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Seasonal variation in reproductive physiological status in the Iberian ibex (*Capra pyrenaica*) and its relationship with sperm freezability

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Abstract

The present work examines the relationship between seasonal changes in testicular function, accessory gland size, and horn growth in Iberian ibexes, as well as the relationship between these changes and the resistance of ibex spermatozoa to freezing-thawing. The size of the bulbourethral glands and seminal vesicles showed pronounced monthly variation ($P < 0.001$), which was correlated positively with the plasma testosterone concentration ($P < 0.001$) and scrotal circumference ($P < 0.001$). The size of the accessory sex glands peaked during the autumn. Overall, semen quality was markedly improved during autumn and winter. When horn growth was at a minimum during autumn and winter, semen quality and accessory gland size were all increased compared to in spring and summer. However, increased plasma testosterone levels in the autumn were strongly associated with reduced sperm freezability; thus, the cryosurvival of spermatozoa collected during the autumn was poorer than at other times of the year. In winter, however, when the plasma testosterone concentration fell to baseline, the negative effects of cryopreservation on the percentage of motile spermatozoa and on the integrity of the plasma membrane of frozen-thawed sperm cells were significantly less intense ($P < 0.05$). These findings show a clear relationship between the functional and morphological status of the different parts of the reproductive tract that optimises reproductive function during the breeding season in the ibex male. They also show that winter is the most suitable season for the collection and cryopreservation of ibex spermatozoa.

Keywords: Accessory sex glands; Breeding season; Cryopreservation; Horn growth; Semen quality; Testosterone

1. Introduction

The response of sperm cells to the freezing-thawing process is affected by season in several mammals [1–4], including humans [5], possibly because of changes in its fresh quality. Species that have adopted seasonal breeding undergo coordinated changes in their reproductive systems during the mating season. For example, the resumption of endocrine activity by the hypothalamic-pituitary-gonadal axis [6–8] leads to an increase in testosterone secretion, which in turn optimises spermatogenic activity [9,10]. During this period the quality of the ejaculates of wild ungulates such as the roe deer (*Capreolus capreolus*) [10] and fallow deer (*Dama dama*) [11,12] is markedly better; semen production is greater and higher percentages of morphologically normal spermatozoa are produced. Further, the increased testosterone secretion favours the function of the acces-

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sory sex glands, helping to optimise the fertilization capacity of the spermatozoa [13]. Testosterone is also necessary for the adequate development of secondary sexual characteristics in ungulates, such as horns [14]. Horns are sexual appendages used by the males of many ungulate species for competitive combat during the pre-mating period. A relationship between annual variation in horn development and seasonal changes in breeding activity is evident since in wild ruminants greater horn growth rates coincide with the period of sexual inactivity. The annual arrest of horn growth occurs when males are producing their highest concentrations of testosterone [14–16]. Moreover, it has been shown that ibexes with the largest and most symmetrical horns produce the best quality sperm [17]. In cervids [12], a relationship exists between sperm quality, testis activity and the antler cycle, mirroring that seen in bovids.

The Iberian ibex (*Capra pyrenaica*), which has a short breeding season that extends from December to mid-February [18,19], is a mountain goat endemic to the Iberian Peninsula. Its numbers, although stable, require a genetic resource bank be maintained if the preservation of the species' different ecotypes is to be guaranteed. With this in mind, several studies have tried to improve the cryopreservation protocols for ibex spermatozoa [20], and it is now known that beneficial effects of the removal of seminal plasma become more noticeable during the decreasing photoperiod (July to December) [21]. This procedure removes the secretions of the bulbourethral glands (among others), the activity of which may be increased during this time. Certainly, in the domestic goat (*Capra hircus*), bulbourethral secretions have been found to interact with egg yolk-based extenders and negatively affect sperm cryosurvival [22,23]. Nonetheless, if the accessory sex glands of ibexes show a seasonal pattern of secretory activity, it has yet to be demonstrated.

Endocrine status may also alter the resistance of sperm cells to freezing-thawing. It has been suggested that increased plasma testosterone has a negative effect on the cryosurvival of ibex spermatozoa [24]. In the latter study, concentration of testosterone did not appear to affect fresh semen quality, but plasma testosterone concentrations were not recorded over the entire year. Assessment of the relationship between sperm freezability and the seasonal pattern of testosterone secretion over the year ought to provide additional information in this respect.

The aim of the present work was to examine the relationship between seasonal changes in testicular function, accessory gland size and horn growth in Iberian ibexes, as well as the relationship between these changes and the resistance of ibex spermatozoa to freezing-thawing.

2. Material and methods

2.1. Animals and experimental procedure

Six adult ibex males aged 4–10 y were examined monthly over the period of one year. All animals used in this work were obtained from the Serranía de Ronda Game Reserve (36 °N latitude, Málaga, Spain) and the Sierra Nevada National Wildlife Park (37 °N latitude, Granada, Spain), and transferred to the Animal Reproduction Department of the INIA (40 °N latitude, Madrid, Spain). They were kept in a sand-floor stable (250 m^2) with a partial roof cover adapted for the maintenance of ibexes. To alleviate stress during the experimental procedures, a period of at least eight months was allowed for the animals to become used to routine restraint and handling. In this time they became accustomed to entering a small restraining stall (2 m^2) in which they would eventually be anaesthetised before undergoing all manipulations. During handling the eyes were covered with a mask to reduce stress. All experimental procedures (blood sampling, measurement of scrotal circumference and horn growth, sexual skin flush evaluation, ultrasonography of the accessory sex glands and electroejaculation) were performed on the same day under general anaesthesia. Animals were anaesthetised with intramuscular detomidine 0.27 mg/kg (Domosedan®, Pfizer Inc., Amboise Cedex, France) plus ketamine hydrochloride 1.40 mg/kg (Imalgene-1000®, Rhône Mérieux, Lyon, France). Once all manipulations were complete, the effects of the anaesthetic were reversed by intramuscular administration of 0.25 mg/kg atipamezol (Antisedan®, Pfizer Inc., Amboise Cedex, France). All animals were fed a balanced diet (Visan K-59, Visan Ind. Zoot., S.A., 28500 Arganda, Madrid, Spain) supplemented with barley grain, barley straw and dry alfalfa. Free access was provided to water and vitamin/mineral blocks. All handling procedures were approved by the INIA Ethics Committee and were performed in accordance with the Spanish Policy for Animal Protection RD1201/2005 which conforms to the European Union Directive 86/609 regarding the protection of animals used in scientific experiments.

2.2. Testicular activity

To evaluate testicular endocrine activity during the experimental period, blood samples from the jugular vein were recovered in lithium heparin collection tubes