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Evaluation of testicular hemodynamics following gonadotropin-releasing hormone administration with the aid of pulse wave Doppler in rams and their relation to hormonal response

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Abstract

The vascularization of the testis through testicular artery is of great importance to maintain its normal function. The vascular disruption due to inadequate arterial blood flow of the testis negatively affects testicular function and semen quality. This study aimed to determine the efficacy of gonadotropin-releasing hormone (GnRH) administration on testicular vascularity in relation to testosterone hormonal response. Five clinically healthy adult ossimi rams 18- to 30-months-old were used. Testicular arteries Doppler examination, blood sampling following GnRH administration and testosterone hormonal assay were conducted. Both pulsatility index (PI) and resistance index (RI) significantly decreased in all treated rams starting from 1 hr till 120 hrs after single GnRH administration, both Doppler indices returned to their pre-treatment values at 144 hours after GnRH administration. Doppler peak systolic velocity (PSV) did not change in response to GnRH administration. Testosterone hormone concentrations negatively correlated with PI and RI but not PSV. In conclusion, GnRH would be useful a beneficial therapy for the treatment of testicular dysfunction in rams by increasing testosterone concentrations and testicular blood flow. And pulse wave Doppler ultrasonography would be a useful non-invasive clinical tool for evaluation of the efficacy of novel therapeutic treatments in rams.

Keywords: Fertility, GnRH, Testicular artery, Testicular blood flow, Testosterone.

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Introduction

The function of the testis is exceptionally subjected to appropriate testicular perfusion. The testicles are the site of sperm production as well as the male sex hormone, testosterone. Testicular tissue, a high metabolic activity tissue, is highly sensitive to nutrient supply disruption, which is mainly through the hematogenic pathway. The testis receives its blood supply through a testicular artery which is unusually long (Bergh and Damber, 1993), with high flow resistance resulting in lower intra-testicular capillary pressure than other organs and slightly higher than venous pressure (Sweeney et al., 1991). The high metabolic demands of the seminiferous tubules exposed to these specific conditions of low pressure and low oxygen tension are usually ensured by the vasculature that in ordinary conditions can supply the testis with adequate quantities of nutrients and oxygen (Bergh et al., 2001). Most evidence suggests that this primary reproductive organ is particularly susceptible to vascular system disruption and that testicular dysfunction can result from moderate blood supply disruption (Damber and Bergh, 1992; and Bergh and Damber, 1993). As in every other organ control in blood stream is along these lines significant and might be especially basic for the testis since the oxygen concentration in the seminiferous tubules is very low (Setchell, 1990). Therefore, any decrease of blood stream causes ischemic harm that leads to sperm deterioration. Partial restriction of the testicular artery has been reported to have an adverse effect on the development, volume and histological structure of bull testes, resulting in complete or incomplete arrest of spermatogenesis (Kay et al., 1992).

Previous studies in humans (Battaglia et al., 2001; Biagiotti et al., 2002; and Tarhan et al., 2003) and rats (Bergh et al., 2001) have shown a correlation between testicular blood flow and quality of sperm as a significant decrease of testicular arterial blood flow in varicocele impair the process of spermatogenesis. Several researchers have studied the effect of reductions in testicular blood flow in spermatogenesis. Additionally, partial arteriosclerosis in the testicular arteries negatively affected the seminiferous tubules in rams, this is likely due to the decrease in testicular blood flow from the affected arteries (Markey et al., 1995).

Hormones appear to be involved in regulating testicular blood flow in addition to local mediators (Bergh and Damber, 1993). In testicular microvasculature of rats, receptors for LH, and a transendothelial transport mechanism for human chorionic gonadotropin (hCG) were found (Ghinea et al., 1994).

Different treatments aimed to improve testicular blood flow and consequently increasing testicular function should be of great value to male fertility. Gonadotropin-releasing hormone (GnRH) is an important hormone used in reproduction and valuable tools for testing the function of the male reproductive endocrine system (Gábor et al., 1998; and Parlevliet et al., 2001). The administration of hCG results in increased testosterone concentration and testicular blood flow in laboratory rodents (Damber et al., 1985). A recent study performed in bucks, demonstrated that melatonin administration significantly increases in live/dead ratio, motility, and normal sperm morphology percentage (Samir et al., 2020), such positive effect would be mediated by the increase in GnRH. There is also a rise in plasma testosterone concentrations in stallions following the administration of hCG (Roser, 1995; and Zwain et al., 1989). Plasma testosterone levels were required for evaluating animal subfertility, as the determination of testosterone concentrations has played a role in identifying subfertility problems originating from hypospermia (Ball, 2008). However, the effect of GnRH analogs on

testicular blood flow as well as the possible relation between hormonal response and testicular blood flow in rams has been lacking in knowledge.

Previously, more complicated and time-consuming techniques were used to measure the testicular hemodynamics. The testicular blood flow in rams was evaluated using continuous infusion of certain chemical substance such as sodium p. aminohippurate. Then the concentrations of such chemical substance were measured in blood samples collected from above the pampiniform plexus, and the testicular blood flow was calculated using a special equation (Mieusset et al., 1990). The need to more simple and accurate applicable method to assess testicular blood flow in short time was necessary.

Ongoing specialized advances of ultrasound applications have empowered new perspectives in the basic structural and functional investigation of testicular tissue and in consequence male fertility. Color and pulsed wave Doppler ultrasound can evaluate blood flow and quickly determine and measure the blood flow parameters. Doppler modes are considered the most accurate measures for detecting any changes in blood. Medically, both peak systolic velocity (PSV, a specific Doppler velocity parameter for the detection of delicate changes in testicular perfusion) and resistance index (RI) of testicular arteries could be useful in distinguishing different causes of dyspermia, and particularly, PSV values clearly differentiated obstructive from non-obstructive azoospermia (Biagiotti et al., 2002). In veterinary practice, the use of Doppler ultrasound is increasing, however, there is paucity in its use especially in male reproduction. Doppler sonography was used in dogs not only to show and assess the blood flow of the testicular arteries but also as a possible marker for semen consistency (Zelli et al., 2013). Evaluation of testicular blood flow by Doppler ultrasonography in camelids has been used to determine male fertility (Kutzler et al., 2011). In stallion, Doppler ultrasonography was useful to characterize blood flow in the testicular artery and to determine certain pathological issues conditions as testicular varicocele (Pozor and McDonnell, 2004; and Pozor, 2007). Recently, Samir et al. (2020) demonstrated that the decrease in the RI and PI was associated with significant increases in sperms viability and percentage of normal morphology in bucks.

Moreover, Doppler Sonography would be a useful tool in the study of seasonal variations in the gonadal function in bucks (Strina et al., 2016). In rams, no relation was found between sperm motility and testicular hemodynamic characteristics, but an association was found between the total sperm abnormalities percentage and Doppler indices parameters (Batissaco et al., 2014). To the best of our knowledge, the available data on the utility of color pulsed Doppler ultrasonography in male small ruminants are still lacking and mainly concerned with male goat (Samir et al., 2015). A recent study using color Doppler ultrasonography in Barki rams found that there were no differences in Doppler indices (RI and PI) values between pre-pubertal and post-pubertal rams, despite the increase in testosterone concentrations (Elbaz et al., 2019). However, there is still a lack of information regarding the interaction between hormones and testicular hemodynamics in rams.

Therefore, the present study aimed to evaluate the vasculature of the testicles using pulse wave Doppler ultrasonography and testosterone concentrations after administration of GnRH in rams.

Materials and methods

Ethical approval:

The study protocol was approved by the Ethical Committee of Assiut University, Faculty of Veterinary Medicine.

Study location:

This study was conducted in Assiut governorate, Egypt, located at 70 m above mean sea level, latitude 27.10° N and longitude 31.11° E. The study was performed during the high-breeding season (December–February) when the low (minimum) and high (maximum, °C) temperature ranged between 8-22°C and 8-23°C, respectively.

Animals and management:

Five clinically healthy Ossimi (Egyptian native fat-tailed breed) adult rams, 18- to 30-months-old and weighing 55 to 75 kg were used in this study. The experiment was conducted during the high breeding season (December to February). The animals were kept indoor at the teaching hospital of the Faculty of Veterinary Medicine, Assiut University without any notable difference in the environmental conditions. Veterinary clinical examination confirmed that all rams were clinically normal and healthy. All rams were subjected to complete reproductive and ultrasonographic examinations to ensure the absence of any reproductive abnormalities. All rams were held under the appropriate conditions and fed in a regular manner, they were fed a daily ration of concentrate and wheat straws with ad libitum water and salt licks for the duration of the study.

GnRH administrations:

All rams received intramuscular administration of 100 µg GnRH analogue buserelin acetate (Receptal; Intervet, Unterschleissheim, Germany) (Jordan et al., 2009) at the start of the experiment (0 hr). GnRH administration was performed 6:00−7:00 AM, in all treated rams.

Blood sampling:

Five-ml blood samples were drawn from all rams by jugular venipuncture into plain tube at 0,1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168 hrs time-points where 0 is the time of GnRH administration. The blood samples were centrifuged at 3.000 rpm for 15 min within 1hr to separate serum. Sera aliquots were kept at –20 °C until hormonal analysis.

Hormonal assay:

Serum testosterone concentrations were measured using an enzyme immunoassay test kit (Cat. No. BC-1115, BioCheck Inc., San Francisco, CA, USA) In brief, 10 µl of serum, 100 µl of testosterone-HRP Conjugate Reagent, and 50 µl of rabbit anti-testosterone reagent were thoroughly mixed (30 seconds) and incubated at 37°C for 90 min. The micro-wells were washed and 100 µl of TMB Reagent was added into each well before incubation at room temperature for 20 min. The reaction was stopped, and the absorbance was assessed at wavelength 450 nm within 15 min.

The sensitivity of the testosterone assay was 0.05 ng/ml. The intra- and inter-assay coefficients of variation for testosterone were 6.4% and 8.4%, respectively. Cross-reactions of various steroids were: testosterone (100%), dihydrotestosterone (0.86%), androstenedione (0.89%), progesterone, and cortisol (<0.0001%), androsterone (1.0%), 17β estradiol (0.05%) and progesterone, epitestosterone, 17-OH-progesterone, estriol, cortisol, DHEA-sulphate (<0.05%).

Ultrasound examinations:

Doppler ultrasonographic scans were conducted using a duplex B-mode (grayscale) and color Doppler ultrasound instrument (ESAOTE Pie Medical MyLabOneVET device, Firenze, Italy) equipped with linear array broadband

transducer with frequencies ranging from 2.5 to 10 MHz. The ultrasonographic examinations were carried out by the same operator to avoid variation. The rams were restrained without tranquilization or sedation. To eliminate the presence of air spaces, the hairs on both sides of the scrotum were thoroughly shaved and the transducer was covered with enough amount of gel to improve ultrasonographic imaging.

In the rams, the spermatic artery approaching the testis convolutes to form a convoluted structure known as the testicular artery. The testicular veins surround the testicular artery forming the pampiniform plexus. Therefore, before the assessment of Doppler parameters of the artery, a clear differentiation between the testicular artery and the testicular veins was done. The testicular artery has a typical spectral waveform on the spectral graph corresponding to the arterial pulse in each cardiac cycle (systole and diastole), while the flow in the veins is almost constant without a pulse (Fig. 1). Doppler analysis was conducted by identifying the vascular structures using grayscale ultrasonography and locating the largest and possibly the longitudinal or oblique section of the testicular artery (Fig. 1). The angle between the Doppler beam and the long axis of the vessel was never more than 60 in the direction of blood flow with the high-pass filter set at 50 Hz. The size of the Doppler gate was adjusted during each examination to obtain a sequence of spectral Doppler graphs with symmetrical and distinct systolic and diastolic cardiac cycles. Color Doppler scans were performed at a constant gain setting, filter setting, and velocity range setting.

The testicular blood flow was assessed using the following parameters: two Doppler indices; pulsatility index (PI), and resistance index (RI) in addition to peak systolic velocity (PSV) as a velocimetry parameter. Peak systolic velocity was automatically calculated and expressed in cm/s. The formulas of the RI and PI were well established and have been previously reviewed (Ginther, 2007). To obtain good results, a sequence of at least three successive symmetric blood flow waves was required to register the measurements during one cardiac cycle using an automatic trace. The measurements were repeated three to five times for each parameter at the same point in different locations along the path of the testicular artery. Following data collection, the Doppler images were transferred to a personal computer. To minimize the variations in recording, the ultrasound settings (focus, gains, brightness, and contrast) were standardized, fixed, and used equally during all examinations. In this study, all calculations were conducted automatically offline and stored on a flash memory device.

Statistical analysis

All results were expressed as means ± All results were expressed as means ± standard mean of error (Mean ±SEM). The data collected have been analyzed statistically using SPSS statistics 21 for Windows (IBM SPSS, 2017). The Kolmogorov-Smirnov test was used to check the normality of the data and the data was distributed normally. General linear models-ANOVA for repeated measures was used to determine the main effects of the time (hours of treatment) on the blood flow parameters (PI, RI and PSV), Fisher's least significant differences (LSD) test was used as a post hoc analysis to detect the mean differences among the hours before or after treatment. The level of significance was set at $P< 0.05$. Testosterone data were

analyzed using one-way ANOVA followed by a post-hoc Tukey's test. P-values less than *0.05* were considered statistically significant. **P < 0.05, **P < 0.001, *** P < 0.0001*.

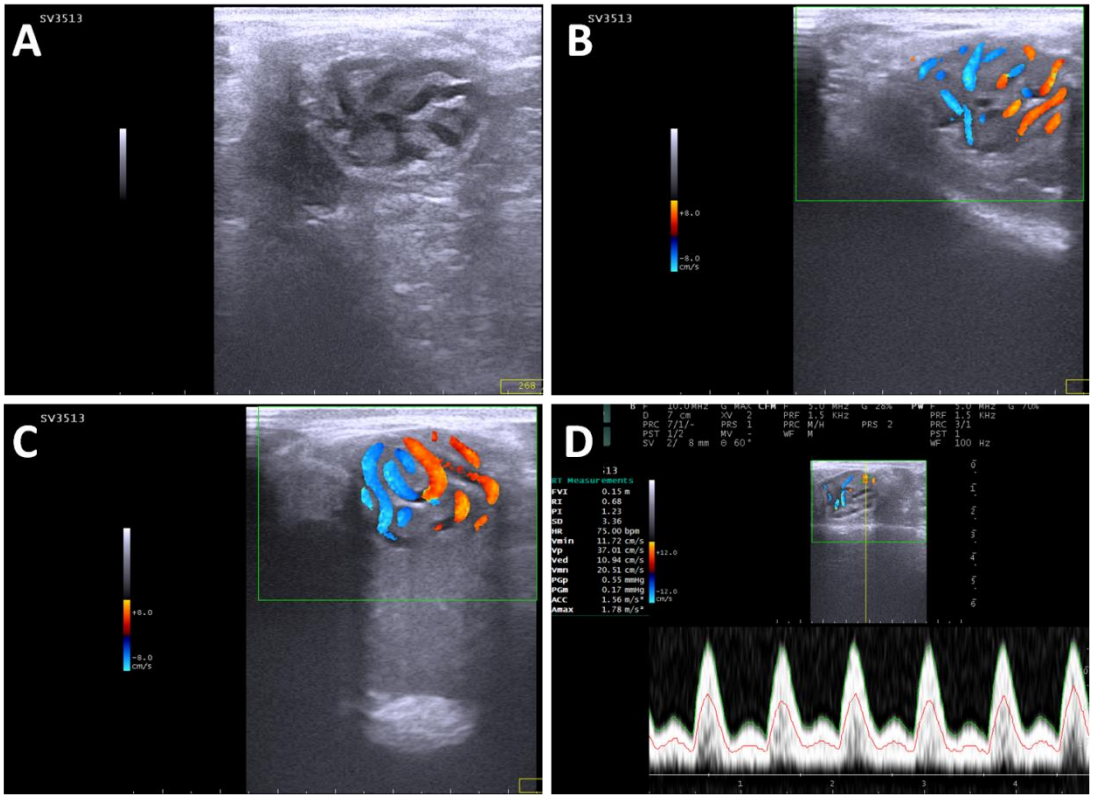

Fig. 1. Representative ultrasound sonogram of the pampiniform plexus in ram showing grey-scale image of the supratesticular artery by B-mode (A); blood flow inside supratesticular artery by color Doppler mode indicated by red and blue colors before GnRH administration (B); and after GnRH administration (C); and blood flow within the supratesticular artery revealed a spectral waveform pattern by pulsed wave Doppler mode (D).

Results

Testicular blood flow:

There were no significant differences between the left and right testicular arteries (LTA and RTA, respectively) with respect to all blood flow parameters PI, RI and PSV (*P>0.05*), Thus, the means of the LTA and RTA were used for further analysis of these variables.

The PI values decreased significantly in all treated rams after single GnRH injection starting from 1 hr and continued till 120 hours after treatment (*P< 0.0001*) and then started to increase reaching pre-treatment values 144 hours after treatment (Fig. 2, and Fig. 3)

The RI values decreased significantly in the all treated rams after single GnRH injection starting from 1 hr and continued till 96 and 120 hours after treatment (*P*< 0.0001 and *P*< 0.005) respectively and then started to increase reaching pre-treatment values 144 hours after treatment (Fig. 3, and Fig. 4)

The PSV showed that there were no significant differences between the PSV values in all treated rams during the different hours after treatment. The PSV minimum mean values were 34.75 ± 0.59 cm/s and the maximum mean values were 38.90 ± 0.81 cm/s (Fig. 3, and Fig. 5).

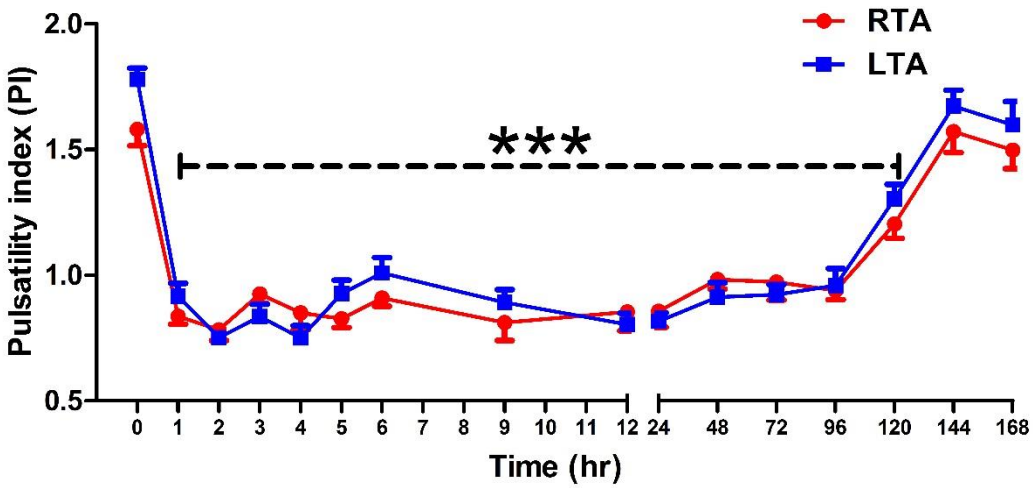

Fig. 2. Pulsatility index (PI) values of the right (RTA) and left testicular artery (LTA) at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168 time-points (hrs) in rams (n= 5). *Values presented as means ± SEM, ***P < 0.0001 (GLM ANOVA followed by post-hoc Fisher LSD test),* and *0 time of GnRH administration.*

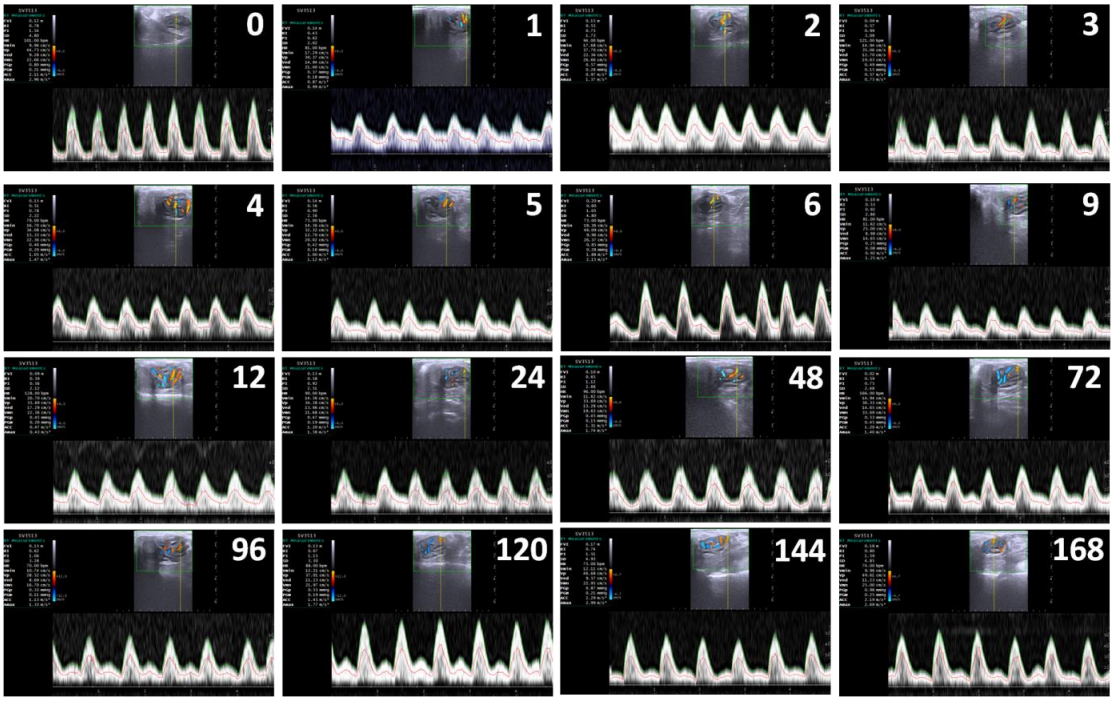

Fig. 3. Representative ultrasonographic scan images of ram testis using color pulsed-wave Doppler ultrasonography showing Pulsatility index (PI), Resistance index (RI) and peak systolic velocity (PSV) of the supratesticular artery at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168 time-points (hrs). 0 is time of GnRH administration.

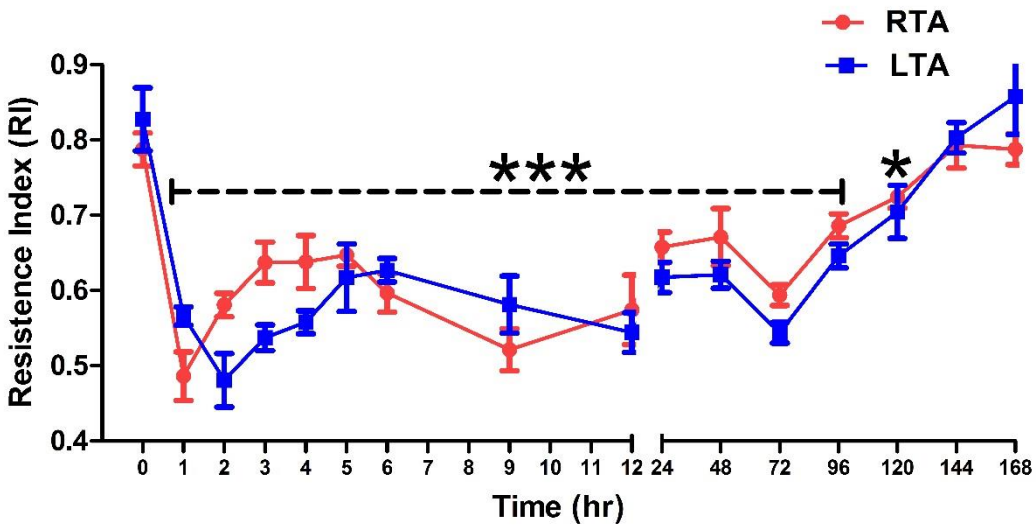

Fig. 4. Resistance Index (RI) values of the right (RTA) and left testicular artery (LTA) at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168 time-points (hrs) in rams (n= 5). *Values presented as means ± SEM, *P < 0.005, ***P < 0.0001 (GLM ANOVA followed by post-hoc Fisher LSD test)*, and *0 time of GnRH administration.*

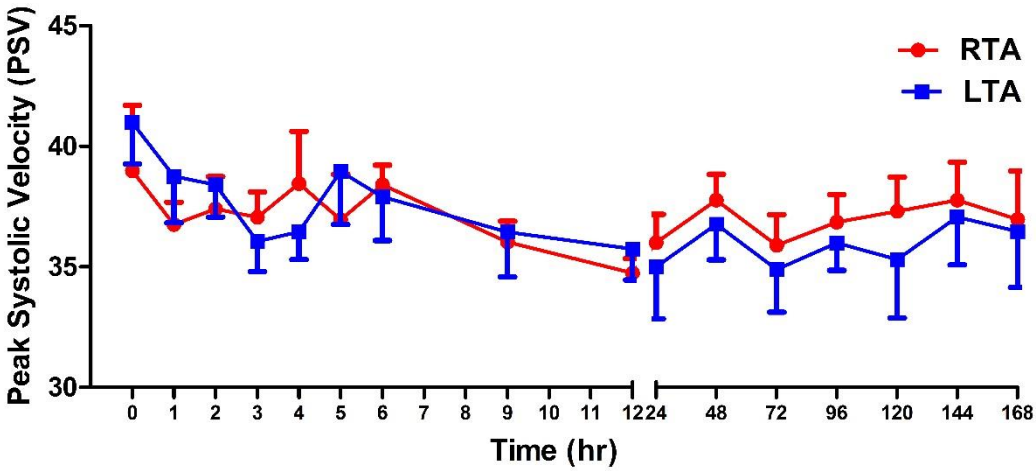

Fig. 5. Peak systolic velocity (PSV) values of the right (RTA) and left testicular artery (LTA) at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168 time-points (hrs) in rams (n= 5). Values presented as means ± SEM. 0 is time of GnRH administration. *Values presented as means ± SEM, Statistical analysis using GLM ANOVA,* and *0 time of GnRH administration*.

Serum concentrations of testosterone:

Serum testosterone levels (mean ±SEM) were significantly higher at 2, 3, 4, and 5 hrs after GnRH administration with 16.10 ±1.00, 13.43 ±3.84, 16.80 ±0.92, 14.07 ±1.85 ng/mL, respectively in compare to serum testosterone level at 0 hr timepoint before GnRH administration (2.83 ±0.73 ng/mL) (Fig. 6).

There were significant negative correlations between serum testosterone concentrations and both the Doppler indices values; PI and RI but not with the PSV (Table 1, and Table 2).

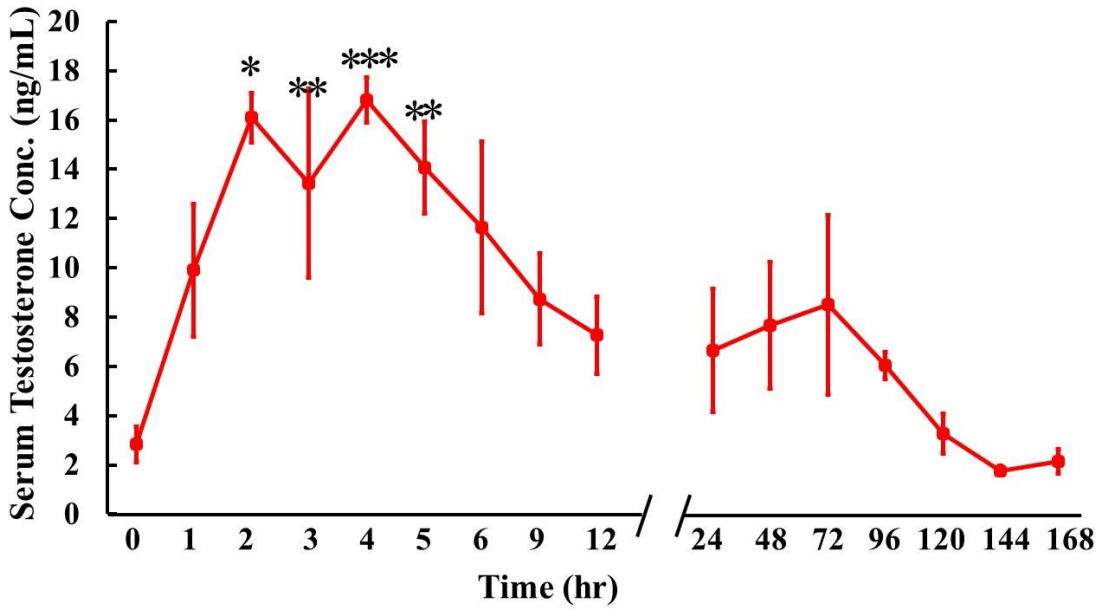

Fig. 6. Serum testosterone concentrations at 0, 1, 2, 3, 4, 5, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168 time-points (hrs) in rams (n= 5). *Values presented as means ± SEM, *P < 0.005, **P < 0.001, ***P < 0.0001 (One-way ANOVA followed by post-hoc Tukey's test)*, and *0 time of GnRH administration*.

Table 1. Pearson correlation matrix for testosterone, Doppler indices, and Doppler velocity parameters:

Correlation coefficient	Testosterone conc	Pulsatility Index	Resistance Index	Peak Velocity
Testosterone conc		-0.479***	-0.422**	0.130^{ns}
Pulsatility Index	-0.479***		0.847***	0.233 ^{ns}
Resistance Index	-0.422**	0.847***		0.216 ^{ns}
Peak Velocity	0.130 ^{ns}	0.233 ^{ns}	0.216 ^{ns}	

- *ns not significant, **P < 0.001, and ***P < 0.0001*

Table 2. P value for testosterone, Doppler indices, and Doppler velocity parameters:

P value	Testosterone conc	Pulsatility Index	Resistance Index	Peak Velocity
Testosterone conc		0.0009	0.004	0.394
Pulsatility Index	0.0009		< 0.0001	0.124
Resistance Index	0.004	< 0.0001		0.154
Peak Velocity	0.394	0.124	0.154	

Discussion

This study successfully evaluated the efficacy of GnRH administration on the testicular arterial blood flow using pulse wave Doppler ultrasonography in relation to serum testosterone concentrations.

Because there is a paucity in the studies of blood flow in the testicular arteries in small ruminants (Camela et al., 2017; Samir et al., 2015; and Zelli et al., 2013) and there is no available data up to date on the blood flow of testicular arteries after GnRH administration in rams. In addition, Doppler ultrasound is a superb device for tracking therapeutic outcomes following medical or surgical procedures (Gracia-Calvo et al., 2015; and Pozor et al., 2011). Therefore, the primary objectives of the current study were to assess the blood flow of testicular arteries by pulsed wave ultrasonography and testosterone concentrations in rams after injection of GnRH analogue. Doppler indices are used in the present study as alternatives to the Doppler velocity measurements to assess the blood flow of the testicular artery. As they are particularly helpful when testing small, convoluted vessels in which a straight portion isn't accessible to assess the Doppler angle. Doppler indices are velocity ratios measurements and accordingly are independent of the Doppler angle. In addition, the indices are interpretable and relatable to the hemodynamics, proximal or distal to the point of blood vessel assessment (Ginther and Utt, 2004). The RI and PI are commonly used in clinical analysis of testicular blood flow perfusion in humans and animals in physiological (Biagiotti et al., 2002; Carrillo et al., 2012; Dubinsky et al., 1998; Gumbsch et al., 2002; Pozor and McDonnell, 2004; and Zelli et al., 2012) and pathological condition (Bumin et al., 2007; Dudea et al., 2010; and Gunzel-Apel et al., 2001).

In the present study there were no significant differences between the LTA and RTA with respect to all blood flow parameters PI, RI and PSV, Thus, the means of both the LTA and RTA arteries were used for further analysis of these variables. Similar results were recorded in arterial blood flow parameters (PSV, end-diastolic velocity; RI and PI) associated with the attainment of puberty in Dorper rams raised in a subtropical climate (Camela et al., 2018). On the other hand, the effect of laterality on the testicular hemodynamics has been recently studied by Hedia et al., (2020) who found that Doppler indices of RI and PI were slightly higher in the RTA rather than the LTA in rams

The post-treatment increases in the testicular hemodynamics determined by the decrease in both the Doppler indices; PI and RI values, as well as, the significant increase in the serum testosterone concentrations support the clinical application of GnRH in treatment of testicular dysfunction and improvement of the testicles normal function in rams. Our results showed that the PI values decreased after single GnRH administration and consequently the blood perfusion increased for the first 120 hours after treatment. This result is partially in accordance with Samir et al., (2015). As they reported that the PI of the supratesticular artery decreased after injection of both hCG and GnRH in shiba goat but after 6 hours while in the present study the PI decreased after one hour. The rapid decrease in the present study may be attributed to the different GnRH analogue or dose or the difference in the animal species. Similar results reported in

stallion after injection of hCG (Bollwien et al., 2008) but the PI decreased for only the first 72 hours. They found that the PI decreased to below the baseline value by one hour after hCG administration but then increased until 12 h after treatment. Adding that, The PI was below the baseline value at 24 h post hCG and equal to the baseline value at 72 h post hCG. The difference in the results might be attributed to the difference in the drug used or the difference in the animal species.

In the present study, the RI values decreased significantly in all treated rams after single GnRH injection for the first 120 hours after treatment. Samir et al., (2015) found that the RI of the supratesticular artery significantly decreased in both hCG and GnRH treated shiba goat groups, a faster decrease in RI was observed in the hCG group (1 hr) compared with the GnRH group (2 hr). While in the present study the RI started to decrease from the first hour after treatment, the difference in response is likely due to the different GnRH analogue or the different dose. The testicular artery RI is considered a valuable indicator of human testis sperm production rate score (Biagiotti et al., 2002) and dog semen quality (Zelli et al., 2013). In the present study, because the RI and PI of the testicular artery significantly decreased after the injections of GnRH could induce an improvement of testicular blood flow. Subsequently, improvement in the spermatozoa production capacity and sperm quality in ram could be performed in rams by injection of GnRH analogues

In addition to increased blood flow in the testis, hCG is known to have a number of other effects on the testis, such as increased vascular permeability and increased lymph flow in the testis (Sharpe, 1977; Setchell and Sharpe, 1981; and Damber et al., 1985). All these studies had examined the impact of hCG on blood flow to the testis after a single dose of hCG. Similarly, the testicular blood flow increased on day 5 after hCG was administered every other day for three doses (Geesaman et al., 1992). On the contrary, vascular permeability and testis lymph flow have been evaluated following multiple hCG injections every two to three days, and these two factors increased after the first hCG injection but not following the second and subsequent injections of hCG (Maddocks et al., 1987).

The PSV showed that there were no significant differences between the PSV values in all treated rams during the different hours after treatment with minimum mean values 34.745 ± 0.591 cm/s and a maximum mean value 38.90 ± 0.813 cm/s. in camels supratesticular arteries, the mean PSV was higher in fertile male camel compared to infertile males (21.41 ± 1.11 cm/s versus 1 5. 0 9 ± 1. 0 9 cm/s) (Kutzler et al., 2011). As there are no reference values for the PSV in animals or rams used in the present study. When we compared the present result with values recorded in camel bulls, the minimum mean values recorded in the present study will be markedly higher than those recorded in the fertile camel bulls. This might add an explanation or evidence for the beneficial effect of GnRH injection on testicular blood flow in rams**.** On the other hand, it was reported that the mean PSV was significantly different

among the groups of patients and controls; men with varicoceles had the highest value but there were no differences among the three groups with varicocele (associated or not with oligoasthenospermia or male accessory gland inflammation). The mean PSV in those with oligoasthenospermia, male accessory gland inflammation and in normal controls was not significantly different. Those with unexplained oligoasthenospermia had significantly lower PSVs than normal controls. In addition, PSV is a reliable indicator for routine clinical use to identify infertile/dyspermic men as PSV clearly differentiated obstructive from non-obstructive azoospermia (Biagiotti et al., 2002).

The clinical use of pulse wave Doppler ultrasonography as a non-invasive tool for evaluation of the efficacy of novel therapeutic treatments would be of great value. This is supported by the significant negative correlation between the Doppler indices (PI and RI) and serum testosterone concentrations reported in this study.

Conclusion

In conclusion, the results of this study indicated that a single administration of GnRH could have a beneficial therapeutic effect on testicular blood flow in rams. These beneficial effects can lead to an increase in male reproductive performance and fertility due to positive influences on testicular blood flow and consequently spermatogenesis. And that pulse wave Doppler ultrasonography would be clinically useful for evaluation of the efficacy of novel therapies in rams. It should be noted that, further studies using large number of animals and different hormonal therapies are required.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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