)	2	24	Sig
Motility (score)	3.34± 0.36	2.87± 1.34	$p > 0.05$

Knowing that motility is the most affected, the membrane and acrosomal integrity seemed to withstand the post-mortem conditions better (Martınez-Pastor *et al.,* 2005). Indeed, sperm motility

is a very important indicator of the individual reproductive potentialities of the ram. Prolonged preservation of the epididymis leads to a severe decrease of the epididymis pH, knowing that there is a correlation between motility and the pH of the epididymis lumen, and also a pycnosis of the epithelial cells of the epididymis which influences the quality of the semen after storage of the latter (Barati *et al.*, 2009).

The results obtained are in agreement with those obtained by Belkadi *et al.* (2013), who found a decrease in motility of 4.55 ± 0.12 (0 h) and 3.13± 0.06 (72 h), while the average percentage is 50% after 72 h. Allaoui (2019) found in the Ouled Djellal breed, a proportion of actively motile spermatozoa evaluated with a computer-assisted semen analyser generally ≥ 70%. Our results are also comparable to those of Guérin *et al.* (2003), who preserved spermatozoa in the intact epididymis or in vitro at 4°c for 24, 48 and 72h.

Kaabia *et al.* (2003) used ram sperm collected from the testis and stored at 5°C for 3 days for AI and in vitro embryo production. However, Martınez-Pastor *et al.* (2005) showed that the deleterious effect of storage at 5°C appeared to be acceptable in deer, but only for 2 days, and that undiluted epididymal sperm in 1.5 *ml* tubes, closed and stored at 4°C, and after 24 h, 48 h and 72 h, showed no significant difference in motility. In contrast, Karja *et al.* (2011), concluded that viability and in vitro fertilisation decreased with increasing time between death and semen collection (up to 40 h at room temperature) in deer (*Cervus elaphus*) and mouflons (*Ovis musimon*).

Nevertheless, Barati et al. (2009) have shown that mobile sperm can be collected after 24 h and even after 72 h when stored in cold (4°c) epididymis or in undiluted tubes in bulls (Bubalus bubalis). Testicular storage at (4.9 - 6) °C for up to 72 h gave excellent results for different sperm parameters (motility, concentration, abnormal spermatozoa ...) (Lone et al., 2011).

Vitality

The vitality rate decreases with time varying from 75± 4.08% at 0 h to 60±1.67% at 24 h (Table 2). The mean percentage of live and dead sperm collected 2 h after slaughter and after 24 h of storage at 5°c did not show significant difference (*p> 0.05*). While some semen samples showed sperm viability up to 48 h after slaughter, although their quality decreased significantly with increasing post-mortem time (Karja *et al.*, 2010).

Table 2. Variation in mass motility and viability (%) of epididymal sperm from testes preserved for 24 hours at 5°C.

Storage time (h)	2	24	Sig
% Sperm viability	75 ±4,08	60±1,67	*p> 0.05*

The majority of anomalies involve the flagellum, which is either folded or rolled up. Tibary *et al.* (2018), showed that the main abnormalities often missed by practitioners are those of the head (vacuoles, diadems), acrosome defects (folded or embedded) and the presence of other cells (spheroid cells, medusa cells). However, for Belkadi *et al.* (2015), the percentage of abnormal sperm is inversely proportional to age. For Haye *et al.* (2004), the specific anomalies most frequently encountered in Djallonké rams are decapitated spermatozoa, the anomaly rate is 2.65 ± 0.92% in young and 2.37 ± 0.92% in old rams, for an overall average of 5.00 ± 1.46%.

In citing the age factor, do not forget to recall that the average age of rams is 8-12 months, knowing that the semen of older rams is of better quality than that of younger rams. There is also a correlation between age and spermatogenetic activity at the level of seminiferous tubules, this is revealed by the relationship between weight and testicular diameter with sperm concentration by Boussena *et al.* (2014) and Belkhiri *et al.* (2017) in Ouled Djellal ram and Sahi *et al.* (2019) in the local billy goat, who indicated that scrotal circumference measurements increase with age, providing a valuable and useful estimate of testicular growth.
It is necessary to mention that the genetic potential and adaptability (Rashamol *et al.*, 2018) of each animal play a more important role than other environmental factors in determining reproductive performance and seminal production (Martin *et al.*, 2010). An excellent ram is one that meets

stricter requirements for scrotal circumference, motility and morphology, it should have excellent health, scrotal circumference of more than 33 for less than 14 months of age, semen morphology≥ 90% normal and semen motility > 50% progressive (Tibary et *al.,* 2018).

Conclusion

In sheep, the collection of epididymal sperm from animals intended for slaughter is an additional means of preserving genetic material from the progeny of pre-selected breeding stock and provides freezable, fertilizing gametes. In the Berber ram, the survival and fertility of epididymal sperm stored at 5°C is remarkable and illustrates the degree of protection of the epididymal environment.

Acknowledgments

We gratefully acknowledge all the butchers and staff of the Annaba slaughterhouse.

Reference

Ameur Ameur A., Ata, N., Benyoucef, M.T., Djaout, A., Azzi, N., Yilmaz, O., Cemal, İ., Gaouar, S.B.S. **2018**. New genetic identification and characterisation of 12 Algerian sheep breeds by microsatellite markers. *Italian Journal of Animal Science* 17, 38-48.

Abdel-Khalek A-E, El-Harairy M-A, Shamiah SH-M, Abu El-Hamd M-A et Khalil W-A. 2009. Some physical characteristics and response to hypo-osmolaity levels of epididymal camel spermatozoa stored at 25° C or 5° C. *Prod. Dept fac. agric, Mansoura Univ. Egypt.anim. Prod. Res. Institute, Egypt, p (8, 34, 57,66, 86).*

Allaoui A. 2019. Study on some factors of variation in the fertility of Ouled Djellal breed breeding rams. PhD thesis in veterinary medicine. University of Batna. Algeria.

Barati F-I, Khaksary H, Mahabady M-E et Mohammadi GH, 2009. Cryopreservation and thawing procedures- Cryopreservation of *in situ* cool stored buffalo (*Bubalus bubalis*) epididymal sperm. *Iranian Journal of Veterinary Research, Shiraz University)*, Vol. 10, 4, Ser 29, 2009, p 339.

Belkadi S., Alloui N., Aissi A., Belkacem L., Aissi A., Safsaf B., Djaaba M., Idir K. 2013. Study of the survival and mobility of the epididymal spermatozoïdes preserved at 4°c in Ouled Djellel rams. *Renc. Rech. Ruminants,* 20.

Belkadi S., Alloui N., Aissi A., Belkacem L., Safsaf B., Idir K. 2015. Influence of age on epididymal sperm parameters and testicular measurements of the Ouled Djellal Algerian sheep breed. *Renc. Rech. Ruminants,* 22.

Belkhiri, Y., Bouzebda, Z., Mouffok, C. 2017. Age and Season Effects on Sexual Parameters in Mature Rams Used in Artificial Insemination Centre (Algeria). *Global Veterinaria*. 18, 31- 40.

Benhenia, K., Lamara, A., Fatmi, S., Iguer-Ouada, M. 2016. Effect of cyclodextrins, cholesterol and vitamin E and their complexation on cryopreserved epididymal ram semen. *Small Ruminant Research* 141, 29-35.

Benyoucef, M.T., Madani, T., Abbas, K., 2000. Systèmes d'élevage et objectifs de sélection chez les ovins en situation semi-aride algérienne, in: Gabiña, D. (Ed.), Analysis and definition of the objectives in genetic improvement programmes in sheep and goats. *An economic approach to increase their profitability, Zaragoza: CIHEAM*, pp. 101-109.

Boussena S., Aimeur, R., Hireche, S., Bouaziz, O., Tainturier, D. 2014. Control of reproductive performance (bodily, testicular and spermatic) in Ouled Djellal breed antennae from puberty to breeding. *Journal of veterinary medicine* 165, 289-296.

David, I., Kohnke, P., Lagriffoul, G., Praud, O., Plouarboué, F., Degond, P., Druart, X. 2015. Mass sperm motility is associated with fertility in sheep. *Science of animal reproduction* 161,75, 81.

Deghnouche, K., Aissaoui, M., Toufik, M., Majid, T. 2017. Reproductive performance of Ouled Djellal ewes in arid area of Algeria. Journal of new sciences, *Agriculture and*

Biotechnology, CSIEA 29, 2815-2819.

Djaout, A., Afri-Bouzebda, F., Chekal, F., El-Bouyahiaoui, R., Rabhi, A., Boubekeur, A., Benidir, M., Gaouar, S. 2017. Status of the biodiversity of Algerian sheep "breeds". *Genetic And Biodiversity Journal* 1, 11-26.

Garcıa-Alvarez O, Maroto-Morales a, Martınez-Pastor F, Garde J-J, Ramon M, Fernandez-Santos M-R, Esteso M-C, Perez-Guzman M-D ET Soler A-J, 2009.Sperm characteristics and in vitro fertilization ability of thawed spermatozoa from Black Manchega ram: Electro ejaculation and postmortem collection. *Theriogenology* (72), 60-168.

GuérinY, Locatelli Y, Comizolli P, Mauget R, Mermillod P, Xavier Legendre X, Jean-Luc Gatti J-L and Jean-Louis Dacheux J-L. 2003. Conservation and use of epididymal sperm from sheep and deer in artificial insemination and in vitro fertilisation. *Les Actes du BRG*, (4) 173- 183.

Hajirezaee S., Bagher M., Amiri, A. and Reza M. 2009. Effects of Stripping Frequency on Semen Quality of Endangered Caspian Brown Trout, Salmo trutta caspius. American Journal of Animal and Veterinary Sciences, 4 (3): 65-71.

Haye A., M'Betiegue C., Nazaire L.G. and Tanon B. 2004. Evaluation of the sperm quality of Djallonke rams in the humid savannah region of Côte d'Ivoire. *Agronomie Africaine* 16 (3): 37 - 46.

Julienne Posière S-C-G. 2002. Collection of cat *(felis catus)* semen by electro ejaculation and epididymal dissection. Comparison of the methods, cryopreservation trial with a canine dilutor. Thesis for the veterinary doctorate. National Veterinary School of Alfort. 31-34.

Kaabia M, Pazb P, Alvareza M, Anela E, Boixoa J-C, Rouissic H, Herraezb P et Anela L. 2003. Effect of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. *Theriogenology* (60), 249-1259.

Karja N-W-K, Respaty E-M-A, Nuraini1 I, Prihatno S-A and Gustari S. 2010. Characteristic of frozen-thawed epididymal spermatozoa and refrigerated storage of ram spermatozoa, J.Indonesian Trop.Anim.Agric. 35(1), 63-67.

Khalil Rezk W-A. 2009. Studies on In Vitro Fertilization in Camels (Camelus dromedaries) B.Sc. Agric. Sci, Animal Prod, Mansoura University, 2001M.Sc. Agric. Sci., Animal Prod. (Anim. Physiol); THESIS, submitted in Partial Fulfillment of the Requirements for The Degree of Doctor of Philosophy in Agriculture Sciences (Animal Production- Animal Physiology) Researcher of Biotechnology Dept. *Animal Production Research Institute*, 14-23.

Lone F-A, Islam R, Khan M-Z et Sofi K-A. 2011. Effect of transportation temperature on the quality of cauda epididymal spermatozoa of ram division of animal reproduction, gynecology and obstetrics. Animal Reproduction Science (123), 54-59.

MADR. 2017. Ministry of Agriculture and Rural Development. Agricultural Statistics (Areas and Productions Serie B). Algeria.

Martí, J., Aparicio, I., García-Herreros, M. 2011. Sperm morphometric subpopulations are differentially distributed in rams with different maturity age in cryopreserved ejaculates. Theriogenology 76, 97-109.

Martí, J., Aparicio, I., Leal, C., García-Herreros, M. 2012. Seasonal dynamics of sperm morphometric subpopulations and its association with sperm quality parameters in ram ejaculates. Theriogenology 78, 528-541.

Martin, G.B., Blache, D., Miller, D.W., Vercoe, P.E. 2010. Interactions between nutrition and reproduction in the management of the mature male ruminant. *Animal: an international journal of animal bioscience* 4, 1214-1226.

Martınez-Pastor F., Guerra C., Kaabi M., Diaza A.R., Anel E., Herraez P., De Paz P and Anel L.2004. Decay of sperm obtained from epididymes of wild ruminants depending on postmortem time, Cell Biology and Anatomy, and Animal Reproduction and Obstetrics,

University of Le'on, Le'on, Spain, Cap'Le´on 10. Enviado a Theriogenology. 120-140.

Rashamol, V.P., Sejian, V., Madiajagan, B., Krishnan, G., Archana, P., Bhatta, R.2018. Physiological adaptability of livestock to heat stress: an updated review. *J Anim Behav Biometeorol* 6, 62-71.

Rizal M.2009. Viability of Bali bull epididymal spermatozoa preserved at 3-5oC in Tris extender with different lactose concentrations. JITV 14(2), p (142-149).

Sahi S., Afri-Bouzebda F., Bouzebda Z., Ouennes H. and Djaout A.2019. Testicular biometry and its relationship with age and body weight of indigenous bucks (Algeria). *Advance. Animal. Vetenaria. Sci.* 7 (10): 882-887.

Smith, M.F., Geisert, R.D., Parrish, J.J. 2018. Reproduction in domestic ruminants during the past 50 yr: discovery to application. Journal of animal science 96, 2952-2970.

Tamayo-Canula J-b, Alvareza M-B, López-Uruenaa E-B, Nicolasa M, Martinez-Pastora B, Anela F, Anela c et Paza C. 2011. Undiluted or extended storage of ram epididymal spermatozoa as alternatives to refrigerating the whole epididymes, Contents lists available at Science Direct. *Animal Reproduction Science* (126), 76-82.

Tibary A., Boukhliq R., El Allali K. 2018. Review of reproductive fitness in rams and bucks. *Rev. Mar. Sci. Agron. Vet:* 6 (2):241-255.