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Daily administration of a GnRH analogue enhances sperm quality in bucks during the non-breeding season

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ABSTRACT

The aim of this study was to determine if daily administration of a GnRH analogue (buserelin acetate) to bucks during the non-breeding season increases testosterone concentration and improves sperm quality. Five bucks received a daily dose of buserelin for 10 days, starting on Day 0 (first administration), and another five bucks remained as controls. Testosterone concentrations were greater in treated than in control bucks during the first hours after buserelin administration ($P = 0.05$), but greater in controls 10 h later ($P < 0.01$). Sperm mass motility and percentage of motile sperm were greater in treated (3.9 ± 0.6 and 70.1 ± 7.9%, respectively) than in control bucks (1.0 ± 0.6, $P < 0.01$; 45.0 ± 7.9%, $P < 0.05$ respectively) on Day 4. Percentage of sperm with normal morphology tended to be greater in treated than in control bucks (81.8 ± 6.2% compared with 63.5 ± 6.4% respectively, $P = 0.08$). The treatment decreased the percentage of sperm with mid piece defect and with bent tail (7.0 ± 1.5% compared with 12.0 ± 1.5%; 8.0 ± 1.7% compared with 13.5 ± 1.7%, treated and control bucks, respectively, $P = 0.05$ for both). The square root percentage of sperm with loose but heads with normal structures tended to be less in treated than control bucks (1.3 ± 0.3% compared with 0.4 ± 0.3% respectively, $P = 0.06$). It was concluded that daily administration of buserelin during the non-breeding season led to a rapid increase in testosterone concentration and improved sperm quality.

1. Introduction

Most goat breeds have a seasonal reproductive pattern mainly determined by photoperiod (Delgadillo et al., 1993; Dardente et al., 2016). In males of several species, gonadotropin and testosterone concentrations are maximal (Delgadillo and Chemineau, 1992), and sperm of the greatest quality, when photoperiod is decreasing (Zarazaga et al., 2009; Giriboni et al., 2017). During the non-breeding season, however, testosterone has a negative feedback at the hypothalamus, decreasing the frequency of GnRH pulses and thus, gonadotropin (FSH and LH) concentration (Tilbrook et al., 1991). In several livestock management systems, it is important to induce out-of-season parturitions, however, male fertility may be an important limitation unless techniques to modify seasonal reproductive pattern are used. Treatments with melatonin implants (Chemineau et al., 1992) or with light regimens (Delgadillo and Chemineau, 1992) have been effectively used in goats, but these techniques cannot be easily used in extensive productive systems, are economically costly, and require several weeks after initiation to have an effect (see review: Menchaca and Ungerfeld, 2017). It,

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therefore, is important to develop other alternatives to improve reproductive capacity of males during the non-breeding season.

The GnRH agonists can be used to induce a rapid increase in the synthesis and secretion of gonadotropins, and thus in androgen secretion (Schanbacher and Lunstra, 1977; Fraser and Lincoln, 1980). These hormones may be used to increase the reproductive capacity of males. In females, GnRH administration has been included in estrous synchronization (Pierson et al., 2003) and multiple ovulation and embryo transfer (Menchaca et al., 2009) treatments. In males, the stimulatory effects of the administration of GnRH are transient and the information in these regards is limited and results inconsistent. In stallions, treatment with two daily doses of a GnRH analogue (buserelin acetate) for 6 weeks improved sexual behavior and increased the quality of frozen-thawed semen during the non-breeding season (Sieme et al., 2004). Similarly, the administration of three doses of another GnRH analogue (gonadorelin diacetate tetrahydrate) every 2 days increased testosterone concentration, led to having a shortened semen collection period and increased the sperm concentration in the ejaculate of camels (Monaco et al., 2015). Administration of GnRH increased testosterone concentration and testicular blood flow in bucks (Samir et al., 2015) and seminal fluid content in rams (Ungerfeld and Fila, 2011). The administration of two daily doses of GnRH for 7 weeks in rams resulted in an increase in testosterone concentration, scrotal circumference and the percentage of sperm with progressive motility in the ejaculate (Schanbacher and Lunstra, 1977). A shorter treatment (21 days), however, induced an initial increase in gonadotropins and testosterone secretion, but the response was not sustained (Lincoln et al., 1986). The sustained administration of GnRH, therefore, suppresses the pituitary-gonadal axis secretions of gonadotropins and testosterone, respectively (Xue et al., 1994; Junaidi et al., 2007) as it exerts a GnRH receptor downregulation (Lincoln et al., 1986) and thus, reduces LH, FSH and testosterone secretion (Fraser and Lincoln, 1980; Lincoln et al., 1986). Thus, it seems that the effectiveness of the administration of GnRH to improve the reproductive capacity is related to the administration protocol selected.

Considering all this information and based on the testosterone response to buserelin treatment (Damián et al., 2015), the protocol included the administration of the dose used in this previous study for 10 consecutive days. To produce positive effects on spermatogenesis with the administration of GnRH, the treatment should be longer, compromising its effectiveness due to the GnRH receptor downregulation that occurs when its administration is continued for a period. A short-term protocol, therefore, may be useful to enhance sperm quality as a result of effects at the epididymis and the sexual glands. The aim of the present study, therefore, was to determine if daily administration of a GnRH analogue for 10 days to bucks during the non-breeding season increases testosterone concentration and improves semen quality.

2. Materials and methods

2.1. Animals and experimental design

All the procedures were approved by the Ethical Committee of Aydın Adnan Menderes University (ADU-HADYEK 64583101/2018/097). The study was performed at the Çine Vocational School (Adnan Menderes University, latitude 37 37′ 48″ N, longitude 28 02′ 27″ E) during the non-breeding season (June, end of spring) with 10 bucks (six Alpine and four Hair bucks; 1–2 years old; 57.3 ± 3.5 kg; mean ± SEM). All animals were allocated in the same pen (9 m × 5.5 m) and received alfalfa hay (1.8 kg/day/animal) and concentrate (1.5 kg/day/animal), and had free access to water. Animals were allocated to two experimental groups and were of homogeneous breeding, as well as having a similar age and body weight. Five bucks received a daily i.v administration of a GnRH analogue (4.2 μg/animal of buserelin acetate, Buserin, Alke, Istanbul, Turkey) for 10 days, a dose that was used because of the response in a previous study (Damián et al., 2015). Buserelin was administrated at 09:00 h beginning on Day 0 (12 June; day of first administration). The other five bucks remained untreated as controls.

2.2. Blood samples

Blood samples were collected twice daily on Day -7 and -4 and from Day 0 to 10, at 9:00 (immediately before buserelin administration) and 19:00 (10 h after buserelin administration). Additionally, on Day 0 and 10, blood samples were collected every 30 min during 7 h beginning immediately before buserelin administration. Samples were allowed to clot for 1 h at room temperature before centrifugation for 15 min at 1500 *g* and were stored at -20 °C until testosterone concentration was quantified by radioimmunoassay in plasma aliquots as previously described (Santiago-Moreno et al., 2005). All the samples were analyzed for testosterone concentration in a single assay. The detection limit of the assay was 0.2 nmol/L and the intra-assay coefficient of variation was 7%.

2.3. Scrotal circumference, semen collection and fresh sperm evaluation

Scrotal circumference was measured on Days -7, -4, 1, 4 and 7, and semen was immediately collected on the same days. Semen was collected by electroejaculation using a rectal probe of 13 cm length x 2 cm width with longitudinal electrodes (Mark IV, Olvet, Ruakura, New Zealand). After insertion of the probe coated with carboxymethyl cellulose into the rectum, electrical pulses were applied for 4 s alternated with rest periods of 4 s until ejaculation. Semen was collected from all animals at all times that collections were scheduled to occur. Immediately after semen was collected, sperm motility mass and sperm vigor, as well as the percentages of motile sperm and sperm with progressive motility were assessed subjectively under phase contrast microscopy (Olympus, model CX31) on a warmed (37 °C) glass slide. Sperm mass motility (wave motion) and sperm vigor (the quality of sperm movement) were scored both on a scale of 0 to 5 (Evans and Maxwell, 1987). Sperm mass motility (0: no motion and 5: numerous rapid waves) was

assessed in fresh samples at 10 × . Sperm vigor, the percentages of motile sperm and sperm with progressive motility were assessed in semen diluted with UHT skim milk (1:10 v/v) at 400 × . Sperm vigor was scored as: 0, no motility; 1, weak tail movement, no forward progression; 2, slow forward movement, often in a circular pattern; 3, moderate forward movement; 4, rapid forward movement; and 5, very rapid forward movement. The percentage of sperm with a functional membrane was determined with use of the hypo-osmotic swelling test (HOST) at 400 × as previously described by Jeyendran et al. (1984). Sperm concentration was determined using a Neubauer chamber. Morphological abnormalities were assessed by phase contrast microscopy at 400 × using 10 µl of fixed samples (1:500 v/v in formol citrate) counting 200 cells. Sperm cell morphology was categorized as either normal or cells having an abnormal head, mid-piece defect, coiled tail, bent tail, broken tail, proximal or distal cytoplasmic droplet, or loose but normal head structure (Boe-Hansen et al., 2018). All sperm analyses were performed by the same operator.

2.4. Statistical analysis

Normal distribution of data of testosterone concentration, scrotal circumference and all sperm characteristics was confirmed with the Shapiro-Wilk test and then compared with the mixed model of SAS software (University Edition), considering treatment (administration or not of buserelin), time (day of collection), and the interaction between treatment and time as main effects. Time was included in the model as a repeated data. Percentage of sperm with loose but normal head structure was normalized with square root transformation before the statistical analysis. A mean value of data collected on Days -4 and -7 was used as a single pretreatment value. Testosterone concentrations during the entire experiment in the morning and during the entire experiment in the afternoon were analyzed independently. The testosterone response to buserelin administration on Day 0 and 10 was compared with a mixed model, including the treatment (administration or not of buserelin), the response to buserelin administration (Day 0 or 10), the time after buserelin administration and the interactions as main effects. The animal in the group, and the breed were included as random effects in the model. Data are presented as the mean ± SEM. Differences were considered as significant when $P \leq 0.05$, and tendencies when $0.1 < P < 0.05$.

3. Results

3.1. Testosterone concentration

Testosterone concentration was not different between groups, however, after the buserelin treatment there was an interaction between treatment and response to buserelin treatment ($P < 0.001$). In the control group, testosterone concentration on Day 0 (8.7 ± 2.4 nmol/L) and 10 (18.0 ± 2.4 nmol/L) was not different, however, in the buserelin-treated bucks, testosterone concentration on Day 0 (18.0 ± 2.4 nmol/L) was greater than on Day 10 (8.8 ± 2.3 nmol/L, $P < 0.001$). Also, on Day 0, testosterone concentration was greater for buserelin-treated (18.0 ± 2.4 nmol/L) than control bucks (8.7 ± 2.4 nmol/L, $P < 0.05$), but on day 10 testosterone concentration was not different between groups (7.5 ± 2.3 and 8.8 ± 2.3 nmol/L, control and buserelin-treated bucks, respectively).

On Day 0,in the samples collected during the first 7 h after the buserelin administration, LSmean values of testosterone concentration were greater in buserelin-treated than control bucks (17.7 ± 3.4 nmol/L compared with 6.4 ± 3.6 nmol/L respectively, $P = 0.05$). There was an interaction between treatment and time ($P < 0.001$): testosterone concentration was greater in buserelin-treated than in control bucks 30 ($P < 0.05$), 60 ($P < 0.01$), 90 ($P < 0.001$), 120 ($P < 0.001$), 150 ($P < 0.01$) and 180 min ($P < 0.05$) after buserelin administration (Fig. 1A). In buserelin-treated bucks testosterone concentration increased and remained greater from 30 to 300 min after the administration of buserelin and subsequently there was a return to basal concentrations.

On Day 10, in the samples collected during the first 7 h after the buserelin administration, testosterone concentration was not affected by the treatment but there was a interaction between treatment and time ($P < 0.001$): testosterone concentration was greater in buserelin-treated than in control bucks at 60 ($P < 0.001$), 90 ($P < 0.01$), 120 ($P < 0.05$) and 180 ($P < 0.05$) after administration. At 300 min after buserelin administration, control bucks had a greater testosterone concentration than buserelin-treated bucks ($P < 0.05$; Fig. 1B). In buserelin-treated bucks, testosterone concentration remained greater from 30 to 240 min after the administration of buserelin, and subsequently there was a return to basal concentrations.

Considering the entire experiment, testosterone concentration before buserelin administration (morning concentrations) was greater in the control than buserelin-treated bucks (9.3 ± 1.0 nmol/L compared with 3.0 ± 0.9 nmol/L, respectively; $P < 0.01$). There was also a tendency for an interaction between treatment and time for the samples collected in the morning ($P = 0.1$; Fig. 2A). In the samples collected during the afternoon, testosterone concentration tended to be greater in the control than buserelin-treated bucks (7.8 ± 1.3 nmol/L compared with 4.3 ± 1.2 nmol/L, $P = 0.08$), and there was no treatment and time interaction (Fig. 2B).

3.2. Scrotal circumference and sperm characteristics

Scrotal circumference was not affected by buserelin administration (30.9 ± 0.9 cm and 29.5 ± 0.9 cm, buserelin-treated and control bucks, respectively) and there was no interaction between treatment and time.

Sperm mass motility was not affected by treatment, but there was an interaction between treatment and time ($P < 0.05$): on Day 4 sperm mass motility was greater in buserelin-treated than in control bucks (3.9 ± 0.6 compared with 1.0 ± 0.6, respectively, $P = < 0.01$; Fig. 3A). The percentage of motile sperm was not affected by buserelin administration. There, however, was also an interaction between treatment and time ($P = 0.05$). On Day 4, the percentage of motile sperm was greater in buserelin-treated than in

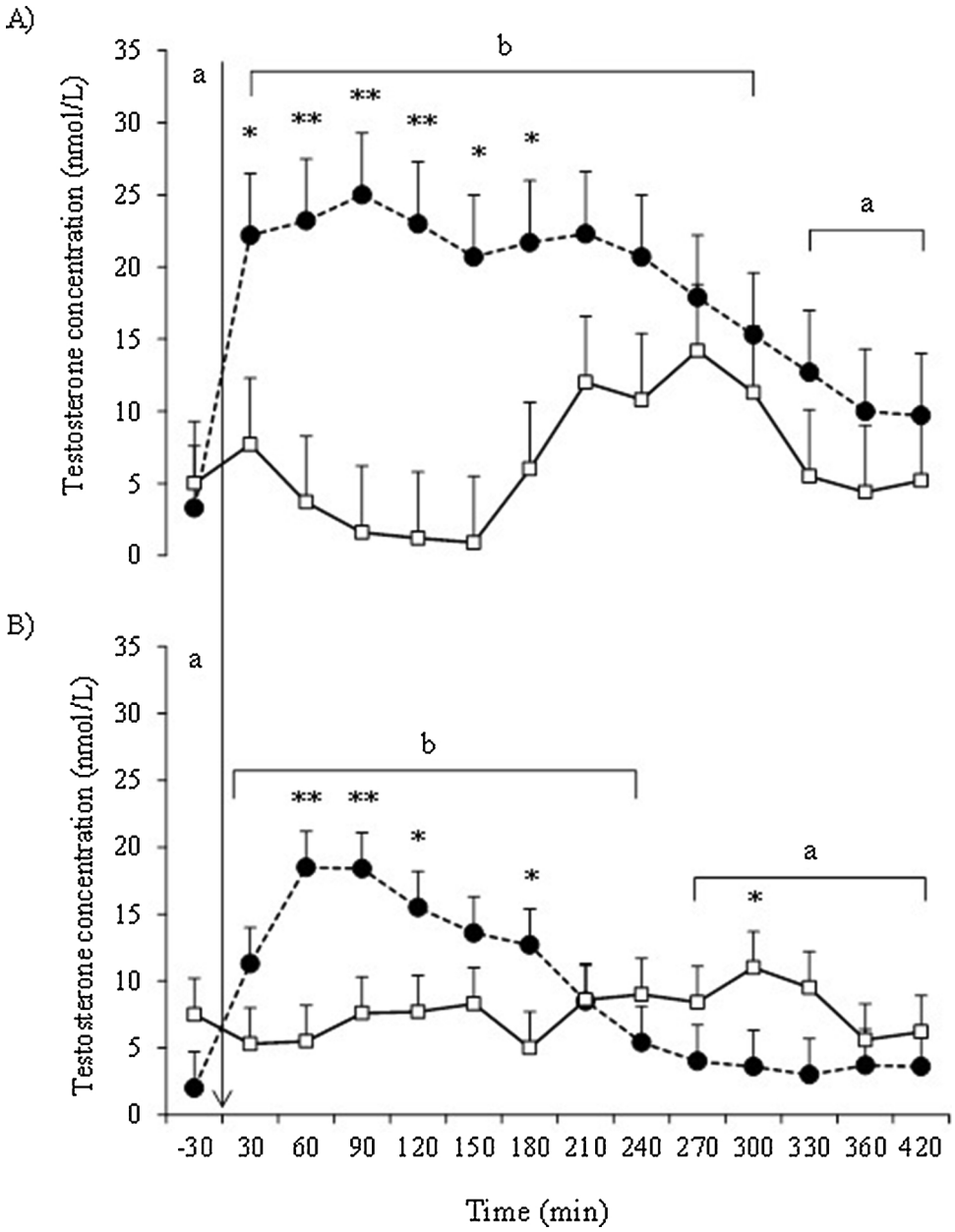

Fig. 1. Serum testosterone concentration (mean ± SEM) in bucks that received a single dose of 4.2 μg of buserelin acetate (n = 5, -●-) or untreated bucks (n = 5, -□-) for A) the first and B) last day of administration.
Different letters indicate differences over time; differences between groups in the same time are indicated *($P < 0.05$) and ** ($P < 0.01$); arrow indicates time of buserelin administration

control bucks (70.1 ± 7.9% compared with 45.0 ± 7.9% respectively, $P < 0.05$; Fig. 3B). The percentage of sperm with progressive motility and sperm vigor were not affected by buserelin administration, but there was a tendency for an interaction between treatment and time ($P = 0.08$ and $P = 0.07$, respectively). The percentage of sperm with normal morphology tended to be greater in buserelin-treated than in control (81.8 ± 6.2% compared with 63.5 ± 6.4% respectively, $P = 0.08$) bucks, and there was no interaction between treatment and time. For morphological abnormalities, the percentages of sperm with a midpiece defect and with a bent tail were affected by treatment. Treatment decreased the percentage of spermatozoa with those abnormalities: percentage of sperm with midpiece defect: 7.0 ± 1.5% compared with 12.0 ± 1.5% for buserelin-treated and control bucks, respectively ($P = 0.05$). Percentage of sperm with a bent tail was 8.0 ± 1.7% compared with 13.5 ± 1.7% for buserelin-treated and control bucks, respectively ($P = 0.05$). The square root percentage of sperm with loose but normal head structures tended to be less in buserelin-treated than in control bucks (1.3 ± 0.3% compared with 0.4 ± 0.3% respectively, $P = 0.06$). The sperm concentration

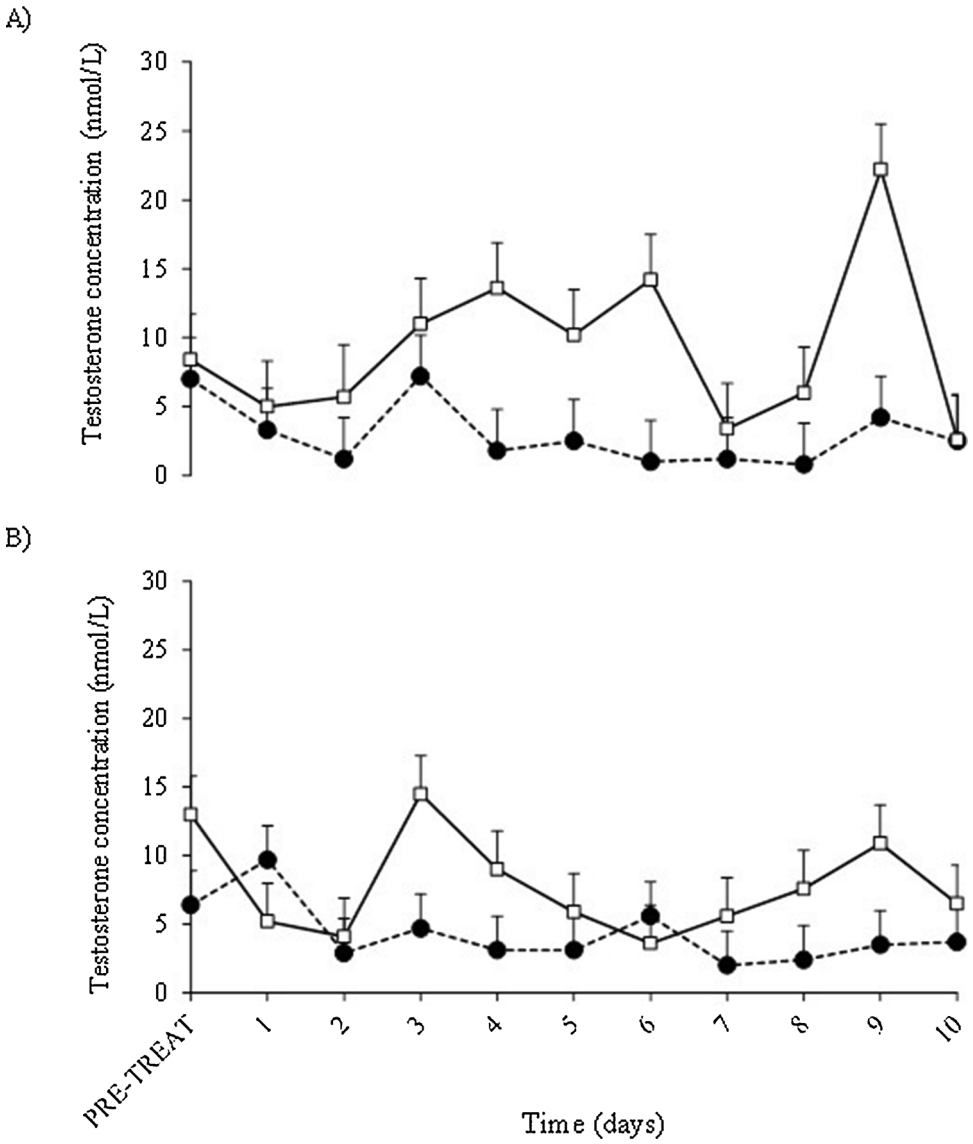

Fig. 2. Serum testosterone concentration (mean ± SEM) in bucks that received a daily dose of 4.2 μg of buserelin acetate for 10 consecutive days (n = 5,-●-) or untreated bucks (n = 5,-□-); samples were collected: (A) in the morning, before buserelin administration (24 h after the previous administration); (B) in the afternoon, 10 h after buserelin administration.

(5.0 ± 0.6 × 10^6 sperm/mL and 4.0 ± 0.6 × 10^6 sperm/mL, buserelin-treated and control bucks, respectively) and the percentage of sperm with functional membranes (75.2 ± 6.4% and 66. 3 ± 5.9%, buserelin-treated and control bucks, respectively) were not affected by buserelin administration, and there was not an interaction between treatment and time.

4. Discussion

Administration of buserelin to bucks during the non-breeding season induced an immediate increase in testosterone concentration, and there was a rapid and transient improvement in sperm quality. To the best of our knowledge, there are no previous reports where there were similar aims, therefore, there are possibilities for evaluation of other protocols, including different GnRH analogues, doses, and frequency of administration for treatment of bucks to enhance their capacity for out-of-season breeding. It should be important to maximize the stimulus with the minimum risk in desensitizing the response to treatment with GnRH or its analogues.

These effects were obvious for enhanced sperm motility and morphology, even considering the small number of animals used in the study. The improvement in sperm quality was observed in a short time after initiation of buserelin treatments, so the positive effects are probably not explained by effects on spermatogenesis, and are more likely related to enhancement of the conditions for sperm stored in the epididymis and/or seminal plasma composition. In effect, gonadotropins and testosterone secreted in response to

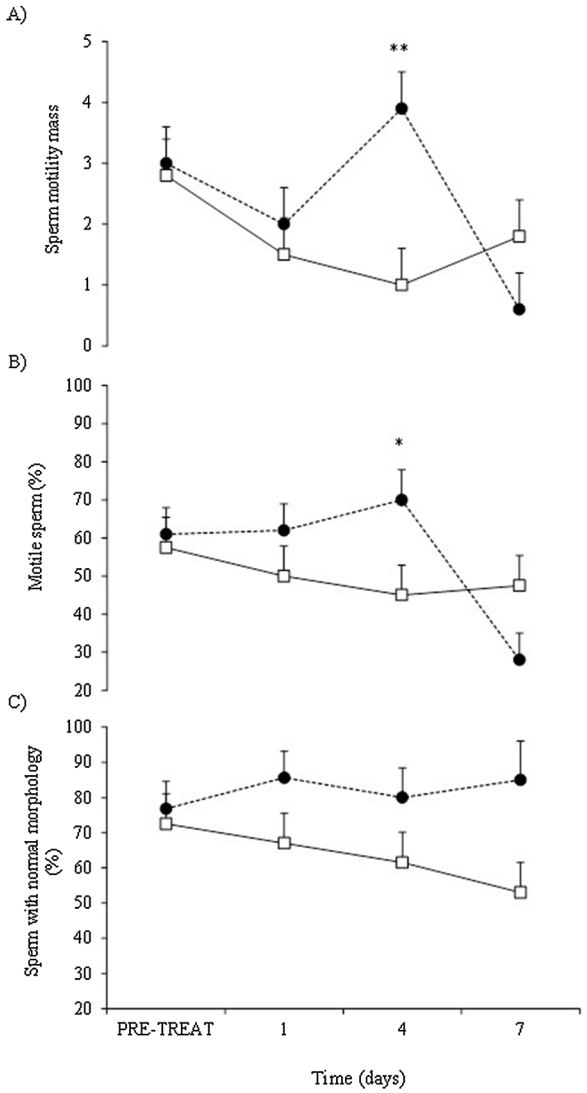

Fig. 3. (A) Sperm motility mass (0–5 scale),(B) percentage of motile sperm (%) and (C) percentage of sperm with normal morphology in bucks that received a daily dose of 4.2 µg of buserelin acetate for 10 consecutive days (n = 5, -●-), and untreated bucks (n = 5, -□-); data are presented as (mean ± SEM).
Differences between groups in a time point are indicated with * ($P < 0.05$) and ** ($P < 0.01$)

buserelin administration may function directly at the epididymis (Parlevliet et al., 2006; Swider-Al-Amawi et al., 2007, 2010,) and accessory sex glands (Tao et al., 1995, 1998; Bilinska et al., 2005), modifying the composition of the seminal plasma. Androgens regulate the epididymal function (Pujol and Bayard, 1979; Pearl et al., 2007), stimulating the synthesis and release of proteins and vesicular secretions into the seminal plasma (Cooper, 1998), and are also associated with the content of fructose, citric acid and some ions and enzymes released by the accessory sex glands into the seminal plasma (Borque and Vázquez, 1999; Matsuoka et al., 2006; Zamiri et al., 2010). All of these components in the seminal plasma interact with the sperm membrane and exert positive effects on sperm metabolism, motility, capacitation and fertilization capacity (Duan and Goldberg, 2003; Shore et al., 2003). Considering that the response was rapidly observed it is not likely related to spermatogenesis, however, it possibly could be explained by modifications in the seminal plasma that enhanced sperm quality. Also, in a recent study in bucks a sustained increase in gonadotropins and/or testosterone concentrations for 20 days improved sperm quality and cryo-resistance to the freezing-thawing process (Beracochea et al., 2018). In addition, FSH and LH are detected in the seminal plasma, with its concentration being positively related to sperm mass motility and sperm concentration in bulls (Tuli et al., 1984). The administration of buserelin probably led to an increase in FSH and LH in the seminal plasma, which might explain the improvement on the sperm quality. Although the mechanisms may be still unclear, the positive effects on sperm morphology were evident on the morpho-abnormalities that are produced in the epididymis, as sperm with loose but normal head structures and with a bent tail, providing greater support to the role of the epididymis in the response to treatment in the present study.

The increase in testosterone concentration during the first day of the experiment (Day 0) was followed by lesser concentrations during the following days. During the first day, the testosterone response was directly related to buserelin administration, remaining elevated for 3 h. During the following days and until the end of the experiment, however, testosterone concentrations decreased to basal. This is probably due to a downregulation of the pituitary GnRH receptors. In effect, although the sustained administration of GnRH agonists leads to an initial increase in LH secretion (Aspden et al., 1997) it is followed by an abolition of its pulsatile release as a consequence of the desensitization on the pituitary gland (D'Occhio et al., 2000). There should also be consideration that testosterone was quantified 10 h after the administration of buserelin, so lesser concentrations may also be consequence of the negative feedback of the testosterone increase at the hypothalamic -pituitary axis.

The positive effects on sperm motility were transient and observed near the mid-treatment period, so it would be interesting to study protocols with more frequent but smaller doses of buserelin, which may maintain the positive effects for a longer time. The administration of multiple small GnRH doses induces a sustained increase in testicular blood flow (Ungerfeld and Fila, 2011). This type of administration will likely be more effective in inducing a response similar to what occurs with physiological conditions and, therefore, to induce a more sustained response. It would not be practical, however, to use this administration pattern in field conditions. Considering that GnRH analogues are commercially readily available, the type of treatment approach used in the present study might be useful for improvement of the reproductive capacity of males during the non-breeding season with a low-cost, rapid and easily applicable treatment. Furthermore, the approach used in the present study could provide a means for extending the periods when there is greater semen quality and thus be useful when different reproductive technologies are utilized such as artificial insemination.

Overall, it is concluded that daily administration of a GnRH analogue to bucks enhanced testosterone secretion and transiently improved sperm quality during the non-breeding season. This approach, therefore, may result in development of other protocols for use of GnRH analogue administration to improve reproductive capacity of males during the non-breeding season.

Declarations of interest

None.

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