Morphological and Structural Investigations of Egyptian Water Buffalo (*Bubalus Bubalis*) Sertoli Cells

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Abstract: Buffaloes are essential part of the economy in many countries and provide sustainable food in addition to being working animals. Inefficiency in reproduction has become problematic in recent years due to a number of factors and although much research concentrates on the female, very little is known about the male buffalo reproductive system. To address this deficiency in the literature, testes were obtained from 20 clinically healthy water buffalo (*Bubalus Bubalis*) bulls aged 3 years old. Scanning electron microscopy showed that the Sertoli cells were columnar to triangle shaped with many processes. In the middle portion of the seminiferous tubules, the Sertoli cell had two types of processes with sheet like and slender cord like appearances. The sheet like processes had simple smooth margins originating from Sertoli cells, surrounding the surfaces of spermatogonia and spermatocytes. The slender cord like processes formed networks around other spermatogenic cells. Transmission electron microscopy showed that the Sertoli cells contained a large irregular shaped nucleus with deep nuclear membrane indentations, few mitochondria, aggregates of ribosomes and few rough endoplasmic reticulum which were observed within the indentations. Each nucleus contained a multivesicular nuclear body, containing vesicles, tubules and ribosome like dense structures. The work herein describes the structure and location of key reproductive cells within the water buffalo. Understanding the features of the male reproductive system is essential in order to advance studies into the reproductive decline of this species and the Bovidae family.

Keywords: Egypt, Testis, Buffalo, *Bubalus Bubalis,* morphology, Sertoli Cells.

INTRODUCTION

The water buffalo (*Bubalus Bubalis*) plays an important role in the economics and food supply of many countries. These include both tropical and subtropical regions including the Mediterranean, Australia, Indian sub-continent, South-East Asia, China, Africa, South and Central America, and numbers are significantly increasing in Europe too. The world population was estimated at 199 million animals in 2012 [1]. There are two distinct types of water buffalo which are classified as either swamp or river buffaloes. The river buffalo has many uses but is mainly used in the dairy industry in comparison to the swamp buffalo which is more commonly used for meat and as a working animal [2]. The areas in which the two types are distributed also differ slightly as the river buffalo is more commonly situated in the Caribbean, Africa, Indian sub-continent, Mediterranean regions, and South America, in comparison to the swamp buffalo which is predominantly found in South-East Asia and China [3]. The reproductive abilities of the water buffalo have become increasingly important, and a point of concern, due to its many roles throughout the world and its economic importance. The water buffalo has become less efficient reproductively due to a number of reasons. Late onset puberty, lower numbers of ovarian follicles, ovarian quiescence, silent heat/silent estrous, variable ovulation timings, seasonal breeding, long postpartum anestrus and low conception rates [4-6] have all been highlighted as potential aggravating factors. Whilst optimised nutritional regimes and management regimes have helped fertility levels [7,8], pregnancy rates are routinely observed throughout the world at just 30-50% [1].

While much scientific research has concentrated on the reproductive system and potential problems in the female, the male has received little attention in this regard. Studies carried out in 1990 and 1992 highlighted some of the features of the testis using transmission electron microscopy in buffalo from Japan and Malaysia [7,9] but studies using scanning electron microscopy have not been carried out and fertility has rapidly declined since these studies 27 and 25 years ago. Artificial insemination techniques have been used in an effort to increase fertility but despite advances in technology and increases in efficiency over the last few years, cryopreserved semen remains less fertile [10-12].

Sertoli cells are known as 'nurse-like' cells as they aid with structural support and sustain nutrition to

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developing germ cells [13,14]. Additionally, Sertoli cells form the blood–testis barrier (BTB), which divides the seminiferous epithelium into basal and adluminal compartments [15-17]. Morphologically, Sertoli cells are columnar-shaped, non-spermatogenic cells that extend centripetally from the basal lamina towards the lumen of the seminiferous tubule. The lateral contour of the cell carries numerous cellular processes and multiform recesses that trap germ cells either partially or completely inside [18-22]. Sertoli cells also contain long and thin mitochondria, lipofuscin and lipid droplets at the base of their cytoplasm [23,24].

The present study provides essential morphological information at the ultrastructural level in order to further understand the reproductive system of the male water buffalo. This data is essential in order to start elucidating whether the reproductive efficiencies of the species could be monitored or enhanced and is an important addition to the research within the Bovidae family.

MATERIALS AND METHODS

The specimens were collected from Itay El-Broud slaughterhouse in the El-Behera province. Institutional ethical permission was granted by both Alexandria University and The University of Nottingham (non-ASPA, ethical review number 1911 161206) and national and government ethics were adhered to. Specimens were grossly examined for any pathological abnormalities and samples were only collected from healthy males where abnormalities were not observed resulting in testes from 10 Egyptian water buffalos (*Bubalus Bubalis*) aged three years old from 20 originally sampled. The testes were dissected free from the surrounding tissues immediately following slaughter, ensuring that the same portion was collected from each animal; the middle portion.

Scanning electron microscopy (SEM) specimens (n=10) were immediately immersed in a fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2) at 4°C for 6 hrs. Once fixed, the samples were washed in 0.1 M sodium cacodylate containing 5% sucrose, processed through tannic acid, and finally dehydrated in a series of increasing concentrations of ethanol. The samples were then critical-point dried in carbon di-oxide, fixed on stubs with colloidal carbon and coated with gold palladium in a sputtering device. Finally, specimens were examined and photographed with a Jeol scanning electron microscope operating at 15 kilovolts (work carried out at the EM unit, Faculty of science, Alexandria University).

For transmission electron microscopy (TEM) 1 mm thick tissue samples were extracted from each testis (n=10), fixed in 6% phosphate buffered glutaraldehyde solution (pH 7.4) for 6 hrs at 4ºC [25]. Following initial fixation, the tissues were washed in several changes of cold (4ºC) 0.1 M phosphate buffer solution every 15 minutes for 2 hrs. The tissues were then post fixed in1% solution of osmium tetroxide in cold (4ºC) 0.1 M buffer (pH 7.2) for 2 hrs, rapidly dehydrated through ascending grades of ethyl alcohol, transferred to propylene oxide and placed in a 1:1 mixture of propylene oxide and epoxy araldite [26]. Semi-thin serial sections (1 µm) were stained with toludine blue and under visualised under a light microscope in order to select areas suitable for electron microscopy examination. Ultra thin sections (60-100 nm) were cut using a glass knife and microtome and stained with uranylacetate followed by lead citrate [26]. These sections were examined with Joel 100 cx transmission electron microscope operating at 100 kilovolts.

RESULTS

Sertoli cells of Egyptian water buffalo were tall columnar cells extending from the basement membrane to the lumen of the seminiferous tubules surrounding the other spermatogenic cells (Figure **1**).

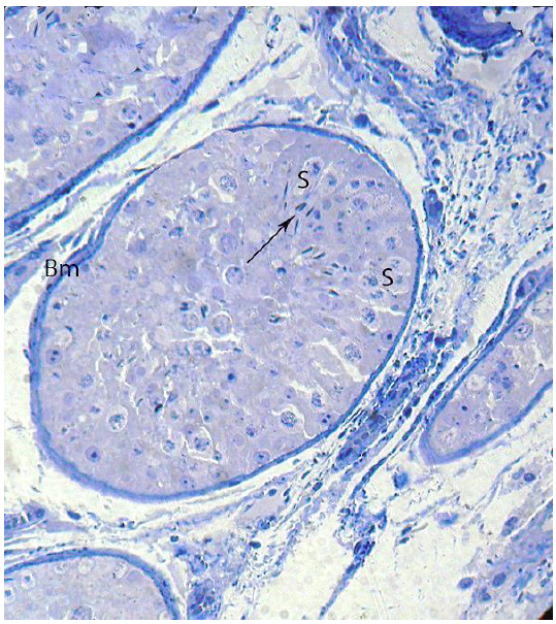

Figure 1: Light photomicrograph of toluidine blue stained Egyptian water buffalo seminiferous tubule showing Sertoli cells (S), basement membrane (Bm) and spermatids (example depicted by arrow). Mag x400.

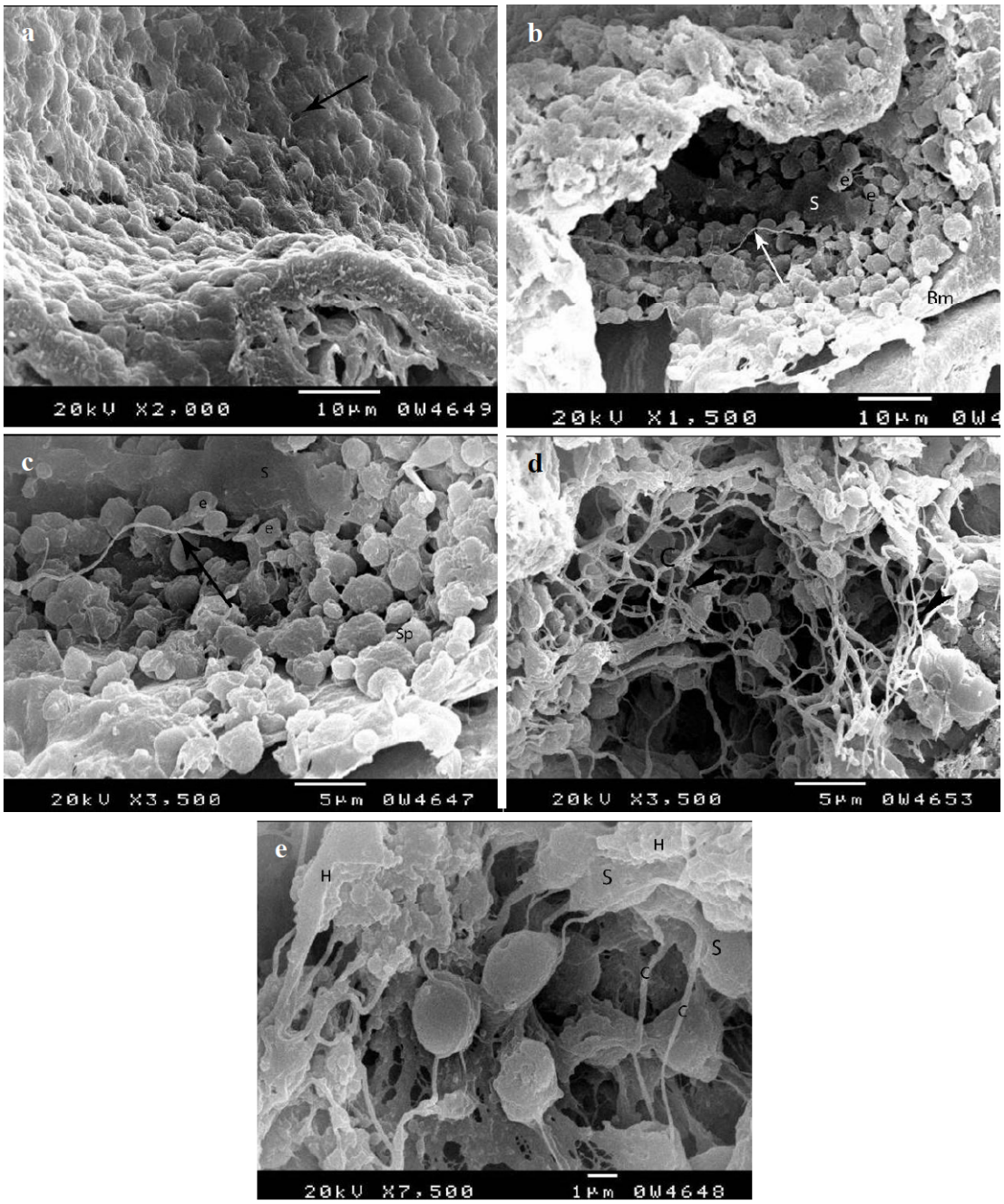

Figure 2: Scanning electron photomicrographs of the Egyptian water buffalo testis. **a**) Basal surface of seminiferous tubules showing polygonal compartment of basement membrane with a 'tiled floor' appearance and pits (arrow). **b**) Interior of seminiferous tubules depicting processes of Sertoli cells (arrow). **c**) Higher magnification of (**b**) depicting Sertoli cell processes (arrow). **d**) Middle portion of seminiferous tubules showing cylinder like process forming a network (marked by arrow head). **e**) Higher magnification photomicrograph of the middle portion of seminiferous tubules. Letters indicate Sertoli cells (S), early spermatids (**e**), basement membrane (Bm), spermatogenic cells (Sp), cylinder like process (C) and sheet like processes (H).

SEM showed that the basal surface of the basement membrane appeared either smooth or had a slightly undulated appearance. There were many polygonal compartments on the basement membrane arranged in close proximity to each other with an appearance similar to a tiled floor (Figure **2a**). Around the seminiferous epithelium, small pits were seen on the basal surface of basement membrane (Figure **2a**). The spermatogenic cells were situated in compartments enclosed by adjacent Sertoli cells, on occasion Sertoli cells were obscured by the other spermatogenic cells. The Sertoli cells were columnar to triangle shaped with many visible processes (Figures **2b+c**). In the middle portion of the seminiferous tubules, the Sertoli cells had two types of processes, sheet like and slender cord like processes (Figures **2d+e**). The sheet like processes

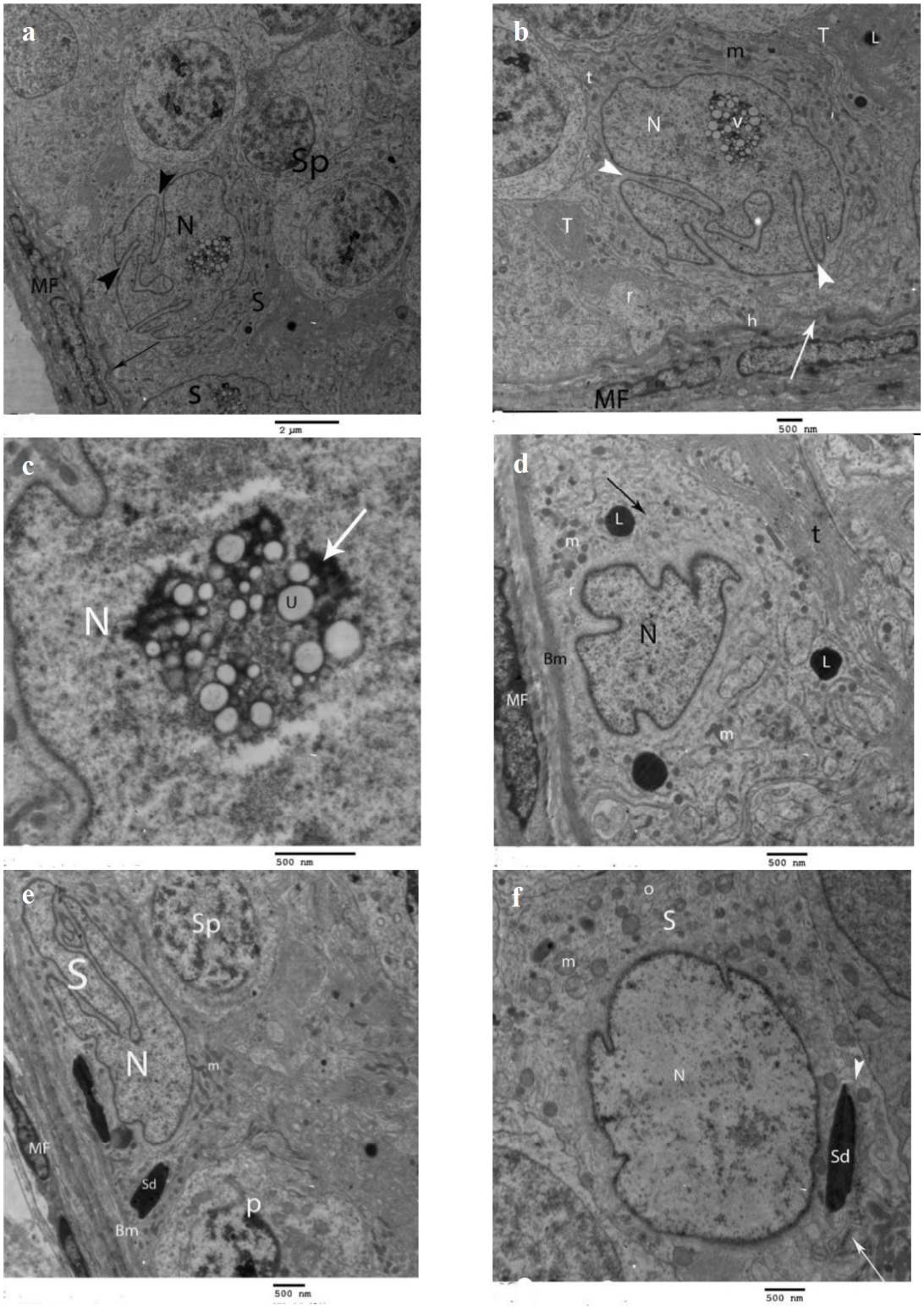

Figure 3: Transmission electron photomicrographs showing the morphology of the Egyptian water buffalo testis. **a**) Seminiferous tubules showing an indentation (arrow head) and a basement membrane (arrow) Mag x1000. **b**) Higher magnification of seminiferous tubule shown in (**a**) highlighting indentations (arrow head) containing free ribosomes and rough endoplasmic reticulum (astrik) and showing a basement membrane (arrow) Mag x1500. **c**) Sertoli cell morphology showing vesicles containing flocculent material (U) Mag x5000. **d**) Sertoli cell showing lamellated (t) and sporadic (arrow) smooth endoplasmic reticulum Mag x2500. **e**) Sertoli cell with neighbouringspermatogenic cell nucleus (N) and adjacent spermatogonia (P) Mag x2000. **f**) Spermatid (Sd) showing sheath (arrow head) and dense fiber (arrow) Mag x2500. Unless otherwise stated, letter indicate the following structures: Sertoli cell (S), spermatogenic cell (Sp), Sertoli cell nucleus (N), myofibroblast (MF), mitochondria (m), lamellated (T) and sporadic (t) smooth endoplasmic endoplasmic reticulum, multivesicular bodies (v), lysosomes (L), hemidesmosomes (h), ribosomes(r) and basement membrane (Bm).

had simple smooth margins originating from Sertoli cells, surrounding the surfaces of spermatogonia and spermatocytes. The slender cord like processes were observed in the basal and middle portions of seminiferous tubules and formed networks around other spermatogenic cells (Figure **2d**).

TEM imaging of the samples showed that the buffalo Sertoli cells contained a large irregular shaped nucleus with deep nuclear membrane indentations. Few mitochondria, aggregates of ribosomes and few rough endoplasmic reticulum were seen within the membranous indentations (Figures **3a+b**). Each nucleus contained a multivesicular nuclear body, which consisted of not only numerous vesicles, but also tubules and ribosome like dense structures. The vesicles had flocculent substance appearance and varied in size and number (Figure **3c**). The cytoplasm of the Sertoli cells had numerous mitochondria ranging from round through to elongated shapes, and also contained ribosomes and lysosomes. Smooth endoplasmic reticulum existed in two forms, sporadic and laminated which included several layers and occupied a wide area within the cytoplasm (Figures **3c+d**). Spermatids were seen in the cytoplasm of Sertoli cells, occasionally these were surrounded by a sheath and dense fibre was surrounded by mitochondrial spermatid (Figures **3e+f**). There were no clear junctions between each spermatid and the Sertoli cell. Myofibroblasts were seen around basement membranes of seminiferous tubules, and appeared elongated through to ovoid in shape and presented with a contracted nucleus each (Figures **3a+b**).

DISCUSSION AND CONCLUSIONS

The present study showed that by SEM the basement membrane of the Egyptian water buffalo (*Bubalus Bubalis*) testis had polygonal compartment arranged in a compact manner, rather like a tiled floor, around seminiferous epithelium. It is proposed that this membrane plays an important role in ensuring fluid regulations from the intertubular medium toward the lumen of seminiferous tubules [27]. The Sertoli cells of Egyptian water buffalo in this study had two types of processes, sheet like and slender cord like processes, similar results were found in the Korean Native goat [28]. Research in other species (canine and rat) has shown that these slender cord like processes participate in disengagement of the residual body from the head of the spermatid just before spermiations [29,30]. This is the first time that the water buffalo testis has been examined using SEM, this therefore gives additional insight into the structure and morphology of the male reproductive system.

Transmission electron microscope investigations in this study revealed that the Sertoli cell nucleus contained a multivesicular nuclear body (MNB), which itself contained tubules, ribosome like dense structures and a number of vesicles. The ruminant Sertoli cells differed from other species by presence of these atypical MNB structures [31-37]. It has been suggested that MNBs are similar to the nucleolar channel system found in the human endometrial glandular cell as they have a similar morphology [22, 31, 38, 39]. The nucleolar channel system periodically occurred and disappeared during the menstrual cycle, whereas the MNB continued was in situ in both the fetus and the adult. It has been suggested that the MNB might be able to communicate between the cytoplasm and perinuclear space [40]. It has also been hypothesised that the vesicles in MNB assist in the exchange of materials between the nucleus and cytoplasm [32]. We found that the vesicles of MNBs contained a flocculent material, which was surrounded by electron dense aggregates of ribosome-like particles, these structures were often associated with the nuclear pores and cytoplasm suggesting that they may be involved in the synthesis and transport of substances vital to the differentiation of germ cells [9]. Our study also revealed that there were numerous smooth endoplasmic reticulum existing in two forms, sporadic and laminated, this is further evidence that lipid and/or steroid metabolism are assisted by these cells [24].

Understanding the basic structure and ultrastructure of the water buffalo (*Bubalus Bubalis*) testis is an essential step towards understanding the fertility problems in this species and undertake comparative analysis with other mammals. Little is known about the male reproductive capacity and further studies can concentrate on whether the male buffalo structure and function are being affected and therefore adding to the decline in reproduction rates observed in this economically important animal. Advances in comprehension of the basic biology, structure and function of both the male and female assist with developing newer technologies that may help to overcome the present fertility crisis observed in the buffalo. Making advances in technologies such as progeny testing, sire evaluation, *in vitro* embryo production and artificial insemination can all be tailored in order to increase fertility rates worldwide.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. All authors contributed toward analysis and writing the manuscript. AD, KM and SAAE undertook sample collection, staining and microscopy.

REFERENCES

[1] Warriach HM, McGill DM, Bush RD, Wynn PC, Chohan KR. A review of recent developments in buffalo reproduction - a review. Asian-Australasian Journal of Animal Sciences 2015; 28(3): 451-5. https://doi.org/10.5713/ajas.14.0259

[2] Bongso TA, Hilmi M, Basrur PK. Testicular cells in hybrid water buffaloes (*Bubalus bubalis*). Research in Veterinary Science 1983; 35(3): 253-8.

[3] Kumar S, Nagarajan M, Sandhu JS, Kumar N, Behl V. Phylogeography and domestication of Indian river buffalo. BMC Evolutionary Biology 2007; 7: 186. https://doi.org/10.1186/1471-2148-7-186

[4] Luktuke SN, Ahuja LD. Studies on Ovulation in Buffaloes. J Reprod Fertil 1961; 2(2): 200-201.

[5] Nam NH. Characteristics of reproduction of the water buffalo and techniques used to improve their reproductive performance. J Sci Dev 2010; 8(1): 100-10.

[6] Gordon I. Controlled reproduction in cattle and buffaloes. UK: CABI 1996.

[7] Qureshi MS, Khan S, Ahmad N. Pregnancy depresses milk yield in Dairy Buffaloes. Ital J Anim Sci 2007; 6: 1290-3. https://doi.org/10.4081/ijas.2007.s2.1290

[8] Usmani RH, Dailey RA, Inskeep EK. Effects of limited suckling and varying prepartum nutrition on postpartum reproductive traits of milked buffaloes. Journal of Dairy Science 1990; 73(6): 1564-70. https://doi.org/10.3168/jds.S0022-0302(90)78826-1

[9] Azmi TI, Bongso TA, Harisah M, Basrur PK. The Sertoli cell of the water buffalo--an electron microscopic study. Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire 1990; 54(1): 93-8.

[10] Neglia G, Gasparrini B, Di Palo R, De Rosa C, Zicarelli L, Campanile G. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean Buffalo cows. Theriogenology 2003; 60(1): 125-33. https://doi.org/10.1016/S0093-691X(02)01328-6

[11] Rossi P, Vecchio D, Neglia G, Di Palo R, Gasparrini B, D'Occhio MJ, *et al*. Seasonal fluctuations in the response of Italian Mediterranean buffaloes to synchronization of ovulation and timed artificial insemination. Theriogenology 2014; 82(1): 132-7. https://doi.org/10.1016/j.theriogenology.2014.03.005

[12] Kumaresan A, Ansari MR, Garg A. Modulation of post-thaw sperm functions with oviductal proteins in buffaloes. Anim Reprod Sci 2005; 90(1-2): 73-84. https://doi.org/10.1016/j.anireprosci.2005.01.009

[13] Bardin CW, Cheng CY, Musto NA, Gunsalus GL. The Sertoli cell. In: Knobil E, Neill JD, Ewing LL, Greenwald GS, Markert CL, Pfaff DW, editors. The Physiology of Reproduction. New York, NY, USA. Raven Press 1988; pp. 933-74.

[14] Griswold MD, McLean JD. The Sertoli cell. In: Neill JD, Editors. Physiology of Reproduction. New York, NY, USA. Elsevier 2006; pp. 949-75. https://doi.org/10.1016/b978-012515400-0/50024-5

[15] Dym M, Fawcett DW. The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. Biology of Reproduction 1970; 3(3): 308-26.

[16] Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocrine Reviews 2004; 25(5): 747-806. https://doi.org/10.1210/er.2003-0022

[17] Hedger MP, Hales DB. Immunophysiology of the male reproductive tract. In: Neill JD, Editor. Physiologyof Reproduction. New York, NY, USA. Elsevier 2006; pp. 1195-286. https://doi.org/10.1016/b978-012515400-0/50030-0

[18] Russell L. Observations on Rat Sertoli Ectoplasmic (Junctional) Specializations in Their Association with Germ-Cells of Rat Testis. Tissue Cell 1977; 9(3): 475-98. https://doi.org/10.1016/0040-8166(77)90007-6

[19] Weber JE, Russell LD, Wong V, Peterson RN. 3-Dimensional Reconstruction of a Rat Stage V Sertoli-Cell .2. Morphometry of Sertoli Sertoli and Sertoli Germ-Cell Relationships. Am J Anat 1983; 167(2): 163-79. https://doi.org/10.1002/aja.1001670203

[20] Russell LD, Tallondoran M, Weber JE, Wong V, Peterson RN. 3-Dimensional Reconstruction of a Rat Stage-V Sertoli-Cell .3. A Study of Specific Cellular Relationships. Am J Anat 1983; 167(2): 181-92. https://doi.org/10.1002/aja.1001670204

[21] Vogl AW, Soucy LJ, Foo V. Ultrastructure of Sertoli-Cell Penetrating Processes Found in Germ-Cells of the Golden-Mantled Ground-Squirrel (Spermophilus-Lateralis). Am J Anat 1985; 172(1): 75-86. https://doi.org/10.1002/aja.1001720106

[22] Fawcett D. Ultrastructure and function of the Sertoli cell. Male reproductive system. In: Hamilton DW, Greepa RO, Editors. Handbook of Physiology. Washington, DC, USA. American Physiology Society 1975; pp. 21-55.

[23] Johnson L. Spermatogenesis. In: Cupps PT, Editor. Reproduction in Domestic Animals. 4th ed. San Diego, CA, USA. AcademicPress Inc. 1991; pp. 173-219. https://doi.org/10.1016/b978-0-08-057109-6.50010-0

[24] Russell LD. Form, dimensions, and cytology of mammalian Sertoli cells. In: Russell LD, Griswold MD, Editors. The Sertoli Cell. 1st ed. Clearwater, FL, USA. Cache River Press 1993.

[25] McDowell AM, Trump F. Histologic fixatives suitable for diagnostic light and electron microscopy. Archives of Pathology and Laboratory Medicine 1976; 100: 405-515.

[26] Hayat M. Basic Techniques for Transmission Electron Microscope. 2nd ed. Baltimore, USA. Academic Press 1986.

[27] Evan AP, Dail WG, Dammrose D, Palmer C. Scanning electron microscopy of cell surfaces following removal of extracellular material. The Anatomical Record 1976; 185(4): 433-45. https://doi.org/10.1002/ar.1091850405

[28] Kwon GO, Park YS, Lee SH, Yamamoto E, Hosaka Y, Ueda H, *et al*. Ultrastructure and function of Sertoli cell processes

in the Korean native goat. Okajimas Folia Anatomica JAPONICA 2002; 79(4): 121-7.
https://doi.org/10.2535/ofaj.79.121

[29] Hamasaki M, Murakami M. Three-dimensional profiles of Sertoli cell processes and associated appendices as viewed by SEM. Journal of Electron Microscopy 1986; 35(2): 132-43.

[30] Park YS, Abe M, Takehana K, Iwasa K. Three-dimensional structure of dog Sertoli cells: a computer-aided reconstruction from serial semi-thin sections. Archives of Histology and Cytology 1993; 56(1): 65-73.
https://doi.org/10.1679/aohc.56.65

[31] Terzakis JA. The nucleolar channel system of human endometrium. The Journal of Cell Biology 1965; 27(2): 293-304.
https://doi.org/10.1083/jcb.27.2.293

[32] Zibrin M. Multivesicular Nuclear Body with Nucleolar Activity in Sertoli Cells of Bulls - Ultrastructural Study. Z Zellforsch Mik Ana 1972; 135(2): 155-64.
https://doi.org/10.1007/BF00315123

[33] Osman DI, Ploen L. Fine-Structure of Sertoli Cells in the Camel (Camelus-Dromedarius). Anim Reprod Sci 1986; 10(1): 37-46.
https://doi.org/10.1016/0378-4320(86)90138-7

[34] Curtis SK, Amann RP. Testicular development and establishment of spermatogenesis in Holstein bulls. Journal of Animal Science 1981; 53(6): 1645-57.
https://doi.org/10.2527/jas1982.5361645x

[35] Kurohmaru M, Yamashiro S, Azmi TI, Basrur PK. An ultrastructural study of the Sertoli cell in the water buffalo (*Bubalus bubalis*). Anatomia, Histologia, Embryologia 1992; 21(1): 82-90.
https://doi.org/10.1111/j.1439-0264.1992.tb00323.x

[36] Jurado SB, Kurohmaru M, Hayashi Y. An Ultrastructural-Study of the Sertoli-Cell in the Shiba Goat. J Vet Med Sci 1994; 56(1): 33-8.
https://doi.org/10.1292/jvms.56.33

[37] Andriana BB, Ishii M, Kanai Y, Kimura J, Fukuta K, Kurohmaru M, *et al.* Multivesicular nuclear body in sertoli cells of the lesser mouse deer, Tragulus javanicus. Okajimas Folia Anatomica Japonica 2003; 80(2-3): 35-9.
https://doi.org/10.2535/ofaj.80.35

[38] More IAR, Armstrong EM, Mcseveney D, Chatfield WR. Morphogenesis and Fate of Nucleolar Channel System in Human Endometrial Glandular Cell. J Ultra Mol Struct R 1974; 47(1): 74-85.
https://doi.org/10.1016/S0022-5320(74)90027-6

[39] More IA, McSeveney D. The three dimensional structure of the nucleolar channel system in the endometrial glandular cell: serial sectioning and high voltage electron microscopic studies. Journal of Anatomy 1980; 130(Pt 4): 673-82.

[40] Osman DI, Ploen L. Fine-Structure of the Modified Sertoli Cells in the Terminal Segment of the Seminiferous Tubules of the Bull, Ram and Goat. Anim Reprod Sci 1979; 2(4): 343-51.
https://doi.org/10.1016/0378-4320(79)90021-6

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