NIGERIAN VETERINARY JOURNAL

ISSN 0331-3026

Nig. Vet. J., March 2016 **Vol. 37 (1): 1-8.**

ORIGINAL ARTICLE

Morphometric and Histological Characteristics of the Uterus of Rusa Deer (*Rusa timorensis*) during Oestrus

Mahre, M.B.[*1], Wahid, H.[2], Rosnina Y.[2], Jesse, F.F.A.[2], Jaji, A.Z.[3], Ojo, N.A.[1] and Umaru, B[1]

[1]Department of Veterinary Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria. [2]Department of Veterinary Clinical studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. [3]Department of Veterinary Preclinical studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. *Corresponding author: Email: mbmahre@gmail.com; Tel No:+2348095002402.

SUMMARY

The purpose of this study was to provide baseline data on the histological characteristics of the uterus of an uncommon tropical deer species, Rusa deer (*Rusa timorensis)* in captivity. Samples (1cm^3) of the the entire parts of the uterus (horn, body and neck) from seven (n = 7) *Rusa timorensis* hinds in oestrus (exhibiting receptivity to the stag the day before slaughter) were obtained from Universiti Putra Malaysia deer breeding unit and fixed in 10% formalin and 4% gluteraldehyde for light and scanning electron microscopy respectively. In the utero-tubal junction, simple tubular glands, similar to endometrial glands were observed. In the endometrium, ciliated cells were scattered among the secretory cells with proliferation of straight endometrial uterine glands which are lined by tall columnar cells with plasma cells at the basement membrane. These glands were extremely well developed and their histological structure indicates increased secretion preparatory to pregnancy. The study demonstrated that the Rusa deer showed increased reproductive parameters as well as parameters manifested in venison production. The microstructure of uterus of Rusa deer shows extremely positive characteristics for nidation of the egg cell and normal development of the embryo. From these investigations, it is clear that the microstructure of the uterus shows favourable conditions that allow for proper development of a healthy embryo even in captivity.

Key words: Captivity, histology, oestrus, *Rusa timorensis*, uterus.

INTRODUCTION

Rusa deer *(Rusa timorensis)* farming began in 1970 in New Zealand and in 1977 in Australia (Standing Committee on Agriculture, 1991). The form of *R. timorensis* farming is pastoral. In 2001, the population of farmed *Rusa timorensis* in Australia was 25, 000 (ANZCCART, 2001). The species is also commercially farmed in New Caledonia, Mauritius, Réunion, and Thailand (De Vos 1982) and has been farmed experimentally in Indonesia, Malaysia and Papua New Guinea (De Vos 1982; Tapari 1990; Semiadi, 2006). Farming is mainly for venison and velvet. *Rusa timorensis* is also a valued trophy animal in New Caledonia, New Guinea, New Zealand and Australia (Tapari, 1990).

The *Rusa timorensis* hind (female deer) are seasonal polyestrous breeders (Mahre *et al.,* 2012; 2013). Both the hind and the stag (male deer) attained sexual maturity at 18 months old (Food and Agriculture Organization, 1982). Under appropriate environmental conditions and proper nutrition, the major breeding activity observed in *Rusa timorensis* in Malaysia is between the months of March and July (Mahre *et al.,* 2012; 2013; 2014). Successful oestrus synchronization protocol using prostaglandin analogue ($PGF_2\alpha$) has been achieved in *Rusa timorensis* and all pregnancies were singleton (Mahre *et al.,* 2015). The reproductive cycle of *R. timorensis* (Mahre *et al.,* 2013) differs from other deer species (Asher, 2011).

Successful domestication of animals is associated with a collection of genes that confer a propensity to tameness (Clive, 2007) – a genotype that the *Rusa timorensis* perhaps share with any species that could be domesticated.

The feasibility of the propagation and genetic management of several endangered species has been demonstrated, but success relies upon understanding of both male and female reproductive biology (Abdul Hakeem *et al.*, 2012). The knowledge of the anatomy of the female reproductive system is essential for the development of a protocol for artificial insemination as well as pregnancy diagnosis and dealing with infertility problems (Jaji *et al.,* 2012). Understanding the unique histological characteristics of the *Rusa timorensis* uterus will be useful in the development of appropriate reproductive techniques in order to increase its population in captivity and also enable easy detection of its reproductive anomalies, thus strategies to propagate and conserve the species can therefore be established. The histological characteristics of the normal uterus of other wild species such as the red brocket deer, White-lipped Peccary and Arabian orynx have been well documented (Mayor *et al.,* 2009; 2011; 2012) and Abdul Hakeem *et al.,* 2012). However, little information is available on the histological characteristics of the normal uterus of *Rusa timorensis*. Therefore, the objective of this study was to observe the histological characteristics of the uterus of *Rusa timorensis* in captivity during the breeding season by means of light and scanning electron microscopy.

MATERIALS AND METHODS

Animals and Management

Description of the morphology of the uterus was performed on seven uniparous hinds, aged between four-and eight-years-old. The hinds were aged using their date of birth records. The reproductive status of all the hinds (uniparous with 2 to 4 singleton pregnancies) was obtained from the farm record. Five of the hinds were killed by wild dogs and the other two hinds were slaughtered as described by Dahlan (2009). The hinds were raised at the Universiti Putra Malaysia deer breeding unit (2.995^{o}N, 101.729^{o}E). Each hind was fed 3.4 kg of commercial hays (16% crude protein), 1.1 kg of concentrates (15% crude protein) daily and fresh water was provided *ad libitum* (Mahre *et al.,* 2016).

Examination of the gross anatomy of the uterus

The uterus was examined *in situ* and then removed. The uterine body and uterine horns from each animal were measured using a pair of calipers (Danyang Youngshun Tools Co. Ltd. China) and a long string as described by Mahre *et al.,* (2016).

Ethical Consideration

This study was undertaken with the approval of the Animal Care and Use Committee (reference number: UPM/FPV/PS/3.2.1.551/AUP-R141), Universiti Putra Malaysia.

Light Microscopy

Samples ($1cm^3$) of the the entire parts of the uterus (horn, body and neck) from seven (n = 7) *Rusa timorensis* hinds in oestrus (exhibiting receptivity to the stag the day before slaughter) were obtained according to the methods of Mahre *et al.,* (2016) from Universiti Putra Malaysia deer breeding unit and fixed in 10% formalin for light microscopy. Twenty-four hours following fixation, the tissues were washed thrice with 70% ethanol and processed automatically using the histokinette machine (Leica ASP 300 tissue processor, Germany). The tissues were then embedded in liquid paraffin and sectioned at 4µm thick using a microtome (Leica, model RM 2155 rotary microtome, Germany). Three sections from each tissue of each female were mounted on a glass slide. Sections were stained with haematoxylin and eosin mixture at a concentration of 5 g/L for the Harris haematoxylin and 10 g/L for the eosin stain. The slides were examined under a light microscope.

Scanning electron microscopy

Samples for electron microscopy were obtained from the same uterine sections of the same hinds used for light microscopy. The samples were cut into small pieces and fixed by immersion in 4% Gluteraldehyde and 0.1M sodium cacodylate for 16 hours at 4^oC and then washed in three changes of 0.1M sodium cacodylate buffer with 10 minutes interval between each wash. Samples were subsequently post fixed in aqueous osmium tetroxide for 2 hours. The samples were again washed in 3 changes of cacodylate buffer with 10 minutes interval in between wash. Dehydration in increased concentrations of acetone (35, 50, 75, 95 and 100%) was followed by critical point drying using a critical point dryer (CPD 030, Baltec, Switzerland) (Mahre *et al.,* 2014). Following mounting on aluminum stubs, the specimens were sputter-coated with 20 µm gold palladium (SEM Coating Unit ES100, Polaron, Equipment, England) and viewed under a scanning electron microscope (Leo 1455 VP, Cambridge, England).

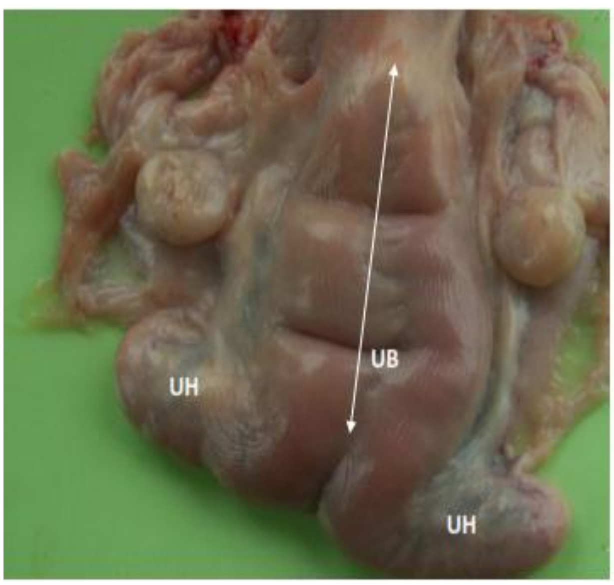

Figure 1: A non-gravid reproductive tract of *R. timorensis* hind showing the paired uterine horns (UH) and a uterine body (UB) (Adapted from Mahre *et al.,* (2016)

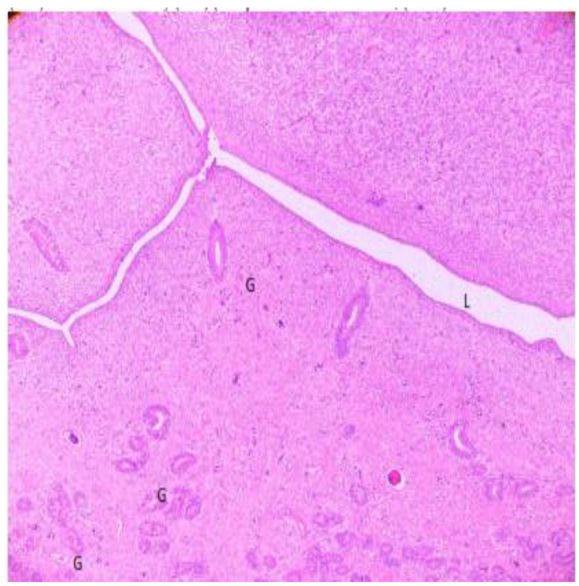

Figure 2: Photomicrograph of the uterus of *Rusa timorensis* showing the uterine lumen (L) and endometrial glands (G) (×40 Magnification)

Statistical analysis

Data were analyzed using the statistical software IBM SPSS Statistic 22. Mean and standard error of mean for each uterine body, left and right uterine horns were calculated.

RESULTS

Gross anatomy of the uterus

The uterus of the *Rusa timorensis* consisted of a small uterine body and two short uterine horns (Figure 1). The average length of the uterine body, left uterine horns and right uterine horns were 4.4 ± 0.4 cm, 11.0 ± 0.5 cm and 10.6 ± 0.7 cm respectively.

Histological characteristics of the uterus

The general view of the histological characteristics of the uterus is shown in Figure 2 and Figure 3. The entire mucosa of the lumen of the uterine horn was lined by tall columnar epithelial cells (Figure 4). In the utero-tubal junction, simple tubular glands similar to endometrial glands were observed (Figure 5). The uterine wall has a thick outer muscular myometrium and a thin inner glandular endometrium or mucosa (Figure 6). The endometrium shows proliferation of endometrial uterine glands (Figure 6). The uterine glands are not coiled but straight and are lined by tall columnar cells with plasma cells at the basement membrane (Figure 7). The endometrium consisted of two cell types: ciliated cells (Figure 8) and secretory cells (Figure 9). In the endometrium, ciliated cells were scattered among the secretory cells (Figure 9).

DISCUSSION

This study has provided baseline data on the histological characteristics of the uterus of *Rusa timorensis* which can be valuable not only in the development of appropriate reproductive techniques but also in the detection of its reproductive anomalies which will subsequently aid the conservation and propagation of this species in captivity.

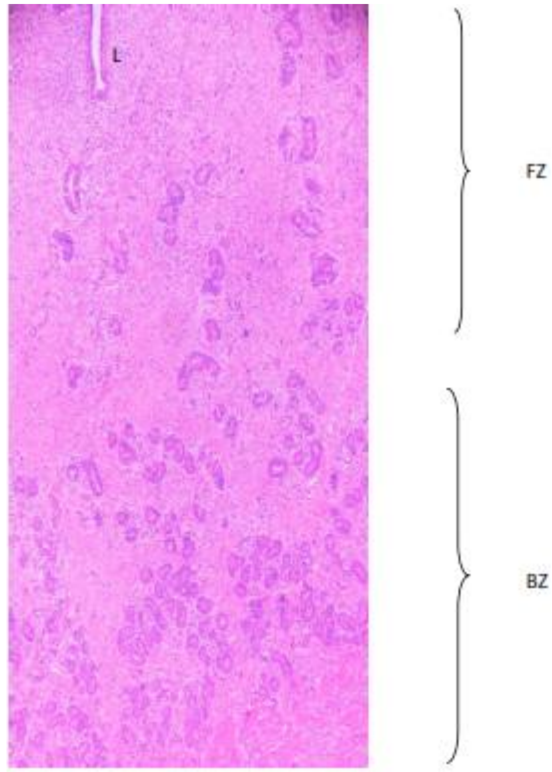

Figure 3: Photomicrograph of the uterus of *Rusa timorensis* showing the uterine lumen (L) and endometrial glands. The histology of the endometrium was grouped into basilar zone (BZ) and functional zones (FZ). The former gives rise to the later (×40 Magnification)

The general morphology of the uterus of *Rusa timorensis* was similar to that of other deer species such as the red brocket deer *(Mazama americana)*, pampas deer *(Ozotoceros bezoarticus)* and mouse deer *(Tragulus javanicus)* (Mayor *et al.*, 2012; Ungerfield *et al.*, 2008; Kimura *et al.*, 2004). However, unlike those of the red brocket deer and mouse deer, the Rusa deer uterus did not have an interconual ligament and this implies that the uterine horns are anchored in such a way that sperm deposited into only one uterine horn of the Rusa deer will be transported to the other uterine horn (Mahre *et al.,* 2016). Similarly, as in other deer species (Ungerfield *et al.*, 2008; Kimura *et al.*, 2004), the uterus of the *Rusa timorensis*

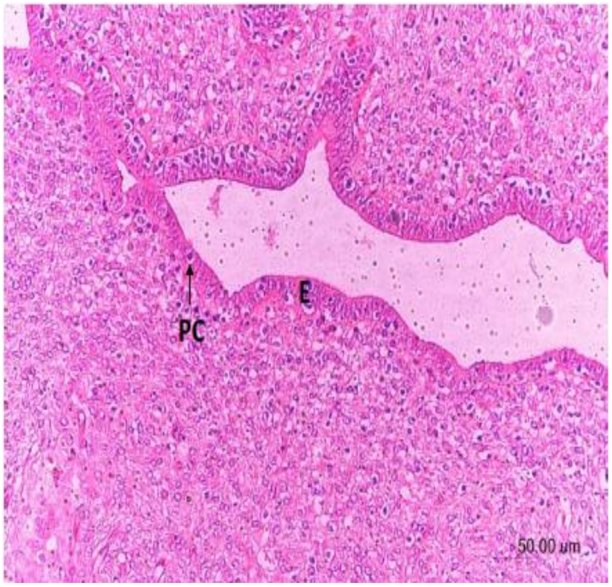

Figure 4: Photomicrograph of endometrium of Rusa timorensis. Epithelium (E) of the endometrium during breeding season; haematoxylin eosin; (400X). The uterine epithelium of *Rusa timorensis* is in the form of high prismatic cells with clearly visible oval nuclei (PC)

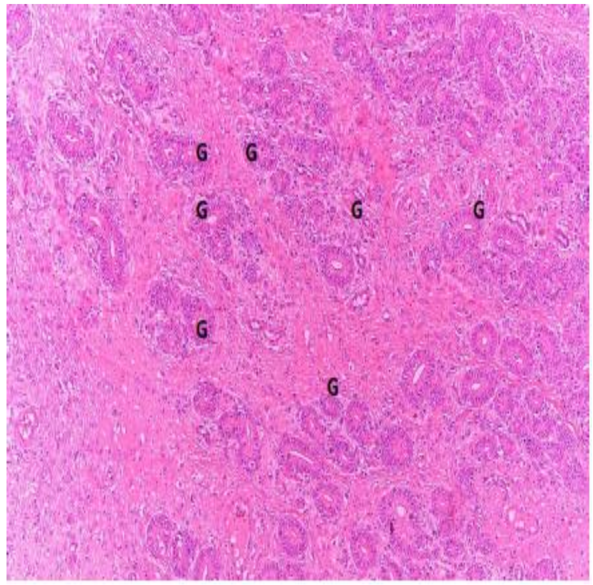

Figure 5: Photo micrograph of the utero-tubal junction of *R. timorensis* showing simple tubular glands (G) (400 X)

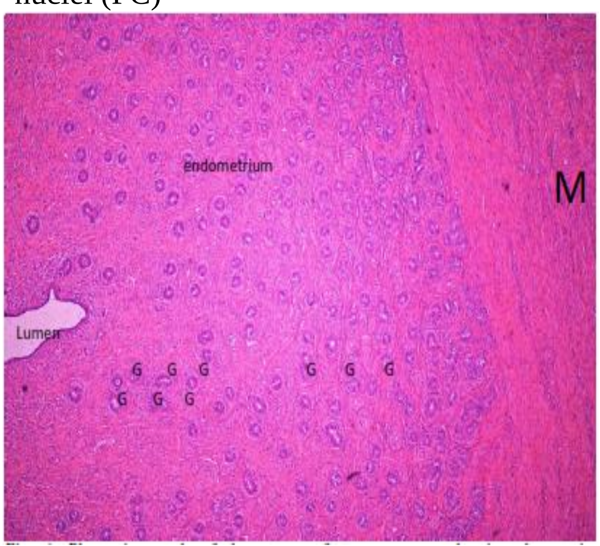

Figure 6: Photomicrograph of the uterus of *R. timorensis* showing the uterine lumen, endometrial glands (G) and myometrium (M) (40 X)

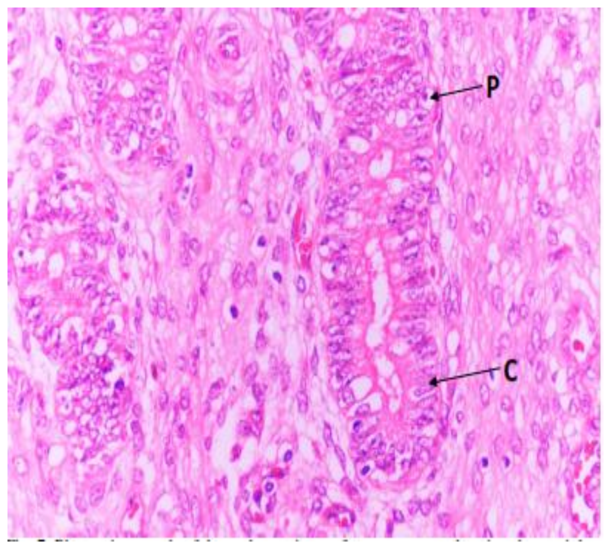

Figure 7: Photomicrograph of the endometrium of *R. timorensis* showing the straight uterine glands. The glands are lined by simple columnar epithelial cells (**C**) with plasma cells (P) at the basement membrane (600 X)

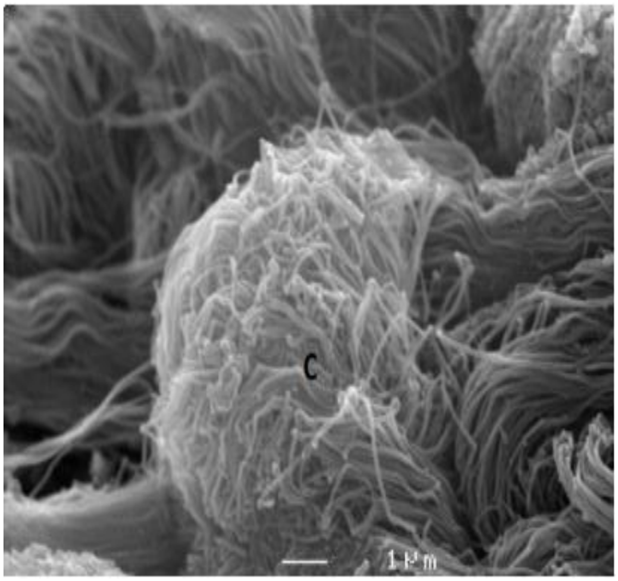

Figure 8: Scanning electron micrograph of ciliated (C) in the endometrium of *Rusa timorensis* (× 9000 magnification)

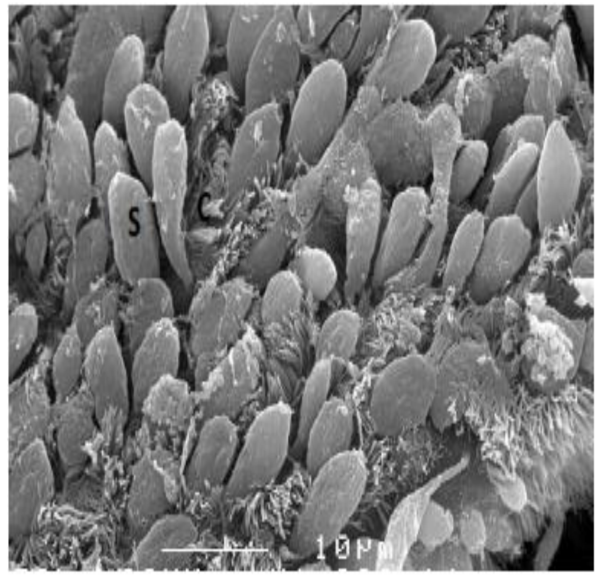

Figure 9: Scanning electron micrograph of ciliated (C) and secretory (S) cells in the endometrium of *Rusa timorensis* (× 1, 900 magnification)

consisted of a small uterine body and two short uterine horns. The uterine horns continue directly into the oviducts and its lumen was continuous cranially with the lumina of the uterine horns through two discrete openings. The lengths of the non-gravid uterus recorded in this study were higher than the values recorded by Mayor *et al.* (2012) in the red Brocket deer. The discrepancy may be due to species variation (Mahre *et al.,* 2016).

Histological structure of the uterus consisted of ciliated cells which were scattered among the secretory cells. The secretory cells provide nutrients which are essential for the development of embryos during their preimplantation stage. The directional beat of the cilia propels fluid currents toward the oviduct (Mahre , 2014).

Histological structure of the *Rusa timorensis* uterus during breeding season points to excellent characteristics for nidation of a fertilized egg cell and smooth development of the embryo. Among the dominant high prismatic cells, there are also stem basal cells and the full structure of the endometrial surface indicates excellent cell activity. These observation is in agreement with the findings of Mayor *et al.*, (2012; 2011; 2009), Abdul Hakeem *et al.,* (2012) and Intan-Shameha (2009) who also reported that breeding season in red brocket deer, White-lipped Peccary, Arabian orynx and sheep is characterised by significant cellular activities of the endometrium.

The functional zone of the endometrium contains uterine glands and contributes most of the endometrial thickness. The basilar zone attaches the endometrium to the myometrium and contains the terminal branches of the tubular glands. In the *Rusa timorensis*, the endometrial gland morphology varies throughout the oestrus cycle. The endometrial glands in the present study are not coiled but straight which is an indication that the animal is at the follicular stage of the oestrous cycle (Intan-Shameha, 2009).

The structure of the basilar zone remains relatively constant over time, but that of the functional zone undergoes cyclical changes in response to oestrogen and progesterone

(Mahre 2014). In the cycling females, probably under the influence of progesterone during the luteal phase, the endometrium transforms from a proliferative to a secretory type, showing a significant proliferation of endometrial uterine glands (Mahre, 2014). The role of progesterone is paramount to transforming the non-pregnant uterus into an enriched environment specifically suited for the developing embryo and for maintenance of the pregnant state (Spencer and Bazer, 2004).

CONCLUSION

The light and scanning electron microscopic investigations of the *Rusa timorensis* uterus during the breeding season concludes that, the microstructure of the uterus shows favourable conditions that allow for proper development of a healthy embryo. This study has also provided baseline data on the histological characteristics of the uterus which can be valuable not only in the development of appropriate reproductive techniques but also in the detection of its reproductive anomalies which will subsequently aid the conservation and propagation of this species in captivity.

ACKNOWLEDGEMENTS

This research was supported by a grant from Research University Grant Scheme (RUGS), Universiti Putra Malaysia Project 9301400. The authors are grateful for this support.

REFERENCES

ABDUL HAKEEM HE, MOHAMMAD BA, KHALEEL J, ZUHAIR A, BANI I, MUSTAFA MA, ASHRAF NE, & MAEN MA (2012). Female reproductive tract anatomy of the endangered Arabian orynx *(Orynx leucorynx)* in Jordan. *Italian Journal of Anatomy and Embryology* 117(3): 167 – 174.

ASHER GW (2011). Reproductive cycles of deer. *Animal Reproduction Science,* 124 (3 – 4):170 – 175.

ANZCCART (2001). Australian and New Zealand Council for the Care of Animals in Research and Teaching. Fact Sheets. Available at *www.adelaide.edu.au/ ANZCCART/Publications*. Accessed 20 December 2014.

CLIVE R (2007). Domestication. Greenwood Publishing Group, *Science*. p. 122 – 126.

DAHLAN I (2009). Characteristics and Cutability of Farmed Rusa Deer (Cervus timorensis) Carcasses for Marketing of Venison. *Asian-Australian Journal of Animal Science* **22** (5): 740 – 746.

DE VOS (1982). Deer Farming: Guidelines on Practical Aspects. *FAO Animal Production and Health Paper* No. 27, Rome, Italy.

FOOD AND AGRICULTURE ORGANIZATION (1982). *Animal Production and Health Paper* 27, 3-4.

INTAN - SHAMEHA AR (2009). Morphology and mucosal immunity of the oviduct and uterus during follicular and luteal phases in ewes. PhD Thesis, Universiti Putra Malaysia. p. 117.

JAJI AZ, BOYI N, GAMBO B, MAHRE MB, LUKA J, & KACHAMAI WA (2012): Pregnancy Related Biometrical Changes in the ovaries and uterus of the red Bororo Cattle in Maiduguri, Nigeria. *Nigerian Veterinary Journal* 33:3, 592 – 599.

MAHRE MB, WAHID H, ROSNINA Y, JESSE, FFA, & AZLAN CA (2012). Exfoliative vaginal cytology during the oestrous cycle of Rusa deer *(Cervus timorensis). Proceedings of the International Conference on one health and VAM*

Congress 24, 345 – 47.

MAHRE MB, WAHID H, ROSNINA Y, JESSE FFA, AZLAN CA & YAP KC (2013). Plasma Progesterone Changes and Length of Oestrous Cycle in Rusa Deer (*R. timorensis*). *Animal Reproduction Science* 141: 148 - 153.

MAHRE, MB, WAHID H, ROSNINA Y, JESSE FFA, AZLAN CA, KHUMRAN AM, AZ, JAJI (2014). Sperm attributes and morphology on *Rusa timorensis*: Light and scanning electron microscopy. *Animal Reproduction Science* 148 (2014): 245 – 250.

MAHRE MB (2014). Oestrus cycle, female reproductive system morphology and temperament and sperm attribute of Rusa deer *(Rusa timorensis)* in captivity. PhD Thesis, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

MAHRE MB, WAHID H, ROSNINA Y, JESSE FFA (2015). Estrus Response and Pregnancy Rate of *Rusa timorensis* Following Estrus Synchronization with a Prostaglandin Analogue. *Malaysian Journal of Animal Science* 18 (1), 45 – 53.

MAHRE MB, WAHID H, ROSNINA Y, JESSE FFA, JAJI AZ, OJO NA, UMARU B, AZMI, TI (2016). Anatomy of the Female Reproductive System of Rusa deer *(Rusa timorensis). Sokoto Journal of Veterinary Sciences,* 14 (1): 15 – 20.

MAYOR P, BODMER E, SCHETTINI L, MARIN O & LO´ PEZ-BE´JAR M (2009). Anatomicohistological characteristics of the female white-lipped Peccary *(Tayassu pecari)* in the Peruvian Amazon. *Anatomia, Histologia & Embryologia* 38: 467–474.

MAYOR P, BODMER E, LO´ PEZ-BE´JAR M & LO´ PEZ-PLANA C (2011). Reproductive biology of the wild red brocket deer *(Mazama americana)* female in the Peruvian Amazon. *Animal Reproduction Science* 128: 123–128.

MAYOR P, LŌPEZ – PLANA C &LŌPEZ – BĒJAR M (2012). Anatomicohistological characteristics of the tubular genital organs of the female Red Brocket deer *(Mazama americana)* in the Peruvian Amazon. *Anatomia, Histologia, Embryologia,* 41: 436 - 444.

SEMIADI G (2006). Biologi rusa tropis [The biology of tropical deer*]. Puslit Biologi LIPI Press.*

SPENCER TE & BAZER FW (2004). Conceptus signals for establishment and maintenance of pregnancy. *Reproductive Biology and Endocrinology*. 2: 49.

STANDING COMMITTEE ON AGRICULTURE (1991). Farming deer. Animal Health Committee, CSIRO Australia.

TAPARI B (1990). A reappraisal of the development of the Rusa deer resource at Bensbach, Western Province: success and failures after 25 Years. *Yagl-Ambu* 15(4): 32-42.

UNGERFELD R, GONZĀLEZ-PENSADO S, BIELLI A, VILLAGRĀN OLAZABAL D & PE´REZ W (2008). Reproductive biology of the pampas deer *(Ozotoceros bezoarticus)*: a review. *Acta Veterinaria Scandinavica* **50 (1)**:16.