Animal Reproduction Science 132 (2012) 88–95

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effect of semen collection method (artificial vagina *vs.* electroejaculation), extender and centrifugation on post-thaw sperm quality of Blanca-Celtibérica buck ejaculates

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ARTICLE INFO

Article history:
Received 25 January 2012
Received in revised form 4 April 2012
Accepted 11 April 2012
Available online 19 April 2012

Keywords:
Blanca-Celtibérica buck
Artificial vagina
Electroejaculation
Cryopreservation
Freezing extender
Breeding season

ABSTRACT

The aim of this study was to evaluate the effect of semen collection method (artificial vagina compared to electroejaculation), season in which the semen was collected (breeding season compared to non-breeding season), freezing extender (Biladyl®, Andromed® and skim milk based extender) and pre-treatment procedure (washing compared to non-washing) on post-thaw semen quality in buck. Ejaculates from seven bucks of the Blanca-Celtibérica breed were collected by artificial vagina and electroejaculation during the breeding (July to December) and non-breeding season (January to June). Samples were split in two aliquots and one of them was washed. Three freezing extenders were evaluated on washing and non-washing sperm samples. Ejaculates collected by artificial vagina had a greater sperm quality after thawing, with greater values ($P \leq 0.05$) for SM (sperm motility), NAR (acrosome intact), YO-PRO-1−/PI− (intact spermatozoa), and Mitotracker+/YO-PRO-1− (spermatozoa with active mitochondria) and lower % DFI (DNA fragmentation index). Thawed sperm samples which were collected during the breeding season had greater values ($P \leq 0.05$) for NAR, intact spermatozoa and spermatozoa with active mitochondria, than those semen samples obtained during the non-breeding season. Semen freezing with Biladyl® and Andromed® resulted in a greater sperm quality ($P \leq 0.05$) after thawing in relation to milk-based extender. Washing procedure had no effect on sperm parameters assessed at thawing. Results from the present study suggest that the success of semen cryopreservation in Blanca-Celtibérica goat depends on semen collection method and season, as well as on the extender used. Thus, the post-thaw sperm quality will be greater ($P \leq 0.05$) when samples are collected by artificial vagina during the breeding season and when Biladyl® or Andromed® are used as freezing extenders.

1. Introduction

The Blanca-Celtibérica goat is an autochthonous breed from Spain considered as an endangered breed. Endangered breeds must be preserved by conservation methods. One of the conservation procedures consists of the development of genetic resource banks, cryopreserving gametes and embryos, thus allowing the storage of genetic resources indefinitely (Watson and Holt, 2001).

The first step for the creation of a sperm cryobank is the use of an effective method for the collection of the ejaculates. For domestic males, the artificial vagina (AV) procedure is the preferred method (Leboeuf et al., 2000),

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http://dx.doi.org/10.1016/j.anireprosci.2012.04.005

but this technique requires a previous training period (Wulster-Radcliffe et al., 2001). Another method of collection such as the electroejaculation (EE) involves an alternative when males are not trained to AV or for wild species, and may be a viable method of repeatedly collecting ejaculates from individual specimens without causing death (Santiago-Moreno et al., 2009). The latter method could be an adequate alternative to collect ejaculates in Blanca-Celtibérica bucks because of there are few herds and they live under a extensive production system, being difficult to train them for AV. However, differences on sperm characteristics between ejaculates collected by AV and EE have been found, obtaining more desirable results when AV is used (Greyling and Gobbelaar, 1983). Besides, substantial differences in seminal plasma composition may exist between ejaculates obtained by AV and EE (Marco-Jiménez et al., 2008).

Sperm cryopreservation causes ultrastructural, biochemical and functional impairment to the spermatozoa (Aitken et al., 1998; Purdy, 2006; Watson, 2000). Egg yolk is a common component of sperm cryopreservation extender as a protector of the plasmatic and acrosomal membrane against temperature-related damaged (Purdy, 2006). The Tris–egg yolk–glucose and non-fat dried skimmed milk extenders are most commonly used for cryopreserving buck spermatozoa (Purdy, 2006). A specific problem in the preservation of goat semen has been its limited tolerance to the inclusion of egg yolk in the freezing medium. Seminal plasma has a detrimental effect on the viability of buck spermatozoa during cryopreservation when extender containing egg yolk is used (Sariözkan et al., 2010). This fact is due to seminal plasma contains egg yolk coagulating enzyme (EYCE), which hydrolyzes egg yolk lecithin into fatty acids and lysolecithin (Iritani and Nishikawa, 1961, 1963) which is highly detrimental to buck spermatozoa. Similarly, it has been identified the protein SBUIII from the goat bulbourethral gland which decreased survival of cooled or frozen sperm diluted in milk-based media. This protein could hydrolyze residual triglycerides in skim milk and produce fatty acids exhibiting toxic effects toward spermatozoa (Pellicer-Rubio et al., 1997). Currently, other extenders based on soybean have been used as an alternative to egg yolk in different species, obtaining for both extenders similar results (Aires et al., 2003; Forouzanfar et al., 2010).

In addition, many studies have reported that the washing procedure of goat semen for removing the seminal plasma from ejaculate is necessary to increase motility, membrane integrity, and fertility after freeze–thaw procedure (Kozdrowski et al., 2007; Machado and Simplicio, 1995). However, other studies have demonstrated that there was no effect of seminal plasma removing on post-thaw sperm quality (Cabrera et al., 2005; Daskin and Tekin, 1996).

Finally, significant seasonal variation in the semen quality of small ruminants living at high or mid-levels altitudes have been demonstrated (Ritar, 1993; Roca et al., 1992) and the chemical composition and volume of the ejaculate may be different depending of season (Maxwell et al., 2007).

With this background, we have evaluated in Blanca-Celtibérica bucks the effects of the collection method (AV compared to EE), season in which the semen was collected (breeding season compared to non-breeding season), freezing extender (Biladyl®, Andromed® and a skim milk-based extender) and pre-treatment procedure (washing compared to non-washing), on sperm quality after cryopreservation.

2. Materials and methods

2.1. Animals and reagents

All animal procedures were performed in accordance with Spanish Animal Protection Regulation, RD 1201/2005, which conforms to European Union Regulation 2010/63. Seven males of Blanca-Celtibérica goat breed (age > 1.5 years) were used. Males were maintained and managed at the Regional Center of Animal Selection and Reproduction (CERSYRA) located in Valdepeñas (Spain). The thawing procedure was conducted at laboratories from Group of Biology of Reproduction in Albacete (Spain).

Chemicals were of reagent grade and were purchased from Sigma (Madrid, Spain). Biladyl® and Andromed® were purchased from Minitüb (Tiefenbach, Alemania). Fluorescence probes were purchased from Invitrogen (Barcelona, Spain), except for propidium iodide (PI) which was purchased from Sigma. Chromatographically purified acridine orange was purchased from Polysciences Inc. (Warrington, PA, USA).

2.2. Semen collection

For each male, the collection of ejaculates was performed first using artificial vagina (AV) and later by electroejaculation (EE), both on the same day. Males were trained to conduct collections by AV. Ejaculates were routinely collected once per week. The procedure of EE was carried out using the protocol described by Garde et al. (2003). Males were sedated with xylacine (0.2 mg/kg Rompun® 2% i.m.; Bayer S.A., Barcelona, Spain), then the rectum was cleaned of faeces and the prepucial area was shaved and washed with physiologic saline serum. For EE, we used a three electrode probe connected to a power source that allowed voltage and amperage control (P.T. Electronics, Boring, OR, USA). Probe diameter, probe length and electrode length was 3.2, 35.0 and 6.6 cm, respectively. The EE regime consisted of consecutive series of 5-s pulses of similar voltage, each separated by 5-s break. Each series consisted of a total of four pulses. The initial voltage was 1 V and was increased in each series until a maximum of 5 V. Two ejaculates per buck and collection method were obtained during the breeding (July to December) and non-breeding season (January to June), on a weekly basis.

2.3. Evaluation of ejaculates

Immediately after the collection of ejaculates, volume, sperm concentration, wave motion, subjective sperm motility (SM) and the percentage of spermatozoa with intact acrosomes (NAR) were determined. The volume of the ejaculates was measured in a conical graduated tube. Sperm concentration was calculated using