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# Decay of sperm obtained from epididymes of wild ruminants depending on postmortem time

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**Abstract**

We have carried out a study on the effect of postmortem time (PT) in some characteristics of epididymal sperm salvaged from hunted Iberian red deer and roe deer. Testis were collected, identified, refrigerated down to 5 °C, and sent to our laboratory by the wardens of the hunting reserves. This way, samples were delivered at different times postmortem. Sperm were extracted from the cauda epididymis by means of cuts. Analyzed parameters were: osmolality, pH, motility—both subjectively and with CASA, HOS test reactivity, acrosomal status and viability (assessed with propidium iodide). Osmolality and pH rose with prolonged postmortem time, possibly due to tissue decomposition. Most sperm quality parameters negatively correlated with PT. Besides, when comparing PT classes (groups of 24 h for red deer and 30 h for roe deer), we could appreciate that motility was more affected by PT than other quality variables. Progressive motility was especially impaired. We also classified the samples in high, medium and low quality for each PT group (considering progressive motility, intact acrosomes and reactivity to the HOS test), and it was clear that after 2 days the number of high quality samples was testimonial, and after several days, we almost found only low quality samples. In conclusion, epididymal sperm from Iberian red deer and roe deer undergo a decrease of quality with PT, but it could stay acceptable within many hours postmortem. There are implications for wildlife conservation programs, as epididymal sperm is a good source of germplasm. If valuable animals die and it is not possible to process their sperm immediately, it may still be possible to obtain viable spermatozoa many hours later.


*Keywords:* Red deer; Roe deer; Postmortem recovery; Epididymal sperm; Refrigeration; Epididymal storage

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## 1. Introduction

The cervid species Iberian red deer (*Cervus elaphus hispanicus*) and roe deer (*Capreolus capreolus*) are appreciated trophies in Spain and are subjected to controlled hunting, both in state hunting reserves and in private properties. Populations are frequently constricted to small areas and separated by fences and other barriers, thus inbreeding and loss of genetic variability are a recurrent hazard [1]. There is also a great interest in keeping good trophies and autochthonous subspecies. Consequently, the interest in developing artificial reproduction techniques and genetic resource banks for these species has been increasing, considering the possibilities that this kind of approach offers [2–4]. Since males are hunted in numbers around the rut season, there is an available source of epididymal sperm.

Postmortem sperm recovery is an useful strategy for germplasm banking [5]. This technique allows to use the epididymal sperm reserves of deceased or hunted males, especially when semen collection by other ways would be difficult or impossible. Sperm stored in the cauda epididymis have usually good quality and a high level of maturation, being able to fertilize oocytes. To date, many studies have demonstrated that it is possible to obtain viable gametes postmortem. Furthermore, successful pregnancies have been achieved in many species using epididymal sperm for artificial insemination [6–10].

However, in order to get good quality samples, sperm collection and processing should be carried out immediately after the death of the animal. This is not always possible, especially regarding wild species. In these circumstances, animal death is generally unpredictable or it happens far away from laboratories and technicians. In the case of Iberian red deer and roe deer in Spain, samples sometimes cannot be delivered immediately to the laboratory, since hunting often takes place in the wild. Even though sperm cells can survive for some time in the epididymes of dead animals, their quality deteriorates with time, because of the changes related to body death and decomposition [8,11].

Therefore, to determine the quality and decay of sperm stored in epididymes postmortem, some studies have been carried out in a few species, such as mice [8,12–15], boar [16], dog [17], some African wild species [18–20], mouflon [6] and Iberian red deer [21–23]. In general, these works agree that there is a general deterioration in sperm quality depending on time postmortem, specially marked in the first hours, and that refrigeration of the epididymes down to around 5 °C is the best strategy to lower this damage. However, there are many dissimilarities between species, possibly due to differences on cold shock endurance of epididymal sperm.

In this work we have evaluated the quality of sperm samples obtained from Iberian red deer and roe deer epididymes, which were delivered to our laboratory at different times postmortem. Since refrigeration devices are widely available, testicles of hunted animals could be kept at 5 °C during its storage. Our objective was to determine the effect of postmortem time on sperm quality and the characteristics of its decline, in the same conditions that often occur when samples are collected in the wild and immediate transport to the laboratory is not always possible. This work is included in a long-term plan dedicated to setting up a germplasm bank for wild ruminant species in the North of Spain, obtaining sperm samples from hunted animals.