Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 66 (2006) 1847–1856
Theriogenology
www.journals.elsevierhealth.com/periodicals/the

Seminal plasma improves cryopreservation of Iberian red deer epididymal sperm

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Received 20 December 2005; received in revised form 28 March 2006; accepted 10 April 2006

Abstract

We tested the protective action of seminal plasma on epididymal spermatozoa from Iberian red deer, especially considering cryopreservation, as a means for germplasm banking improvement. We obtained seminal plasma by centrifuging electroejaculated semen, and part of it was thermically inactivated (denatured plasma; 55 °C 30 min). Epididymal samples (always at 5 °C) were obtained from genitalia harvested after regulated hunting, and pooled for each assay (five in total). We tested three seminal plasma treatments (mixing seminal plasma with samples 2:1): no plasma, untreated plasma and denatured plasma; and four incubation treatments: 32 °C 15 min, 5 °C 15 min, 5 °C 2 h and 5 °C 6 h. After each incubation, samples were diluted 1:1 with extender: Tes-Tris-Fructose, 10% egg yolk, 4% glycerol; equilibrated for 2 h at 5 °C, extended down to 10^8 spz./mL and frozen. Sperm quality was evaluated before 1:1 dilution, before freezing and after thawing the samples, assessing motility (CASA) and viability (percentage of viable and acrosome-intact spermatozoa; PI/PNA-FITC and fluorescent microscopy). Plasma treatment, both untreated and denatured, rendered higher viability before freezing and higher results for most parameters after thawing. The improvement was irrespective of incubation treatment, except for viability, which rendered slightly different results for untreated and denatured plasma. This may be due to the presence of thermolabile components. We still have to determine the underlying mechanisms involved in this protection. These results might help to improve the design of cryopreservation extenders for red deer epididymal sperm.

Keywords: Red deer; Epididymal sperm; Seminal plasma; Cryopreservation; Wildlife preservation

1. Introduction

Conservation of biodiversity is a difficult but essential task that must be approached using diverse strategies. One of the most promising ones is the use of artificial reproductive techniques and germplasm banks, which provide flexible means of management, and allow to indefinitely store genetic material from whole populations [1]. However, obtaining germplasm from wild animals is generally problematic. Thus post-mortem collection – either from hunted or accidentally killed animals – constitutes the best source of germplasm, especially in areas of regulated hunting.

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doi:10.1016/j.theriogenology.2006.04.036

Post-mortem semen samples are obtained from the cauda epididymis, where mature spermatozoa in the male genital tract are stored. However, it has been shown that the fertility of epididymal spermatozoa diminishes notably if they are submitted to stressing conditions during the cryopreservation process [2], and there is evidence that sperm DNA is altered in this situation [3]. Although spermatozoa from the cauda epididymis have been compared to ejaculated spermatozoa in terms of functionality and fertility [4], there are many differences, the most important being the different environment surrounding them: epididymal fluid vs. seminal plasma. Seminal plasma is known to exert many effects on spermatozoa, many of them by the direct action of seminal plasma proteins [5–10]. Some of these effects are negative for sperm storage and cryopreservation; thus, Dott et al. [11] found that the incubation of epididymal spermatozoa in seminal plasma was detrimental for survival (dog, ram and bull). These effects are due to a capacitation-inducing effect of the seminal plasma in many species [6,7,12–16]. Moreover, a recent study on the addition of bull seminal plasma to African buffalo epididymal sperm before cryopreservation [17] reported negative results. We must consider that this effect could be due to the enhancing effect of some bovine seminal plasma proteins on capacitation [6], or to a species effect, expressed through a differential sensinivity to the seminal plasma from a different species. Besides, Tecirlioglu et al. [18] found that the addition of seminal plasma to mouse epididymal sperm decreased motility and prevented fertilization.

Nevertheless, seminal plasma has shown positive effects in many studies, both on washed ejaculated spermatozoa and epididymal spermatozoa. In contrast to the capacitating action of some proteins, others regulate sperm function, including suppression of capacitation and acrosome reaction [5,10,19–22]. Moreover, seminal plasma proteins modulate the interaction of spermatozoa with the female genital tract and exert an immunosuppressive action [20,22–27]. Furthermore, it has been demonstrated that seminal plasma improves, and even reverses, cold shock in washed ejaculated spermatozoa from ram [8,28], and also cryopreservation results in this species [29]. Fertility trials have shown that ovine AI results can be improved by addition of seminal plasma, both with cooled [30] and cryopreserved semen [31]. Other beneficial effects of seminal plasma supplementation have been noted in bovine [32], boar [21,33] and human semen [34–37].

Apart from the effect of proteic factors on sperm functions, the beneficial effect of seminal plasma is due to the presence of reactive oxygen species (ROS) scavengers, not only enzymatic (superoxide dismutase, catalase, glutation peroxydase) but also non-enzymatic (α-tocopherol, ascorbic acid, glutation, etc.) [36–46]. Although it has been demonstrated that the epididymis possesses an antioxidant system [47], the low volume of epididymal fluid and the high dilution undergone by epididymal spermatozoa during the collection process could increase their vulnerability to ROS, whereas whole semen might provide a more efficient antioxidant environment, due to the secretions of the accessory sex glands [44,45]. In fact, Braun et al. [48] showed that flushing and storing epididymal spermatozoa with seminal plasma was beneficial for motility.

The objective of the present study is to evaluate the effect of seminal plasma on epididymal sperm obtained from Iberian red deer, especially during the cryopreservation process. This species has a high value in Spain, both ecological and economical, being the most appreciated hunting species. Creation of germplasm banks would greatly enhance management of these populations and preserve its genetic wealth, threatened by inbreeding and hybridization [49]. In this case, a major source of sperm for germplasm banking consists on post-mortem epididymal samples from controlled hunting. However, although many studies on cryopreservation of post-mortem sperm samples from red deer have been carried out [50–54] and successful pregnancies have been achieved [55,56], there are still many improvements to accomplish on the cryopreservation and application of these samples. Indeed, several studies on this species have shown important loss of quality pertaining manipulation and cryostorage of epididymal samples [2] and, as indicated above, Esteso et al. [3] showed that these changes may involve DNA damage. Since this could be caused by the lack of protection of epididymal samples, quality may be better preserved treating the samples with appropriate media, such as seminal plasma.

Nevertheless, there is a general concern on the risk of disease transmission that any assisted reproductive technique conveys [57,58]. Use of seminal plasma from one animal to treat the washed or epididymal spermatozoa of another could incur in contamination with pathogens, especially in wild or half-domesticated species, which cannot be submitted to veterinary control as strictly as the domesticated ones. Another drawback from using seminal plasma is the variability between subjects and collecting seasons. Many studies have demonstrated that seminal plasma composition and