Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Exogenous melatonin influences distribution of French Alpine buck spermatozoa in morphometrically distinct subpopulations during the non-breeding season

Ivona Žura Žaja[a], Silvijo Vince[b,*], Suzana Milinković-Tur[a], Nina Poljičak Milas[c], Marko Samardžija[b], Hrvoje Valpotić[d], Velimir Berta[e], Marinko Vilić[a], Kristina Rakić[f]

[a] Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, 10 000, Zagreb, Croatia
[b] Clinic of Reproduction and Obstetrics, Faculty of Veterinary Medicine, University of Zagreb, 10 000, Zagreb, Croatia
[c] Department of Pathophysiology, Faculty of Veterinary Medicine, University of Zagreb, 10 000, Zagreb, Croatia
[d] Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, 10 000, Zagreb, Croatia
[e] Veterinary Practice Varaždin, 42000, Varaždin, Croatia
[f] Faculty of Veterinary Medicine, University of Zagreb, 10 000, Zagreb, Croatia

A R T I C L E I N F O

Keywords:
Melatonin implants
Spermatozoa subpopulations
Morphometry
French Alpine bucks

A B S T R A C T

The aim of this study was to establish subpopulations of spermatozoa in bucks using the principal component (PC) and cluster analysis according to morphometric head and tail variables, and to determine differences in proportions of subpopulations between exogenous melatonin-treated and control bucks. The bucks ($n = 12$) were assigned to two groups comprising six bucks each. By the end of March, four melatonin implants were inserted in the bucks in the experimental group. Semen was collected weekly using an artificial vagina from March to May (the non-breeding season). Analyses were performed in stained smears by SFORM computer-assisted program for eight head and five tail variables. The PC analysis revealed four components with the most important value for each (head outline, head ellipticity, mid-piece length and width). Cluster analysis indicated there were three subpopulations (average-sized spermatozoa– C_1; small and less-elliptic – C_2; big and elliptic – C_3). Melatonin-treated bucks had a greater proportion of C_1 spermatozoa and a lesser proportion of C_2 spermatozoa during May ($P < 0.05$). To the best of our knowledge, this is the first study where PC and cluster analyses were performed on buck semen with head and tail variables analyzed together, in the same analysis, to evaluate spermatozoa population. Also, this is the first analysis of morphometric variables for assessing the influence of melatonin on spermatozoa subpopulations. The positive effect of melatonin on the proportions of spermatozoa in subpopulations could have been a consequence of the decreasing the proportion of the subpopulation with the least head and tail sizes and ellipticity.

1. Introduction

Small ruminants have a seasonal reproduction pattern that depends on the length of daylight. Sexual activity increases, depending on the breed, as the days get shorter (autumn). An implant, that when inserted, releases sustained amounts of melatonin that is sufficient to maintain physiological plasma concentrations for a period of 70–90 days simulates the shortening of the days. The

* Corresponding author at: Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10 000, Zagreb, Croatia.
E-mail address: svince@vef.hr (S. Vince).

https://doi.org/10.1016/j.anireprosci.2018.03.003
Received 27 January 2018; Received in revised form 23 February 2018; Accepted 2 March 2018
0378-4320/

implant is positioned at the base of the ear and does not need to be removed as it is biodegradable. In small ruminants, it induces estrus and ovarian activity 50–70 days after insertion. In rams and bucks, the use of the implant results in increases in sexual activity during the anestrous season and it increases the quality and quantity of the sperm are increased (Delgadillo et al., 2001, 2004).

In the moderate climate zone, the natural reproductive season of small ruminants is from mid-summer to mid-winter (from August to December), thus, the season begins and continues during the period of the year with a shorter duration of daylight (Rosa and Bryant, 2003; Sarlós et al., 2013). In the past 30 years, slow-release melatonin implants have been used in the control of small ruminant reproduction (Casao et al., 2010a; Egerszegi et al., 2014). The estrous cycle of small ruminants is regulated by endogenous melatonin released by the pineal gland during the dark (night) hours of the day. In addition, estrous cycle regulation is dependent on both genetic and environmental factors (Delgadillo et al., 2001). In seasonal animals, such as small ruminants, melatonin is the chemical messenger which enables the perception of daylight length changes (Chemineau et al., 1996). Accordingly, melatonin has an important role in the adaptation of the circadian rhythm and seasonal changes, owing to its increased concentration in the blood of small ruminants during the dark hours of the day (Malpaux et al., 2001; Cajochen et al., 2003). Ejaculate quality in bucks is significantly less during the non-breeding season (Al-Ghalban et al., 2004). During the non-breeding season, the concentration of testosterone in bucks is much less, resulting in a lesser expression of libido as well as a smaller volume and weight of testes which is consequently reflected in the lesser quality of the ejaculates of these bucks (Al-Ghalban et al., 2004; Delgadillo et al., 2004). Melatonin has a regulatory effect on the hypothalamus-pituitary-testicular axis, modulating pulsatile GnRH release as well as gonadotropin and testosterone secretion (Gallego-Calvo et al., 2015). In addition, the administration of melatonin improves semen quality parameters in rams and bucks during the non-breeding season (Casao et al., 2010a; Egerszegi et al., 2014; Vince et al., 2017). Also, melatonin has directly induced beneficial effects on other spermatozoa characteristics which results from decreased apoptotic-like changes, modulated sperm capacitation, and fertilization rates (Casao et al., 2010b; Gallego-Calvo et al., 2015).

Routine evaluation of semen traditionally included the assessment of normal spermatozoa morphology. An important, but subjective component of such assessment, however, has limited value for practical use (Rodriguez-Martinez, 2006). Other approaches using computer-assisted image analysis have been implemented to diminish subjectivity, improve repeatability, and enhance the sensitivity of spermatozoa morphology assessment (Wibowoa et al., 2013; Sinha et al., 2014; Maroto-Morales et al., 2016). The development of automatic image-processing systems has replaced classical methods, with more accurate, and repeatable measurements of spermatozoa head dimensions in different species, including goats (Gravance et al., 1996a,b; Hidalgo et al., 2005; Hidalgo and Dorado, 2009). Both approaches using computer-assisted image analysis of spermatozoa morphology and multivariate statistical methods, including discriminant and cluster analyses, have been used with great success to morphologically assess spermatozoa head forms in several animal species (Thurston et al., 2001; Buendía et al., 2002; Esteso et al., 2006; Rubio-Guillén et al., 2007; Hidalgo and Dorado, 2009; Valverde et al., 2016). In bulls, the use of spermatozoa head morphometric variables has been considered a reliable criterion for the validation of semen quality (Phillips et al., 2004), and it is recommended as part of the standard spermiogram assessment in domestic animals (Rodríguez-Martínez, 2007; Sousa et al., 2013). To the best of our knowledge, there are no data in the recent literature on the analyses of buck spermatozoa subpopulations regarding the overall morphometric variables such as head, mid-piece and tail measurements. There is a positive correlation between a longer tail and mid-piece may provide a beneficial effect on spermatozoa longevity, although there are inconsistent reports related to these variables (Malo et al., 2006; Firman and Simmons, 2009). Generally, mammalian ejaculates consist of heterogeneous co-existing populations of spermatozoa that are comprised of physiologically and functionally different subpopulations (Peña et al., 2005; Rubio-Guillén et al., 2007). The identification of spermatozoa subpopulations with the most optimal preferential capacity to populate the oviduct and if cells of these subpopulations are involved in fertilization result in normal development of an embryo, might be of the utmost importance to improve the accuracy of semen quality assessments (Muiño et al., 2008).

Recently, morphometric measurements of spermatozoa characteristics in domestic and wild animals have been intensively studied. Data from either the breeding or non-breeding season regarding the effects of exogenous melatonin on morphometric variables of spermatozoa, in general have not been investigated. The aims of the current study, therefore, were: (1) to determine the value of primary morphometric variables of spermatozoa in exogenous melatonin-treated and non-treated bucks, and to use these variables to calculate secondary morphometric parameters of head forms, such as: regularity, rugosity, ellipticity, and elongation; (2) to establish subpopulations of spermatozoa using multi-variate statistical methods, such as principal component and cluster analysis, according to morphometric spermatozoa variables for both the heads and tails, and to determine the differences in the proportions of particular subpopulations between the groups of bucks during the non-breeding season.

2. Materials and methods

2.1. Ethics and welfare approval statement

The Council of the Faculty of Veterinary Medicine, University of Zagreb, Croatia, in accordance with Article No. 31 of the Statute of the Faculty of Veterinary Medicine, University of Zagreb, Croatia, and based on the proposal of the Committee for Ethics in Veterinary Science, approved the current research at the session held on February 22, 2017.

2.2. Bucks

The study was performed on 12 clinically healthy bucks of the French Alpine breed with a proven fertility history, aged 1.5–4 years (M ± SD for the control group: 2.25 ± 0.71, and for the experimental group: 2.64 ± 0.84), and with body weight ranging