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Original article

ECHOGENICITY OF DIFFERENT STRUCTURES OF THE SCROTAL BAG IN RAMS: EFFECT OF AGE, INJURIES AND SEXUAL ACTIVITY

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Summary

Castillo-Hernández, G., M. L. Rodríguez-Villanueva, O. Salvador-Flores, J. de Lucas Tron & J. A. Maldonado-Jáquez, 2023. Echogenicity of different structures of the scrotal bag in rams: effect of age, injuries and sexual activity. *Bulg. J. Vet. Med.* (online first).

The purpose of this research was to characterise the echogenicity of the different structures within the scrotal bag in rams. Three studies that included 43 rams were conducted. Ultrasound sections (US) were made with the Mindray® equipment. It was found that pubertal males showed greater echogenicity ($P<0.05$) in testes and the pampiniform plexus, whereas prepubertal males ($P<0.05$) – in the epididymis and in the right pampiniform plexus. Rams in mating showed the highest echogenicity values ($P<0.05$). In non-mating animals, the highest values were found in the epididymis ($P<0.05$). Rams with injuries presented higher echogenicity in testes ($P<0.05$). In pampiniform plexuses, males without injuries demonstrated the highest values ($P<0.05$). A high and positive correlation ($P<0.0001$) was found between the evaluated variables, specifically the right cranial longitudinal (RCL), right proximal transverse (RPT), right medial transverse (RMT), right distal transverse (RDT) measures. Echotexture in rams was affected by age, presence of injuries and sexual activity. In addition, the high correlation suggests that the evaluation of testicular health in rams could be carried out only with several US.

Key words: image analysis, rams, reproduction, ultrasonography

INTRODUCTION

There is growing pressure for high efficiency in the sheep milk and meat production, so improving the reproductive efficiency of herds is one of the main ways to increase productivity (Ahmadi *et al.*, 2012; Andrade *et al.*, 2012). Hence, the evaluation of content of the scrotal bag, and especially of the ram testicles, is essential to ensure the ewes gestation during the mating season (Boukhliq *et al.,* 2018).

On the other hand, the use of ultrasound techniques has become an important exploration option given its enormous advantage of being non-invasive, relatively easy to perform and offering clinical diagnostic options, as well as being a useful predictor of the fertility of males with satisfactory predictive values (Stålhammar *et al*., 1997; Camela *et al*., 2018). Likewise, research on testicular ultrasound associated with the study of changes in the intensity of pixels has shown that it was correlated with the potential production of semen (Hedia *et al.,* 2020).

In this sense, the pendular anatomy of the testes in sheep facilitates the ultrasound examination, without using sedatives. This, in turn, facilitates the evaluation and analysis of the structures within the scrotal bag, such as the pampiniform plexus, the testes, and the epididymis, allowing for the quantitative determination of normal echogenicity patterns of the testicular parenchyma (Carazo *et al*., 2014). Thus, these technologies are capable to measure characteristics such as testicular volume, echotexture and illustrate elasticity and stiffness. Therefore, these technologies allow visualisation of testicular hypervascularisation and can help in the early diagnosis of anomalies that may affect the reproductive performance of males. This information is clinically relevant because vascularisation and microvessel density, i.e., the number of vessels per mm^2, reflect abnormalities in normal function of testicles (Schurich *et al*., 2009; Lock *et al*., 2011; Orlandi *et al*., 2022). However, the use of these technologies for this purpose has not been widely disseminated, and therefore there is little information on the use of ultrasonography to determine reproductive fitness (Arteaga *et al.,* 2005) and testicular echogenicity parameters of rams at different ages and under different management regimes (El-baz & Razek, 2019).

Therefore, the objective of the present study was to characterise the echogenicity (understood as the intensity of shades of gray measured in pixels) of the components of the scrotal bag content of rams at different ages, with different sexual activity, and with or without presence of apparent clinical injuries in order to expand technology that contributes to developing echogenicity standards for the monitoring and evaluation of testicular health, as well as to assist in the selection of animals in which characteristics associated with precocity and others related to fertility are observed.

MATERIALS AND METHODS

All methods used, as well as the animals handling in this study adhered strictly to the accepted guidelines for the ethical use, care and welfare of animals used in International Research, according to the Federation of Animal Science Societies (ASAS, 2020), National Academy of Medicine (NAM, 2011) and the Universidad Nacional Autónoma de México (UNAM) with the approval of the project "Ultrasound evaluation of male and female reproductive organs in sheep and goats, to determine echogenicity parameters related to productive and reproductive efficiency".

The work was developed in a production unit (n=500) destined to the production of lambs for slaughter, which is constituted by animals in absorption towards the Katahdin breed. The production unit is located in the municipality of Nicolás Romero, Estado de Mexico, Mexico at latitude 19° 38' 11¨ N. The climate is temperate subhumid, with rains in summer, annual precipitation of 800–1000 mm and an average annual temperature of 16 °C (García, 2004).

Three studies were conducted. The first was carried out in February (non-breeding season), for which 13 clinically healthy lambs with no apparent injuries on palpation were randomly chosen. The males were divided into three groups: prepubertal animals (6 months; n=5), pubertal animals (10–12 months; n=4) and adult animals (older than 3 years; n=4). Age was considered as a factor of echogenicity of testicular parenchyma, epididymis and pampiniform plexuses.

The second study was carried out in the months of June-July (beginning of sexual activity), for this, four Katahdin breed rams, clinically healthy and without apparent injuries on palpation were used. Two were chosen for a 35-day mating and two as controls (without sexual activity). In this study, the effect of sexual activity (mating vs non-mating) on the echogenicity of testicular parenchyma, epididymis and pampiniform plexuses was considered.

For the third study, carried out in the month of October (breeding season), 21 crossbred males (Katahdin sire) were used, in the final stage of fattening with an average weight of 41.4 kg and at an average age of 5 months.

All the animals underwent scrotal bag palpation and the presence or absence of injuries and their relationship with the echogenicity of the testicular parenchyma, epididymis and pampiniform plexuses were considered.

At the time of the ultrasound evaluation in each study, the animals were seated and palpation was performed on each side of the scrotal bag, likewise, each testicle was slid into the bag. The testicular perimeter was measured and later both testicles were exposed, regions without hair were located to perform ultrasound sections (US). The sections were made with the Mindray®, model DP-10 (Shenzhen, China) using 5 MHz convex probe and transcutaneous approach. The images were analysed using the free Image J® 1.480 software (Fig. 1). Fourteen content sections were made as followed: two lon-

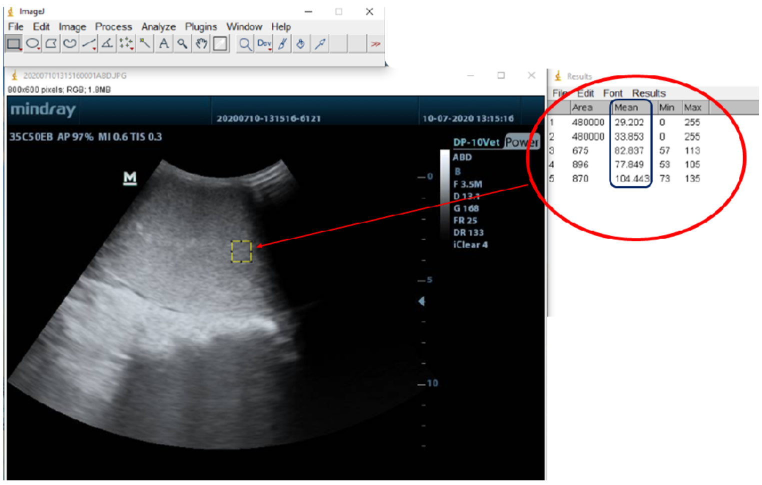

Fig. 1. Ultrasonogram analysed with the Image J software counting the number of pixels per section. Mean indicates the average number of pixels; Min and Max: the minimum and maximum number of pixels in the area under evaluation.

gitudinal sections in each testicular parenchyma, one in the cranial part and the other in the caudal part, as well as three transverse sections in proximal, middle and distal regions. Each epididymis was longitudinally cut in the tail region, and each pampiniform plexus was transected (Fig. 2). The sections made had 4 repetitions each and were listed as right cranial longitudinal (RCL), right proximal transverse (RPT), right medial transverse (RMT), right distal transverse (RDT), right pampiniform plexus (RPP), right epididymis tail (RET), right caudal longitudinal (RCaL), left cranial longitudinal (LCL), left proximal transverse (LPT), left medial transverse (LMT), left distal transverse (LDT), left pampiniform plexus (LPP), left epididymis tail (LET), left caudal longitudinal (LCaL), in addition to testicular diameter (TD).

Statistical analysis was carried out by means of ANOVA considering age, sexual activity and the presence of testicular injuries. Comparison of means was carried out with the Tukey test. In addition, a Spearman correlation analysis was performed between the evaluated structures and the live weight and testicular diameter of the rams from all studies. For data analysis, the statistical package SPSS IBM ® 2018 was used.

RESULTS

Table 1 shows the echogenicity results for the rams by age groups. The pubescent males showed greater echogenicity ($P<0.05$) in both testes, likewise, the prepubertal and adult males showed the lowest values, with no difference between them ($P>0.05$). Greater echogenicity was observed in prepubertal males ($P<0.05$) in both epididymis with respect to pubertal and adult males. On the other hand, in the right pampiniform plexus, prepubertal and pubertal males showed the highest echogenicity ($P<0.05$), with respect to adult males. However, in the left pampiniform plexus, pubertal males had higher echogenicity ($P<0.05$) vs adult males, but without difference from prepubertal males.

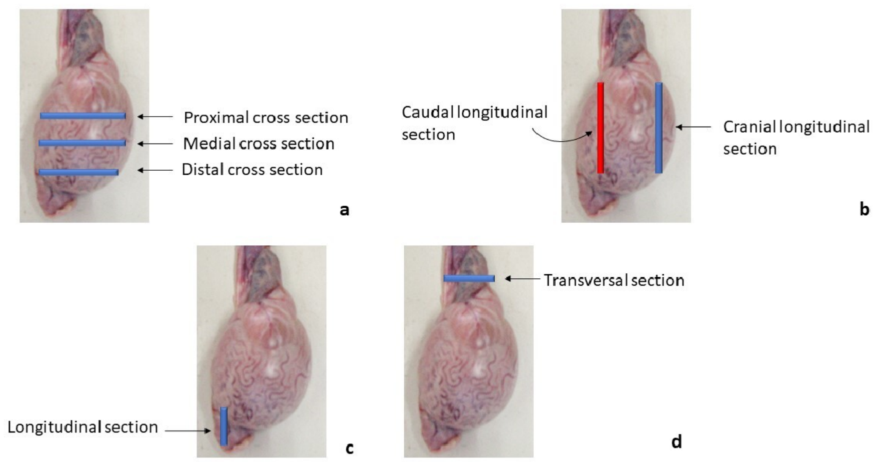

Fig. 2. Ultrasound sections made in ram testicles of different ages, with and without injuries and under different regimen of sexual activity.

The echogenicity given by the effect of sexual activity is shown in Table 2. In this regard, the highest values (P<0.05) were found in both testicles for the rams that underwent mating compared to the non-mating rams. However, regarding the epididymis of both testes, the highest values were found in non-mating animals (P<0.05). On the other hand, no differences were found (P>0.05) in the pampiniform plexuses of both testicles and between the type of sexual activity.

Regarding the echotexture of the components of scrotal content, especially the testicles and pampiniform plexuses with the presence of injuries (Fig. 3), the testicles with abnormalities presented the highest values (P<0.05). On the contrary, the pampiniform plexuses without injuries showed the highest values (P<0.05). In the case of the epididymis, no differences were found between rams with and without injuries (Table 3).

Table 4 shows the correlation analysis between the evaluated variables, proving a strong relationship, specifically of RCL,

Table 1. Pixels per testicular structure (mean±S.E.) in rams at different ages

Structure	Prepubertal	Pubertal	Adults
Right testicle	43.8±4.2[b]	63.0±3.7[a]	40.4±4.4[b]
Left testicle	45.7±4.3[b]	66.3 ±3.8[a]	46.5±4.5[b]
Right epididymis	23.4±4.9[a]	11.6±4.5[b]	9.7±5.1[b]
Left epididymis	18.7±3.2[a]	9.2±2.8[b]	8.8±3.1[b]
Right pampiniform plexus	34.7±5.1[a]	38.9±4.9[a]	17.2±5.6[b]
Left pampiniform plexus	35.1±8.7[ab]	46.3±8.2[a]	21.1±7.2[b]
R^2		0.53	
P Value		<0.0001	

[ab] different letters within rows indicate significant difference (P≤0.05); R^2= determination coefficient.

Table 2. Pixels per testicular structure (mean±S.E.) in adult rams subjected to different regimes of sexual activity.

Structure	Mating	No mating
Right testicle	104±0.2[a]	94.9±0.2[b]
Left testicle	108±0.2[a]	100.9±0.3[b]
Right epididymis	9.8±0.5[b]	18.6±1.1[a]
Left epididymis	11.6±0.3[b]	15.3±1.1[a]
Right pampiniform plexus	33.8±0.6	34.0±1.7
Left pampiniform plexus	37.9±0.8	39.2±1.3
R^2	0.13	
P Value	<0.0001	

[ab] different letters within rows indicate significant difference (P≤0.05); R^2= determination coefficient.

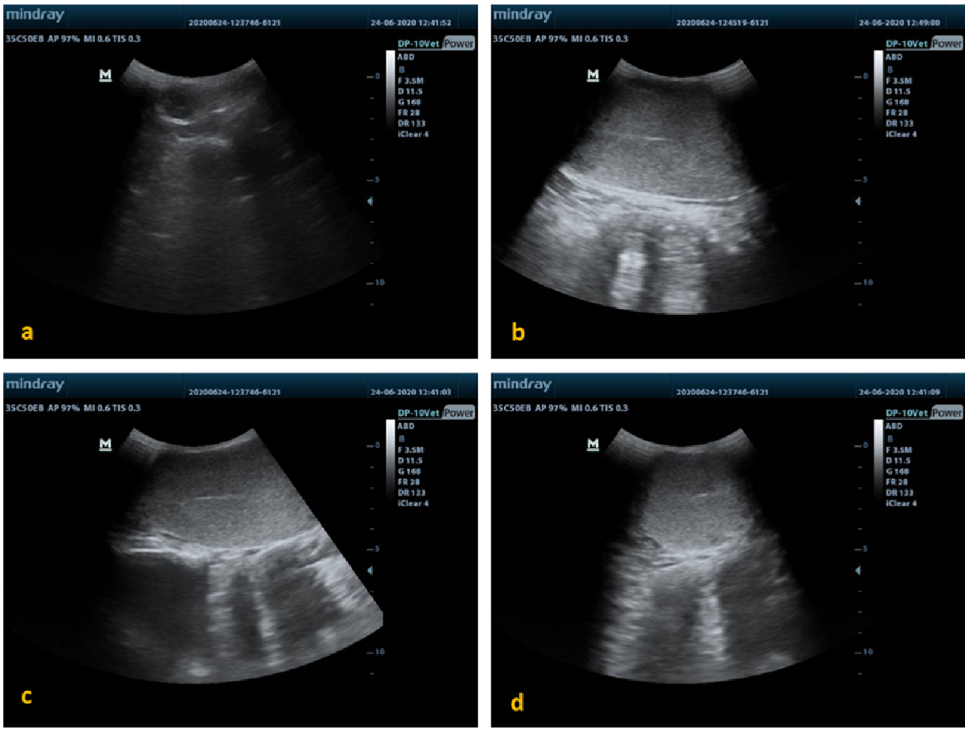

Fig. 3. Ultrasonogram of the medial section in different males showing differences in echogenicity. A) anechoic areas, practically in the entire section indicating fluid accumulation; B) areas with elevated echogenicity indicating fibrosis, probably as a result of a previous blow; C; D) anechoic areas next to areas with elevated echogenicity, which are indicators of early inflammatory processes.

Table 3. Pixels per testicular structure (mean±S.E.) in male sheep with and without injuries on palpation

Structure	No injury	With injury
Right testicle	52.4±1.2[b]	80.5±4.9a
Left testicle	42.1±1.1[b]	68.3±4.5a
Right epididymis	25.5±1.6	20.3±3.7
Left epididymis	25.8±01.4	22.2±2.2
Right pampiniform plexus	49.7±3.0[a]	27.5±2.6[b]
Left pampiniform plexus	40.5±3.1[a]	20.6±5.9[b]
R^2	0.76	
P Value	<0.0001	

[ab] different letters within rows indicate significant difference (P≤0.05); R^2= determination coefficient.

Table 4. Correlation matrix between ultrasound sections of the different testicular structures, live weight and testicular diameter

RCL	RPT	RMT	RDT	RPP	RET	RCaL	LCL	LPT	LMT	LDT	LPP	LET	LCaL	Weight	TD
RCL	1														
RPT	0.79	1													
RMT	0.88***	0.81***	1												
RDT	0.73	0.69***	0.77***	1											
RPP	0.21	0.16	0.18	0.14	1										
RET	–0.13	–0.22	–0.13	–0.03	0.31*	1									
RCaL	0.65***	0.54***	0.61***	0.57***	0.04	–0.05	1								
LCL	0.73***	0.71***	0.76***	0.65***	0.01	–0.23	0.58***	1							
LPT	0.71***	0.74***	0.71***	0.65***	0.05	–0.27*	0.56***	0.68***	1						
LMT	0.73***	0.69***	0.73***	0.69***	0.08	–0.27*	0.52***	0.76***	0.76***	1					
LDT	0.56***	0.53***	0.66***	0.64***	-0.1	–0.16	0.54***	0.71***	0.62***	0.8***	1				
LPP	0.14	0.11	0.07	–0.03	0.49***	-0.03	–0.1	0.09	0.04	0.11	–0.08	1			
LET	0.32*	0.21	0.27*	0.22	0.38**	0.61***	0.33**	0.07	0.03	0.03	–0.03	0.05	1		
LCaL	0.58***	0.52***	0.61***	0.63***	–0.07	–0.25	0.47***	0.55***	0.53***	0.61***	0.62***	-0.1	–0.01	1	
Weight	0.62**	0.67**	0.67**	0.77***	0.19	–0.09	0.47*	0.7**	0.67**	0.7***	0.55*	0.09	0.13	0.41	1
TD	0.43*	0.35	0.49*	0.58*	0.19	0.03	0.25	0.52*	0.5*	0.58*	0.4	0.04	0.35	0.41	0.71***

* P<0.05; ** P<0.01; *** P<0.001; RCL=right cranial longitudinal; RPT=right proximal transverse; RMT=right medial transverse; RDT=right distal transverse; RPP=right pampiniform plexus; RET= right epididymis tail; RCaL= right caudal longitudinal; LCL=left cranial longitudinal; LPT=left proximal transverse; LMT=left medial transverse; LDT= left distal transverse; LPP=left pampiniform plexus; LET=Left epididymis tail; LCaL=left caudal longitudinal; TD=testicular diameter.

RPT, RMT and RDT measures in a positive way (P<0.0001) with practically all the other variables considered, except for RPP, RET and LPP. The variables RCaL, LCL, LPT and LMT were related (P<0.0001) with practically all variables except for LPP and LET. The variable that was negatively related to practically all the variables was RCaL, but not significantly.

DISCUSSION

Some studies have shown that echogenicity in testicles increase in the most active stage of growth of the seminiferous tubules, in which a greater number of germ cells are produced (Arteaga *et al*., 2005; Giffin *et al*., 2014). Likewise, it has been described that testicular echotexture increases as testicles grow and develop, as a result of increase in cell proliferation and increase in diameter of the seminiferous tubules at puberty (Kastelic *et al*., 2001) in line with the report of Cardili *et al*. (2014), indicating greater echogenicity in animals that reached puberty, similar to results found in the present study. Rodrigues *et al*. (2020) found out that ultrasound characteristics of testicles, epididymis and accessory sex glands were modified by age in addition to being useful to obtain information on progression of sexual maturation.

On the other hand, the greater epididymal echogenicity in the prepubescent males can be attributed to the fact that during sexual maturation, the cellular content of seminiferous tubules is modified and light appears them with the beginning of fluid secretion. In this sense, testicular and epididymal development, in this phase, is associated with an increase in epithelial height of the seminiferous tubules, the number of tubules within the lumen and size of this lumen, which would give rise to an increased fluid in the tissue, in other words, the more fluid content is, the less echogenic the tissue is (Correia, 2015) providing another explanation for lower values found in adult rams.

About sexual activity, Wu *et al*. (2010) demonstrated that pixel intensity was closely related to serum testosterone concentration and reproductive status. In addition, Rodrigues *et al*. (2020) concluded that blood flow to the reproductive system in ultrasound evaluations was affected by breed and age.

Brito *et al*. (2012) concluded that testicular echogenicity varied depending on age and demonstrated that the ultrasound characteristics observed in different testicular structures were more strongly related to the development of spermatogenesis than to age.

Regarding the presence or absence of injuries, Kastelic & Brito (2012) showed that the intensity of pixels was associated with testicular abnormalities, in addition, anechoic images (black appearance) or those with decreased echogenicity (dark grayish) were associated with accumulation of fluid within the testicle, while increased echogenicity (lighter gray) was indicative of fibrosis and calcifications. In relation to this, the use of ultrasound has important advantages for diagnosis of anomalies that are not detected on palpation of males, especially future breeders (Gnemmi *et al*., 2020).

Finally, it has been pointed out that the sonographic characteristics of testicular parenchyma were related to the histology and morphology of testicles, as well as the changes in seminiferous tubules, onset and efficiency of spermatogenesis (Giffin *et al*., 2014). The foregoing, for the purposes of this study, is interesting, that as testicles' diameter increases, echogenicity increases, which may be a clear indication of good gonadal health. Likewise, positive correlations have been found between mean values of pixels, the area occupied by seminiferous tubules in testicles and lumen area of the seminiferous tubules (Arteaga *et al*., 2005). These results coincide with our expectation, since it is known that there is a positive relationship between testicular diameter and body weight (Lyndsay, 1977; Braun *et al*., 1980; Cartee *et al*., 1989).

CONCLUSION

In conclusion, echogenicity in rams was affected by factors such as age, the presence of injuries and sexual activity. The high correlation found between the variables suggested that the evaluation of testicular health in rams could be carried out in a simple and practical way with only several ultrasound sections included, making ultrasound technology a tool to determine the general state of the testes, epididymis and pampiniform plexus, to predict the reproductive success of rams in production units.

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