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Effects of buserelin administration on testicular blood flow and plasma concentrations of testosterone and estradiol-17β in rams

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ABSTRACT

This research aimed to examine for the first time the impact of single dose administration of gonadotropin-releasing hormone (GnRH) analog buserelin acetate on the testicular blood flow measurements (peak systolic velocity [PSV], end-diastolic systolic velocity [EDV], resistive index [RI], and pulsatility index [PI]) and the plasma steroids (testosterone and estradiol-17β) concentrations in rams. For this purpose, twelve adult Ossimi rams were randomly assigned into the buserelin group ($n = 8$) and were injected intravenously (iv) with buserelin acetate (0.008 mg/ram), whereas the remaining rams ($n = 4$) were injected with normal saline iv and served as a control group. Blood sampling and testicular pulsed-wave Doppler scanning were conducted immediately before (0) and 1, 3, 6, 24, 48, 72, 120, and 168 h after treatment. The control group did not reveal any substantial changes ($P > 0.05$) in the examined parameters, except for the EDV ($P < 0.05$). In the buserelin-treated group, a marked reduction in RI and PI values ($P < 0.05$) occurred 1 to 3 h after administration of buserelin. Besides, there was a significant increase in testosterone plasma concentrations following buserelin treatment. In conclusion, the administration of buserelin triggered a series of substantial changes in the testicular blood perfusion and steroidogenesis that could have a positive effect on testicular function in rams.

1. Introduction

The testis is a relatively compact reproductive structure in which the highly tortuous seminiferous tubules constitute approximately 70%–80% of the testicular parenchyma with limited oxygen condensation and tightly enclosed by a thick connective tissue capsule. It is known as the most important component in the male reproductive system, as it performs crucial metabolic tasks that include both exocrine (spermatogenesis) and endocrine (steroidogenesis) physiological processes. Thus, maintaining a constant and steady arterial blood supply through the testicular artery is critical for the testis' functionality [1,2]. Several studies in humans [3,4], stallion [5,6], rams [7], and rats [8] reported a significant correlation between testicular blood perfusion and semen quality as well as seminal and plasma concentrations of testosterone. In other words, increased testicular blood flow may result in increased male fertility because of its positive impacts on spermatogenesis [9].

Pulsed-wave Doppler ultrasonography provides a detailed analysis of the blood flow and waveform; it is used to characterize blood flow in the testicular artery of rams [10-12], stallions [13,14], and bucks [15]. Parameters such as peak systolic velocity (PSV), end diastolic velocity (EDV), and indices such as resistive index (RI) and pulsatility index (PI) are the most used blood flow measures. However, PSV and EDV can directly reflect blood flow velocities in arterial vessels throughout the cardiac cycle, and their values are highly variable and inconsistent across measurements

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[16]. Since the RI and PI values are negatively associated with blood flow, they have been used mainly as markers for testicular blood flow in rams [9] and indicators for fertility in camel bulls [17] and dogs [18].

Several treatments were tested to modify the testicular blood flow and, in turn, improve testicular function and boost male fertility. Gonadotropin-releasing hormone (GnRH) is known to be an essential reproductive hormone and a valuable endocrine tool to modulate the function of the male endocrine reproductive system [19,20]. It has been shown that in Shiba bucks, GnRH analogs can improve testicular blood flow and testicular volume [15]. Moreover, plasma testosterone concentrations and motile sperm percent have been increased after injection of GnRH analog in a Beagle dog with azoospermia [21].

To the best of our knowledge, the impacts of buserelin administration on testicular blood flow and plasma concentrations of steroid hormones (testosterone and estradiol -17β) are not previously examined in rams. We hypothesize that administering a GnRH analog (buserelin) to Ossimi rams will improve their reproductive performance via its possible effect on the testicular blood perfusion and steroid hormones' production. Therefore, the present study aimed to investigate how a single dose of buserelin affects testicular hemodynamics as measured via pulsed wave-Doppler ultrasonography. Furthermore, it aimed to study whether or not there are changes in the concentrations of testosterone, and estradiol-17 after buserelin administration.

2. Material and methods

The present study was conducted during the breeding season between November 2020 and January 2021 at the Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University. The experimental protocol concerning the care and handling of the rams was approved by the Animal Care and Ethical Use Committee of the Faculty of Veterinary Medicine, Cairo University (VetCU28042021264).

2.1. Animals and management

Twelve adult fat tailed Ossimi rams were used in the present study. Based on clinical, andrological, and ultrasonographic examinations, rams were deemed healthy and free from any cardiovascular or reproductive problems. Rams were 2–4 years old and weighing 45–60 kg. They were housed under normal daylight, ambient temperature, and humidity, receiving a balanced ration according to the NRC recommendations (each ram consumed daily 1.25 kg ration consisting of 400 g pelleted concentrates and 850 g tibn and green forage), and they had free access to fresh water and salt licks. Rams were routinely vaccinated and dewormed against parasites.

2.2. Experimental design

The animals were randomly allocated into two groups. Those in the treated group (n= 8) were subjected to a single intravenous (jugular vein) injection of GnRH analog buserelin acetate (0.008 mg/ram; Receptal inj.®; Intervet, Angers, France), whereas those in the control group (n= 4) were injected with 2 ml of normal physiological saline 0.9%.

At the same time of the day, venous blood sampling (jugular vein) and pulsed-wave Doppler ultrasonographic examination of the right and left testicular artery were conducted immediately before 0 min and 1, 3, 6, 24, 48, 72, 120, and 168 h after intravenous injection of buserelin and saline [15]. The collection of blood and Doppler examination were conducted for the buserelin and saline group, respectively by the same investigator.

2.3.1. Blood sampling and hormonal analysis

Shortly before the ultrasonographic testing, a venous blood sample was drawn from the jugular vein into an empty EDTA tube. All samples were centrifuged at 1207 x g for 15 min. Afterward, plasma was recovered and preserved at - 20°C before any further laboratory procedures.

The plasma testosterone and estradiol-17β concentration, respectively, were determined using commercial ELISA kits (BioCheck, Inc. Foster City, USA) and (BIOS, Microwell Diagnostic Systems, South San Francisco, USA).

The intra- and inter-assay variance coefficients were, respectively, 3.3% and 4.8% for testosterone and estradiol -17β, whereas the test sensitivity was 0.05 ng/ml and 20 pg/ml for testosterone and estradiol -17β, respectively.

2.3.2. Testicular ultrasonographic examination

The ultrasonographic examinations were conducted by the same operator once after the collection of blood samples. All examinations were conducted using an ultrasound device (SonoScape E1V, SonoScape Medical Corp., Guangdong, China; Xcelsitas AG, Berlin, Germany) fitted with a linear array transducer (7–14 MHz).

Without sedation, all the animals were gently restrained. The fine wool on both sides of the scrotum was trimmed well to remove any imaging artifacts. The transducer was coated with a large amount of scanning gel before ultrasonographic scanning, to prevent any air bubbles and improve the clarity of the image.

All the examination settings for the ultrasound system (frequency, brightness, depth, and contrast) were standardized, and fixed uniformly for all examinations. For pulsed-wave Doppler measurement, the angle between the Doppler beam and the long axis of the testicular artery was never greater than 60 with a high-pass filter set at 50 Hz. The Doppler gate was kept stable at 0.5 mm.

The transducer was positioned longitudinally on the sidewall of the scrotum and gently guided until the sonographic presentation of the testicular artery within the vascular network at the proximal pole of the testis (pampiniform plexus). Following the presentation of the spectral layout of the testicular artery (Fig. 1), the parameters analyzed were peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI= (PSV−EDV)/PSV), and pulsatility index (PI = (PSV−EDV)/mean velocity). For each parameter, two to four measurements were reported in different areas of interest along the testicular artery path [8,20].

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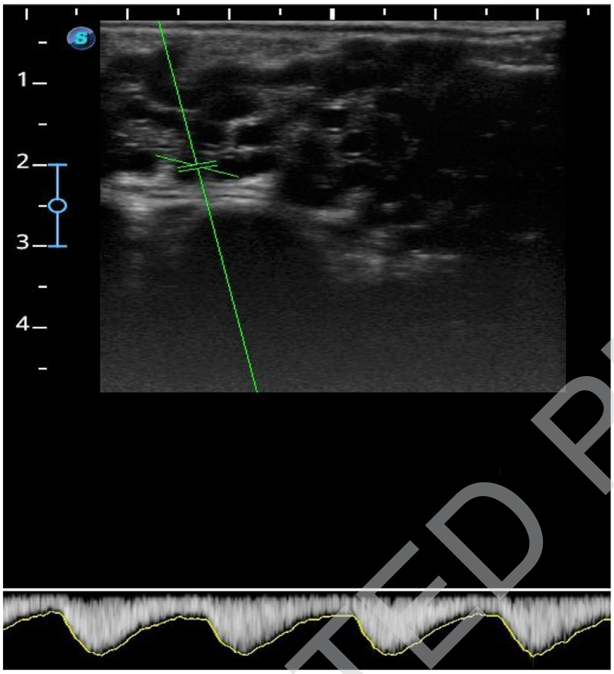

Fig. 1. Ultrasonographic examination of the Ossimi ram testis by pulsed-wave Doppler mode. The spectral pattern within the testicular artery appeared as monophasic and nonresistive waveforms.

2.4. Statistical analysis

Temporal changes in blood flow parameters (PSV, EDV, RI, and PI) along with plasma hormone concentrations (testosterone and estradiol-17β) were presented as mean ±SD of the number of observations measured in each parameter for all animals. Results were checked for normality using the Shapiro-Wilk test. Statistical analysis was done using the general linear model for repeated measures followed by Fisher's least significant difference test (LSD). The means of right and left testicular Doppler indices were tested using a t-test. The relationship between plasma concentrations of hormones and pulsed-wave Doppler measurements was tested by Pearson's correlation coefficient. All statistical analysis was conducted using the Statistical Package for Social Sciences SPSS® version 26.0 (SPSS Inc., Chicago, Illinois, USA). Values of less than 0.05 were deemed to be significant.

3. Results

Regarding the testicular blood flow measures (PSV, EDV, RI, and PI), there was no significant variation between the right and left testes, hence, the means of both testes were used for subsequent analysis.

No significant variation in plasma testosterone concentrations was recorded in the control group (Table 1) during the experiment. However, the testosterone concentrations increased ($P < 0.05$) 1 to 3 h after buserelin administration (Fig. 3). Then, testosterone concentrations decreased gradually from 6 to 72 h ($P < 0.05$) to reach nearly 60% of the baseline value at 120-168 h post-treatment ($P > 0.05$), whereas the plasma concentrations of estradiol-17β did not exhibit any significant differences in either control or treated group (Table 1, and Fig. 2).

The testicular blood flow values for PSV, RI and PI of the control group did not indicate any changes during the study ($P > 0.05$) (Table 1). In comparison, EDV values improved ($P < 0.05$) 3 h after the start of the experiment.

Regarding the treated group, the PSV measures did not reveal any substantial differences during the experiment; conversely, the EDV values were a significantly increase 1 h followed by a steady decline ($P > 0.05$) until 24 h post-treatment. Again, EDV values transiently increased ($P < 0.05$) at 48 h, and then it slightly decreased at 72 h until the end of the study ($P >0.05$), (Fig. 3).

The RI measurements displayed a significant decline 1 to 3 h post- buserelin injection, followed by a transit increase at 6 and 24 h ($P > 0.05$), once again RI values exhibited a significant decrease at 48h and finally, it displayed an increased values ($P > 0.05$) till the end of the experiment. In the same manner, The PI measures demonstrated a significant decrease at 1,3, and 48 h after administration of buserelin, while PI values increased on the other time points during the study ($P >0.05$) (Fig. 3).

As illustrated in Table 2, there was a positive correlation between the PSV and EDV; PSV and RI; PSV and PI (r =0.4, 0.5, and 0.6, respectively. $P < 0.01$). By contrast, there was a negative association between EDV and both RI and PI (r = -0.4 and -0.3, respectively. $P < 0.01$). There were no correlations between estradiol-17β and any of Doppler measurement ($P >0.01$). While there were negative correlations between the concentration of testosterone and both RI and PI (r= - 0.3 and -0.2, respectively. $P <0.01$). Moreover, the RI was in a strong positive association with PI (r = 0.9, $P < 0.01$).

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Table 1
Mean ±SD of testicular blood flow measures and plasma hormonal concentrations in the control group (n =4).

Parameters	0h	1h	3h	6h	24h	48h	72h	120h	168h
PSV (cm/s)	26.8±14.7	25.9±6.3	26.2±8.3	24.6±10.8	26.3±6.1	27.9±5.4	24.7±10.1	26.5±7.3	27.5±6.9
EDV (cm/s)	8.9±4.2^b	9.6±2.8^b	17.2±4.5^a	11.4±3.4^b	11.9±2^b	9.8±2.8^b	9.9±2.9^b	11.9±3.2^a	10.5±2.2^b
RI	0.6±0.1	0.5±0.1	0.4±0.1	0.6±0.1	0.5±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.7±0.1
PI	0.9±0.3	0.8±0.2	0.7±0.2	0.8±0.2	0.8±0.2	0.8±0.2	0.9±0.3	0.8±0.2	0.8±0.2
Testosterone (ng/ml)	7.8±5.2	9.9±2.1	10.7±2.8	8.2±2	7.3±0.6	8.3±1	7.1±0.9	6.3±1.7	9.6±2.3
Estradiol -17β (pg/ml)	30.7±15.9	27.4±13.6	29.1±14.1	29.4±15.1	30.4±12.9	31.3±16.8	32.9±19.9	31.9±22.3	32.4±18.3

Values with different superscripts (a, b) within the same row are significantly different (P < 0.05).

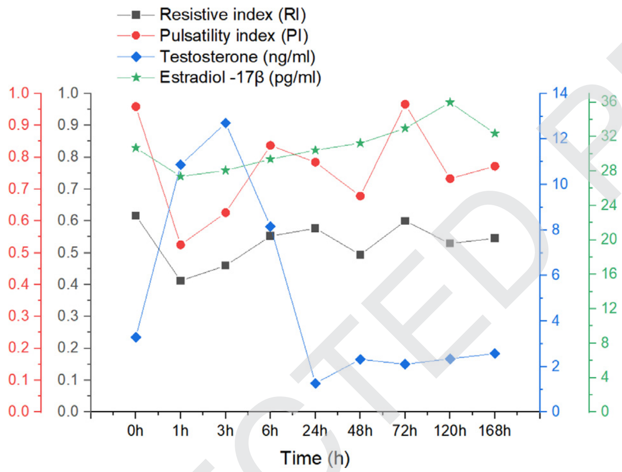

Fig. 2. Temporal changes in plasma hormonal profiles (testosterone and estradiol -17β) and intratesticular blood flow measures (resistive index RI, pulsatility index PI) in the treated group (n = 8).

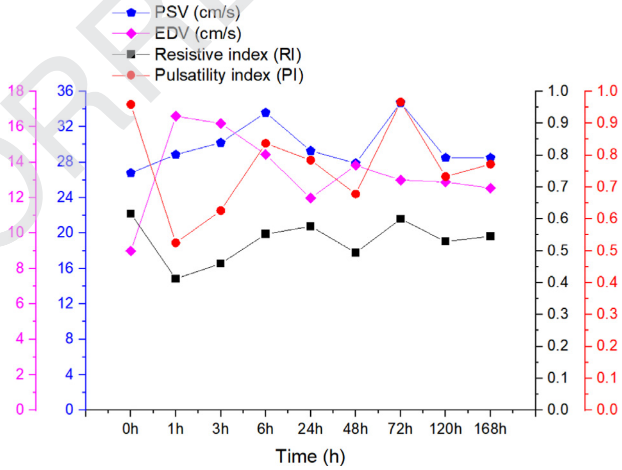

Fig. 3. Temporal changes in the intratesticular blood flow measures (peak systolic velocity PSV, end diastolic velocity EDV, resistive index RI, and pulsatility index PI) in the treated group (n=8).

4. Discussion

GnRH is a beneficial endocrine resource for modulating the endocrine function of the male reproductive system [17,18]. To the best of the authors' knowledge, this is the first research to look at the impact of GnRH analog administration on plasma concentrations of steroid hormones as well as testicular hemodynamic measures in rams. The current study's findings supported the hypothesis that a single dose of buserelin has a significant effect on testicular hemodynamics and alerts the steroid hormones (testosterone, estradiol-17β) profiles in Ossimi rams. The availability of such knowledge is essential to improve animal productivity as a potential solution to various problems associated with ram fertility.

The previous studies on males of domestic animal species tried to enhance the fertility and general reproductive performance (libido and semen quality) through the administration of exogenous agents include for example

Table 2
Correlation coefficients between intratesticular hemodynamic parameters (PSV, peak systolic velocity, EDV, end diastolic velocity, RI resistive index, T_4 testesterone and PI, pulsatility index) in Ossimi rams.

Paired measures	Correlation coefficients
PSV × RI	0.5**
PSV × PI	0.6**
EDV × RI	- 0.4**
EDV × PI	- 0.3**
T_4 × RI	-0.3**
T_4 × PI	-0.2**
RI × PI	0.9**

** Significant at 1% level.

GnRH analog [15], human chorionic gonadotropin (hCG) [6], or melatonin [23,24].

There was a significant alteration in plasma testosterone concentrations following buserelin treatment in the present study. These changes reflect the indirect role of buserelin in the biosynthesis of testosterone through its effect on the release of the luteinizing hormone (LH) and consequently on the steroidogenesis function of the Leydig cells [25]. We demonstrated a considerable transit increase in plasma testosterone concentrations from 1 to 3 h after the buserelin administration. In agreement with our investigation, Monaco et al. 2015 [26] reported that administration of GnRH analog temporarily increased the plasma testosterone concentrations (peak 140 min after the treatment) and enhance the overall camel bulls' reproductive performance. In the same pattern, the testosterone peak had been observed after 2 h in GnRH analog-treated cattle bulls [27] and GnRH analog -treated stallions [28]. Although Samir et al. 2015 [15] reported that the peak of plasma testosterone profile in bucks was 6 h post-GnRH analog intramuscular injection, the variation in results may be attributed to the different route of GnRH analog injection, as the intravenous injection in our study was absorbed immediately, resulting in a rapid rise in testosterone concentrations compared to the intramuscular administration in the other study. Moreover, a different GnRH analogs were used in both studies [15].

In the present study, the buserelin administration did not show any remarkable changes in the plasma concentrations of the estradiol-17β. In males, estradiol synthesis is primarily due to the conversion of testosterone inside the Sertoli cells into estradiol -17β [29,30] which is mostly dependent on the bioavailability of adequate testosterone resources and/or the activity of aromatase enzymes, as documented in rams [11] and stallions [6]. In line with our findings, nonsignificant changes were reported in the concentrations of 17β-estradiol in the GnRH analog-treated bucks [15] and stallions [31]. Conversely, Schanbacher and Echternkamp [32] reported a significant increase in the plasma estrogen concentrations of cow bulls after multiple GnRH analog injections. This disagreement may be due to the dose of GnRH analog and/or species variation. In the present study, buserelin appeared to have a direct effect on Leydig cells via increasing testosterone production, but this was not accompanied by the same stimulatory effect on Sertoli cell function (i.e., estrogen production). Therefore, the authors speculate these concentrations of estradiol might be due to an issue with the aromatase enzyme activity or testosterone uptake in Sertoli cells.

A previous study in bulls [19] recorded an increase in the scrotal surface temperature measured with infrared thermography after the GnRH analog administration. Furthermore, the injection of multiple small doses of buserelin triggered a rapid increase in testicular fluid content in rams [33].

In the present work, the waveforms of testicular blood flow (supratesticular artery) had a monophasic and nonresistive pattern. These results were consistent with the cardiac cycle rhythm of testicular blood flow reported in various species [4,34,35]. On contrary, the spectral pattern of testicular blood flow in stallions is distinguished by biphasic and resistive waveforms, this variation could be related to the vertical orientation of rams' testes compared with the horizontal alignment in the stallions [14].

Two of the Doppler measurements recorded in this experiment (PSV and EDV) directly revealed the velocity of arterial blood flow during the cardiac cycle, however, their values are distinctly irregular and not constant between measurements of the same organ [14]. On the other side, pulsed-wave Doppler indices (RI and PI) are more precise measures of arterial blood flow, representing information on testicular vasculature, not just blood velocity. The RI and PI of the testicular artery are an excellent indicator of spermatogenesis rate in human testis [3] and semen quality in dogs [18]. The present study revealed a substantial decrease in the RI and PI values following the buserelin administration. Since there is a drop in RI and PI values in the present study reflects a consequent rise in testicular blood perfusion due to the lack of resistance of the internal arterial wall to blood flow, which is beneficial for testicular functionality [36,37]. Numerous studies have shown that the decline in RI and PI values is accompanied by an improvement in the function of the testicles (both steroidogenesis and spermatogenesis) [11,15]. In the same sense, Brito et al. 2017 [38] have recorded a significant decrease in the RI measures of the ovarian blood flow after the GnRH analog administration in mares.

The majority of interesting findings in the present study occurred shortly after buserelin injection, which could be attributed to the compound's short half-life and rapid elimination from blood circulation, particularly after the i.v. administration [39].

There was a strong positive correlation between RI and PI values after the buserelin injection, these clear associations might reflect the vasodilatory effect of buserelin in Ossimi rams. in addition, there was negative association between testicular blood flow indices (RI and PI) and plasma concentrations of testosterone, which might be due to the fact that the reduction in the RI and PI values is accompanied with a marked increase in the testicular blood flow and in turn the testicular functions (testosterone production).

In conclusion, the pulsed-wave Doppler application is a trustable noninvasive diagnostic technique with high utility in ram reproductive practices. The administration of Busrelein elicits several changes in the testicular hemodynamics and testicular endocrine activity in rams. Buserelin improves testicular blood and testosterone

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production. Thus, we recommend using buserelin to boost reproductive performance in Ossimi rams.

Conflict of interest

The authors declare that they do not have any conflict of interest.

Author contribution

Amr El-Shalofy: Conceptualization, Methodology, Validation, Review and Formal analysis. Mohamed Hedia: Conceptualization, Validation, Investigation, and Writing.

Uncited Reference

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