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Effect of melatonin treatment on semen parameters and endocrine function in Black Racka rams out of the breeding season

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ABSTRACT

The influence of melatonin implantation on scrotal circumference, semen quantitative and qualitative parameters, plasma melatonin as well as basal and provoked testosterone concentration was evaluated in Black Racka rams in the non-breeding season. Twelve rams were used in the 60-day-long trial; six of them were implanted (M) with Melovine® (Ceva, Libourne, France) subcutaneously on day 0 (10th May) and 30 days later again, while the other six rams remained untreated (C). Scrotal circumference (SC) was measured and semen was collected weekly and assorted to two 30-day-long periods. Blood samples were collected on days 0, 30 and 60 and basal testosterone level and GnRH-induced testosterone response were evaluated. Ejaculate volume (VOL) increased in both groups from the minimum value at the beginning of the trial (0.52 ± 0.08 ml vs. 0.44 ± 0.06 ml) to the maximum at 60 days after the first implantation (0.82 ± 0.07 ml vs. 0.73 ± 0.05 ml, $P < 0.05$). Significant differences were found in SC, VOL and total spermatozoa number from day 30 to day 60 after the first melatonin implantation between the treatment groups ($P < 0.05$). The statistical analyses revealed no difference between treated and control groups regarding total motility % (TM%) and progressive motility % (PM%) at the first implantation and the last sampling. However, at the second implantation melatonin treatment had a positive effect on TM% ($P < 0.05$) and PM% ($P < 0.001$) compared to group C. Basal testosterone (T_b) concentrations were not different between groups at the beginning of the treatment; nevertheless, 60 days later a significantly elevated T_b level was measured in group M compared to C ($P < 0.05$). Testosterone response after the GnRH test was not different between groups at the first and second implantation, while at the third sampling elevated T_b was detected and the provoked testosterone concentrations (T_{incr}) were also higher in group M at 30 and 60 min after GnRH treatment ($P < 0.05$). Plasma melatonin level was increased by implantation to 445.3 ± 91.55 pmol/L measured at day 30 compared to 92.4 ± 9.15 pmol/L on day 0 ($P < 0.05$), and a further increase of melatonin concentration was recorded after the insertion of the second implants at day 60 (699.45 ± 163.91 pmol/L; $P < 0.05$). In conclusion, melatonin implantation in the non-breeding season (at the beginning of May) significantly improved the endocrine and exocrine function of testicles and some quantitative as well as quality parameters of the ejaculate in rams of the Hungarian native breed Black Racka.

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1. Introduction

Among farm animals living in the temperate climate zone, sheep are regarded as a strongly seasonal species. This phenomenon is controlled primarily by cyclic changes of daylight as well as several other factors, i.e. temperature, feeding, contact with males, lambing time, and lactation length (Rosa and Bryant, 2003). These seasonal changes of reproductive performance can be observed also in rams, in a degree decreasing according to the level of domestication and intensive breeding (Lincoln et al., 1990). However, these changes are less marked in rams than in ewes because spermatogenesis and sexual activity never stop, while females have a definite anoestrous period (Pelletier and Almeida, 1987; Casao et al., 2010a). In seasonal animals, melatonin is the chemical messenger which allows the perception of daylight length changes (Chemineau et al., 1993). Melatonin synthesized by the pineal gland plays important roles in several fields of physiology, e.g. in the nervous system, antioxidant defence mechanism, immune system and gastrointestinal tract, as has been reviewed extensively in recent years (Reiter et al., 2009; Hardeland et al., 2011; Carpentieri et al., 2012). The pattern of melatonin secretion conveys information about light/dark cycles to the physiological centres of the body to enable the organization of seasonal and circadian rhythms. Commercial products have been developed for the manipulation of seasonal breeding in sheep (Arendt, 1998).

Melatonin can be used alone as an implant or intravaginal device in ewes, and it is recommended to combine it with other hormonal treatments eventually after an artificial light (photoperiodic) treatment to attain better results (Chemineau et al., 1996; Rathbone et al., 1997). In several studies concerning ewes of different breeds melatonin administration has been found to advance the onset of reproductive activity or prolong sexual activity (English et al., 1986; Kusakari and Ohara, 1997; Abecia et al., 2006; Papachristoforou et al., 2007); furthermore, melatonin has been reported to exert beneficial effects on oocyte quality, ovulation and conception rates (Stellflug et al., 1988; Chemineau et al., 1996; Forcada et al., 2006; deNicolo et al., 2008; Tsiligianni et al., 2009).

In young rams under a long daylight period, melatonin administration increased gonadotropin and testosterone concentrations and reduced prolactin level in the blood plasma but did not affect puberty, in contrast to ewes (Kennaway and Gilmore, 1985). In mature rams, the insertion of melatonin implants facilitated testicular growth with an elevated testosterone concentration and improved semen characteristics in different breeds (Chemineau et al., 1996; Garde López-Brea et al., 1996; Kaya et al., 2000; Casao et al., 2010c). Furthermore, melatonin treatment strengthens ram effect and net lamb production of ewes under field conditions (Fitzgerald and Stellflug, 1991; Rosa et al., 2000; Palacín et al., 2008). A direct beneficial action of melatonin on sperm motility (Casao et al., 2010c) and on other sperm characteristics during the non-breeding season has been demonstrated recently with decreased apoptotic-like changes and modulated capacitation and fertilization rates (Casao et al., 2010b). Data of other authors indicate that if no pre-treatment with long days was applied, melatonin treatment did not have any effect on testis size and semen parameters (Rosa et al., 2012). The Hungarian Black Racka is a native sheep breed which is mainly kept under extensive conditions and bred strictly seasonally (from August to December; Dunka, 2002). Ram lambs reach puberty at the age of 7 months, whilst ram and virgin ewes are first bred at the age of 1.5 years, and the lambing percentage is 110%. It was observed by progesterone profile investigations that the first ovulation occurred in ewes at the end of August, and open ewes had cyclic ovarian features in late January (Becskei, 2002). The reproductive activity of Black Racka rams runs parallel with the seasonal oestrous patterns of ewes (Sarlós et al., 2013). The freezability of ram semen depends on the season (D'Alessandro and Martemucci, 2003); furthermore, inter-breed differences were observed (El-Alamy and Foote, 2001; Joshi et al., 2005). Despite several weak points of the method, semen freezing has been used in conservation programmes of numerous native sheep breeds (Marco-Jimenez et al., 2005; Nel-Themaat et al., 2006; Sabev et al., 2006). Nowadays there is an increasing demand for ex situ in vitro gene preservation by semen cryopreservation from ejaculates obtained outside the breeding season.

The aim of this study was to determine how melatonin treatment affects the endocrine and exocrine functions of the testicles in Hungarian native Black Racka rams in the non-breeding season, with regard to its possible use in conservation programmes.

2. Materials and methods

The studies were conducted at the Experimental Farm of the Research Institute for Animal Breeding and Nutrition in Herceghalom (northern latitude: 47° 29′, eastern longitude: 18° 44′) during the non-breeding season (from 10 May to 10 July).

2.1. Animals

Twelve Black Racka rams (age: 38–48 months, body weight: 55–70 kg) were included in the trial. The animals were housed in groups and fed alfalfa hay and concentrate. They were turned out to pasture daily. The animals had free access to fresh water and mineralized salt blocks.

2.2. Melatonin treatment and GnRH test

The rams were randomly assigned to two equal groups. The melatonin group (M) was implanted subcutaneously twice with a single melatonin capsule (18 mg melatonin implant; Melovine®, Ceva, Libourne, France) first at the beginning of the trial (d0) and then 30 days (d30) later. The control group (C) received no implant. The GnRH test was performed on the days of implantation (d0, d30) and 30 days after the last melatonin treatment (d60) with an intravenous injection of 0.008 mg buserelin (Receptal inj.®, Intervet, Angers, France). Blood samples were collected from the jugular vein for the determination of basal hormone levels after sperm collection during the daytime and 30, 60, 90, 120 min after GnRH application. Samplings were repeated according to the timing of the GnRH tests.

2.3. Plasma metabolites

To prove the similar energy status of rams, basal samples of GnRH challenges collected on d0, d30 and d60 were also assayed for β-OH-butyrate (BHB) and non-esterified fatty acid (NEFA) concentrations.

2.4. Data collected

The following parameters were measured in all experimental animals during the 60-day trial.

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