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Original article

Chios ram testicular blood flow and echotexture changes depending on age, season and ejaculation process

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Abstract

The present study aimed to investigate the impact of age, season and ejaculation on ram testicular blood flow and echotexture. The survey was conducted biweekly on 7 Chios rams for one year, including breeding and non-breeding periods. The rams were divided into 2 age groups: 3 rams 2-6 years old (mature) and 4 rams 9-13 years old (old). Hemodynamic indices [Pulsatility index (PI), Resistive index (RI), End-diastolic velocity (EDV), testicular artery Diameter (D), Time-averaged maximum velocity (TAVM), Blood flow volume (BFV)] and echotexture parameters [Mean value (MV), Contrast (Con), Gray value distribution (GVD), Run length distribution (RunLD), Long run emphasis (LRunEm), Entropy (Ent), Correlation (Cor), Standard deviation (StD), Gray variance (GV) and Gradient mean value (GMV)] were evaluated in each testis before and after ejaculation. Ejaculation did not affect testes blood flow or echotexture ($p>0.05$). PI and RI were higher in the breeding period compared to the non-breeding period, for both testes ($p<0.001$). Left testis GV and Cor before ejaculation were lower ($p=0.01$) and higher ($p=0.03$), respectively, in the breeding compared to the non-breeding period. Left testis D ($p=0.005$) and BFV ($p<0.001$) were higher in old compared to mature rams after ejaculation. Right testis Con ($p=0.03$) and Cor ($p=0.05$) before ejaculation were higher in old rams, whereas right testis Ent after ejaculation was higher in mature rams ($p=0.05$). In conclusion, testicular blood flow and echotexture are affected by season and ram age, but not by ejaculation.

Key words: Chios breed, ram, ram age, seasonality, testicular blood flow, testicular echotexture

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Introduction

Ram selection for reproduction is based on their genetic material, as well as on clinical and laboratory evaluation of their fertilizing ability. Previous research stated that ultrasonographic examination of testes could assist the process of ram selection (Batissaco et al. 2013, Ntemka et al. 2018). In fact, both B-mode and Doppler are non-invasive contemporary techniques that can be applied as a selection method to locate and identify physiological or pathological alterations in testicular parenchyma and blood flow, with limited likelihood of a misdiagnosis (Cook and Dewbury 2000, Gouletsou et al. 2003, DesCôteaux 2010).

Ram testicular blood flow and echotexture can be affected by many factors, such as temperature, administration of medicines or hormones, diseases and stress (Chandolia et al. 1997, Gouletsou 2017). Even though ejaculation is a stressful process, previous studies reported that testicular echogenicity didnot change after semen collection, either using an artificial vagina (Gouletsou et al. 2003), or by electroejaculation (Ahmadi et al. 2012). On the other hand, there are no published data about ram ejaculation effects on testicular blood flow.

Physiological testicular echotexture and blood flow have not been widely studied in rams. Consequently, there are just a few data about the ultrasonographic progressive changes that could be observed over increasing ram age or while the rams are entering the breeding season. Specifically, echotexture has been reported as increasing as the ram age increased (Chandolia et al. 1997). Concerning the attainment of puberty, decreased testicular echogenicity was noticed in rams just before puberty, but it was recorded as moderateafter puberty (Tapping and Cast 2008, Andrade et al. 2014, Camela et al. 2019). Moreover, Ahmadi et al. (2012) found that mean numerical pixel values and pixel heterogeneity of ram testes were higher during the non-breeding season, whereas Gouletsou (2017) considered that testicular echotexture was lower during the breeding period, after qualitative ultrasonographic evaluation. Regarding previous studies on testicular blood flow, it was not affected by the attainment of puberty (Camela et al. 2019), whereas it was higher during the breeding season (Hedia et al. 2019).

The Chios breed is one of the most widespread in Greece, adjusted to regional climatic conditions. This breed attains puberty earlier than others, and high fertility, prolificacy and high milk production are its most important characteristics, which are particularly significant for the regional dairy industry. However, no studies have been conducted and no data have been obtained about the testicular blood flow and echotexture of Chios rams.

The limited data available and the great interest in high productivity on Greek sheep farms motivated the annual recording of Chios ram testicular blood flow and echotexture data, as well as the investigation of the contribution of age, season and ejaculation on ram testicular blood flow and echotexture.

Materials and Methods

The present study was approved by the Research Ethics Committee of the School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece.

The survey was performed on 7 Chios breed rams, separated into two age groups: 3 rams between 2 and 6 years old (mature) and 4 rams between 9 and 13 years old (old). They were housed at the Department of Reproduction and Artificial Insemination, Directorate of the Veterinary Centre of Thessaloniki, Greek Ministry of Rural Development and Food, Thessaloniki, Greece (longitude: 22°58′ E, latitude: 40°37′ N, altitude: 32 m). The rams were kept indoors in individual pens, under natural daylight and temperature and received a daily diet according to NRC guidelines (1985).

The investigation was conducted biweekly for a whole year, including both breeding (May to September) and non-breeding periods (October to April) (Vosniakou et al. 1989). B-mode and Pulsed Wave Doppler examinations were applied using the Esaote MyLab™One ultrasound equipped with a linear probe, 7.5 MHz. Semen was collected by artificial vagina. Testicular ultrasonographic imaging was performed on rams in the standing position, on each testis separately. Although spermatogenesis relies on the function of both testes, Batissaco et al. (2013) approached their research objectives by estimating blood flow to each testis separately. Testicular asymmetry inside the scrotum is usual, as one testis is located lower than the other due to the different anatomy of the vascular system. Previous studies have confirmed this difference in testicular size/position in other species (Mittwoch 1988, Davies Morel 2008). In addition, unilateral diseases of the ram testes, such as inflammation or hernia, are often detected, and have an adverse effect on semen quality. Taking into account the above-mentioned data, ultrasonographic examination during the present study was performed separately on each testis.

Testicular ultrasonographic imaging was performed 24 hours prior to, and immediately after ejaculation, maintaining such an interval in order to limit the induced stress to the examined rams (Marai et al. 2007). In total, 52 measurements were performed for each parameter on each ram. The probe was placed vertically on the marginal part of the testicular artery, on the caudal pole of the testis at the level of epididymal tail. Pulsed Wave Doppler was performed on the testicular artery to measure Pulsatility index (PI), Resistive index (RI), End-diastolic velocity (EDV), testicular artery Diameter (D), Time-averaged maximum velocity (TAVM) and Blood flow volume (BFV).

Images by B-mode trans-scrotal scanning of the testicular parenchyma (longitudinal and transverse sections) were analyzed using Echovet v2.0 software.

Analysis of a digital image aims to extract both qualitative and quantitative data. Actually, two-dimensional images are a table of pixels. In the case of black and white images, the information extracted from the displayed structures is based on the echotexture, which is the level of brightness of an organ or structure in relation to the grayscale gradation.

EchoVet 2.0 software, used in the present study, enables the analysis of the texture of digital images with the method of statistical data/sizes, which is the simplest method. The numerical data resulting from the analysis of the images are mathematically equated with statistical quantities, which include:

- First-order gray-level statistics based on the histogram of the image.
  - Mean value (MV), which refers to the mean value of the brightness of an image.
  - Standard deviation (StD), which refers to the deviation of brightness values around their mean value.
- Second-order gray-level statistics based on the co-occurrence matrix (Pratt 1978; Allison et al. 1994; Aschkenasy et al. 2005, Nailon 2010).
  - Contrast (Con), which refers to the fluctuation of the brightness of an image.
  - Entropy (Ent), which refers to the level of homogeneity of an image.
  - Correlation (Cor), which refers to the correlation between two pixels of an image.
- Higher order statistics based on route length (run-length matrix) (Valckx and Thijssen 1997; Nailon 2010; Pitas 2010).
  - Gray value distribution (GVD), which refers to the way gray levels are distributed in an image.
  - Run length distribution (RunLD), which refers to the way route lengths are distributed in an image.
  - Long run emphasis (LRunEm), which refers to the long routes of an image.
- Statistics related to the gradient of brightness (Galloway 1975, Nailon 2010, Pitas 2010).
  - Gray variance (GV) which refers to the variation of brightness differences between adjacent pixels of an image.
  - Gradient mean value (GMV) which refers to the mean value of brightness differences between adjacent pixels of an image.

Statistical analysis

Statistical analysis was performed using SPSS® 24.0. Data are presented as mean ± SD. A paired t-test was applied to compare the mean values of hemodynamic indices before and after ejaculation. A further t-test was applied to compare the mean values of echotexture parameters before and after ejaculation. A mixed linear repeated measurements model using two factors (age and breeding period) with the Bonferroni correction was used to evaluate the effect of ram age on hemodynamic and echogenicity indices, and the effect of season on the abovementioned indices and their interaction. In cases where the interaction between the two factors was insignificant, the main effects of each factor were investigated. The assumptions about either variability or the form of population distribution were assessed with or without transformed data. A value of $p \leq 0.05$ was considered statistically significant.

Results

The effect of season on ram testicular blood flow is presented in Table 1. PI and RI of both testes were higher before and after ejaculation, in the breeding season compared to the non-breeding season ($p<0.001$). Moreover, testicular artery D and BFV of the left testis were higher during the non-breeding season compared to the breeding season, before ejaculation ($p=0.012$ and $p<0.001$, respectively). The left testis EDV both before and after ejaculation, and the right testis EDV only before ejaculation were higher in the non-breeding season, compared to the breeding season ($p<0.001$, $p<0.001$ and $p=0.02$, respectively). The effect of season on raw testicular echotexture is presented in Table 2. The value of left testis GV before ejaculation was lower in the breeding period compared to the non-breeding period ($p=0.01$). Left testis Cor before ejaculation was higher in the breeding period compared to the non-breeding period ($p=0.03$).

The analytical results indicated that the interaction between age and breeding season was insignificant for all the variables and, hence, the main effects of each factor were investigated.

The effect of ram age on testicular blood flow is presented in Table 3. Testicular artery D of the right testis was wider in old rams, both before and after ejaculation ($p=0.038$ and $p<0.001$, respectively), whereas for the left testis, it was observed only after ejaculation ($p=0.005$). Moreover, left testis BFV was higher after ejaculation in old rams, compared to mature rams

Table 1. Effect of season on ram testicular blood flow.

Parameter	Testis	Ejaculation	Season				p-value
			Breeding (n=22)	Breeding (n=22)	Non-breeding (n=30)	Non-breeding (n=30)	
PI	left	before	1.23[a] ± 0.32	1.75-0.56	0.86[b] ± 0.39	1.83-0.15	<0.001
		after	1.25[a] ± 0.38	1.95-0.46	0.89[b] ± 0.37	1.88-0.18	<0.001
	right	before	1.17[a] ± 0.41	1.94-0.34	0.93[b] ± 0.79	1.9-0.17	<0.001
		after	1.17[a] ± 0.36	2.12-0.37	0.85[b] ± 0.38	1.82-0.19	<0.001
RI	left	before	0.67[a] ± 0.11	0.87-0.41	0.53[b] ± 0.17	0.84-0.14	<0.001
		after	0.67[a] ± 0.12	0.91-0.38	0.54[b] ± 0.16	0.86-0.16	<0.001
	right	before	0.64[a] ± 0.14	0.88-0.28	0.53[b] ± 0.15	0.84-0.16	<0.001
		after	0.65[a] ± 0.12	0.89-0.35	0.52[b] ± 0.16	0.84-0.17	<0.001
EDV	left	before	0.09[a] ± 0.03	0.18-0.04	0.11[b] ± 0.04	0.28-0.04	<0.001
		after	0.1[a] ± 0.03	0.18-0.03	0.11[b] ± 0.04	0.26-0.04	<0.001
	right	before	0.1[a] ± 0.03	0.17-0.04	0.11[b] ± 0.03	0.22-0.05	0.02
		after	0.1 ± 0.04	0.22-0.04	0.17 ± 0.58	0.26-0.05	0.26
BFV	left	before	16.31[a] ± 13.35	52-1	25.07[b] ± 21.18	89-1	<0.001
		after	18.86 ± 16.26	63-1	22.9 ± 21.64	109-1	0.15
	right	before	23.21 ± 19.08	79-1	23.3 ± 20.03	112-1	0.78
		after	24.19 ± 19.51	88-1	23.67 ± 19.68	103-1	0.94
D	left	before	1.32[a] ± 0.6	2.5-0.06	2.01[b] ± 2.71	27-0.27	<0.001
		after	1.42 ± 0.65	2.9-0.38	1.55 ± 0.66	3.8-0.27	0.16
	right	before	1.57 ± 0.71	2.9-0.27	1.94 ± 2.71	27-0.38	0.2
		after	1.59 ± 0.66	2.9-0.27	1.58 ± 0.66	3-0.38	0.97
TAVM	left	before	0.17 ± 0.04	0.29-0.10	0.16 ± 0.04	0.29-0.01	0.49
		after	0.17 ± 0.04	0.30-0.10	0.16 ± 0.04	0.33-0.09	0.78
	right	before	0.17 ± 0.04	0.32-0.10	0.16 ± 0.04	0.26-0.09	0.15
		after	0.17 ± 0.04	0.29-0.10	0.16 ± 0.04	0.29-0.09	0.39

Values are mean ± SD. Different indicators a and b in the same row denote statistically significant differences between seasons. Values are also presented as range (max-min)(p≤0.05).
PI: Pulsatility index, RI: Resistive index, EDV: End-diastolic velocity (m/s), BFV: Blood flow volume (ml/min), D: Diameter (mm), TAVM: Time-averaged maximum velocity (m/s).

(p<0.001). The effect ram age on testicular echotexture is presented in Table 4. Right testis TAVM, before and after ejaculation, were higher in mature rams compared to old rams (p=0.04 and p<0.001, respectively). A similar result was noticed for left testis TAVM only before ejaculation (p<0.001).The old rams had higher values of right testis Con and Cor before ejaculation, and a lower value of right testis Ent after ejaculation, compared to mature rams (p=0.03, p=0.05 and p=0.05, respectively).

Ejaculation did not significantly affect either hemodynamic or echogenicity values (p>0.05).

Discussion

Alterations of testicular blood flow could be associated with seasonal fluctuation of testosterone, which has been positively correlated to testicular artery diameter and blood flow (Pozor 2007). Consequently, higher blood flow could be observed during the breeding season, when testicular volume is higher (Boyd et al. 2006, Sarlós et al. 2013). Nevertheless, the present study revealed that PI and RI values were higher during the breeding season, so testicular blood flow was lower. In agreement with our findings, Samir et al. (2018) reported decreased testicular blood flow during spring and summer, even though young (<2 years) non-seasonal breeder goats were included in their study. In contrast, Hedia et al. (2019) recorded lower values of these indices during the breeding season. These different findings could be attributed to different breeds, local geographical conditions, and study design.

Testicular echotexture has been positively correlated to seminiferous tubule diameter (Giffin et al. 2014). As testicular tissue consists of seminiferous tubules at a rate of 70-80% and testicular volume is associated

Table 2. Effect of season on ram testicular echotexture.

Parameter	Testis	Ejaculation	Season				p-value
			Breeding (n=22)	Breeding (n=22)	Non-breeding (n=30)	Non-breeding (n=30)	
MV	left	before	15.87 ± 0.35	16.12-15	15.84 ± 1.1	16.11-5.1	0.89
		after	27.35 ± 101.45	16.09-14.02	15.65 ± 1.41	16.08-5.07	0.24
	right	before	15.59 ± 1.78	16.1-5.03	25.14 ± 95.96	16.1-6.03	0.37
		after	15.46 ± 1.79	16.09-4.99	15.82 ± 1.03	16.1-16.09	0.10
Con	left	before	15.86 ± 0.61	16.8-13.68	15.77 ± 0.58	16.8-12.84	0.28
		after	15.85 ± 0.60	16.78-12.69	15.81 ± 0.42	16.8-11.53	0.72
	right	before	15.88 ± 0.61	16.79-12.76	15.78 ± 0.55	16.77-13.22	0.26
		after	15.86 ± 0.55	16.79-13.82	15.85 ± 0.52	16.79-13.76	0.86
GVD	left	before	493 ± 4189.12	16.64-5.57	56.83 ± 419.06	16.63-14.54	0.28
		after	15.86 ± 1.21	16.64-6.6	54.13 ± 391.74	16.68-6.59	0.38
	right	before	118.63 ± 633.6	16.64-13.62	15.89 ± 1.18	16.64-5.59	0.21
		after	63.87 ± 422.72	16.63-5.59	15.80 ± 1.19	16.63-5.55	0.38
RunLD	left	before	90.03 ± 655.81	15.83-5.76	15.67 ± 0.38	15.83-13.79	0.39
		after	15.61 ± 0.75	15.83-9.79	634.03 ± 637.44	15.88-6.78	0.25
	right	before	15.71 ± 0.25	15.83-14.73	15.70 ± 0.27	15.83-14.74	0.57
		after	15.50 ± 1.45	6.78-15.83	15.63 ± 0.91	15.83-6.75	0.56
LRunEm	left	before	15.74 ± 1.81	16.28-4.28	15.99 ± 1.13	16.29-5.28	0.42
		after	15.98 ± 1.07	16.29-8.28	15.96 ± 1.23	16.29-4.27	0.94
	right	before	15.99 ± 0.54	16.29-14.27	15.96 ± 0.53	16.29-14.27	0.89
		after	15.84 ± 1.93	16.29-4.27	16.05 ± 0.49	16.29-14.27	0.35
Ent	left	before	15.98 ± 0.64	16.51-13.50	15.90 ± 0.64	16.52-14.49	0.47
		after	15.97 ± 0.60	16.51-13.5	15.99 ± 0.57	16.55-14.5	0.85
	right	before	16.05 ± 0.55	16.53-14.49	15.97 ± 0.57	16.56-14.5	0.28
		after	15.90 ± 0.71	16.51-13.49	15.98 ± 0.57	16.53-14.49	0.50
Cor	left	before	11.29^{a} ± 0.64	12.01-9.93	11.12^{b}± 0.65	12-9.94	0.03
		after	11.28 ± 0.69	12-8.98	11.21 ± 0.69	12-8.98	0.56
	right	before	11.24 ± 0.68	12-8.98	11.21 ± 0.53	12.03-9.98	0.23
		after	11.17 ± 0.65	12-9	11.23 ± 0.57	12.01-9.95	0.43
StD	left	before	15.88 ± 0.32	16.03-14.99	15.86 ± 0.35	16.02-14.99	0.57
		after	15.79 ± 0.59	16.02-12	15.87 ± 0.39	16.02-14	0.33
	right	before	15.91 ± 0.29	16.03-14.99	15.91 ± 0.33	16.03-14.01	0.93
		after	15.86 ± 0.35	16.02-14.99	15.89 ± 0.38	16.02-13.99	0.71
GV	left	before	15.74^{a} ± 0.52	16.07-14.02	15.92^{b} ± 0.33	16.08-15.01	0.01
		after	15.84 ± 0.51	16.09-13.02	15.85 ± 0.45	16.07-13.05	0.98
	right	before	15.86 ± 0.45	16.08-14.03	15.83 ± 0.41	16.07-15.02	0.84
		after	15.87 ± 0.38	16.09-15.02	15.85 ± 0.39	16.05-15.02	0.66
GMV	left	before	15.85 ± 0.49	16.2-14.17	15.90 ± 0.49	16.2-14.18	0.46
		after	15.81 ± 0.56	16.2-14.14	15.78 ± 0.55	16.18-14.16	0.75
	right	before	15.94 ± 0.49	16.2-14.15	15.93 ± 0.46	16.19-14.16	0.78
		after	15.81 ± 0.63	16.21-13.16	15.90 ± 0.49	16.18-14.68	0.27

Values are mean ± SD. Different indicators a and b in the same row denote statistically significant differences between seasons. Values are also presented as range (max-min) ($p \leq 0.05$).

MV: Mean value, Con: Contrast, GVD: Gray value distribution, RunLD: Run length distribution, LRunEm: Long run emphasis, Ent: Entropy, Cor: Correlation, StD: Standard deviation, GV: Gray variance, GMV: Gradient mean value.

Table 3. Effect of ram age on testicular blood flow.

Parameter	Testis	Ejaculation	Ram age				p-value
			Mature rams (n=26)	Mature rams (n=26)	Old rams (n=26)	Old rams (n=26)	
PI	left	before	1.03 ± 0.38	1.77-0.16	1.01± 0.42	1.83-0.15	0.86
		after	1.05 ± 0.43	1.95-0.23	1.03 ± 0.40	1.85-0.18	0.86
	right	before	1.08 ± 0.90	1.94-0.32	1.00 ± 0.41	1.9-0.17	0.45
		after	0.98 ± 0.42	1.91-0.21	0.99 ± 0.39	2.12-0.19	0.92
RI	left	before	0.60 ± 0.15	0.87-0.15	0.58 ± 0.17	0.87-0.14	0.39
		after	0.60 ± 0.15	0.88-0.20	0.59 ± 0.16	0.91-0.16	0.90
	right	before	0.58 ± 0.16	0.88-0.28	0.57 ± 0.16	0.86-0.16	0.81
		after	0.57 ± 0.17	0.88-0.20	0.58 ± 0.15	0.89-0.17	0.85
EDV	left	before	0.11 ± 0.04	0.2-0.04	0.10 ± 0.04	0.28-0.04	0.18
		after	0.11 ± 0.03	0.19-0.04	0.10 ± 0.04	0.26-0.03	0.33
	right	before	0.11 ± 0.04	0.22-0.04	0.11 ± 0.03	0.17-0.04	0.34
		after	0.12 ± 0.04	0.26-0.04	0.16 ± 0.58	0.24-0.05	0.58
BFV	left	before	20.47 ± 19.58	89-1	22.03 ± 18.16	81-1	0.45
		after	15.44$^{\alpha}$ ± 15.16	91-1	25.5$^{\beta}$ ± 21.43	109-1	<0.001
	right	before	21.09 ± 18.27	88-1	24.48 ± 20.44	112-1	0.11
		after	23.49 ± 21.37	103-1	24.19 ± 18.17	83-1	0.73
D	left	before	1.54 ± 1.27	3-0.06	1.86 ± 2.57	2.7-0.38	0.24
		after	1.26^{a} ± 0.54	2.8-0.38	1.67^{b} ± 0.69	3.8-0.38	<0.001
	right	before	1.46^{a} ± 0.65	3.5-0.27	2.03^{b} ± 2.71	2.7-0.38	0.005
		after	1.48^{a} ± 0.68	3-0.27	1.67^{b} ± 0.63	3-0.27	0.03
TAVM	left	before	0.17^{a} ± 0.04	0.27-0.10	0.16^{b} ± 0.04	0.29-0.1	<0.001
		after	0.17 ± 0.04	0.26-0.09	0.16 ± 0.05	0.33-0.1	0.31
	right	before	0.17^{a} ± 0.04	0.32-0.10	0.16^{b} ± 0.04	0.29-0.09	0.04
		after	0.18^{a}± 0.04	0.29-0.11	0.16^{b} ± 0.04	0.28-0.09	<0.001

Values are mean ± SD. Different indicators a and b in the same row denote statistically significant differences between ram age groups. Values are also presented as range (max-min) ($p \leq 0.05$).
PI: Pulsatility index, RI: Resistive index, EDV: End-diastolic velocity (m/s), BFV: Blood flow volume (ml/min), D: Diameter (mm), TAVM: Time-averaged maximum velocity (m/s).

with spermatogenesis (Sarlós et al. 2013), the increased testicular volume in the breeding season could induce an increase in testicular echotexture. However, the results of the present study showed that GV value was higher in the non-breeding season but Cor was higher in the breeding season, which reflects increased echotexture in the non-breeding season. In agreement with our results, Ahmadi et al. (2012) observed higher echotexture in the non-breeding season, recording increased mean numerical pixel values and pixel heterogeneity. Allthough Gouletsou (2017) used a qualitative assessment of ultrasound images, she also stated that testicular echotexture could possibly decrease during the breeding season.

Concerning age, the present study indicated increased blood flow in old ram testes. Regarding testicular echotexture, Con and Cor values were higher but Ent was lower in old rams. As has already been mentioned, testicular blood flow and echotexture have been studied in rams just before or after puberty (Chandolia et al. 1997, Andrade et al. 2014, Camela et al. 2019), but there are no data about old rams. In general terms, it is supposed that testicular volume is decreased in very old males since degenerative changes of the seminiferous tubules can be observed. However, according to Turner et al. (2007) these processes may not be reflected in the ultrasonographic evaluation of testes. Moreover, the finding of our study that old Chios rams preserve high semen quality supports their use in flocks up to the age of 13 years old, a fact which is of considerable importance for Greek farms, where artificial insemination is not widely applied (Ntemka et al. 2019).

Ejaculation is a stressful process, as it induces peripheral vasoconstriction, and an increase in metabolism and body temperature. Consequently, blood flow resistance in the testicular artery could be increa-

Table 4. Effect of ram age on testicular echotexture.

Parameter	Testis	Ejaculation	Ram age				p-value
			Mature rams (n=26)	Mature rams (n=26)	Old rams (n=26)	Old rams (n=26)	
MV	left	before	15.90 ± 0.33	16.12-15	15.82 ± 1.11	16.11-5.1	0.59
		after	27.06 ± 100.82	16.09-5.07	15.76 ± 0.97	16.09-7.04	0.25
	right	before	15.59 ± 1.78	16.1-5.03	25.21 ± 96.42	16.1-6.03	0.37
		after	15.75 ± 1.28	16.09-4.99	15.60 ± 1.50	16.09-503	0.54
Con	left	before	15.74 ± 0.6	16.77-12.84	15.87 ± 0.59	16.8-13.68	0.15
		after	15.85 ± 0.47	16.79-14.79	15.82 ± 0.69	16.8-11.53	0.70
	right	before	15.71[a] ± 0.6	16.77-12.76	15.9[b] ± 0.54	16.79-13.22	0.03
		after	15.79 ± 0.55	16.79-13.82	15.91 ± 0.51	16.79-13.76	0.09
GVD	left	before	487.17 ±4162.15	16.62-5.57	57.04 ± 421.09	16.64-5.6	0.28
		after	15.9 ± 1.22	16.63-6.6	54.47 ± 393.62	16.68-6.59	0.38
	right	before	16.01 ± 0.57	16.64-13.62	91.86 ± 546.15	16.64-5.59	0.22
		after	15.72 ± 1.76	16.63-5.55	51.45 ± 363.72	16.63-6.59	0.38
RunLD	left	before	15.49 ± 1.19	15.83-5.76	70.86 ± 564.27	15.83-6.78	0.38
		after	848.07 ± 732.95	15.83-9.79	15.64 ± 0.91	15.88-6.78	0.25
	right	before	15.69 ± 0.27	15.83-14.74	15.71 ± 0.25	15.83-14.73	0.85
		after	15.48 ± 1.44	15.82-6.75	15.65 ± 0.90	15.83-6.78	0.42
LRunEm	left	before	15.94 ± 1.30	16.29-5.28	15.84 ± 1.57	16.29-4.28	0.49
		after	15.95 ± 0.98	16.29-8.28	15.98 ± 1.29	16.29-6.27	0.68
	right	before	15.98 ± 0.52	16.29-14.27	15.97 ± 0.54	16.29-14.27	0.93
		after	15.92 ± 1.41	16.29-4.27	15.99 ± 1.23	16.29-4.28	0.75
Ent	left	before	15.86 ± 0.66	16.51-13.5	15.99 ± 0.62	16.52-14.49	0.14
		after	16.01 ± 0.55	16.52-14.5	15.95 ± 0.6	16.55-13.5	0.48
	right	before	15.98 ± 0.58	16.53-14.49	16.02 ± 0.56	16.56-14.5	0.70
		after	16.05[a] ± 0.55	16.51-14.49	15.87[b] ± 0.68	16.53-13.49	0.05
Cor	left	before	11.2 ± 0.72	12.01-9.93	11.19 ± 0.60	12-9.95	0.61
		after	11.27 ± 0.64	12-8.98	11.22 ± 0.72	12-8.98	0.75
	right	before	11.07[a] ± 0.62	12-9.98	11.25[b] ± 0.57	12.03-8.98	0.05
		after	11.16 ± 0.64	12-9	11.24 ± 0.58	12.01-9.95	0.30
StD	left	before	15.87 ± 0.34	16.03-14.99	15.87 ± 0.34	16.02-14.99	0.83
		after	15.83 ± 0.44	16.02-13.99	15.84 ± 0.52	16.02-12	0.95
	right	before	15.87 ± 0.37	16.03-14.01	15.94 ± 0.25	16.03-14.99	0.19
		after	15.86 ± 0.42	16.01-13.99	15.89 ± 0.32	16.02-14.99	0.72
GV	left	before	15.85 ± 0.40	16.08-15.02	15.83 ± 0.45	16.07-14.02	0.78
		after	15.86 ± 0.48	16.09-13.05	15.84 ± 0.48	16.06-13.02	0.67
	right	before	15.80 ± 0.46	16.06-14.03	15.88 ± 0.40	16.08-14.03	0.18
		after	15.88 ± 0.36	16.06-15.02	15.86 ± 0.39	16.09-15.02	0.31
GMV	left	before	15.91 ± 0.47	16.2-14.17	15.86 ± 0.49	16.2-14.08	0.48
		after	15.77 ± 0.57	16.2-14.15	15.81 ± 0.54	16.18-14.14	0.55
	right	before	15.91 ± 0.50	16.19-14.16	15.95 ± 0.44	16.2-14.15	0.64
		after	15.85 ± 0.57	16.19-13.16	15.86 ± 0.54	16.21-13.16	0.85

Values are mean ± SD. Different indicators a and b in the same row denote statistically significant differences between ram age groups. Values are also presented as range (max-min) ($p \leq 0.05$).

MV: Mean value, Con: Contrast, GVD: Gray value distribution, RunLD: Run length distribution, LRunEm: Long run emphasis, Ent: Entropy, Cor: Correlation, StD: Standard deviation, GV: Gray variance, GMV: Gradient mean value.

sed. However, the present study demonstrated that ejaculation did not affect ram testicular echotexture and blood flow. This is in accordance with previous studies where semen was collected either by artificial vagina or electroejaculation (Gouletsou et al. 2003, Ahmadi et al. 2012). This finding could be attributed to the complex structure of the pampiniform plexus which coils around testicular artery loops, reducing blood pressure and enhancing temperature regulation.

In conclusion, testicular blood flow and echotexture are affected by season and ram age, but not by ejaculation.

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