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RESEARCH ARTICLE

Stereological Measurement of Testicular Volume in Kivircik Rams

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Summary

In this study, measurement of testis volume by stereology in Kivircik rams, Turkey's traditional sheep breed, was purposed. Right and left testes with epididymis were taken from a total of 42 Kivircik rams whose carcass weights were measured previously. Fifteen, 14, and 13 of them were 5, 6, and 7 months old, respectively. Weights and morphometric measurements (length, width, and thickness) of the testis and epididymis were taken. Theirs volumes were estimated according to the Archimedes principle. According to Cavalier's principle, 0.6 cm thick 8-11 parallel sections were taken. In these sections for whole testis (testis + epididymis) volume 0.8 cm, and for testis and epididymis volumes, grids with 0.4 cm intervals randomly placed on sections and convergent points were counted. By using Microsoft Excel (XP version), contained volume, volume ratio and coefficient error estimation formulas were used for estimating values automatically. In the study, statistical difference was not observed ($P>0.05$) between right and left testes volumes values acquired from Archimedes and Cavalier's methods. The highest increase in epididymis, testis, and whole testis volumes, which were measured separately, was observed between rams at 6-7 months of age. In correlation analyses, estimated according to months by using mean values, it was recorded that there was a correlation between carcass weight and epididymis volume ($P<0.05$). Correlation was observed by ratio of $P<0.01$ between epididymis volume and testis thickness for 7 months old rams. Although there was no correlation observed between testis volume and epididymis volume for 7 months old rams. Instead of above evaluations, correlation ratio of $P<0.01$ was observed between morphometric measurements and stereological data of all analysis. Regarding features, showed higher correlations, regression analyses were made. In the presence of some morphometric data, regression equations, which required us to get stereological data, were calculated. Our thought is the collected data of this study can support the further experimental animal studies.

Keywords: *Epididymis, Kivircik rams, Stereology, Testis, Testicular volume*

Kıvırcık Koçlarında Testis Hacminin Stereolojik Ölçümleri

Özet

Türkiye'nin ulusal bir koyun ırkı olan Kıvırcık koçlarında testis hacmini stereolojik bir yöntemle hesaplama amaçlandı. Karkas ağırlıkları alınmış 15 adet 5 aylık, 14 adet 6 aylık, 13 adet 7 aylık olmak üzere 42 adet Kıvırcık koçtan sağ ve sol testisler epididymis ile birlikte alındı. Testislerin ağırlık ve morfometrik ölçümleri (uzunluk, genişlik, kalınlık) alındı. Testis ve epididymis'lerin Arşimet prensibine göre hacimleri hesaplandı. Cavalieri prensibine göre 0.6 cm kalınlığında olmak üzere 8 ile 11 adet paralel kesitler alındı. Bu kesitlerde tüm testis (testis+epididymis) hacmi için 0.8; testis ve epididymis hacmi için 0.4 cm sonda aralıklı gridler kesitler üzerine rastgele atılarak üzerinde çakışan noktalar sayıldı. Hacim, hacim oranı ve hata katsayısı hesaplama formüllerini içeren Microsoft Excel (XP sürümü) programı kullanılarak değerler otomatik olarak hesaplandı. Çalışmada Arşimet ve Cavalieri metotları kullanılarak elde edilen hacim değerleri ile sağ ve sol testis hacim değerleri arasında istatistiksel fark gözlenmedi ($P>0.05$). Cavalieri metodu kullanılarak aylara göre ayrı ayrı hesaplanan epididymis, testis ve tüm testis hacimlerindeki en hızlı artış 6 aylık ile 7 aylık koçlar arasında gözlendi. Ortalama değerler kullanılarak aylara göre yapılan korelasyon analizinde 6 aylık koçlarda karkas ağırlığının epididymis hacmi ile korelasyon gösterdiği saptandı ($P<0.05$). 7 aylık koçlarda ise epididymis hacminin testis kalınlığı ile arasında $P<0.01$ oranında korelasyon tespit edildi. 7 aylık koçların testis hacmi ile epididymis hacmi arasında ise korelasyon görülmediği tespit edildi. Bu değerlendirmeler dışında tüm morfometrik ölçümler ile stereolojik veriler arası analizde $P<0.01$ oranında korelasyonların olduğu gözlendi. Aralarında yüksek korelasyonların bulunduğu özellikler dikkate alınarak regresyon analizleri yapıldı. Testislerin bazı morfometrik verilerinin bilinmesi halinde stereolojik verilerini elde edebileceğimiz regresyon denklemleri hesaplandı. Bu çalışmadan elde edilen tüm verilerin yapılacak olan deneysel hayvan çalışmalarına katkı sağlayacağını düşünmekteyiz.

Anahtar sözcükler: *Epididymis, Kıvırcık koç, Stereoloji, Testis, Testis hacmi*

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INTRODUCTION

Kivircik is a national sheep breed raised in Turkey and constitutes 6-7% of Turkey's sheep population [1]. This breed, which has pretty good meat and milk yield, is the main sheep breed of the Thracia region [2].

In rams, puberty begins with increased libido and the production of sperm cells having the capacity of fertilization. These kind of sperm cells are produced in 112-185 days and this period may change with nutrition, climate, body weight and race [3].

Testicular volume measurement is a significant criterion in spermatogenesis prediction and evaluating testis functions in human [4-6]. Because testis volume is composed of tubuli seminiferi contorti and tubuli seminiferi recti, any change in testis measures reflects the changes in this tissue mass and also spermatogenesis [6].

Besides human studies [4-8], there are also many testis volume studies had been conducted on stallion [9], donkey [10], dog [11,12] and a couple of rams included Chios and Serres rams [13], Awassi rams [14], Libyan fat-tailed rams [15].

Rams have great importance in the improvement of the fertility of a sheep herd. Consequently, knowing testis measurements and spermatologic properties of rams that are going to be selected as breeding purposes takes an important place in evaluating highly productive breeds and providing continuance of genotypes produced [16].

Because the relation between the size of the testis and reproductive activity is important, biometric analyses on testis are also significant in young rams. Every study on testis helps in the better understanding of this organ and system, and accordingly assists in going one step forward in genetic studies. In this study, our purpose is to report the relationship between the volumetric enlarging of testes and epididymis, and the morphometric measures of whole testes in Kivircik rams by using the stereological method, to be a contributor to veterinary and other experimental studies of animals.

MATERIAL and METHODS

In our study, right and left testes with epididymis were taken from 42 Kivircik rams from Cekmece Meat and Integrated Plant and their carcass weights were also recorded. Fifteen, 14, and 13 of the Kivircik rams were 5, 6, and 7 months of age, respectively (born in August-September-October). Fixation of taken materials was provided by steeping in 10% formalin solution at 10 days. After pulling out the solution, membranes of testes were peeled and ductus deferens was removed. The whole testis (testis and epididymis) were weighed on 1 mg digital assay balance. After this process, morphometric measures (testis length, width, and thickness) were taken by calipper (Mitutoya, Eng). Testis length were taken from extremitas capitata to extremitas caudata of testis, testis widht were taken from margo medialis to margo lateralis at the widest point of the testis and also testis thickness were taken from margo epididymalis to margo liber at the widest point of the testis.Then, according to Archimedes principle, the volume of testis and epididymis were calculated regarding the volume raised in a graduated bowl. Afterwards, with slicer (Bosch), 0.6 cm parallel sections were obtained *(Fig. 1)*. For volume estimation, according to testis size 8-11 sections were sliced. These sections were superimposed by providing the same direction sides upwards and then sections were enumerated.

Fig 1. The whole testis (testis and epididymis) slices cut in paralel sections

Şekil 1. Tüm testis'in (testis ve epididymis) paralel dilimlere ayrılmış kesitleri

Volume Estimation

In order to estimate whole testis volume, and testis and epididymis volumes, the following procedure was undertaken. Grids with 0.8 cm prob intervals for whole testis and grids with 0.4 cm prob intervals for testis and epididymis were randomly put on sections and the points fall on the related sections that belong to material volumes we measure were counted *(Fig. 2)* and testis volume estimated via following formula.

$V = t \times a(p) \times \sum P$

V : Volume

t : Section thickness (0.6 cm)

a (p) : Area represented by each point on pointed area measurement ruler (0.64 cm^2 for whole testis, 0.16 cm^2 for testis and epididymis)

$\sum P$: Total point count on section surface area

Volume Fraction Estimation

The area measurement ruler, as shown in *Fig. 2*, was thrown on section cites randomly. Circled points hit on the whole testis, and small points hit on testis and epididymis, were counted. Since the small points/big points proportion

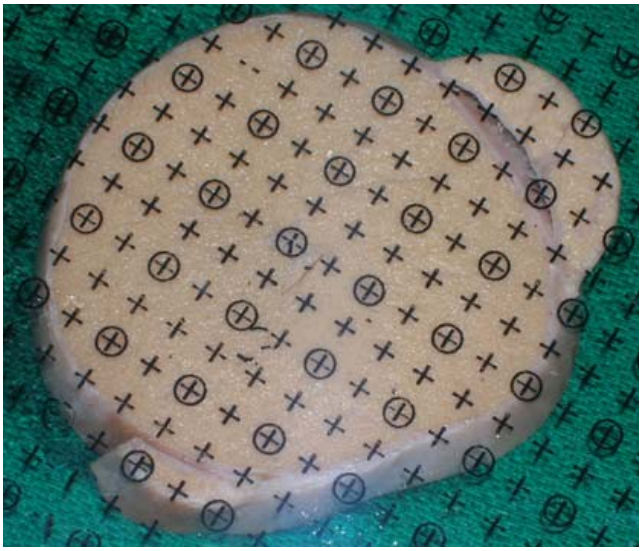

Fig 2. Superimposition of the grid covering testis and epididymis slice
Şekil 2. Testis ve epididymis kesitlerinin üzerine noktalı alan ölçüm cetvelinin atılması

was 4/1, testis or epididymis volume/whole testis ratio was calculated by the formula below.

$$V_V(whole\ testis) = \frac{\sum P\ testis\ or\ epididymis}{4 \times \sum P\ whole\ testis}$$

Volume ratio varies between 1 and 0, and generally expressed as (%) percentage [17].

In order to question the sufficiency of the quantity of sections and point frequency on pointed area measurement ruler that used in this study, CE was calculated according to method reported by Gundersen and Jensen [18].

In the study, statistical differences evaluated by months between the right and left testis were examined by Archimedes and Cavalier's principles. By ignoring carcass weight, correlation analysis was applied monthly between morphometric measurement values of testis and stereological data. By referring to data obtained from correlation analyses, on the basis of highly correlated properties, regression analyses was carried out.

In order to control the significance of difference between mean values of all measurements, a Student-t test was performed. In statistical data estimation (mean value, standard deviation, correlation, and regression analyses), SPSS 8.0 and Minitab 12 programs were used. Nomina Anatomica Veterinaria was used for the anatomical nomenclature [19].

RESULTS

Mean carcass weights of rams used in the current study were 15.9±1.45 kg, 19.5±1.23 kg, and 24.9±2.47 kg for 5, 6 and 7 month-old rams, respectively.

Volumetric and Volumetric Fraction Data

By ignoring the age variation, the mean value of whole testis volume estimation of rams used in this study (calculated according to Archimedes principle) was found as 70.5±32.9 cm^3 (right: 71.4±34.6 cm^3, left: 69.6±31.6 cm^3).

Stereologically, the mean value of whole testis volume was 67.00±32.9 cm^3 (right: 67.7±34.0 cm^3, left: 66.2±32.3 cm^3). A statistical difference was not observed between values obtained using Archimedes and Cavalier's principles (P>0.05) *(Table 1)*. A statistical difference was not determined between right and left testis volumes, which were obtained from Archimedes and Cavalier's principles (P>0.05).

Table 1. *Total volume of whole testis (cm^3) using Archimedes and Cavalier's principles*
Tablo 1. *Arşimet ve Cavalier prensibi kullanılarak hesaplanan tüm testisin (cm^3) toplam hacmi*

Features	Section	n	Mean±SD
Whole testis (Archimedean Principle)	Right	42	71.4±34.6
	Left	42	69.6±31.6
	Total	84	70.5±32.9
Whole testis (Cavalier's Principle)	Right	42	67.7±34.0
	Left	42	66.2±32.3
	Total	84	67.0±32.9

Whole testis parts (testis and epididymis) values obtained by using Cavalier's principle were shown in *Table 2*. There was no statistical difference between right and left values of testis and epididymis volumes, estimated month-to-month according to Cavalier's principle (P>0.05). Besides, for 5 months, mean testis volume was 41.6±22.1 cm^3 (88%); for 6 months it was 52.6±26.3 cm^3 (88%); and for 7 months it was 90.2±19.9 cm^3 (90%). For epididymis the values were 4.82±1.36 cm^3 (12%); 6.02±2.17 cm^3 (12%); and 9.45±2.38 cm^3 (10%), respectively.

According to Cavalier's principle, coefficients of error for whole testis, testis and epididymis, were 2%, 4%, and 1%, respectively.

Correlation Data

Correlation ratio of P<0.05 was observed between carcass weight and epididymis volume for 6 months old rams where correlation analysis was evaluated according to months by using mean values but no other correlations were observed for carcass weight with neither morphometric nor stereological values for other months. Epididiymis volume of 7 months old rams showed correlation (P<0.01) with only testis thickness in all other morphometric measurements. Again while there was no correlation between epididiymis volume and testis volume of stereological datas, correlation ratio of P<0.01 was

observed for other morphometric an stereological datas at 7 months old rams. Instead of above evaluations, correlation ratio of P<0.01 observed between morphometric measurements and stereological data of all analysis *(Table 3)*.

Regression Analyses

By using correlation analyses, regression analyses, based on highly related properties, was evaluated. Thus, regression equations, maintaining stereological data under available morphometric data, were estimated *(Table 4)*.

DISCUSSION

Although use of technically improved equipment and methods, for comparing testis and epididymis volumes and confirming the results, direct measurement and estimations are needed. Because of this, we thought and followed a different way of direct measurement by using stereological methods on testis and epididymis of Kivircik rams.

Sakamoto et al.[4] and Karaman et al.[5] indicated that

Table 2. *Right, left and mean volumes and volume percentages of testis and epididymis*
Tablo 2. *Sağ, sol ve ortalama testis ve epididymis hacim ve hacim oranları*

Component	Section	5 Month (n=15)		6 Month (n=14)		7 Month (n=13)	
		Mean±SD	%	Mean±SD	%	Mean±SD	%
Testis volume (cm^3)	Right	41.7±22.1	89	53.7±27.7	89	91.3±22.2	90
	Left	41.4±22.9	88	51.4±25.8	88	89.0±18.0	90
	Total	41.6±22.1	88	52.6±26.3	88	90.2±19.9	90
Epididymis volume (cm^3)	Right	4.70±1.36	11	5.98±2.29	12	9.45±2.42	10
	Left	4.95±1.39	12	6.07±2.13	12	9.45±2.45	10
	Total	4.82±1.36	12	6.02±2.17	12	9.45±2.38	10

Table 3. *Correlation analyses according to month between morphometric measurement values of testis and stereological data*
Tablo 3. *Testisin morfometrik ölçüm değerleri ile stereolojik veriler arasında aylara göre yapılan korelasyon analizleri*

Features	Age (Month)	Carcass Weight	Testis Weight	Testis Length	Testis Width	Testis Thickness	Whole Testis Volume	Epididymis Volume	Testis Volume
Testis weight	5	0.03^{NS}							
	6	0.46^{NS}							
	7	0.23^{NS}							
Testis length	5	0.00^{NS}	0.85^{**}						
	6	0.40^{NS}	0.86^{**}						
	7	0.26^{NS}	0.94^{**}						
Testis width	5	0.17^{NS}	0.93^{**}	0.89^{**}					
	6	0.53^{NS}	0.98^{**}	0.91^{**}					
	7	0.28^{NS}	0.94^{**}	0.95^{**}					
Testis thickness	5	0.02^{NS}	0.97^{**}	0.86^{**}	0.94^{**}				
	6	0.45^{NS}	0.99^{**}	0.85^{**}	0.98^{**}				
	7	0.32^{NS}	0.90^{**}	0.82^{**}	0.83^{**}				
Whole testis volume	5	0.04^{NS}	1.00^{**}	0.84^{**}	0.93^{**}	0.98^{**}			
	6	0.47^{NS}	1.00^{**}	0.85^{**}	0.98^{**}	0.99^{**}			
	7	0.32^{NS}	0.99^{**}	0.95^{**}	0.93^{**}	0.89^{**}			
Epididymis volume	5	0.02^{NS}	0.87^{**}	0.84^{**}	0.86^{**}	0.88^{**}	0.88^{**}		
	6	0.56^{*}	0.91^{**}	0.73^{**}	0.88^{**}	0.87^{**}	0.90^{**}		
	7	0.18^{NS}	0.52^{NS}	0.51^{NS}	0.48^{NS}	0.69^{**}	0.51^{NS}		
Testis volume	5	0.03^{NS}	1.00^{**}	0.83^{**}	0.92^{**}	0.98^{**}	1.00^{**}	0.87^{**}	
	6	0.45^{NS}	1.00^{**}	0.85^{**}	0.98^{**}	0.99^{**}	1.00^{**}	0.89^{**}	
	7	0.32^{NS}	0.98^{**}	0.95^{**}	0.92^{**}	0.86^{**}	1.00^{**}	0.42^{NS}	

^{NS} *Not significant,* * *P<0.05,* ** *P<0.01*

Table 4. *Regression analyses on highly related properties*
Tablo 4. *Yüksek ilişkinin bulunduğu özellikler üzerinde yapılan regresyon analizleri*

Testis	Regression Equations	R^2	P
Whole testis volume	Whole testis volume = (0.74 x Testis weight) - 1.95	R^2 = 99.5%	P<0.001
	Whole testis volume = (2.28 x Testis lenght) - 123	R^2 = 86.1%	P<0.001
	Whole testis volume = (3.75 x Testis thickness) - 99.2	R^2 = 94.9%	P<0.001
	Whole testis volume = (3.77 x Testis width) - 118	R^2 = 93.3%	P<0.001
	Whole testis volume = (1.07 x Testis volume) + 2.22	R^2 = 99.8%	P<0.001
Testis volume	Testis volume = (0.69 x Testis weight) - 3.76	R^2 = 99.3%	P<0.001
	Testis volume = (2.11 x Testis lenght) - 116	R^2 = 85.8%	P<0.001
	Testis volume = (3.48 x Testis width) - 94.0	R^2 = 94.5%	P<0.001
	Testis volume = (0.93 x Whole testis volume) - 1.95	R^2 = 99.8%	P<0.001
Epididymis volume	Epididymis volume = (0.27 x Testis widht) - 6.77	R^2 = 72.4%	P<0.001
	Epididymis volume = (0.07 x Whole testis volume) + 1.87	R^2 = 75.3%	P<0.001

testicular volume measurement enable the estimation of spermatogenesis and testicular functions. Many measuring methods, such as compass [9], orchidometer [4-7,11], ultrasonography [4,6,7,9,12], have use for the estimation of testis volume. Karaman et al.[5] indicated that the Prader orchidometer is an objective, reliable, and cheap measuring method. But Paltiel et al.[11] reported that the ultrasound (US) method give more faultless and correct measures than Prader and Rochester orchidometers.

All above mentioned methods are practicaly used methods on live animals and human beings. However by using stereological technique in our research, we tried to determine the most realistic volume values by direct measurement on testes and epididymis of animals. Although the stereological technique is not a method used in clinic practice, it has been one of the preferred method used in volumetric evaluation of materials study by pathology and anatomy and obtained images by imaging techniques as it gives reliable results both for human and veterinary usage.

The volumetric contraction might occur about 5%-15% by the effect of fixation liquids used [20]. In this study, testes and epididymides were collected from slaughterhouses and instantly placed in fixation liquid (10% formalin) in order not to putrify, and were kept 10 days in the solution. The amount of volumetric contraction in testis and epididymis was not determined.

In experimental studies, Avdi et al.[13], who report that the seasonal variations in testis volume, indicated that testis volumes of Chios rams and Serres rams are minimum in March and April, and in February and March respectively. They have also reported that testis volumes are maximum for both breeds in July and August. Nipken and Wrobel [10] have report that testis volume changes according to age between the adolescence and senescence period. In their study, they indicate that tubular volume, and thus related testis volume show rapid increase (approximately 3-fold) until the middle of the sexual maturity period, and later phase out in the regression period. In our study, since only volume estimation was planned to be examined, testis volume variations of Kivircik rams (obtained in March) were not determined.

Rapid enlargement of testes indicates mitosis in the germinal epithelium and the beginning of spermatogenic activity, and rams reach puberty in this period. In Awassi rams, highest values are measured between the 7th and 9th months [14]. Even in Libyan fat-tailed rams, the most rapid growth occurre between 85 and 180-200 days of age [15]. In our study, the volume of testis and epididymis showed a rapid increase between the 6th and 7th months. As a result it was concluded that puberty began during this period in Kivircik rams.

In order to estimate the testes volumes by special computer programs containing mathematical equations and formulas, volumes as cm^3, volume rates, and coefficients of error were easily and rapidly acquired [20]. In stereological methods, estimation of the coefficient error is effective in deciding the appropriate point density on grids. In a study, a coefficient error smaller than 5% is an acceptable ratio [8,18,21]. With the computer program used in our study, coefficients of error for whole testis, testis and epididymis volumes were found as 2%, 4%, and 1%, respectively. In this study, by concluding the sufficiency of sections count and point frequency on a pointed area measurement ruler, the reliability of study was presented.

Including of epididymis measures in the testis volume measurements, for estimations, caused volumetric increase errors. Since it is impossible to ignore the measures of skin and epididymis in measurements especially held by the Prader orchidometer, testis volume is observed greater than it is. Kabay [8] reported that US and MRI methods give more appropriate results than the Prader orchido-

meter, depending on the testis and epididymis tissue differentiation properties. In our study, we easily estimated the volumes and volume ratios in sections acquired by the stereological technique.

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