Testicular morphometry and epididymal sperm qualities of donkeys in Bolgatanga, Ghana

A.J. Ayang[1], J. Atawalna[1], Banjamin O. Emikpe[1], D. Abiliba[1], E.P. Dongbataazie[1], E. Adom[3], A.D. Asare[4], M.P. Amponsah[2]

[1] School of Veterinary Medicine, KNUST, Kumasi, Ghana

[2] Veterinary Disease Investigative Laboratory, Amakom, Ghana

[3] Department of Pharmacognosy and Herbal Medicine, UDS, Ghana

[4] School of Veterinary Medicine, Legon, Accra, Ghana

SUMMARY

The application of artificial insemination in donkeys must be based on a knowledge of breeding soundness, while other reproductive physiology of the donkey including testicular morphometric parameters and sperm production is scanty in literature. This study sought to assess the testicular morphometry and epididymal sperm qualities of donkeys in Bolgatanga and to determine the effect of season on spermatogenesis and epididymal sperm parameters. Twelve sexually mature donkeys, aging 10 to 15 years old, had their testes and epididymis surgically harvested immediately after slaughter, semen parameters were assessed thereafter and testicular volume calculated. Epididymal sperm was harvested by retrograde flushing technique and sperm parameters were obtained. This study showed no significant difference (p-value >0.05) between the length, width and volume of the left and right testes, and that the rainy season showed greater values in seminiferous tubule diameter, epithelial height, and luminal diameter. The study revealed that donkeys showed a greater level of spermatogenesis in the rainy season compared to the dry season, indicating that donkeys are seasonal breeders.

Key words: Testis – Morphometry – Donkeys – Epididymal – Spermatogenesis

INTRODUCTION

Donkeys are members of the family *Equidae*, along with horses and zebras (Nasr et al., 2021). More than 40 million donkeys live in Asia, Africa, and Latin America, mostly in dry and semi-arid areas (Starkey and Starkey, 1996). Donkeys are adaptable to the temperature changes of the desert regions of Africa and Asia (Nasr et al., 2021). Globally they are essential to farmers and traders, because they can provide draught power and rural transportation at minimal cost (Starkey and Starkey, 1996). The donkey population in Ghana is estimated to be 14,500 (FAOSTAT, 2015), and they are often used for work like transportation, pack transport, or pulling carts in Northern Ghana (Chiarini-Garcia et al., 2009).

Corresponding author:
Benjamin Emikpe. School of Veterinary Medicine, KNUST, Kumasi, Ghana. E-mail: banabis2001@yahoo.com

Submitted: May 18, 2023. **Accepted:** July 18, 2023

https://doi.org/10.52083/KVOV3346

Donkey hides are a crucial component of the traditional Chinese Medicine treatment e'jiao, and manufacturers of e'jiao need four to ten million donkey skins annually (Matlhola et al., 2020). The demand for donkey hides in China has triggered a global increase in the illegal slaughter of donkeys leading to a drastic decline in the population of working donkeys in low-and middle-income countries (Zhu et al., 2017; McLean et al., 2018). Donkeys are therefore at risk of extinction; hence the need to take drastic steps towards their conservation.

The population of Donkeys can be increased using assisted reproductive techniques such as artificial insemination, which has been successfully implemented in livestock and poultry production (Hagstrom et al., 2004). The application of artificial insemination in donkeys must be based on a knowledge of breeding soundness (Atawalna et al., 2015), while other reproductive physiology of the donkey including testicular morphometry parameters and sperm production is scanty in literature. Unfortunately, the influence of season (rainy and dry) on testicular morphometry has never been reported in Donkeys in Ghana. In view of this gap, this study sought to investigate the testicular morphometry and epididymal sperm qualities of donkeys in Bolgatanga and to determine the effect of season on spermatogenesis and their overall epididymal sperm parameters.

MATERIALS AND METHODS

Study Area

The study samples were taken in the Bolgatanga Central Municipality, which is the regional capital of the Upper East region. It is located on the north-eastern corridor of Ghana between longitude 1°W and 0°E and 10°N and 11°N and covers an area of 1,509 km² (Ghana Statistical Services, 2015/2022). The municipality is bordered to the North by the Bongo District, South and East by Talensi-Nabdam District and Kassena-Nankana District to the West. Bolgatanga is about 775.4km from Accra, the capital of Ghana. It has a tropical climate with two distinct seasons – a wet season that runs from May to October and a long dry season that stretches from October to April; with hardly any rains (Ghana Meteorological Services Report, 2021).

Study Population

The study population included adult donkeys aged 10-15 years. The ages of the donkeys were estimated by comparing the appearance of their unique dentition as described by Muylle et al. (1999). A sum total of 12 donkey testicle pairs of the age ranging from 10 to 15 years old were used for this study. Six (6) donkey testicle pairs were used in the rainy season and six (6) donkey pairs were used in the dry season making a sum total of 12 donkey pairs for the study.

Study Duration

The study lasted from January to July 2022. Samples were taken in January to represent the dry season, and in July to represent the rainy season.

Sample Collection

The testes samples were obtained with the scrotum intact from apparently healthy animals immediately after slaughter. The collected samples were wrapped in plastic bags and placed on ice packs and transported to the laboratory using Coleman boxes. In the laboratory, the testes and epididymis were then dissected and separated.

For each sample, the length and width of right and left testis were measured using a measuring tape. Three measurements were taken per sample and the mean value was calculated and recorded. The volume of the testes was estimated as described by Mohammed et al. (2018) using the formula $V=0.5236LW^2$ where; V= volume of testes, L= Length of testes, W = width of testes. This procedure was repeated for the rainy season.

Histological Processing

The parenchyma of the left testis was fixed in 10% neutral buffered formalin solution for histological examination. The samples were first immersed in the fixative 10% formalin for 24-48 hours. The testicular tissue was then thoroughly washed using running tap water to remove fixative and then treated in ascending grades of ethanol concentrations

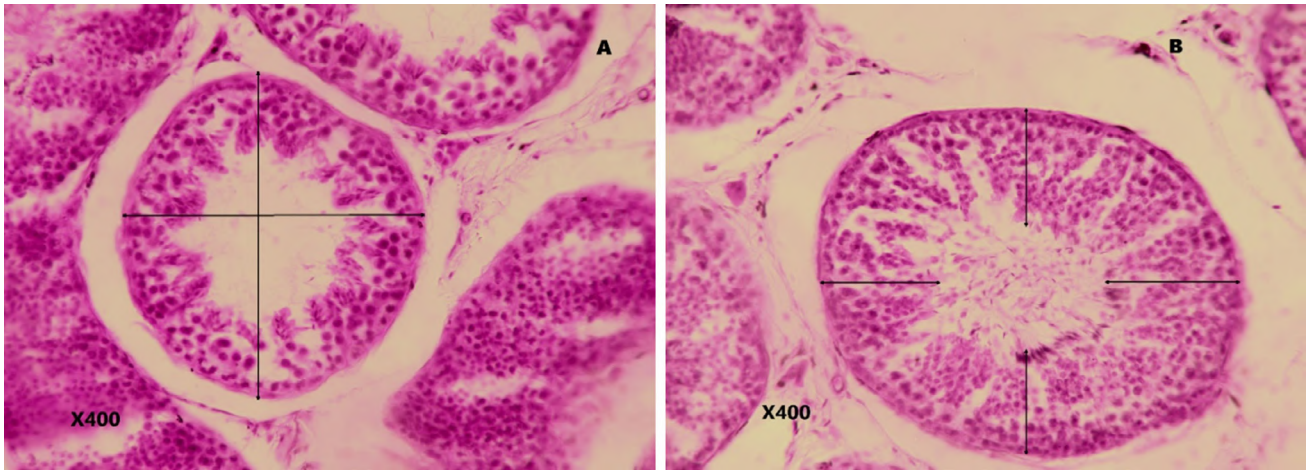

Fig. 1.- Measurement of diameter (A) and epithelial height of seminiferous tubules (B).

(70, 80, 90 and 100%), cleared in xylene to remove alcohol and to permit the fixed tissues to be miscible with paraffin wax. The tissue was then embedded in paraffin and the testicular tissue was cut 5µm thickness and stained with Haematoxylin and Eosin (H&E), as described by Burry et al., (2010).

Measurement of seminiferous tubules parameters

The diameter, height of epithelium and luminal diameter of seminiferous tubules were the histological measurements that were taken from the prepared slides. These measurements were made from randomly selected 50 round and nearly round seminiferous tubules per sample using Imaging light microscopy Amscope Software (Yalçin et al., 2020).

As is seen in Fig. 1A, for each tubule, the diameter is calculated by taking the averages of the five diameters perpendicular to each other measured in µm (Kazemi et al., 2016).

The height of the epithelium was measured by measuring the thickness of the epitheliums of at least 30 tubules from the randomly selected different areas of each sample at x40 magnification, measuring from the four sides and from the four angles as seen in Fig. 1B, and the calculation was completed by taking their averages (Kazemi et al., 2016).

The luminal diameter of the seminiferous tubules was then calculated by subtracting the epithelial heights from the diameters obtained.

Testicular Biopsy Score

Testicular Biopsy Score was performed using the Johnsens Score. This is a semi-quantitative method that was used to determine the degree of spermatogenesis. For the method, fifty (50) round and nearly round seminiferous tubules per testicular sample at x40 magnification were randomly selected and graded on a score of 1 to 10, based on the criteria in Table 1 below. The level of spermatogenesis in both the dry and rainy season was then extrapolated using the scores from the Johnsens Mean Testicular Biopsy Score (MTBS).

Table 1. Johnsens testicular biopsy score.

Score	Description
10	There is full spermatogenesis
9	Incomplete spermatogenesis with many late spermatids
8	There are less than 5 spermatozoa per tubules and a few late spermatids
7	There are many early spermatids but no spermatozoa or late spermatids.
6	There are few early spermatids but no spermatozoa or late spermatids
5	There are many spermatocytes but no spermatozoa or spermatids
4	There are few spermatocytes but no spermatozoa or spermatids
3	There are only spermatogonia
2	There are only spermatogonia
1	There is no seminiferous epithelium

Source: (Gune et al., 2019).

Table 2. Gross testicular measurements of the left and right testicles of donkeys.

Parameters	Left testis	Right testis	t- value	p-value
Length (cm)	13.65 ± 1.084	14.29 ± 1.220	1.1000	0.2897
Width (cm)	9.620 ± 0.9737	9.938 ± 0.8307	0.7016	0.4944
Volume (cm^3)	659.7 ± 159.2	748.9 ± 174.8	0.068	0.3037

Evaluation of Epididymal Sperm

The Epididymis from samples collected in July were separated from the testis in the left scrotal sac, packed in plastic bags and stored in Coleman vacuum boxes on ice packs. In the Laboratory, the epididymides were allowed to thaw and sperm retrieved from them by the aspiration flotation procedure (Cary et al., 2004; Gloria et al., 2011).

After separation of the epididymis, the vas deferens was clamped to avoid loss of semen; then some semen was aspirated from the epididymis for estimation of concentration and motility, followed by several parallel incisions made on the dissected epididymis, and suspended Phosphate Buffer Solution (PBS). This was allowed for about 15 minutes for the sperm cells to swim into the medium. The retrieved epididymal spermatozoa were evaluated for concentration, motility and morphology. Sperm concentration was determined using a hemocytometer by performing a 1:20 dilution of semen with distilled water (Cary et al., 2004). The 20-ml sample of the immobilized sperm preparation was then transferred to the counting chamber and counted using a light microscope (Sokol et al., 2000). The motility was obtained by viewing each sample under a light microscope with a stage warmer set to 37°C, then placing a drop of semen sample on a glass slide and gently covered with a cover slip. At least 2 x 100 sperm/sample were counted and rated as motile or non-motile (Merkies et al., 2000).

Sperm Morphology was determined by placing a drop of semen on a slide preheated to 40°C and mixed with one part of 5% bluish eosin solution, and four parts of 10% negrosin aqueous solution. The spermatozoa were classified as those with a cell membrane structure unstained (living) and those with a damaged membrane structure stained pink (dead) (Łacka et al., 2016).

RESULTS

Gross testicular parameters

Table 2 shows the gross testicular morphometric parameters. The results showed no significant difference (P> 0.05) between the length, width and volume of the left and right testes. However, the values for the right testes were numerically higher than those of the left testis.

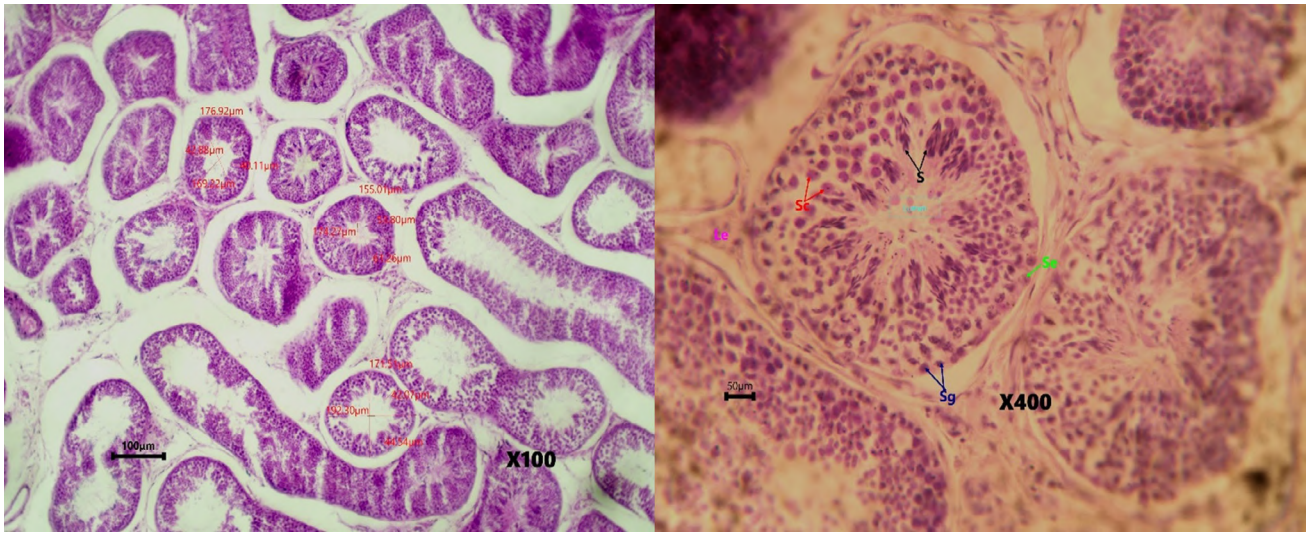

Fig. 2.- Seminiferous tubule showing spermatogenesis in the Rainy Season. Le: Leydig cells, Se: Sertoli cells, Sg: Spermatogonia, Sc: Spermatocytes, S: Spermatozoa.

Table 3. Histo-morphometric parameters of donkeys.

Parameters	Rainy season	Dry season	t-value	p-value
ST Diameter (µm)	199.5 ± 23.03	125.3 ± 3.316	6.422	0.0002*
Epithelial Height (µm)	56.57± 3.987	37 ± 2.970	5.913	0.0004*
Luminal Diameter (µm)	86.81 ± 20.65	48.98 ± 6.053	4.397	0.0023*
Johnsen's score (µm)	8.688 ± 0.3953	8.100 ± 0.5237	6.422	0.0239*

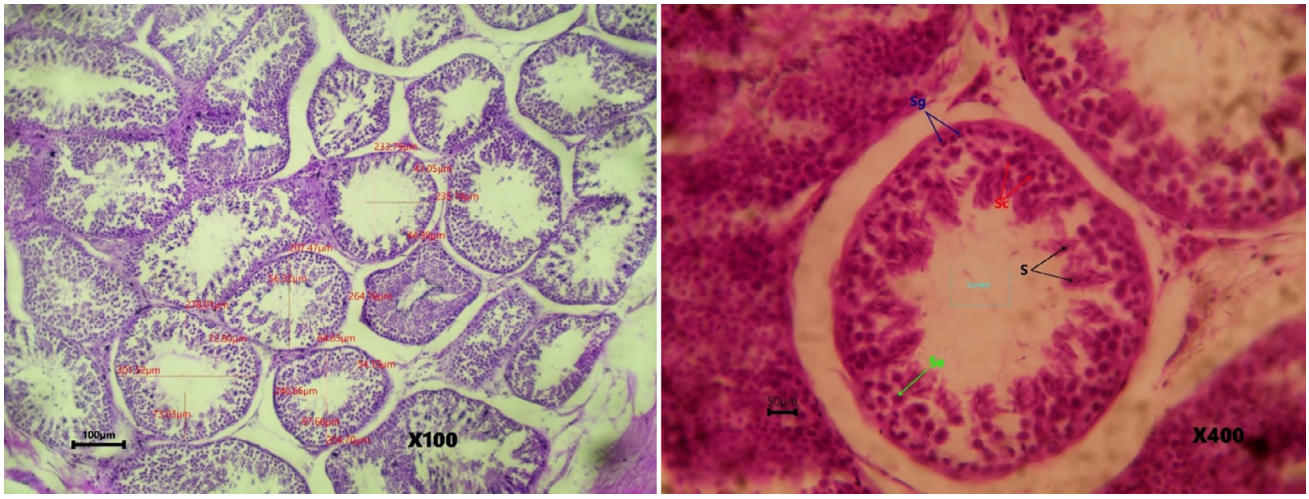

Fig. 3.- Seminiferous tubule showing spermatogenesis in the Dry Season. Le: Leydig cells, Se: Sertoli cells, Sg: Spermatogonia, Sc: Spermatocytes, S: Spermatozoa.

Histo-morphometric parameters

The results of the seminiferous tubular diameters, epithelial height and luminar diameter are shown in Table 3. The histo-morphometric parameters (the Seminiferous tubule diameter, epithelial height, luminal diameter and Johnsen's score) of the testes were significantly (P< 0.05) higher in the rainy season than in the dry season.

Testicular biopsy score

The degree of spermatogenesis in the dry and rainy seasons was assessed using the modified Johnsen's score. The results indicated the Johnsen score was significantly higher in the rainy season than in the dry season. The rainy season (Fig. 2) shows a greater level of spermatogenesis with several layers of spermatocytes than the dry season (Fig. 3) with a scanty layer of spermatocytes.

Epididymal sperm parameters

Table 4 below shows the results of the epididymal sperm parameters obtained during the study. The spermatozoa concentration ((10^6/ml), percentage (%) of motile, live normal cells, abnormal cells, and dead cells for the wet season were 272.12 ± 4.81, 88.46 ± 0.35, 63.84 ± 0.32, 20.75 ± 0.26, 15.41 ± 0.22 respectively, whilst that of

Table 4. Epididymal sperm quality parameters with respect to season.

Sperm Parameter	Rainy Season	Dry Season	p-value
Concentration (106/ml)	272.12 ± 4.81	167.68 ± 1.56	0.003*
Motility (%)	88.46 ± 0.35	54.51 ± 1.86	0.09
Live Normal Cells (%)	63.84 ± 0.32	39.34 ± 0.65	0.186
Live Abnormal (%)	20.75 ± 0.26	12.79 ± 2.34	0.845
Dead Cells (%)	15.41 ± 0.22	9.50 ± 0.71	0.078

the dry season were 167.68 ± 1.56, 54.51 ± 1.86, 39.34 ± 0.65, 12.79 ± 2.34, 9.50 ± 0.71 for spermatozoa concentration ((10^6/ml), percentage (%) of motile, live normal cells, abnormal cells, and dead cells. The epididymal sperm quality parameters were relatively higher in the wet season as compared to the dry season. Nevertheless, the differences in all the sperm quality parameters were not statistically significant ($p>0.05$) except for the sperm concentration ($p=0.003$). Sperm concentration was significantly high in the rainy season as compared to the dry season.

DISCUSSION

This current study assessed the testicular morphometry and epididymal sperm qualities of donkeys in Bolgatanga and also determined the effect of season on spermatogenesis and epididymal sperm parameters. The findings of the study revealed that the donkeys' right testes were slightly longer in length and width as compared to the left testes; however, the differences recorded were statistically insignificant (p-value > 0.05). This finding is in agreement with the findings of Contri et al. (2012), who, upon examination of the testes in light horse breeds, reported that their right testes were longer than their left testes, but the difference was not statistically significant. Carluccio et al. (2013) also found no significant differences in length and width of the testes in their study on the northeast breed of Brazilian donkeys, even though they recorded lower testicular volume compared to this study. The findings from this study, however, differs from previous research work done in Tori breed stallions by Kavak et al. (2003), where they found the left testis to be significantly larger than the right testes.

From this study, some differences existed in the histo-morphometric parameters. The measured seminiferous diameter recorded in the rainy season (199.5 ± 23.03) and dry season (125.3 ± 3.316) were lower values compared to the 222 ± 6 recorded by Contri et al. (2012). Also, the epithelial heights measured were relatively lower as compared to work done by Contri et al. (2012); however, the luminal diameters were higher in this study as compared to work done by Nipken et al. (1997). The findings indicate that there exists some sort of seasonal variation in the seminiferous tubules' diameters. This could be attributed to seasonal variations in the reproductive activity of the donkeys in the year. In addition, seminal tubule activity and sperm production increases during winter and autumn, which is equivalent to the rainy season as reported by Carluccio et al. (2013).

In this study, the mean sperm concentration recorded (272.12×10^6/mL) is in agreement with values reported in donkeys by Miragaya et al. (2018). However, the recorded motility of 88.46% in this study is lower than 90.8% observed by Gloria et al. (2011) in sexually mature Martina Franca jackasses between 5 and 6 years of age, but greater than the 82.5% recorded by Contri et al. (2012) for sexually mature Martina Franca jackasses also between 5 and 6 years of age in the winter. This could be attributed to the differences in geographical areas, as well as possible differences in the breeds and ages of donkeys used in the different studies. This study used donkeys between the ages of 10-15 years. Therefore, it could be possible that sperm production concentration and quality could have been affected by the increasing age of the donkeys. Sperm concentration was significantly ($p<0.05$) affected by the season, as the sperm concentration reduced during the dry season when compared to the rainy season. This contradicts the findings of Carluccio et al. (2013), who found out that the mean sperm concentration was significantly lower in Martina Franca jackasses in the winter period compared with the spring and the summer. This finding ascertains that donkeys are seasonal breeders.

This assertion on age affecting sperm quality has been indicated by Kidd et al. (2001), who suggested that increased male age is associated with a decline in semen volume, sperm motility, and sperm normal morphology, but not with sperm concentration. Several mechanisms have been proposed to explain how aging in males may cause changes in semen parameters. These changes can be related to seminal vesicle inadequacy, which reduces semen volume or prostate atrophy such as reduction in water and protein content, which might affect sperm motility and ejaculate volume (Kidd et al., 2001). Major chang-

es associated with age must be related to testicular degeneration (Nipken and Wrobel, 1997), which impacts spermatogenesis and may justify deterioration of seminal quality, as supported by decreased motility (Miró et al., 2005).

CONCLUSION

The season of year influenced the histo-morphometric parameters of donkeys and that the donkey is a seasonal breeder with peak spermatogenesis occurring in the rainy season. Also, the epididymal sperm parameters of donkeys are of a good quality that can be utilized in artificial insemination programs. Future research should be carried out on the use of locally available materials as semen extenders for the improvement of donkey populations.

REFERENCES

ATAWALNA J, EMIKPE BO, SALLAH EK, SHAIBU W, FOLITSE RD (2015) The health problems, gastrointestinal and blood parasites commonly associated with donkeys in the upper East Region of Ghana. *Afr J Biomed Res*, 18(1): 37-41.

CARLUCCIO A, PANZANI S, CONTRI A, BRONZO V, ROBBE D, VERONESI MC (2013) Influence of season on testicular morphometry and semen characteristics in Martina Franca jackasses. *Theriogenology*, 79(3): 502-507.

CARY JA, MADILL S, FARNSWORTH K, HAYNA JT, DUOOS L, FAHNING ML (2004) A comparison of electroejaculation and epididymal sperm collection techniques in stallions. *Can Vet J*, 45(1): 35-41.

CHIARINI-GARCIA H, ALVES-FREITAS D, BARBOSA IS, ALMEIDA FRLC (2009) Evaluation of the seminiferous epithelial cycle, spermatogonial kinetics and niche in donkeys (*Equus asinus*). *Animal Reprod Sci*, 116(1-2): 139-154.

CONTRI A, GLORIA A, ROBBE D, DE AMICIS I, CARLUCCIO A (2012) Characteristics of donkey spermatozoa along the length of the epididymis. *Theriogenology*, 77(1): 166-173.

GLORIA A, CONTRI A, DE AMICIS I, ROBBE D, CARLUCCIO A (2011) Differences between epididymal and ejaculated sperm characteristics in donkey. *Anim Reprod Sci*, 128(1-4): 117-122.

GUNE AR, PATIL AD, GUNE RP, PATIL RS (2019) Histopathological patterns of testicular biopsies in azoospermic infertile males. *Int J Health Sci Res*, 9(5): 22-27.

HAGSTROM DJ (2004) Donkeys are different: an overview of reproductive variations from horses. *University of Illinois, USA, Department of Agriculture, Local Extension Councils Cooperating*, 1-5.

KAZEMI S, FEIZI F, AGHAPOUR F, JOORSARAEE GA, MOGHADAMNIA AA (2016) Histopathology and histomorphometric investigation of bisphenol A and nonylphenol on the male rat reproductive system. *North Am J Med Sci*, 8(5): 215-221.

ŁACKA K, KONDRACKI S, IWANINA M, WYSOKIŃSKA A (2016) Assessment of stallion semen morphology using two different staining methods, microscopic techniques, and sample sizes. *J Vet Res (Poland)*, 60(1): 99-104.

MATLHOLA DM, CHEN R (2020) Telecoupling of the trade of donkey-hides between Botswana and China: challenges and opportunities. *Sustainability (Switzerland)*, 12(5): 1-10.

MCLEAN AK, NAVAS GONZALEZ FJ (2018) Can scientists influence donkey welfare? Historical perspective and a contemporary view. *J Equine Vet Sci*, 65: 25-32.

MIRAGAYA MH, NEILD DM, ALONSO AE (2018) A review of reproductive biology and biotechnologies in donkeys. *J Equine Vet Sci*, 65: 55-61.

MUYLLE S, SIMOENS P, LAUWERS H, VAN LOON G (1999) Age determination in mini-Shetland ponies and donkeys. *Zentralbl Veterinarmed A*, 46(7): 421-429.

NASR M, RASHED R, SHOGY K, HASSANIN A (2021) Morphometric and morphological characterization of the donkey testis in relation to age. *J Curr Vet Res*, 3(2): 41-54.

NIPKEN C, WROBEL KH (1997) A quantitative morphological study of age-related changes in the donkey testis in the period between puberty and senium. *Andrologia*, 29(3): 149-161.

SOKOL RZ, SHULMAN P, PAULSON RJ (2000) Comparison of two methods for the measurement of sperm concentration. *Fertil Steril*, 73(3): 591-594.

STARKEY P, STARKEY M (1996) Regional and world trends in donkey populations. *Donkeys, People and Development, July*, 10-21. http://www.atnesa.org

TÖPFER-PETERSEN E, EKHLASI-HUNDRIESER M, KIRCHHOFF C, LEEB T, SIEME H (2005) The role of stallion seminal proteins in fertilisation. *Anim Reprod Sci*, 89(1-4): 159-170.

YALÇIN T, KAYA S., KAYA TEKTEMUR N, OZAN İE (2020) The Methods Used in Histopathological Evaluation of Testis Tissues. *Batman University Journal of Life Sciences* Batman Üniversitesi Yaşam Bilimleri Dergisi, 10(1): 148.