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Effects of melatonin administration on seminal plasma metabolites and sperm fertilization competence during the non-reproductive season in ram

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ABSTRACT

The purpose of this study was to investigate the effects of ram melatonin treatment on the sperm quality and metabolite composition of the seminal plasma in the non-breeding season. Four mature rams were treated with 54 mg melatonin in March subcutaneous implants and four untreated rams served as control. At 0, 30, 90 and 120 days semen samples were collected and sperm, separated from seminal plasma, was evaluated for its capacity to fertilize and produce embryos in vitro. Seminal plasma metabolites were extracted and analyzed by capillary electrophoresis/mass spectroscopy. In the resulting electropherograms, the area corresponding to selected metabolites was extracted and quantified. Ram melatonin treatment affected the in vitro fertilization competence of sperm. Blastocyst output increased until 90 days after treatment (27.20 ± 7.35 vs 54.7 ± 4.4% at 0 and 90 days respectively; $p < 0.05$) while the untreated group did not show statistical differences.

In treated rams, the concentration of melatonin in seminal plasma increased from 3.34 ± 1.70 at day 0 −9.65 ± 2.89 AU (Arbitrary Units) after 90 days, then decreased to reach the level of the untreated ram after 120 days ($p < 0.05$). During 90 days after melatonin treatment, an increase ($p < 0.05$) in seminal plasma concentrations of glutamic acid (6.28 ± 1.53 vs 14.93 ± 1.53 AU at 0 and 90 days respectively), glutamine (16.89 ± 4.65 vs 54.51 ± 4.65 AU), carnitine (22.97 ± 9.81 vs 104.30 ± 9.81 AU), acetyl-carnitine (48.15 ± 17.32 vs 217.69 ± 17.32 AU), choline (1.82 ± 1.55 vs 14.16 ± 1.55 AU) and arginine (1.31 ± 1.08 vs 14.25 ± 1.08 AU) was detected. Tyrosine concentration increased during 30 days from melatonin treatment (12.79 ± 3.93 vs 27.08 ± 3.04 AU) but at 90 days its levels were similar to the untreated group. In conclusion, melatonin treatment during the non-breeding season improves the concentration of several metabolites in seminal plasma and sperm fertilization competence in Sarda breed ram.

1. Introduction

Melatonin, an indoleamine synthesized by the pineal gland, plays important roles in several fields of physiology, e.g. in the nervous system, antioxidant defense mechanism, immune system, gastrointestinal tract and reproductive system, as has been largely reviewed [1,2]. A positive relationship between the daily light cycle and the characteristics of the sperm and its fertility potential [3,4] was highlighted. In contrast, no correlation has been found between sperm concentration, motility or morphology, and melatonin, testosterone and estradiol levels in men's blood or seminal plasma [5]. Addition of 2 mM melatonin to human sperm significantly improved the percentage of motile, progressive motile and rapid sperm, decreasing the intracellular nitric oxide concentration but not reactive oxygen species (ROS) [6]. Previous findings have

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reported that the administration of melatonin to Wistar rats negatively influences motility, morphology, and epididymal sperm concentration [7]. On the contrary, it has been recently showed that melatonin treatment does not affect the motility of Wistar rat sperm [8].

Melatonin implants significantly increased the activity of antioxidant enzymes in seminal plasma during the non-reproductive season [9] and positively influenced the percentage of progressive motile sperm in Rasa Aragonesa breed ram. In vitro treatment with melatonin did not affect the kinematic parameters of sperm but increased the fertilization rate of oocytes following IVF.

Treatment with melatonin has been shown to increase the viability, motility, intracellular ATP concentrations and DNA integrity of Sarda breed sperm during the non-reproductive season [10]. This contradictory effect of melatonin could be determined by the difference in sensitivity to seasonality, the duration of treatment and the concentration of hormones [3]. To improve performance during the non-breeding season, slow-release melatonin implants have been widely used to control the reproduction of small ruminants for over thirty years both in highly seasonal [11] and Mediterranean [12] ewes, including the Sarda breed [13]. Commercial melatonin implants have been designed to maintain high levels of the pineal hormone for 40–70 days [14], although they may release melatonin for more than 100 days [15]. However, several issues related to their mode of action are not yet clear [12].

In the ram, although sperm production continues throughout the year, during the non-breeding season the quality of the sperm is low [16] and is associated with a reduction in the diameter of the testis [17] and of the hormonal profiles [12]. It has been reported that melatonin treatment of rams of different breeds during the non-breeding season increases sperm quality and its fertility potential, modulate hormonal interactions and modifies the biochemical composition of the seminal plasma [16–19]. This indolamine interacts with the hypothalamus-pituitary axis which induces an increase in the pulsatile secretion of gonadotropin-releasing hormone (GnRH), low levels of prolactin and increases in luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone secretion [20] and the improvement of the quality of fresh and frozen semen in different breeds during the non-breeding season [10,18,19,21].

Melatonin implants influence sperm capacitation by modifying the activity of the plasminogen activator (PA) and acrosin [22,23] in ram sperm and seminal plasma. These enzymes are essential for sperm capacitation [24] and, whereas PA seems related to testosterone concentration [23], acrosin activity is independent of the fluctuations of this hormone and may be the result of a direct action of melatonin on sperm cells.

The seminal plasma is a complex fluid secreted from rete testis, epididymis, seminal vesicles, prostate and bulb-urethral glands [25]. It provides metabolic support, as an energy source for the sperm cells, and influences the functionality of the sperm [26]. Seminal plasma metabolites are involved in different sperm features related to sperm function, fertilization and embryo development in the female reproductive tract [27–29]. Mammalian testis secretes considerable amounts of amino acids (200 μmoles/day) into the seminal plasma [30] that play a key role in defining the sperm quality. Free metabolites of seminal plasma, including choline, carnitine, acetyl-carnitine, arginine, tyrosine, are involved in several metabolic ways increasing sperm fertility potential [31]. The concentration of seminal plasma metabolites gives several positive actions on the sperm developing the prevention of lipid peroxidation [31], supporting the transport of fatty acids into the mitochondria for β-oxidation [32], promoting ATP synthesis [33], providing ready energy [34], protecting against oxidative damage [35] and repairing ROS insults [35].

Treatment with melatonin has been shown to modify the composition of seminal plasma [5,9,18,36,37]. However, studies evaluating the effect of melatonin administration in the non-breeding season on sperm quality and on the composition of seminal plasma metabolites are scarce.

In the present study, the impact of melatonin implantation in ram during the non-breeding season on the sperm fertilization capacity as index of fertility potential of ram sperm and on the composition of the seminal plasma metabolites season were investigated.

2. Materials and methods

2.1. Reagents and animals

All reagents and media were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

The experimental procedures with Sarda breed ram (*Ovis aries*) were approved by the Animal Care and Use Committee of the University of Sassari, Italy. All procedures were carried out at the experimental facilities of the Regional Research Centre (AGRIS) of Sassari, Italy (latitude 40°40′ N) from March to June during the non-breeding season, described for this breed at this latitude from late January to late March [13]. These facilities meet the requirements of the European Union for Scientific Procedure Establishments. This study followed ethical guidelines for care and use of agricultural animals for research (EC Directive 86/609/EEC for animal experiments).

2.2. Experimental design

Melatonin implants maintain high serum levels of melatonin for 40–70 days [11] but release the hormone for about 100 days [15]. Four times were selected for semen collection to assess whether melatonin treatment affects seminal plasma composition or sperm fertilization competence, and how long its effect is detectable. We chose 30 days after treatment to assess whether this affects the concentration of melatonin and metabolites studied in the seminal plasma and the fertility potential of sperm in the short time, while 90 and 120 days were chosen to assess whether the effect on the analyzed factors lasts when the release of melatonin is minimal. The semen of melatonin-treated ram trained to artificial vagina was collected after 0, 30, 90 and 120 days from treatment.

To evaluate whether melatonin affects metabolite seminal plasma composition and sperm fertility competence, seminal plasma was separated from sperm and treated for analysis. Choline, glutamine, glutamic acid, histidine, carnitine, arginine, tyrosine, acetyl-carnitine and melatonin were extracted from seminal plasma and quantified by Capillary electrophoresis/mass spectrometry.

To estimate sperm fertilization capacity, in vitro matured sheep oocytes were fertilized with separated sperm and development to the 2-cell at 30 h and blastocyst stages at 7 days were detected.

2.3. Semen collection

Eight adult Sarda breed rams aged 3–5 years were maintained in an outdoor environment and received 400 g of commercial concentrate feed per head (crude protein 20.4% and 12.5 MJ ME/kg DM), divided into two times of the day (morning and evening), water and hay (crude protein 11.1% and 7.2 MJ ME/kg DM) ad libitum. Rams, randomly divided into two groups, were kept in separated pens, in visual contact with each other. The treated group, consisting of 4 rams which received three contemporary subcutaneous melatonin implants (Melovine, CEVA VETEM S.P.A., Milano,