Quality of European red deer spermatozoa stored in the epididymides and in a liquid state at 5 degrees Celsius

NICOLETTA MAGDALENA NEUMAN

ANNA DZIEKOŃSKA

UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN, POLAND

FACULTY OF ANIMAL BIOENGINEERING

DEPARTMENT OF ANIMAL BIOCHEMISTRY AND BIOTECHNOLOGY

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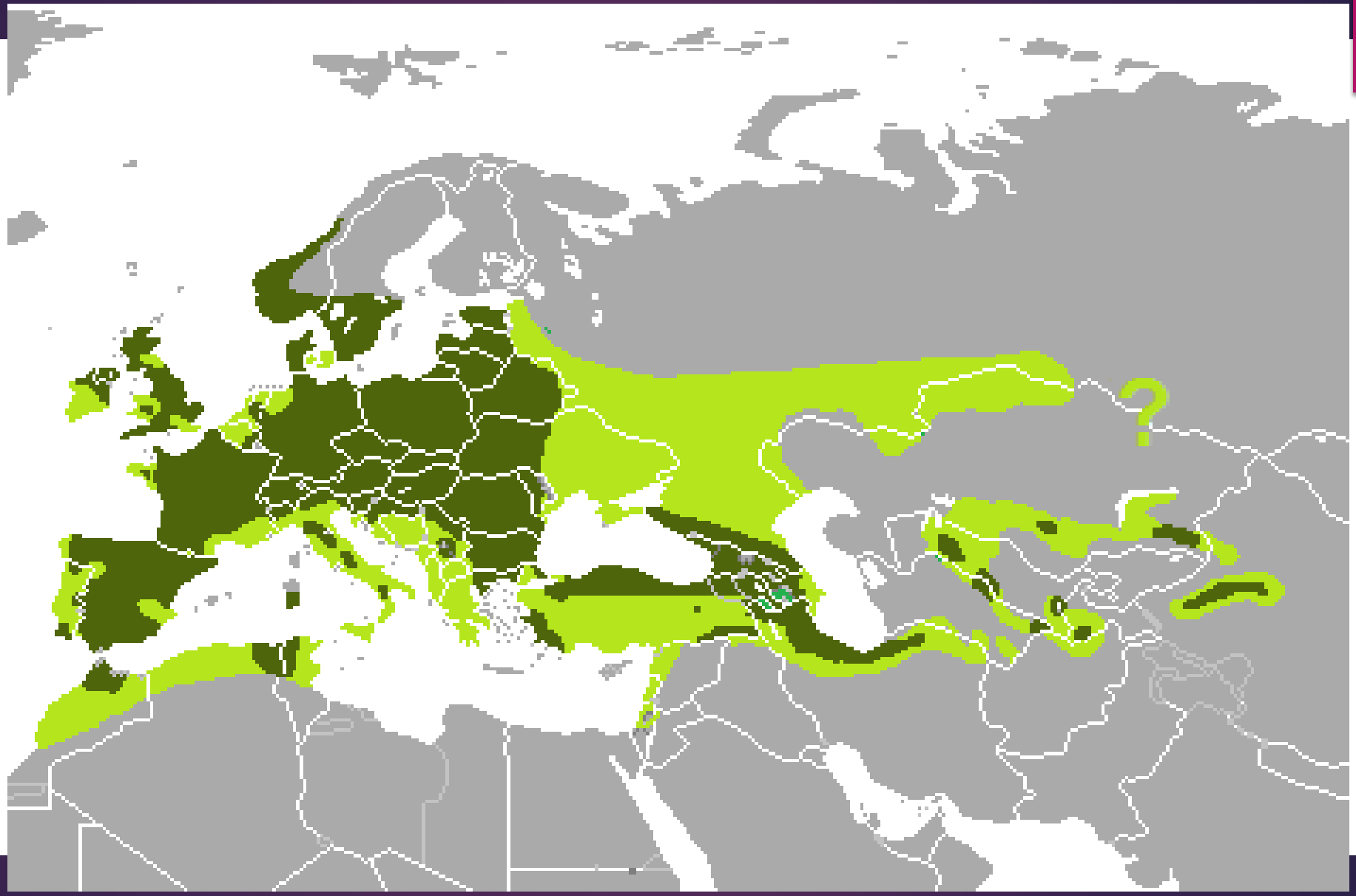


https://pl.wikipedia.org/wiki/Jele%C5%84_szlachetny#/media/Plik:Red_deer_(Cervus_elaphus)_reconstructed_and_recent.png

- Ruminant animals
- Semen is collected in September and October

https://www.lasy.gov.pl/pl/edukacja/lesnoteka-1/ssaki/jelen-szlachetny

- Ejaculated semen (collected by electroejaculation or using an artificial vagina) is best for preservation, but such semen samples are difficult to obtain from free-living animals. Epididymal sperm collected post-mortem may also be used for this purpose. Despite some differences in characteristics between epididymal sperm and ejaculated sperm, which concern, among other factors, the motility and biochemical composition of their membranes, epididymal sperm retain the ability to fertilize and can be used in reproduction.

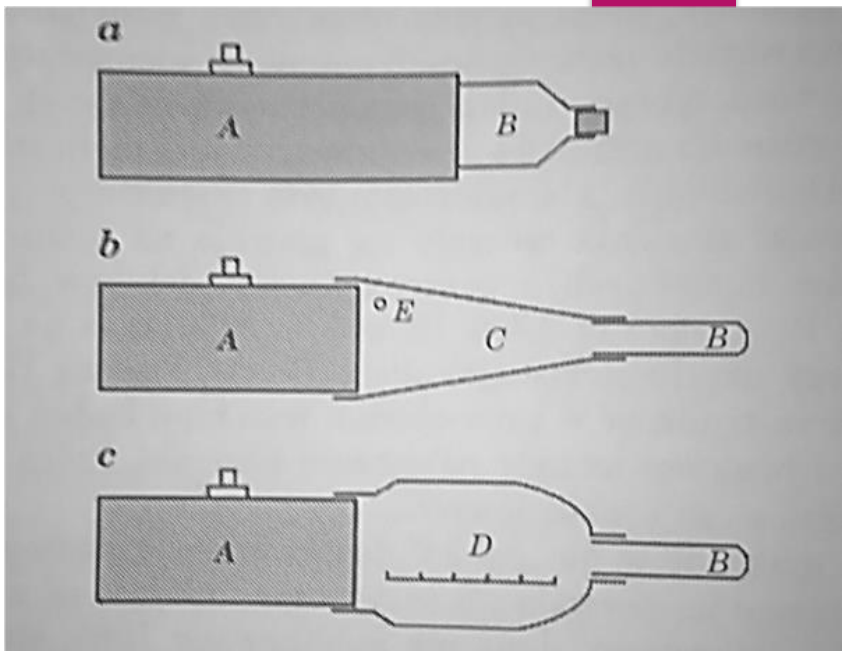
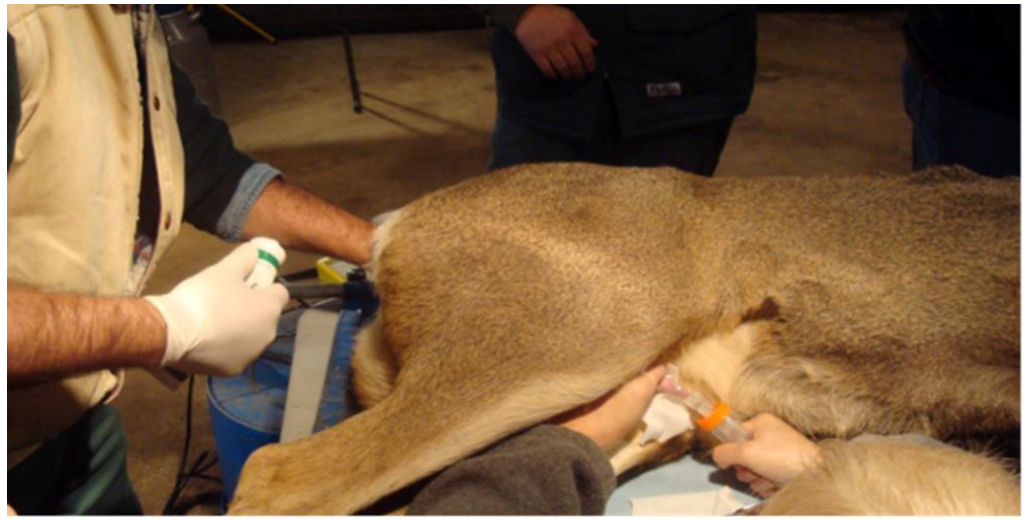
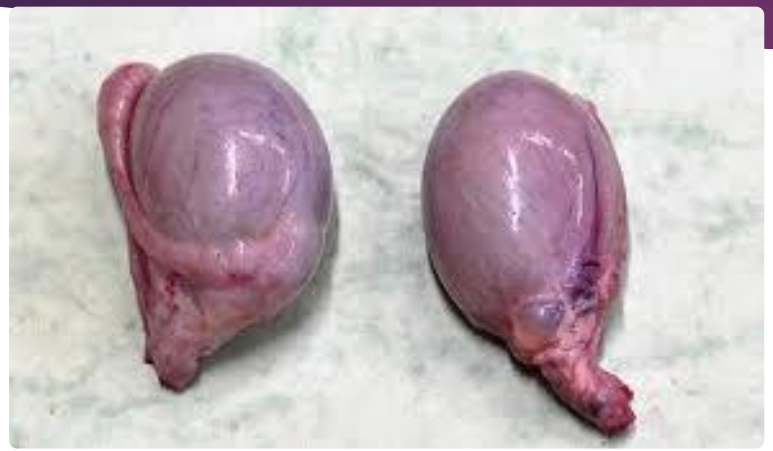


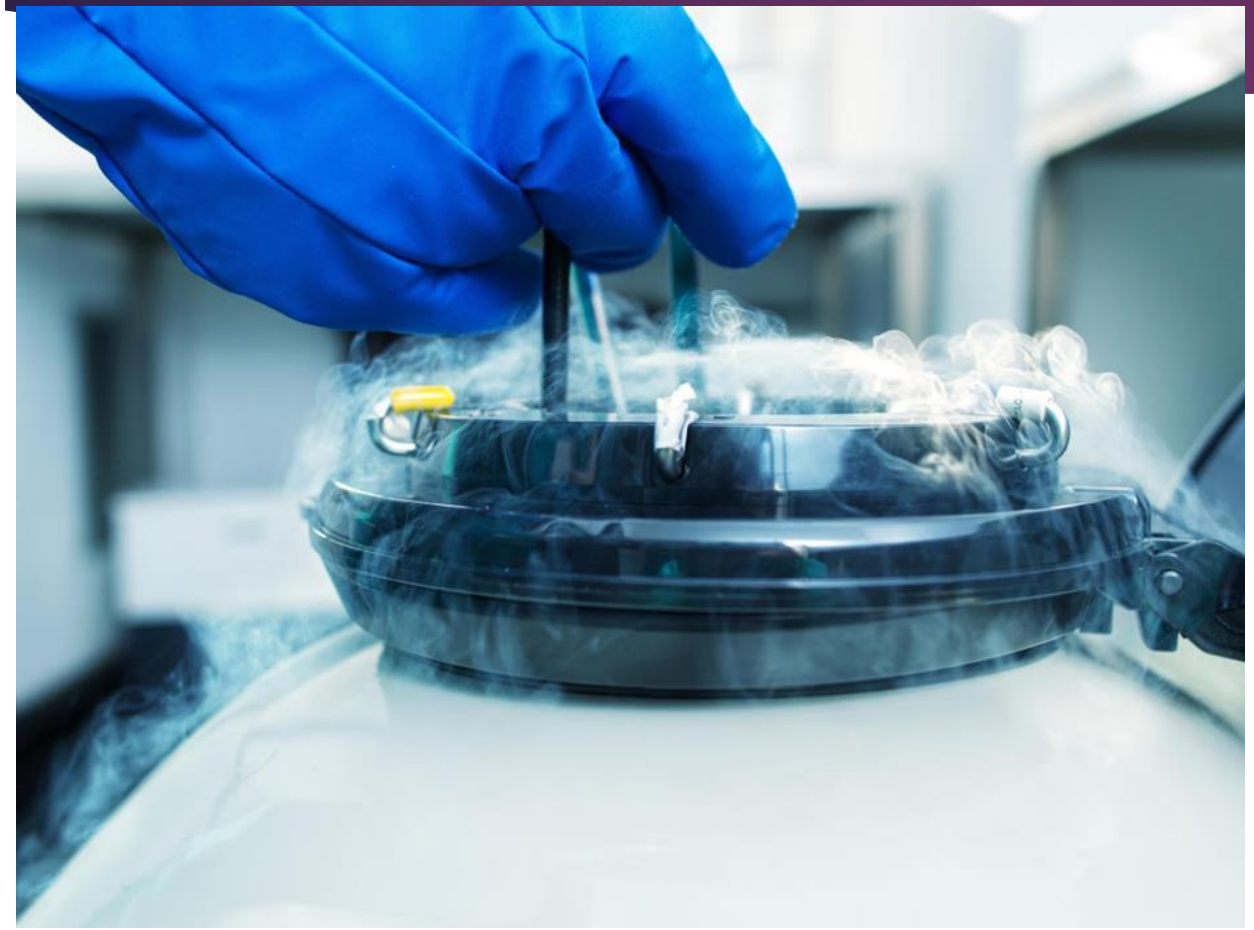
Diagram of the artificial vagina for collecting deer semen: a - original design, b - modified, c - for observing the course of ejaculation; A - shaft, B - semen collector, C - rubber funnel, D - transparent plastic graduated bulb, E - vent. Giżejewski 2002.



http://www.louisianacattleanddeervet.com/newsletter/september2015-newsletter-white-tailed-deer/



- Epididymal sperm collected postmortem, like ejaculated sperm, can be stored in a frozen state (in liquid nitrogen, -196°C), in a liquid state (at a reduced temperature at 5°C) and can also be stored in the epididymis (up to several hours, preferably also at reduced temperature).



Use of liquid nitrogen method is not always possible due to:

- ▶ Technical limitations
- ▶ Economical limitations
- ▶ More time-consuming
- ▶ Reduced fertilizing ability

https://static.independent.co.uk/s3fs-public/thumbnails/image/2018/08/05/10/sperm-storage.jpg

The aim of research

- The aim of the study was to compare the biological properties of European red deer sperm stored in the epididymis and in a liquid state at a temperature of 5°C.

- Assessment of biological properties of stored sperm included assessment of motility, viability and integrity of acrosomal membranes, apoptotic-like changes, mitochondrial activity and DNA integrity.

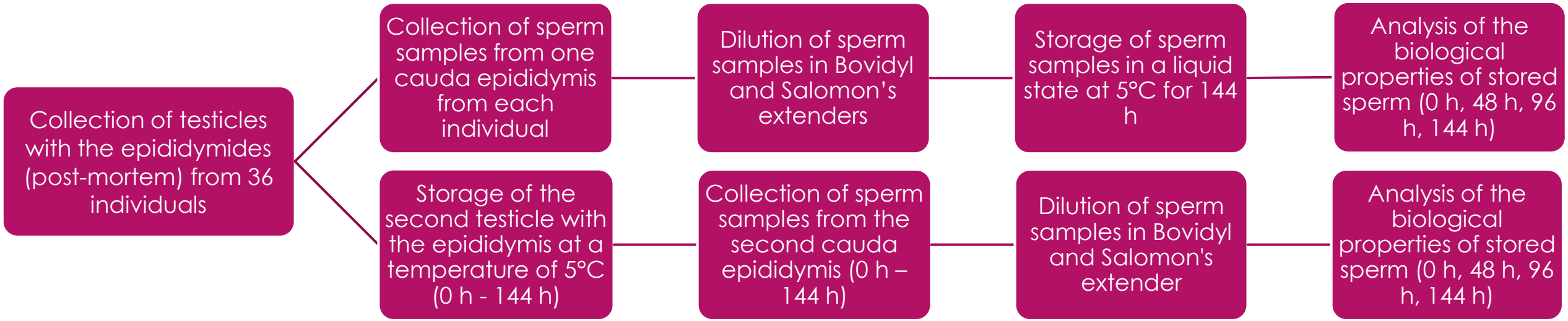
- Epididymal sperm samples were collected postmortem from 36 European red deer stags. The animals were shot during legal hunts conducted in Warmia and Mazury in the rutting season throughout September and October. The hunts were conducted in accordance with hunting laws and wildlife management regulations.
- The material (testicles and epididymis stored in the scrotum sack) was collected from hunted stags and transported to the laboratory. Sperm samples were collected from the cauda of the epididymis by cutting it several times with a scalpel and then squeezing the liquid contents into an Eppendorf tube.

Animal Reproduction Science, 213, 106269. doi:10.1016/j.anireprosci.2019.106269

- After standard analysis, sperm samples were divided into two parts and diluted in two extenders:
  - Bovidyl® (commercially available)
  - Salomon's (containing TRIS (0.22M), citric acid (0.07M) fructose (0.05M) and clarified egg yolk (20% v/v).

- The final sperm concentration in the sample was 100 x 10^6 sperm/mL.

Ist variant



2nd variant

Motility analysis

- The analysis of motility was performed using the CASA system and the Hamilton Thorne IVOS v. 12.3 motion analyzer. The evaluation involved software settings that were recommended by the manufacturer for analyses of gazelle/deer sperm.
- To assess motility, each stored sperm sample was diluted 1:4 in phosphate-buffered saline to a concentration of 20–30 × 10^6 sperm/ml. The diluted samples were incubated at 37°C for 5 min. Aliquots of each sperm sample were placed in the Makler counting chamber of the CASA system. Approximately 1,000 sperm were examined in two repetitions.

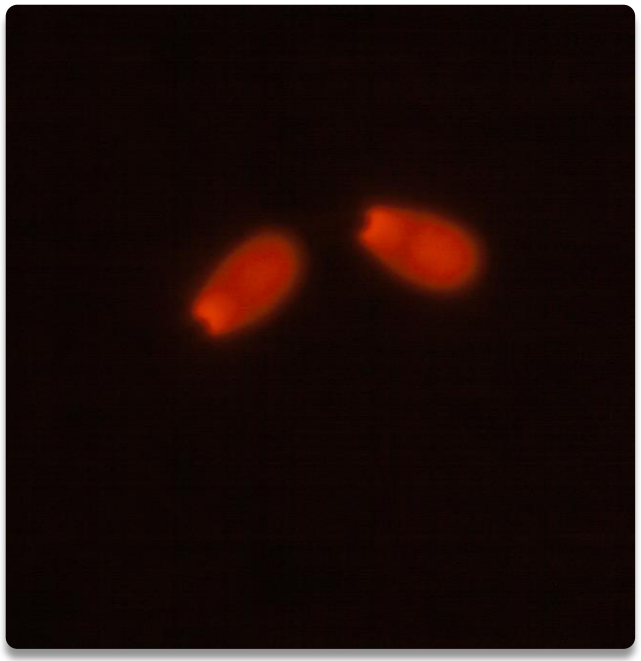
Fluorescence analysis

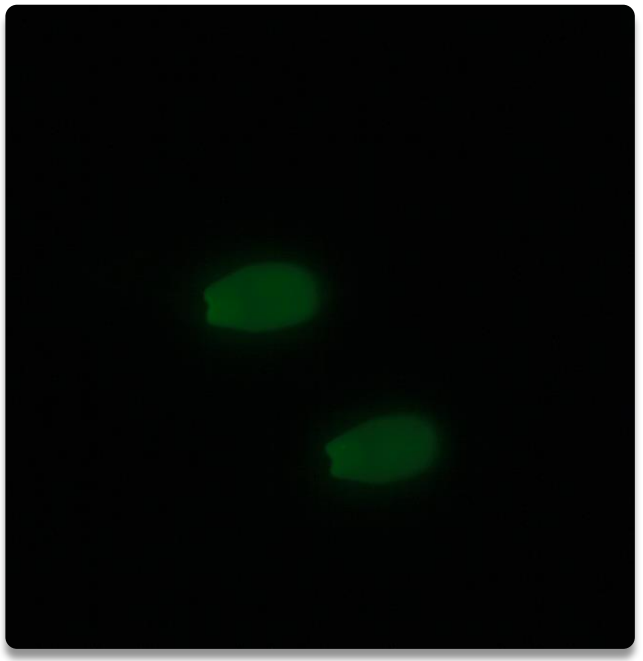
- For fluorescence analyses, all stored sperm samples were diluted with phosphate-buffered saline. Each stained sperm sample was analyzed under a fluorescence microscope (Olympus BX 41, Tokyo, Japan) at 600× magnification. At least 200 sperm were evaluated in each sample.

https://microscopecentral.com/cdn/shop/products/OlympusBX41FaceToFaceDual-PlanAPO-TiltingTelescoping_1e6c63cb-55ba-4c9a-83df-0e0083e398e7.jpg?v=1644032511&width=1445

The integrity of plasma membrane

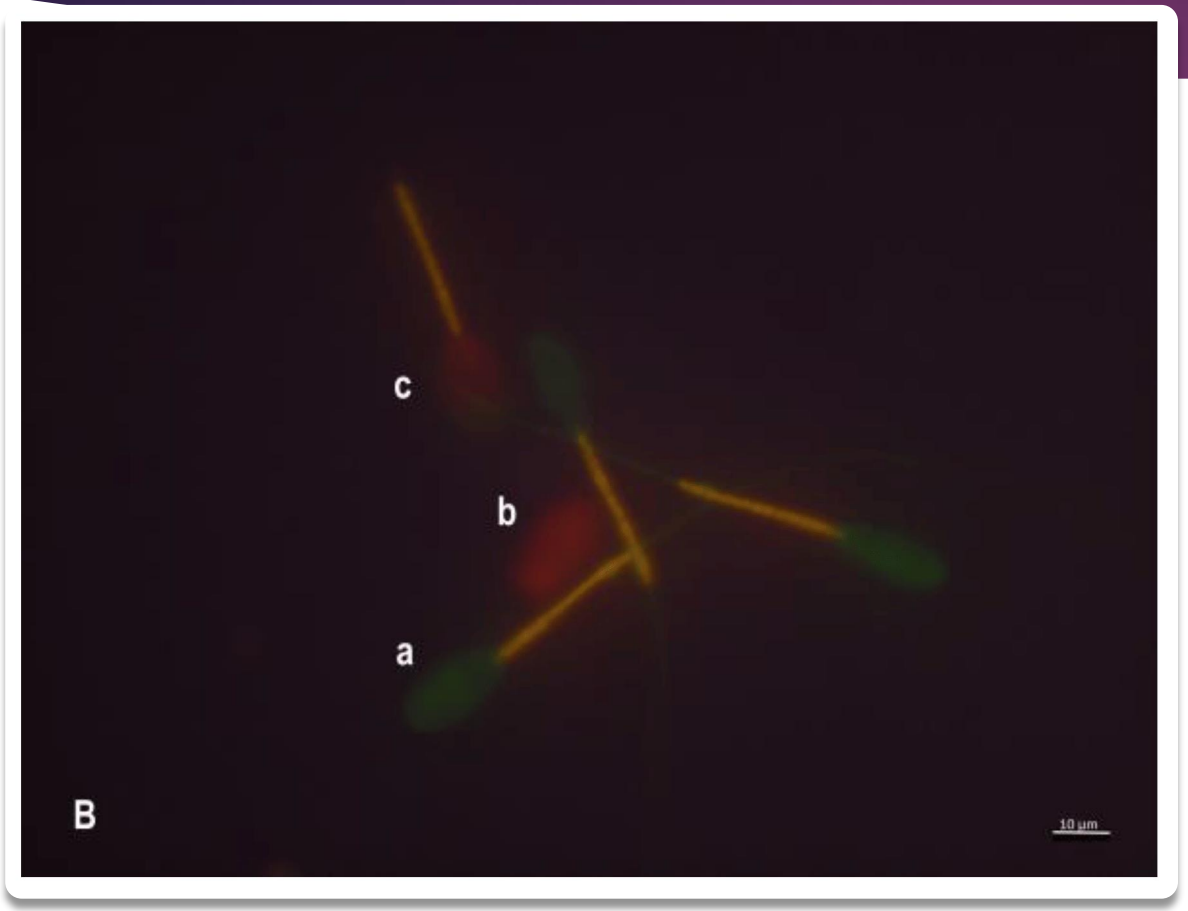
- The integrity of sperm cell membranes was assessed using SYBR-14 and propidium iodide (PI) fluorescent staining.
- Sperm with green fluorescence in the head were considered viable sperm with intact cell membranes, while sperm with red fluorescence in the head were considered dead.





Sperm with red fluorescence in the head - dead.
Sperm with green fluorescence in the head - viable sperm with intact cell membranes.

Sperm mitochondrial activity

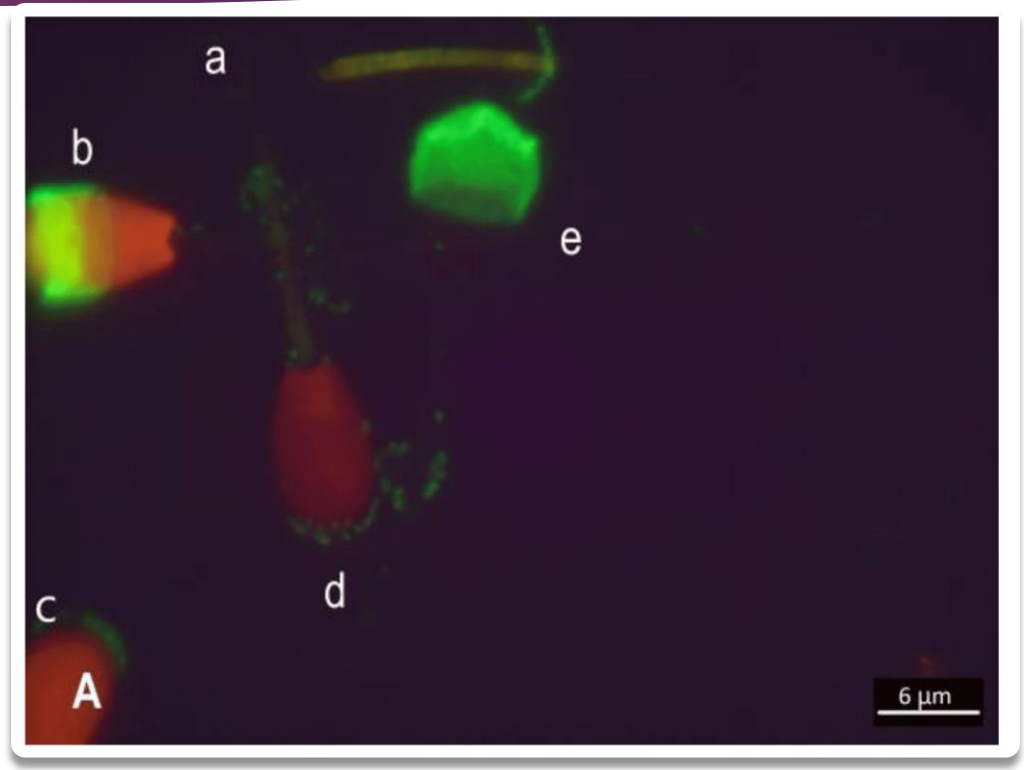


- Sperm mitochondrial activity and viability were assessed using a combination of JC-1 and PI fluorochromes.

(B) Mitochondrial membrane potential assessed with the use of JC-1/PI: (a) sperm with green fluorescence in the head and orange fluorescence in the midpiece - live sperm with high mitochondrial membrane potential; (b) sperm with red fluorescence in the head and green fluorescence or no fluorescence in the midpiece - dead sperm with low mitochondrial membrane potential; (c) sperm with green-red fluorescence in the head and orange fluorescence in the midpiece - early necrotic sperm with high mitochondrial membrane potential. Animals, 2023, 13, 990. doi: 10.3390/ani13060990

The integrity of acrosomal membrane

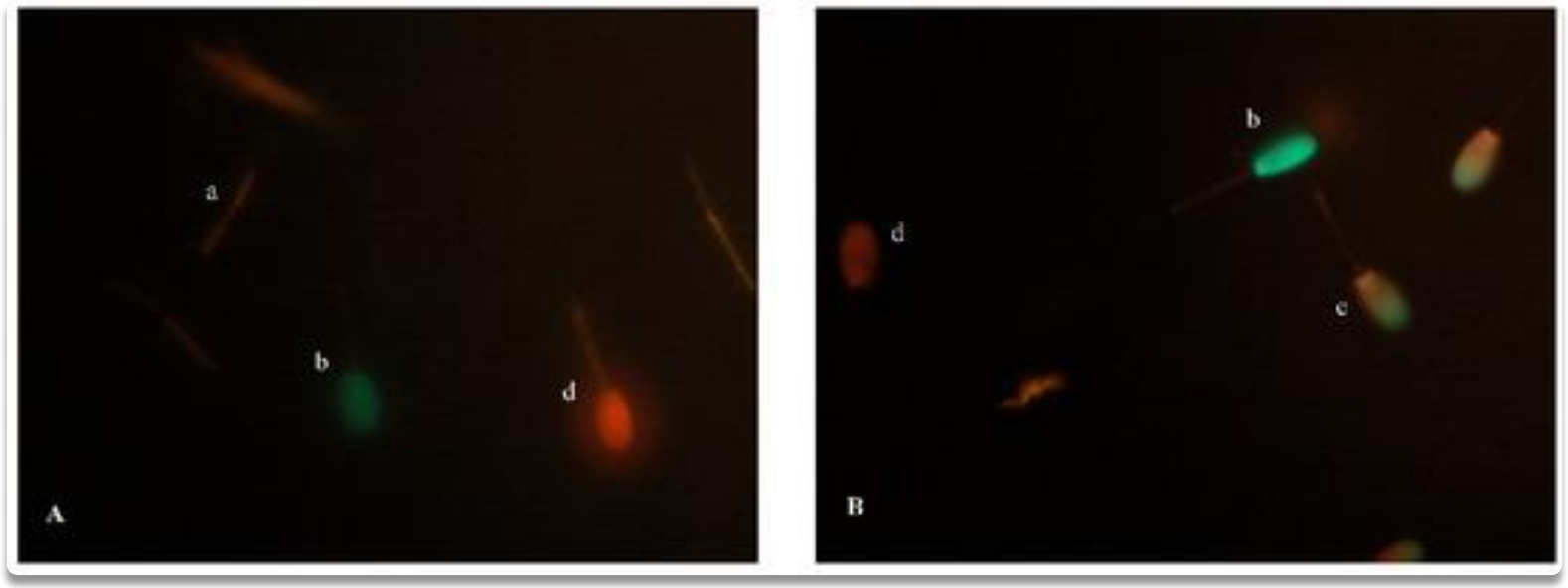
- The integrity of sperm acrosomal membranes was assessed by fluorescence using FITC-labeled peanut agglutinin. Four sperm populations were observed under the microscope.



(A) Acrosome status assessed by FITC-PNA staining: (a) spermatozoa without fluorescence in the head region and with fluorescence in the mid-piece - live sperm with intact acrosomes; (b) sperm with green-red fluorescence in the head - early necrotic, acrosome-reacted sperm; (c,d) sperm with red fluorescence in the head - dead sperm; (e) sperm with green fluorescence in the acrosomal cap - acrosome-reacted sperm.
Animals, 2023, 13, 990. doi: 10.3390/ani13060990

Sperm viability and apoptotic-like changes

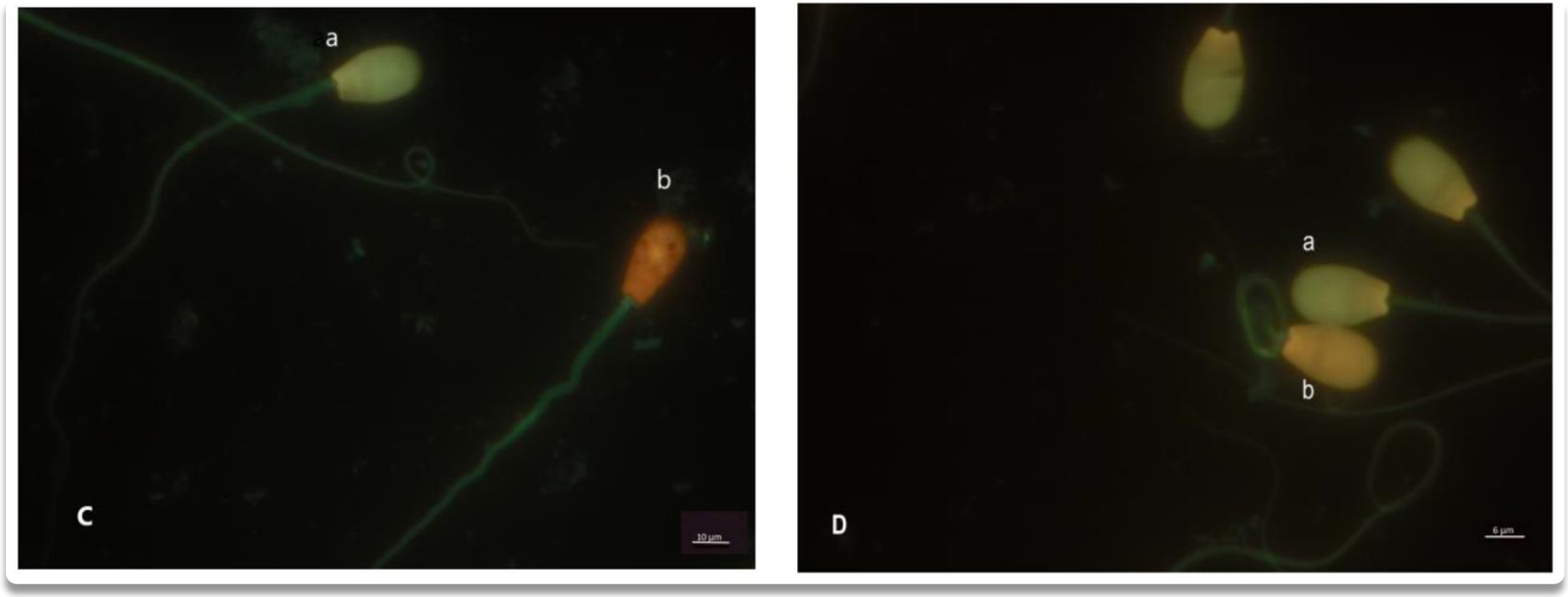
- Sperm viability and apoptotic-like changes were assessed using YO-PRO-1 and PI fluorochromes (available in the Vybrant Apoptosis Assay Kit). To visualize all spermatozoa during fluorescence analysis, the fluorochrome JC-1 was added to the sample, which stains only the sperm midpiece. Four subpopulations of sperm were distinguished.



(A,B) Red deer sperm stained with the fluorescent combination of JC-1 and YO-PRO-1/PI fluorochromes under a fluorescence microscope: (a) Non-apoptotic cells, yellow/orange fluorescence in midpiece. (b) Apoptotic cell, green fluorescence in head region. (c) Moribund/dying sperm, cell with dual fluorescence green and red in head region. (d) Dead sperm, red fluorescence in head region.
Int. J. Mol. Sci. 2022, 23, 14591. doi: 10.3390/ijms232314591

DNA integrity

- DNA integrity was assessed using acridine orange. Sperm with green fluorescence in the head were considered cells with intact DNA, while sperm with orange or red fluorescence in the head were considered cells with damaged DNA.



(C,D) DNA status assessed by acridine orange staining: (a) sperm with green fluorescence in the head - normal sperm; (b) sperm with red or orange fluorescence in the head - sperm with damaged DNA (DNA fragmentation). Animals, 2023, 13, 990. doi: 10.3390/ani13060990

Results

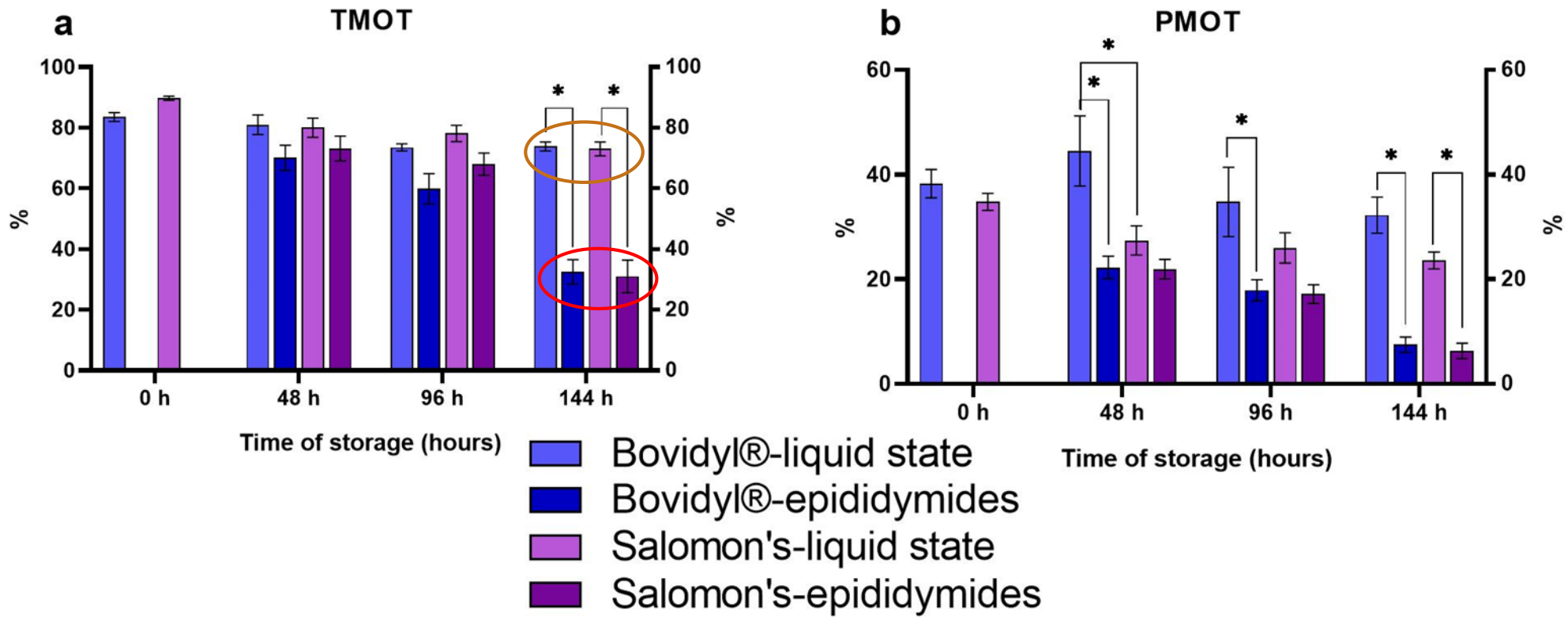


Figure 1. Motility and motility parameters of spermatozoa stored in the epididymis and in a liquid state at 5°C (n = 36). a – TMOT, total motility; b - PMOT, progressive motility. The mean (± SEM) values of stored epididymal spermatozoa are presented. * Significant at $P \leq 0.05$.

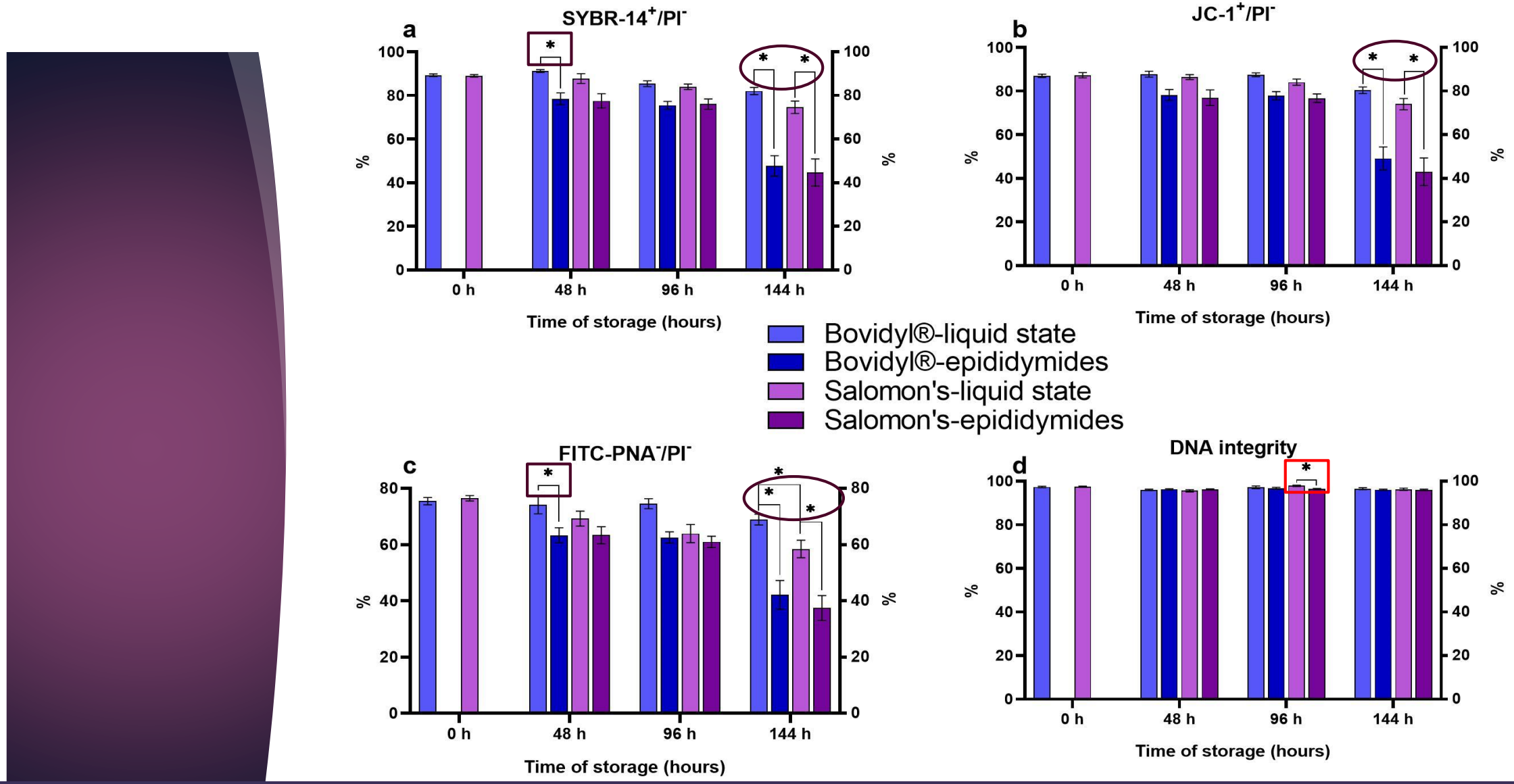
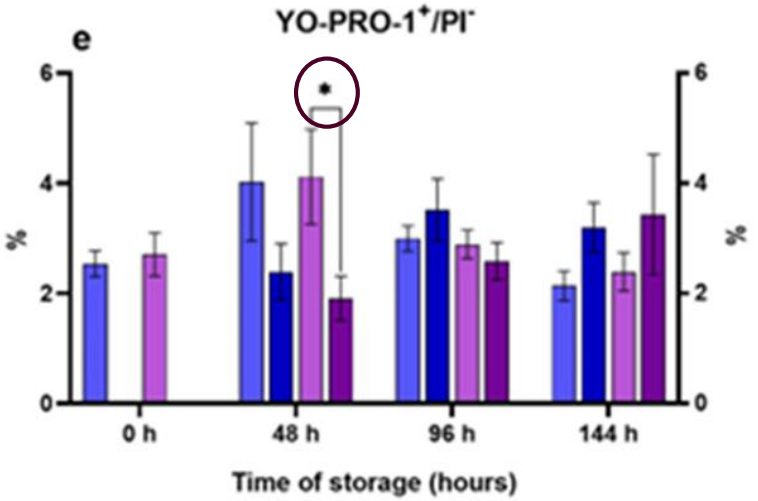
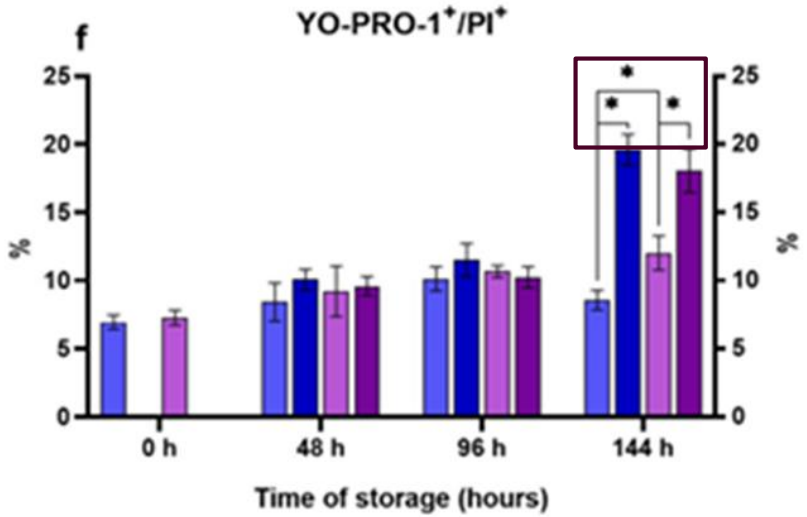
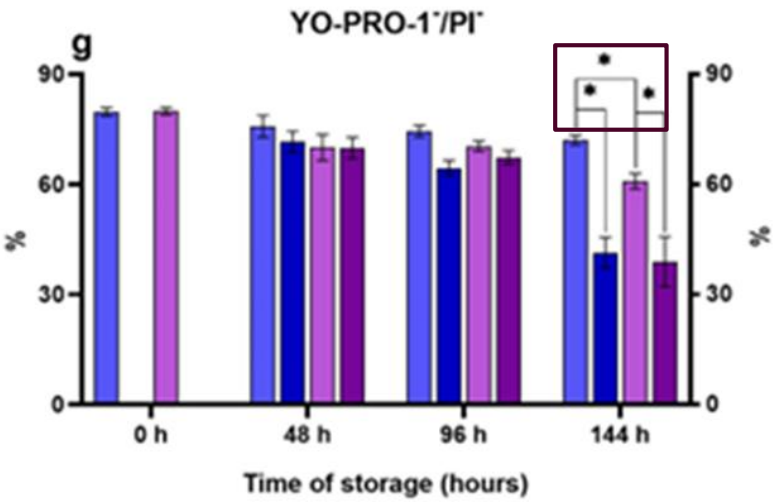


Figure 2. Plasma membrane integrity, mitochondrial membrane potential, acrosomal membrane integrity, DNA integrity in spermatozoa stored in the epididymis and in a liquid state at 5 °C (n = 36). a - SYBR-14^{+}/PI^{-}, sperm with an intact plasma membrane; b - JC-1^{+}/PI^{-}, mitochondrial membrane potential; c - FITC-PNA^{-}/PI^{-}, sperm with an intact acrosomal membrane; d - DNA integrity. The mean (± SEM) values of stored epididymal sperm are presented. * Significant at $P \leq 0.05$.







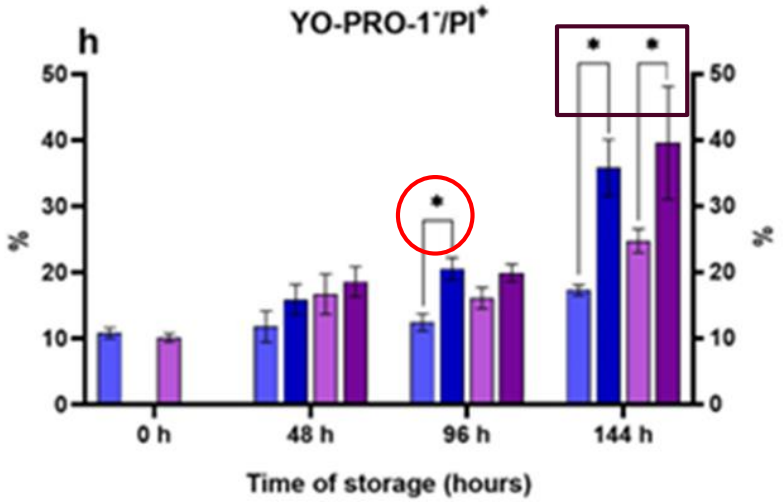


Figure 2. Apoptotic like-changes in spermatozoa stored in the epididymis and in a liquid state at 5 °C (n = 36). e - YO-PRO-1^{+}/PI^{-}, viable spermatozoa with apoptotic-like changes; f - YO-PRO-1^{+}/PI^{+}, moribund spermatozoa; g - YO-PRO-1^{-}/PI^{-}, viable spermatozoa without apoptotic-like changes; h - YO-PRO-1^{-}/PI^{+}, necrotic spermatozoa. The mean (± SEM) values of stored epididymal sperm are presented. * Significant at $P \leq 0.05$.

Conclusion

Storing sperm in the epididymis, compared to storing it in a liquid state, causes earlier structural and functional damage to various cellular structures, which leads to deterioration of their motility.

The biological properties of sperm cells are more effectively preserved during liquid storage, and this method is recommended for short-term preservation of spermatozoa.

Nevertheless, the analysis of the obtained results of total motility, membrane integrity and mitochondrial activity indicates that European red deer spermatozoa can be stored in the epididymides in the scrotum at 5 °C for up to 96 h.

The choice of sperm preservation extender may influence the biological properties of stored epididymal sperm. The use of Bovidyl diluent had a better effect on preserving the progressive movement of sperm than Salomon's extender.

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A comparison of the biological properties of European red deer (*Cervus elaphus elaphus*) spermatozoa stored in the epididymides and in a liquid state at 5 °C

Nicoletta M. Neuman [a], Anna Dziekońska [a,*], Aleksandra Orzołek [a], Przemysław Gilun [b]

[a] *Department of Animal Biochemistry and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland*
[b] *Department of Local Physiological Regulations, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland*

Thank you for your attention!