Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 72 (2009) 1065–1072
Theriogenology
www.theriojournal.com

Artificial insemination of captive European brown hares (*Lepus europaeus* PALLAS, 1778) with fresh and cryopreserved semen derived from free-ranging males

T.B. Hildebrandt [a], K. Roellig [a,*], F. Goeritz [a], M. Fassbender [a], R. Krieg [b], S. Blottner [a], B. Behr [a], R. Hermes [a]

[a] *Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany*
[b] *Kleintierzucht Krieg, Merseburger Str. 3, 06255 Muecheln/Wuensch, Germany*

Received 20 March 2009; received in revised form 22 June 2009; accepted 28 June 2009

Abstract

This study aimed to establish artificial insemination (AI) protocols to predictably initiate pregnancy during the breeding season in the European brown hare (EBH) (*Lepus europaeus* PALLAS, 1778). Semen was collected from seven captive and eight free-ranging males by means of electroejaculation. Semen from the free-ranging males was cryopreserved using directional freezing. Total motility/integrity of fresh and frozen-thawed semen was 91.6%/87.7% and 46.9%/53.8%, respectively. Ovulation was induced in ultrasonographically preselected females using a gonadotropin-releasing hormone analogue. Each female was inseminated with 1 mL fresh (Group A, n = 16) or frozen-thawed semen (Group B, n = 9) at a concentration of 100×10^6 spermatozoa/mL. The use of ultrasonography (10 to 22 MHz) confirmed the intracervical semen deposit, the success of artificial ovulation induction (formation of postovulatory corpus luteum), and permitted the monitoring of individual pregnancies. Although sperm motility/integrity was significantly different between groups, no significant difference was detected in conception rates (A, 87.50%; B, 77.78%). Because of embryonic resorption, there was a slight difference in fertility rate between groups (A, 62.5%; B, 77.78%). Overall, AI in captive EBH using fresh and frozen-thawed semen achieved successful fertility rates. Long-term cryopreserved semen was used to bring new genetic material from the wild into a genetically limited captive population without extensive animal transport. Therefore, AI has the potential to enhance breeding programs for EBH especially when cryopreserved semen from wild donors is used.

Keywords: Assisted reproduction; Directional freezing; Electroejaculation; Lagomorpha; Ultrasonography

1. Introduction

The European brown hare (EBH) (*Lepus europaeus* PALLAS, 1778) is a common representative of the Eurasian fauna. However, feral populations also exist in Eastern Asia, North and South America, and Australia [1] due to introduction by humans in historic times. For centuries, hares have played an important role as game animals. Nowadays, they are part of a multimillion-dollar meat export business in several countries [2]. Nevertheless, the reproductive biology of EBH is still not completely understood. In particular, it remains speculative whether this species regularly exhibits such reproductive phenomena as embryonic resorption [3] or superfetation [4–6]. Thus, new methods to regularly

* Corresponding author. Tel.: +49 30 5168 242; fax: +49 30 5126 104.
E-mail address: kathleen_roellig@gmx.de (K. Roellig).

0093-691X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2009.06.026

explore and facilitate pregnancy in captive EBH are required.

To date, several attempts have been made to develop artificial insemination (AI) for use in EBH as well as in other *Lepus* species. The majority of these attempts used epididymal sperm, which required the castration or even the death of the male individual [7–11]. Given that the EBH is not a domesticated animal, routine handling can be problematic. Therefore, techniques such as use of an artificial vagina, which are routinely used for semen collection in rabbits [12], require a minimum level of cooperation and usually are not suitable for EBH (unpublished data, Krieg et al.). Another method for collecting sperm from live animals is electroejaculation, which has proved to be feasible in several domestic and wildlife species but relatively unsuccessful in EBH [7]. The development of an ejaculatory probe specially designed for hares finally allowed the collection of fresh sperm from live animals under general anesthesia [13–16]. This new method was used to evaluate the reproductive capability of free-ranging EBHs [17]. A further study provides promising results in conventionally freezing EBH semen with the goal of preserving this freshly collected sperm for use in conservation and industry breeding similar to those protocols designed for rabbits [18].

To perform AI, it is necessary to define the optimal time of insemination. Previous studies have reported that there is no definitive estrous cycle in EBH revealed by cytologic evaluation of vaginal smears [19] and monitoring of reproductive hormones [20]. In the past decades, ultrasonography has become a routinely used tool to analyze reproductive function in several wildlife species [13]. This diagnostic technique has also been adapted to monitor EBH reproductive status [21]. Therefore ovarian activity can now be detected routinely in anesthetized animals. As in other Lagomorpha species, ovulation in EBH is induced by mating. Therefore, it is necessary to trigger this mechanism artificially for successful AI. There are three possibilities described for the rabbit [12] that have also been used for the EBH. These include (i) mating with a vasectomized male [10], (ii) administration of human chorionic gonadotropin [7–9,11], or (iii) administration of a gonadotropin-releasing hormone analogue (e.g., buserelin) [16].

There have been several attempts to cross-breed lagomorphs including the EBH to investigate species boundaries [8–11]. However, AI as a breeding and experimental tool in EBH was first intended to be used by Stavy et al. [7]. They recommended the method as a technique to enhance breeding in captive populations. Their protocol required the castration of males to collect spermatozoa, which would ultimately limit the use of this technique on a large scale. Compared with AI in the EBH, AI in rabbits has been used routinely since the 1920s allowing similar or even better pregnancy rates than that of natural breeding [12]. European brown hares and rabbits are taxonomically closely related. Therefore, it is reasonable to apply existing techniques for the rabbit in the EBH. Recently, Kozdrowski and Siemieniuch [16] successfully inseminated EBH with fresh semen.

Captive breeding colonies mainly derive from a few founder individuals. In general, transforming wild hares into cage hares has mostly been unsuccessful and led to death of the individuals. Nevertheless, the need to continually introduce new genetic material into captive populations with limited genetic diversity is required. Taking all this into account, the aim of this study was to establish reliable methods for the application of successful AI in captive EBHs using fresh and also cryopreserved semen obtained from wild males.

2. Materials and methods

2.1. Animals

Artificial insemination was performed in 16 different female EBHs kept under natural climate conditions at the field research station of the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany. They were housed in individual cages ($2 \times 2m^2$), fed with customized hare pellet diet and fresh hay, and were given ad libitum access to water.

The semen samples used for the AI with fresh semen (Group A, n = 16) derived from 10 ejaculates from 7 different captive males (housed under the same conditions as the females). The semen samples for the AI with frozen-thawed semen (Group B, n = 9) were collected in 8 ejaculates from 8 different free-ranging males in April 2003 in North Rhine Westphalia, Germany [17]. This was part of a research project to evaluate the reproductive performance of free-ranging populations [14,15,17,21]. Hares were caught with nets, put in a transportation box, and brought to an examination room. The examination methods performed were the same for captive and free-ranging animals, as described later. Semen was recovered from the free-ranging males, cryopreserved with directional freezing, and stored at the IZW for 3 yr. After reproductive assessment, the wild semen donors were released back to their natural habitat within 5 to 8 h. All semen collections were performed from February to