Testicular morphometric and echotextural parameters and their correlation with intratesticular blood flow in Ossimi ram lambs

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SUMMARY

The present detailed study aimed to record for the first time the testicular dimensions (length, width, thickness, and volume), testicular echotexture (pixel intensity, pixel standard deviation, and colored area pixels) using gray-scale/Doppler ultrasonography and investigating the blood flow indices (resistive and pulsatility index) for testicular artery from one hand, and to explore the possible associations between these parameters in Ossimi ram lambs under subtropical conditions from the other hand. 8 ram lambs (aging 4-5 months and weighing 30-40 kg) were submitted to B-mode testicular ultrasonography (US) for evaluation of the testicular length, width, thickness, and volume. The testicular artery was submitted to the pulsed-wave Doppler examination to measure the resistive index (RI) and pulsatility index (PI). Computer-assisted image analysis was applied for B-mode/color Doppler images. Regarding the testicular dimensions, testicular length, width, thickness, and volume were (63.90±10.42 mm, 34.50±3.70 mm, 36.50±2.52 mm, and 58.80±9.98 mm^3, respectively). Blood flow indices (RI and PI) experienced a significant strong association between each other (r= 0.878, $P \leq 0.01$). Regarding testicular image analysis, testicular parenchyma recorded higher values of testicular echogenicity (82.5 ±12.47), which was directly associated with other testicular morphometric characteristics ($P \leq 0.01$). As we can conclude, the testicular US is of great potential usefulness to assess the testicular dimension and reproductive growth in ram lambs. RI and PI were directly affected by the sexual stage and could be a reflection of the attainment of puberty and testicular function in rams under subtropical conditions.

KEY WORDS

Testes, Doppler, Testicular artery, Ossimi Lambs, Rams.

INTRODUCTION

Livestock production is an important industry in different countries in the world particularly in the developing countries to fulfil the needs of meat, milk, and other products (manure and wool). Egypt has a modest population of ovine species which is estimated to 5.69 million heads, which represents approximately 7.4% of all red meat production in Egypt according to FAOSTAT[1]. The whole red meat-producing farm animals including sheep providing a self-sufficiency ratio of red meat which was estimated as 55.9% in 2017[2]. Besides, farm animals' production contributes to 10-14% of national income in Egypt, which is expected to decline due to the rapidly increased Egyptian population, which exceeds 100 million according to World Population Review (2018). For these clear points, more efforts should be directed through breeding soundness examination in Ossimi lambs via testicular ultrasonographic and Doppler examination of testicular functionality to maximize the efficiency of sheep production especially the Ossimi breed, which is the most common breed reared in Egypt.
Regarding the breeding soundness examination in farm animals, there are different classical methodology concerning with selection of the most appropriate male for breeding. Also, breeders tend to house a limited number of high reproductive qualities to impregnate the largest number of females. These methods consist of routinely used measures as estimating the scrotal circumference, rectal examination of accessory sex glands, traditional physical examination of testicular and preputial contents as well as semen analysis[3-5].
Due to the extensive breeding systems, practitioners have to deeply evaluate the breeding capacity of sires using the most advanced available technologies. Recently, Ultrasonography (US) is the most suitable method to scan the normal morphological features of the primary reproductive organ (testis). US is a non-invasive, available technique to reliably scan the reproductive organs[6]. US provides promising imaging results, where it could be used to verify the normal morphological architecture of the testis and check its main functions (spermatogenesis and steroidogenesis). In addition, US could be used to compare between the normal and abnormal physiological reproductive status, where US allowing andrologists to detect the abnormal pathological testicular contents, which severely deteriorate the breeding capacity of the male[7, 8].
Despite the gray-scale testicular ultrasonographic examination, testicular Doppler analysis is an emerging recent technique to evaluate the testicular functions through investigating its vascular irrigation via testicular artery, which has a promising outcome in the last decade. As a vital organ, testicular blood flow has great communication with its main functions, where disruption of the testicular blood flow is a critical process on the testicular physiological status[9]. For that, the examination of the testicular artery by color Doppler and measuring the blood flow indices (resistive and pulsatility index) have a considerable role to deeply investigate the testicular functions[10]. Several workers in bucks, bulls, dogs, stallions, and rams have detected the direct association between testicular hemodynamics and reproductive quality via investigating the association between tes-

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ticular blood flow indices, sperm characteristics, and plasma androgen concentrations[11-15].

In addition to these important methodologies to evaluate the testicular functions, andrologists have recommended another promising technique to investigate the reproductive status through the application of a computer-based technique to assess pre-saved B-mode images of scrotal contents, especially for testicular parenchyma[16]. Nowadays, several reports have confirmed that there is strong communication between testicular echotextural parameters and sperm quality and quantity characteristics[17-19]. Testicular ultrasonogram is a software-based method for evaluating the testicular parenchyma echotexture[20]. Testicular echogenicity (pixel intensity), testicular heterogeneity (pixel standard deviation) as well as testicular vascular area pixels (testicular artery) have been obtained for the testicular parenchyma to correlate the breeding efficiency with testicular image analysis technique to be more trustful for further breeding soundness examination[21].

According to our knowledge, there is a scarce number of reports concerning using the Doppler ultrasonography to assess the testicular ultrasonographic morphometric, echotextural attributes as well as testicular blood vascular irrigation indices through breeding soundness examination in ram lambs. For that, we hypothesize that the application of such promising techniques has considerable usefulness to evaluate the reproductive status. In addition, sexual maturation could be affect on testicular dimensions, the echotextural appearance of testicular parenchyma as well as blood flow indices of the testicular artery in ram lambs under subtropical conditions.

MATERIALS AND METHODS

Animals and management

The present study was conducted using a total number of 8 prepubertal ram lambs (aging 4-5 months and weighing 30-40 kg). All animals were submitted to routine clinical examination especially for the vascular system (pulse rate and capillary refilling time) to exclude any diseased ram from the study. All animals were kept on a balanced ration as well as free access to fresh water. All study protocol was carried out according to the guidelines of animal care and ethical use committee of Cairo University (CU II S 5 18).

Ultrasonographic examinations

All testicular ultrasonographic scanning was performed by the same investigator. Gray-scale and pulsed-wave Doppler were done using 7.5 MHz linear-array transducer (EXAGO, Echo Control Medical, France). All settings (brightness, frequency, and gain) were kept fixed for all measurements. Rams were kept secured in the lateral recumbency without sedation. The fine scrotal wool was shaved before ultrasound examination. The transducer was covered with an appropriate amount of gel to avoid any imperfections.

Gray-scale testicular ultrasonography

Testicular parenchyma was checked for testicular length (L), width (W) and thickness (T) using electronic calipers (Fig. 1). Testicular volume (TV) was calculated using the following formula, where $TV= L \times W \times T \times 0.71$[22].

Pulsed-wave Doppler scanning

Testicular blood flow dynamics were measured to check the testicular irrigation (Fig. 2). Spectral Doppler examination was carried out by identifying all vascular structures using B-mode ultrasonography as well as color flow mapping for the proximal pole of the testes (pampiniform .plexus). The angle between the Doppler beam and the longitudinal axis of the testicular artery was never more than 60, with a high pass filter set at 50Hz. The spectral Doppler gate was fixed at 1 mm. The studied blood flow indices were resistive index (RI= (peak systolic velocity-end diastolic velocity)/peak systolic velocity) and pulsatility index (PI= (peak systolic velocity-end diastolic velocity)/ mean velocity) according to Batissaco et al.[23].

Image analysis

The pre-saved B-mode/color Doppler images of the testicular parenchyma were used for further computer-assisted image analysis (Fig. 3). Image analysis was performed using image assessment software (Image J, U. S. National Institutes of Health, Maryland, USA). Both testicular echogenicity (pixel intensity) and heterogeneity (pixel standard deviation) were calculated on the area of interest (1 cm^2/ testis) by drawing a rectangle 0.5 to 1.0 cm deep into the homogenous testicular parenchyma[24].

The colored red and blue areas of pampiniform plexus (area of interest) were calculated per pixel using Adope PhotoShop CC softwere (1990-2013, Adope Systems). Colored Doppler im-

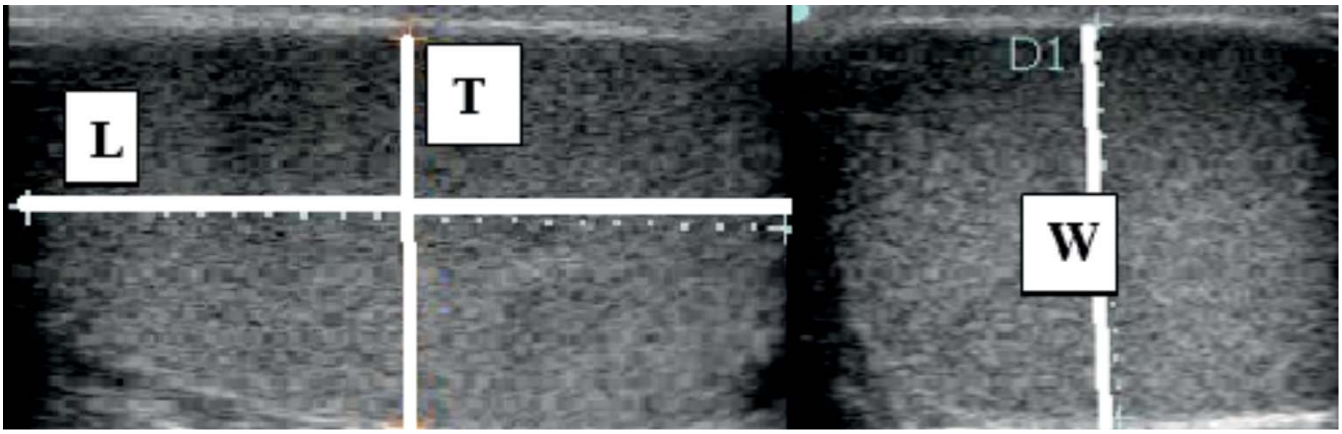

Figure 1 - Ultrasonographic estimation of the studied testicular dimension (L, testicular length, T, Testicular thickness, W, testicular width) using electronic calipers.

Figure 2 - Assessment of blood flow indices (resistive and pulsatility index) for testicular artery in ram lambs.

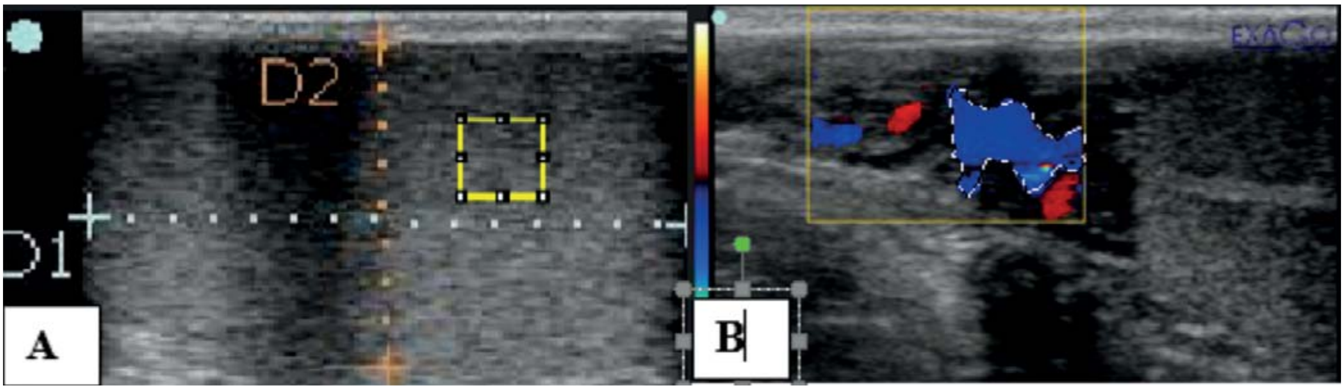

Figure 3 - Computer-assisted image analysis for pre-saved gray-scale/colored testicular images, where A represents the rectangle area of interest within testicular parenchyma, and B represents calculating the area/pixels within the vascular regions (pampiniform plexus) of testicular artery.

ages were exported to a removable hard disk for further computer analysis. A magnetic lasso tool was obtained to identify the colored area, which is followed by counting the pixels in this area of interest according to Batissaco et al.[23].

Statistical analysis

The results of the studied variables (testicular morphometric parameters, testicular length, width, thickness, and volume as well as blood flow indices, resistive index, and pulsatility index) were expressed as mean ±SD. The resulted data were tested for normality using Shapiro-Wilk test, where data showed normal statistical distribution.

A t-test was used to compare means of the right and left testes' measurements. Pearson correlation coefficients were studied between the whole variables. Differences were considered significant at P≤0.05. All the statistical analysis was carried out using SPSS, version 16.0.

RESULTS

As shown in table1, testicular width, thickness, and volume recorded significant differences between the both testes (*P*≤0.05), where the mean values for these parameters were 34.51 mm, 36.55 mm and 58.89 mm^3, respectively. In addition, the left testis showed the highest testicular dimensions compared with the right testis.

Interestingly, there were positive associations (Table 2) between the studied testicular dimensions, where testicular width showed a considerable communication (Table 2) with the testicular thickness (r =0.913, *P*≤0.01), testicular length (0.770, *P*≤0.01) and testicular volume (r =0.935, *P*≤0.01).

However, testicular volume recorded a strong association with testicular length (r =0.933, *P*≤0.01) and testicular thickness (r =0.854, *P*≤0.01).

Table 1 - Mean ±SD of testicular dimensions (length, width, thickness and volume), testicular echotextural attributes (echogenicity, heterogeneity and area/pixels) as well as intratesticular blood flow indices (resistive and pulsatility index) in ram lambs (n=8).

Parameters	Right testis	Left testis	*P*-value	Range	Overall
Testicular length (mm)	60.52±11.38	67.32±10.87	0.623	48.90-81.50	63.92±11.43
Testicular width (mm)	33.67±2.38[a]	35.35±4.64[b]	0.001	29.80-40.20	34.51±3.70
Testicular thickness (mm)	35.25±1.47[a]	37.85±2.73[b]	0.001	33.40-40.70	36.55±2.52
Testicular volume (mm^3)	51.44±13.30[a]	66.33±23.18[b]	0.001	41.62-93.51	58.89±19.99
Resistive index	0.51±0.05[a]	0.74±0.12[b]	0.025	0.43-0.84	0.62±0.15
Pulsatility index	0.72±0.10[a]	1.24±0.23[b]	0.022	0.64-1.60	0.98±0.34
Testicular echogenicity	83.26±10.66	81.83±14.50	0.348	63.75-102.15	82.55±12.47
Testicular heterogeneity	20.63±2.47[a]	21.68±1.00[b]	0.018	17.98-24.46	21.15±1.91
Area/pixels	3037±789.87[a]	2627±178.68[b]	0.011	1477-5579	2832±367.21

[a,b] at the same row are significantly different at $P \leq 0.05$.

Table 2 - Correlation coefficients between testicular morphometric parameters, testicular echotexture measures and testicular blood flow indices in ram lambs.

Paired measures	Correlation coefficient
Testicular length × testicular width	0.770**
Testicular length × testicular thickness	0.623**
Testicular length × testicular volume	0.933**
Testicular length × testicular echogenicity	0.505*
Testicular width × testicular thickness	0.913**
Testicular width × testicular volume	0.935**
Testicular width × testicular echogenicity	0.760**
Testicular width × testicular heterogeneity	-0.438*
Testicular thickness × testicular volume	0.854**
Testicular thickness × testicular heterogeneity	0.668**
Testicular volume × testicular heterogeneity	0.658**
Resistive index × pulsatility index	0.878**
Resistive index × testicular heterogeneity	0.458*

* means significant at $P \leq 0.05$.
** means significant at $P \leq 0.01$.

Regarding the spectral Doppler analysis of the testicular artery (Table 1), both resistive and pulsatility indices experienced considerable changes between the right and left testicular artery ($P \leq 0.05$), where the values for resistive and pulsatility indices were ranged between 0.43-0.84 and 0.64-1.60, respectively. There was a strong positive correlation (Table 2) between both indices ($r = 0.878$, $P \leq 0.01$).

Regarding the testicular computer-assisted image analysis (Table 1 and 2), testicular echogenicity was not significantly different between the right and left testes ($P \geq 0.05$), where the overall measurement was 82.55±12.47. while, there were significant different changes between the testicular heterogeneity and testicular vascular area pixels ($P \leq 0.05$). testicular heterogeneity showed positive associations with the other testicular dimensions as testicular thickness ($r = 0.668$, $P \leq 0.01$) and testicular volume ($r = 0.658$, $P \leq 0.01$).

DISCUSSION

During the last decade, the using of ultrasonography technology to evaluate testicular functionality has noted a great potential impact on the breeding soundness evaluation in farm animals. However, there is a lack of literatures concerning the potential importance of testicular ultrasonographic imaging and their possible role for the prediction of reproductive efficiency in rams[23, 24]. In addition to these clear points, the testicular ultrasonographic examination is a highly valuable and accurate technique to definitely measure the true testicular morphometric dimensions, where the classical scrotal circumference examination is not an accurate technique to define the exact testicular volume owing to the presence of a considerable scrotal skin, subcutaneous fascia and epididymis, which could greatly interfere with the precise determination of testicular size[25].

In the present study, testicular dimensions showed a considerable difference between the right and left testes. However, Martinez et al.[26] had reported that testicular weight, not size, affected on daily sperm production and sperm cells concentration in rams, where there is a constant rate of sperm production by 20 million sperm/1 g of testis/day. In agreement with our results, Iraqi ram lambs recorded similar measurements for testicular length, width, and thickness[27], where animals showed a closely related bodyweight and growth pattern. In contrast, Karadi lambs[28] and Santa Ines lambs[29] experienced clear different testicular biometric parameters, where there was a severe decline in testicular length and width, which might be due to the great smaller bodyweight (21.82 kg and 23 kg, respectively) compared with Ossimi lambs in our study (30-40 kg).

The testis is a vital compact organ with a high metabolic rate function, where its main functions are spermatogenesis (sperm production) and steroidogenesis (testosterone synthesis). To maintain the testicular physiological roles, constant testicular blood flow should be supplied through the main testicular artery. Regarding the testicular blood irrigation in ram lambs, there is a lack in reports dealing with the characterization of testicular hemodynamics and its potential role for reproductive development in lambs[30]. The four common parameters of hemodynamics are peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI). PSV and EDV are concerning with deeply measure the arterial blood velocities, but these measures are not constant and considerably depending on dif-

ferent factors[10]. Resistive index (RI) and pulsatility index (PI) are the most useful blood flow indices to evaluate the testicular vascularity and to predict fertility[31, 32]. In the current study, the spectral Doppler analysis for testicular artery in Ossimi lambs experienced a non-resistive monophasic pattern of the cardiac cycle. Our investigations were in agreement with those reported in dogs[33] and bulls[17]. In addition, resistive index and pulsatility index showed higher values for the left testis compared with the right one. In agreement with this finding, previous reports in human[34] and healthy dogs[35] have explained that the left testicular artery arches over the renal vein, which provide a further pressure on the arterial wall and increasing the blood velocities in this area. In addition, we recorded a significantly higher testicular of the left testis compared with the right testis, where the higher testicular volume should be provided a suitable vascular infusion to achieve its functions.
Computer-based evaluation of testicular ultrasonogram had been used to monitor the sexual development[16, 36]. In our study, we recorded that testicular echogenicity (pixel intensity) was higher in the parenchyma of ram lambs. In previous studies, Riberio et al.[37] and Camela et al.[24] recorded a similar measurement for testicular pixel intensity in Santa Ines and Dorper lambs, respectively. These cellular histomorphological changes are characterized through the increase in the diameter of seminiferous tubules, morphological differentiation of Sertoli cells and the amount of the produces testicular fluids[38, 39]. As far as we know, spermatogenesis is a vital process and is directly associated with the constant blood supply for the testes via the testicular artery[40]. This is in agreement with the declined values of RI and PI in the right testis, which allow less resistance against the passing blood within the testicular artery, which supposed to causing the higher values of colored rea pixels in the pampiniform plexus of the right testis compared with the left testis owing to the increased right testicular vascular irrigation.

CONCLUSION

As we can conclude, testicular ultrasonographic examination is useful to deeply predict the sexual development in Ossimi ram lambs. Gray-scale imaging could be used through the routine breeding soundness evaluation in ram lambs. Testicular pulsed-wave Doppler analysis is a promising technique to investigate the testicular vascular irrigation and prediction of testicular function. Thus, reproductive development is strongly associated with different testicular ultrasonographic and spectral Doppler analysis in Ossimi ram lambs under subtropical conditions.

CONFLICT OF INTEREST

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

AUTHORSHIP CONTRIBUTION STATEMENT

Hedia is the main investigator in data collection, writing and drafting, while El-Belely is the main supervisor of study design and reviewing.

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