*Int. J. Morphol.,*
*31(3)*:1076-1080, 2013.

# Morphological, Stereological and Histometrical Assessment of the Testicular Parameters between Holstein and Simmental Bulls

## Evaluación Morfológica, Estereológica e Histométrica de los Parámetros Testiculares entre Toros Holstein y Simmental

**Murat Sırrı Akosman[*]; Ömer Faruk Lenger[**] & Hasan Hüseyin Demirel[***]**

**AKOSMAN, M. S.; LENGER, O. F. & DEMIREL, H. H.** Morphological, stereological and histometrical assessment of the testicular parameters between Holstein and Simmental bulls. ***Int. J. Morphol., 31(3)***:1076-1080, 2013.

**SUMMARY:** The testicular measurement is important criteria for experimental researches especially in toxicological studies and the prediction of spermatogenesis. In light of this knowledge, we aimed to estimate and compare these parameters in two different kinds of cattle breeds. The gross anatomical measurements were performed by vernier caliper, volume of the testis was estimated by Cavalieri method and seminiferous tubule diameter was measured on the histological sections by software-loaded computer attached to microscope. The mean testis weight, length, width, volume, and tubule diameter of the Simmental bulls and the Holstein bulls measured as 316 g, 12.1 cm, 6.9 cm, 295 $cm^3$ and 226.68 μm and 299 g, 12.1 cm, 6.8 cm, 285 $cm^3$ and 223.44 μm, respectively. In conclusion all investigated parameters were not found statistically important in groups and between the breeds ($p>0.05$). The authors believed that the obtained data will contribute to the literature and facilitate future research.

**KEY WORDS: Bull; Cavalieri method; Seminiferous tubule; Testis; Volume.**

## INTRODUCTION

In Turkey, the Simmental and Holstein bulls are raised for the brood stock and beef aimed. The Turkish breeders keep Simmental cows for their beef and dairy features and the Holsteins cows for their highest-milk production. The adaptation of the Simmental cattles to the environment better than the Holstein cattles (Alpan & Arpacik, 1998). Actually, testicular volume measurement is a significant criterion in spermatogenesis prediction, evaluating testis functions and detected variety of disorders affects testicular growth and development (Sakamoto *et al*., 2007; Karaman *et al.*, 2005; al Salim *et al.*, 1995; Paltiel *et al*., 2002; Setchell & Brooks, 1988; Wing & Christensen, 1982; Sakr & Shalaby, 2012; Al-Damegh, 2012). Because 70-80% of the testis consist of seminiferous tubules and any change in testis measures reflects the changes in this tissue mass and also spermatogenesis (al Salim *et al.*; Paltiel *et al*.; Setchell & Brooks). As a result of these scientific findings, testicular and scrotal measurements are especially in common use as an important factor for the bulls to decided whether brood stock in the herd (Hahn *et al*., 1969; Silva *et al*., 2011).

Recently, there are some measurement techniques, including caliper (Gouletsou *et al.*, 2008), orchidometry (Paltiel *et al*.), ultrasonography (Gouletsou *et al.*; Paltiel *et al.*) and the combination of MR-I and stereological volume estimation tool "Cavalieri method" (Coronado *et al.*, 2011; Kabay *et al*., 2009; Gocmen-Mas *et al.*, 2010) for *in vivo* testis volume measurements, and furthermore water displacement method "Archimedean principle" and individual administration of Cavalieri method (Akosman & Özdemir, 2010; I˙nce *et al.*, 2012; Dorostghoal *et al*., 2011) for in vitro testis volume measurements. The volume estimation method "Cavalieri method" (Gundersen *et al*., 1988, 1999) is unbiased and current way to estimate the volume and volume fractions of the organ (Gundersen *et al*., 1988). If we mentioned the method simply, first of all the testis has to be sliced constant and in parallel form with a random start and then surface area of the obtained slices measured by a transparent test probe consist of the constant distance of points (Evans *et al*., 2004). The morphological and histological parameters mentioned above are really

[*] Department of Anatomy, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.
[**] Department of Medical Biology and Genetics, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.
[***] Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.

important parameters especially on the determination of the future reproduction ability of the herd. In this study, we aimed to investigate and compare these parameters between two cattle breeds of almost same age and same weights. We believe that the data will contribute and be useful for the future scientific researches.

## MATERIAL AND METHOD

This study was performed on 6 Simmental bulls (1-1.5 years old, 400-350 kg) and 6 Holstein bulls (1-1.5 years old, 300-350 kg) paired testis. All testes were removed immediately from their carcass after slaughter in the abattoir.

**Tissue Preparation and Sampling Protocol**. Firstly, the epididymis were removed and leaving only the testis proper and all testes were weighted (Kern, Balingen-Germany). The testis length (from proximal to distal pole) and the width were measured by vernier caliper (Labomar, 304B-01-Turkey). The important point on the width measurements, the testicular shape will affected from the resting on a table and avoid to distortion of testicle shape all testicles leaved to the water and gently hold by two pens from both poles and measured. After these measurements, neutral buffered 10% formalin immersion fixation performed for one week to testis.

**Volume Estimation**. The testes were sliced into 1 cm intervals from a random start and the areas per fractions on the slices were measured by 1 cm point grid.

The total testis volume was calculated by the formula below:

$$V = t \times a/p \times \sum P \ cm^3$$

t; mean slice thickness (1cm),
a/p; area per point (1cm x 1cm),
$\sum P$; counted points.

The coefficient of error (CE) of the volume estimations was calculated according to Nyengaard (1999).

**Seminiferous Tubule Diameter Measurements**. The little tissue pieces were obtained from the different regions of the testes, embedded in paraffin, sliced in 6 µm and stained by Haematoxylin and Eosin. The diameter of the seminiferous tubules were measured by Imagefocus software which was running on a computer connected to a 3.2 mp Cmex camera (Euromex) attached to Olympus BH2 microscope (Orhan *et al.*, 2004; Tas *et al*., 2010; Yenilmez *et al*., 1995).

**Statistics**. All evaluated parameters in groups and between the breeds were compared using Mann-Whitney U statistical test.

## RESULTS

According to the morphological inspection the Simmental bulls were larger than the Holstein bulls even on the same age because of the Simmental cattle beef feature and their testes were in healthy condition. The mean testis weight, length and width were 316 g, 12.1 cm, 6.9 cm and 299 g, 12.1 cm, 6.8 cm for the Simmental and Holstein bulls, respectively (Table I).

Table I. The weight, length and the width of the testes.

| Simmental Bull | Testis Weight (g) | Testis Length (cm) | Testis Width (cm) | Holstein Bull | Testis Weight (g) | Testis Length (cm) | Testis Width (cm) |
|---|---|---|---|---|---|---|---|
| Right | 319 | 12.8 | 6.7 | Right | 256 | 11.5 | 6.3 |
| Right | 371 | 13 | 7.2 | Right | 323 | 12.8 | 6.9 |
| Right | 328 | 12.1 | 6.9 | Right | 331 | 12.4 | 6.9 |
| Right | 306 | 12 | 6.6 | Right | 304 | 12.3 | 6.8 |
| Right | 274 | 10.6 | 6.7 | Right | 309 | 12.9 | 6.6 |
| Right | 324 | 12 | 7.4 | Right | 297 | 12.3 | 6.7 |
| Mean | 320 | 12.1 | 6.9 | Mean | 303 | 12.4 | 6.7 |
| Left | 324 | 12.7 | 6.8 | Left | 288 | 11.3 | 6.9 |
| Left | 361 | 13 | 7.2 | Left | 247 | 11.4 | 6.3 |
| Left | 316 | 12.2 | 7 | Left | 301 | 11.6 | 7 |
| Left | 321 | 12.3 | 6.7 | Left | 307 | 11.6 | 7.2 |
| Left | 284 | 11.4 | 7 | Left | 318 | 12.7 | 7 |
| Left | 258 | 11 | 6.5 | Left | 312 | 12.7 | 6.8 |
| Mean | 311 | 12.1 | 6.9 | Mean | 296 | 11.9 | 6.9 |
| Total Mean | 316 | 12.1 | 6.9 | Total Mean | 299 | 12.1 | 6.8 |

According to stereological volume estimation the average testis volume of the Simmental bull was 295 $cm^3$ while this value for the Holstein bull was 285 $cm^3$ (Table II).

Finally, the mean diameter of the testicular tubules measured as 226.68 µm and 223.44 µm for the Simmental and Holstein bulls, respectively (Fig. 1, Table II).

There were no statistical significance found in groups and between the sexes ($p>0.05$) of the measurements of the testicular weights, lengths, widths, volumes and tubule diameters.

**Coefficient error of the study**. The CE value of the Cavalieri method was calculated as 0.02 for the Simmental bull testis and 0.02 for the Holstein bull testis.

Table II. The average volume of the testis, coefficient of error of the Cavalieri method and the results of the seminiferous tubule diameter measurements.

| Simmental Bull | Testis Volume ($cm^3$) | Coefficient of error | Tubule Diameter (µm) | Holstein | Testis Volume ($cm^3$) | Coefficient of error | Tubule Diameter (µm) |
|---|---|---|---|---|---|---|---|
| Right | 311 | 0.02 | 220.05 | Right | 286 | 0.02 | 210.65 |
| Right | 335 | 0.02 | 215.11 | Right | 304 | 0.02 | 214.10 |
| Right | 292 | 0.02 | 190.39 | Right | 297 | 0.02 | 268.32 |
| Right | 321 | 0.02 | 240.47 | Right | 248 | 0.03 | 220.99 |
| Right | 278 | 0.02 | 230.90 | Right | 301 | 0.02 | 254.20 |
| Right | 256 | 0.02 | 246.72 | Right | 307 | 0.02 | 204.25 |
| Mean | 299 | 0.02 | 223.94 | Mean | 291 | 0.02 | 228.75 |
| Left | 298 | 0.02 | 209.98 | Left | 282 | 0.02 | 263.50 |
| Left | 329 | 0.02 | 233.00 | Left | 295 | 0.02 | 173.63 |
| Left | 248 | 0.02 | 184.76 | Left | 301 | 0.02 | 215.36 |
| Left | 308 | 0.02 | 187.08 | Left | 279 | 0.02 | 210.04 |
| Left | 293 | 0.02 | 272.27 | Left | 288 | 0.02 | 215.10 |
| Left | 265 | 0.02 | 289.47 | Left | 231 | 0.02 | 231.18 |
| Mean | 290 | 0.02 | 229.43 | Mean | 279 | 0.02 | 218.14 |
| Total Mean | 295 | 0.02 | 226.68 | Total Mean | 285 | 0.02 | 223.44 |

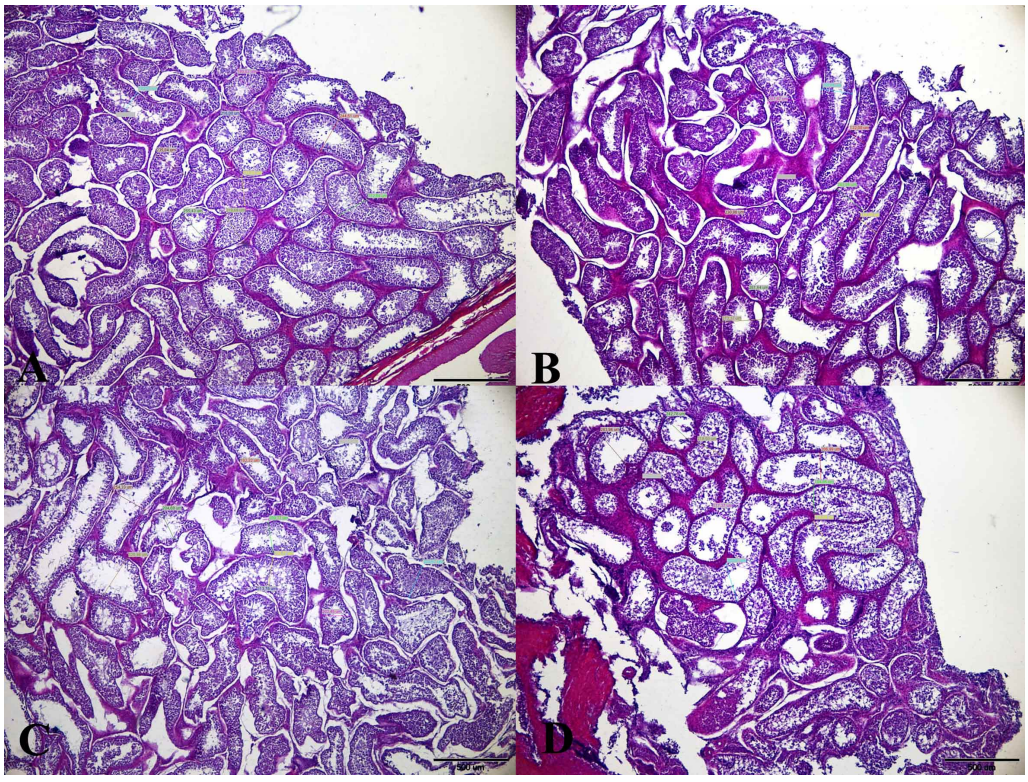


Fig. 1. A histological view of the parenchyma of a A. Holstein bulls left testis, B. Holstein bulls right testis, C. Simmental bulls left testis, D. Simmental bulls right testis.

## DISCUSSION

The *in vivo* testicular measurement could allow to estimate the spermatogenesis and testicular functions (Sakamoto *et al*.; Karaman *et al*.). The scrotal circumference, testicular volume and shape especially important for reproductive potential in young beef cattle as a selection criteria (Hahn *et al*.; Silva *et al*.). These dimensions highly correlated with the total sperm production (Hahn et al.). The measurements of these parameters could easily achieved using caliper in daily practice (Bailey *et al.*, 1998). The scientists mentioned that the ultrasound imaging also use beneficially to estimate the size and volume of the testis (Bailey *et al.*, 1998). The volume estimation technique here we applied, does not individually permit *in vivo* estimations in daily practice. However it is very successful and useful in the *in vitro* studies. It allows to estimate the total and components volume of the organ (Pazvant *et al.*, 2009). Additionally, the Cavalieri methods coefficient of error value was found so appropriate. Generally less than %5 CE is an acceptable value for a study and shows the quality of the sampling procedure (Bolat *et al*., 2011; Pazvant *et al.*).

The result of all investigated parameters include seminiferous tubules, in groups and between breeds were not found statistically important ($p>0.05$). This may depend on animals of almost same age and same weights. However, in a study on 17-22 month-old Holstein bulls, the weights of the testicles and the lengths were measured as 460 g and 14.7 cm (Hahn *et al*.). This difference may originated from the animals age. Because testicular weight increase depends to the age (Hahn *et al.*). The measured length of the seminiferous tubule between the breeds has no statistical significance (Fig. 1). The measurement of the seminiferous tubule and volume estimation of the total testis volume will be valuable especially in the toxicological research and the determination of the environmental pollution. The environmental polluters such as pesticides and heavy metals causes disorders on the reproductive activity and the impairment on the germ cell in the testis of the mammals (Bonde, 2010; Fejes et al., 1991; Meldrum & Ko, 2003; Ince *et al*.; Michael *et al.*, 2007; Bailey *et al*., 2004). In conclusion, in this research we estimated the testicular weight, length, width, volume and the diameters of the seminiferous tubules and could not detected statistically importance in groups or between sexes ($p>0.05$). This kind of measurements on the testis will be beneficial on the evaluation of the reproduction features of the herd, toxicological research and the testicular effects of the environmental polluters.

**AKOSMAN, M. S.; LENGER, O. F. & DEMIREL, H. H.** Evaluación morfológica, estereológica e histométrica de los parámetros testiculares entre toros Holstein y Simmental. ***Int. J. Morphol., 31(3)***:1076-1080, 2013.

**RESUMEN:** La medición testicular es un criterio importante para las investigaciones experimentales sobre todo en los estudios toxicológicos y predicción de la espermatogénesis. El objetivo fue estimar y comparar estos parámetros en dos diferentes tipos de razas de ganado. Las mediciones anatómicas fueron realizadas con un pie de metro, el volumen de los testículos se estimó por el método de Cavalieri y el diámetro de los túbulos seminíferos se midió en las secciones histológicas observadas al microscopio mediante software. La Media del peso testicular, longitud, ancho, volumen y diámetro de los túbulos seminíferos de los toros Simmental fueron 316 g, 12,1 cm, 6,9 cm, 295 cm3 y 226,68 µm y de los toros Holstein fueron 299 g, 12,1 cm, 6,8 cm, 285 cm3 y 223,44 µm, respectivamente. En conclusión, todos los parámetros investigados no tuvieron una importante significación en los grupos y entre las razas ($p> 0,05$). Creemos que los datos obtenidos contribuirán a la literatura y facilitar las futuras investigaciones.

**PALABRA CLAVE: Toro; Método de Cavalieri; Túbulos seminíferos; Testículo; Volumen.**

## REFERENCES

Akosman, M. S. & Özdemir, V. Capability of the Cavalieri method for volume estimation of the dog testis. *Eurasian J. Vet. Sci., 26(2)*:63-7, 2010.

Al-Damegh, M. A. Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E. *Clinics (Sao Paulo), 67(7)*:785-92, 2012.

Alpan, O. & *Arpacik, R. Cattle breeding*. 2nd ed. Ankara, Sahin, 1998.

Al Salim, A.; Murchison P. J.; Rana, A.; Elton R. A. & Hargreave, T. B. Evaluation of testicular volume by three orchidometers compared with ultrasonographic measurements. *Br. J. Urol., 76(5)*:632-5, 1995.

Bailey, T. L., Hudson, R. S.; Powe, T. A.; Riddell, M. G.; Wolfe, D. F. & Carson, R. L. Caliper and ultrasonographic measurements of bovine testicles and a mathematical formula for determining testicular volume and weight in vivo. *Theriogenology, 49(3)*:581-94, 1998.

Bailey, S. A.; Zidell, R. H. & Perry, R. W. Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? *Toxicol. Pathol., 32(4)*:448-66, 2004.

Bolat, D.; Bahar, S.; Selcuk, M. L. & Tipirdamaz, S. Morphometric investigations of fresh and fixed rabbit kidney. *Eurasian J. Vet. Sci., 27(3)*:149-154, 2011.

Bonde J. P. Male reproductive organs are at risk from environmental hazards. *Asian J. Androl., 12(2)*:152-6, 2010.

Coronado, C.; Arriagada, O. & Galdames, I. S. Easy and unbiased determination of the maxillary sinus volume. *Int. J. Morphol., 29(4)*:1375-8, 2011.

Dorostghoal, M.; Sorooshnia, F. & Zardkaf, A. Stereological analysis of wistar rat testis during early post-natal development. *Anat. Histol. Embryol., 40(2)*:89-94, 2011.

Evans, S. M.; Janson, A. M. & Nyengaard, J. R. Q*uantitative Methods in Neuroscience.* New York, Oxford University Press, 2004.

Fejes, J.; Breyl, I. & Salitrosová, H. Detection of residues of foreign substances in the tissues of cows and bulls and in bull sperm. *Vet. Med. (Praha), 36(4)*:203-11, 1991.

Gocmen-Mas, N.; Karabekir, S.; Kusbeci, O. Y.; Sahin, B.; Ertekin, T.; Bas, O.; Yazici, A. C. & Senan, S. Morphometric Analysis of Hemicerebellar Asymmetryn with Central Vertigo Cases: A Stereological Study. *Int. J. Morphol., 28(2)*:637-42, 2010.

Gouletsou, P. G.; Galatos, A. D. & Leontides, L. S. Comparison between ultrasonographic and caliper measurements of testicular volume in the dog. *Anim. Reprod. Sci., 108(1-2)*:1-12, 2008.

Gundersen, H. J.; Bendtsen, T. F.; Korbo, L.; Marcussen, N.; Moller, A.; Nielsen, K.; Nyengaard, J. R.; Pakkenberg, B.; Sorensen, F. B.; Vesterby, A. & West, M. J. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS, 96(5)*:379-94, 1988.

Gundersen, H. J.; Jensen, E. B.; Kieu, K. & Nielsen, J. The efficiency of systematic sampling in stereology--reconsidered. *J. Microsc., 193(Pt. 3)*:199-211, 1999.

Hahn, J.; Foote, R. H. & Seidel, G. E. Jr. Testicular growth and related sperm output in dairy bulls. *J. Anim. Sci., 29(1)*:41-7, 1969.

I˙nce, N. G.; Pazvant, G.; Oto, C. & Kahvecioglu, O. Stereological measurement of testicular volume in kivircik rams. *Kafkas Univ. Vet. Fak. Derg., 18(3)*:379-84, 2012.

Kabay, S.; Yucel, M.; Ozden, H.; Yaylak, F.; Ozbek O. & Gumusalan, Y. Magnetic resonance imaging is a complementary method to stereological measurement of testicular volume. *Urology, 73(5)*:1131-5, 2009.

Karaman, M. I.; Kaya, C.; Caskurlu, T.; Güney, S. & Ergenekon, E. Measurement of pediatric testicular volume with Prader orchidometer: comparison of different hands. *Pediatr. Surg. Int., 21(7)*:517-20, 2005.

Meldrum, J. B. & Ko, K. W. Effects of calcium disodium EDTA and meso-2,3-dimercaptosuccinic acid on tissue concentrations of lead for use in treatment of calves with experimentally induced lead toxicosis. *Am. J. Vet. Res., 64(6)*:672-6, 2003.

Michael, B.; Yano, B.; Sellers, R. S.; Perry, R.; Morton, D.; Roome, N.; Johnson, J. K.; Schafer, K. & Pitsch, S. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicol. Pathol., 35(5)*:742-50, 2007.

Nyengaard, J. R. Stereologic methods and their application in kidney research. *J. Am. Soc. Nephrol., 10(5)*:1100-23, 1999.

Orhan, I.; Hayit, H.; Duksal, I.; Ozercan, I. H.; Firdolas F. & Semercioz, A. Paf antagonist protective effect on ischemic damage of contralateral testes in unilateral testes torsion. *T. Urol. Dergi., 30(1)*:11-6, 2004.

Paltiel, H. J.; Diamond, D. A.; Di Canzio, J.; Zurakowski, D.; Borer, J. G. & Atala, A. Testicular volume: Comparison of orchidometer and us measurements in dogs. *Radiology, 222(1)*:114-9, 2002.

Pazvant, G.; Sahin, B.; Kahvecioglu, K.O.; Gunes, H.; Ince, N.G. & Bacinoglu, D. The volume fraction method for the evaluation of kidney: A stereological study. *Ankara Üniv. Vet. Fak. Derg., 56*:233-9, 2009.

Sakamato, H.; Saito, K.; Oohta, M.; Inoue, K.; Ogawa, Y. & Yoshida, H. Testicular volume measurement: Comparison of ultrasonography, orchidometry and water displacement. *Urology, 69(1)*:152-7, 2007.

Sakr, S. & Shalaby, S. Y. Carbendazim-induced testicular damage and oxidative stress in albino rats: Ameliorative effect of liquorice aqueous extract. *Toxicol. Ind. Health*, doi: 10.1177/0748233712456059, 2012.

Setchell, B. P. & Brooks, D. E. *Anatomy, vasculature, innervation and fluids of the male reproductive tract. In*: Knobil, E. & Neill, J. D. (Eds.). The physiology of reproduction. New York, Raven, 1988.

Silva, M. R.; Pedrosa, V. B.; Silva, J. C.; Eler, J. P.; Guimarães, J. D. & Albuquerque, L. G. Testicular traits as selection criteria for young Nellore bulls. *J. Anim. Sci., 89(7)*:2061-7, 2011.

Tas, M.; Saruhan, B. G.; Kurt, D.; Yokus, B. & Denli, M. Protective role of lycopene on aflatoxin b1 induced changes sperm characteristics and testicular damages in rats. *Kafkas Univ. Vet. Fak. Derg., 16(4)*:597-604, 2010.

Wing, T. Y. & Christensen, A. K. Morphometric studies on rat seminiferous tubules. *Am. J. Anat., 165(1)*:13-25, 1982.

Yenilmez, E.; Yamanturk, P. & Aytekin, Y. The effects of ethanol on rat testis and fertility. *Turk. Patol. Derg., 11(1)*:1-13, 1995.

Correspondence to:
Murat Sırrı Akosman
Department of Anatomy
Faculty of Veterinary Medicine
Afyon Kocatepe University
Afyonkarahisar
TURKEY

Phone: +902722281312-232

Email: akosmans@yahoo.com

Received: 24-12-2012
Accepted: 19-05-2013