Revealing histological and morphological features of female reproductive system in tree shrew (Tupaia belangeri)

Shan Zhong[1#], Shi-yao Zhang[1#], Hui-jie Xing[2#], Xiao-tan Zhang[1], Guang Wang[1], Yong-ping Bao[3], Jiang-nan Fu[2], Xuesong Yang[1]**

[1]*Division of Histology and Embryology, Key Laboratory for Regenerative Medicine of the Ministry of Education, Medical College, Jinan University, Guangzhou 510632, China*

[2]*Insitution of Laboratory Animal; Institute of Integrated Chinese and Western Medicine, Chinese Medical College, Jinan University, Guangzhou 510632, China*

[3]*Norwich Medical School, University of East Anglia, Norwich, Norfolk, UK*

[#]*Contributed to the work equally.*

**Corresponding authors: Xuesong Yang Tel: +86(20)85228316, Email: yang_xuesong@126.com. Or Jiang-nan Fu Tel: +86(20)85224045 Email: fujiangnan126@126.com*

ORCID of the authors:
Shan Zhong: 0000-0002-8079-2428
Shi-yao Zhang: 0000-0003-0175-8369
Hui-jie Xing: 0000-0002-6074-7697
Xiao-tan Zhang: 0000-0001-5480-8660
Guang Wang: 0000-0001-7595-2880
Yong-ping Bao: 0000-0002-6425-0370
Jiang-nan Fu: 0000-0002-0025-3310
Xuesong Yang: 0000-0002-2537-5752

Abstract

The tree shrew has been used as a primate animal model in neuroscience studies but it has only rarely been employed in the study of reproductive systems. This is mainly because we know very little about the histological features of reproductive organs of the tree shrew. In this study, we have systematically analyzed the histology of reproductive organs of tree shrew, in comparison with human organs. The uterus of female tree shrew is uterus biomes unicolis, which is connected with an enveloped ovary through a thin fallopian tube. Histologically, the fallopian tube consists of folded mucosa, muscularis and serosa. Like other mammalian animals, the different developmental stages (primordial, primary, secondary and Graafian follicles) of ovarian follicles including inner oocyte and outer granulosa cells are embedded in the cortex. The luminal endometrium, middle muscular myometrium and serosa constitute the wall of uterus of tree shrew. The uterine endometrium contains simple columnar ciliated cells and goblet cells, and there are rich uterine glands in underlying stroma. Furthermore, these glands of tree shrew are round and smaller during anestrus, and become much longer when they are in estrus. The uterine endometrium in younger animals was less developed when compared to a mature tree shrew. Compared to human uterine endometrium, the histological features of tree shrew are very similar, indicating that it could potentially be good primate animal model for studying the diseases in reproductive system.

Key words: tree shrew, reproductive organ, histological features, endometrium

Introduction

Tree shrews (Tupaia belangeri) are squirrel-like, rat-sized animals inhabiting in tropical shrublandand forests of South and Southeast Asia and South China (Fan et al. 2013). Recently, genomic analysis has confirmed that the tree shrew is closely related to primates (Fan et al. 2013; Fan et al. 2014). Zoologists have completed the domestication of Tupaia belangeri as experimental animal under laboratory condition, and they have an understanding of the basic physiological parameters of domesticated tree shrews (Wang et al. 2013). Hence, tree shrew can be considered as an excellent primate animal model in many research fields of life sciences. Because of close similarity with primate, tree shrew is deemed to be a good alternative for monkey, which is used in the studies of psychology (Fang et al. 2016; Nair et al. 2014), immunology (Glebe and Urban 2007; Li et al. 2011; Yu et al. 2016), physiology (Feng et al. 2015; Wang et al. 2013; Wu et al. 2013) and pathology (Zhang et al. 2016) and human disease research (Cao et al. 2003; Pan et al. 2016; Ye et al. 2016) as a primate experimental animal. However, compared with frequently-used animal models such as mouse and rabbit, little has been known about the morphological characters of tree shrew, which can be employed in many research felids as an emerging animal model. Hence, there is no doubt that studying the morphological characters in various systems of tree shrew is absolutely required to vigorously support the widespread use of tree shrew as primate animal model. The more we know about the histological features of the animal the quicker it could be widely employed as experimental animal.

Reproduction is one of the most important biological processes, and it enables the existence and continuation of species. The female reproductive system undertakes the most important tasks of human reproduction. This system is composed of ovaries, oviduct, uterus and vagina, and these organs implement different roles and works in reproductive processes. The ovaries supply the optimal conditions for ovarian follicles to mature and the synthesis of estrogen and progesterone to control development of follicles and uterine endometrium. An oocyte ovulates from mature follicles and is fertilized with sperm to become a zygote in the oviduct, or will degenerate eventually. The Endometrium of the uterus undergoes monthly cyclic change and menstruation, which is hormonally responsive during the reproductive cycle and pregnancy. After successful fertilization, uterine endometrium will sustain the development of the embryo and the placenta (Cobb 2002; Emerson 2002).

The increased incidence of reproductive diseases and widespread application of IVF, has prompted scientists and doctors to investigate reproductive systems using various animal models including tree shrew. At present, a number of different animal species are used to mimic humane reproductive systems. Serious ethical issues and the size of some larger experimental animals, such as monkey and sheep, make them difficult to use routinely in experiments. Some smaller experimental animals, for example cat, mouse or rabbit, have reproductive systems that are significantly different from human beings. Thus, as a non-monkey primate, tree shrew is small-size animal and could be suitable for the study of reproductive diseases. Female tree shrews give birth to up to three young after a gestation period of 45 to 50 days, reach sexual maturity at about four months, and breed for much of the year, with no clear breeding season in most species (Cobb 2002; Emerson 2002). In order to better validate tree shrew as suitable animal model, a greater understanding or the morphological and histological features of tree shrews compared with other better documented experimental animals and human tissues. In this study, we investigate the histological and morphological description of the reproductive system in tree shrew.

Materials and Methods

Tree shrew (Tupaia belangeri chinensis) and mice

Female tree shrews (120 g ± 10 g) were purchased from the experimental animal center of Kunming Medical College (Kunming, China). The female Kunming mice were obtained from the Laboratory Animal Centre of Sun Yat-sen University (Guangzhou, China). The animals were housed in an alternate light-dark cycle (every 12 hours) room with a temperature of 22 ± 2°C and a relative humidity of 50–60%. Tree shrews and mice were fed a complete formula food and allowed water adlibitum. 12-week and immature (11 day) tree shrews and mice were used in this study after a one-week adaption. All the animal experiments were in accordance with the Guidelines on the Care and Use of Animals for Scientific Purposes (2004, Singapore). The protocols for the animal studies were also reviewed and approved by the Experimental Animal Ethical Committee of Jinan University.

Histology

Briefly, the 12-week (11 day) female tree shrew and Kunming mice reproductive organs including ovary, oviduct and uterus were dissected, and then fixed in 4% paraformaldehyde at 4°C for 24 hours. The specimens were then washed, dehydrated, cleared in xylene and embedded in paraffin wax. The tissue specimens were serially sectioned at 4 μm using a rotary microtome (Leica, RM2126RT). The sections were stained with haematoxylin and eosin (H&E), or Masson's trichrome dyes (Masson staining)(Lazarous et al. 1992; Li et al. 2014; Zappi and Lombardo 1984). The Masson staining was used to reveal the presence of fibrosis and muscle in the tissues.

Photography and Microscopy

For light microscopy, whole mount reproductive organs of female tree shrews were photographed using a stereo-fluorescence microscope and processed using an Olympus software package Image-Pro Plus 7.0. Photographs of the stained histological the sections of tree shrew and mice were captured using by an epi-fluorescence microscope (Olympus IX51, Leica DM 4000B) at 200× or 400× magnification.

For transmission electron microscopy (TEM), the treated uteruses of tree shrew were fixed with 2.5% glutaral in 0.1 M PBS for 2 hours, and then the uteruses were dissected. The samples were sent to the TEM Laboratory of Sun Yat-sen University. The embedding, ultrathin sectioning and staining were performed by professional technicians and examined using a Tecnai G2 Spirit Twin (FEI, USA).

Results

The intact morphology of female reproductive organs in tree shrew.

To clearly observe the female reproductive organs' form in its full spatial completeness, we isolated the ovary, oviduct, and uterus and vagina of 12-week female tree shrew (Fig.1). From the views of dorsal, ventral and lateral sides (Fig. 1A-N), we could see that the segment of lower vagina and partial uterus of tree shrew appears to be an oval cylinder (Fig. 1C-F), while the upper segment is double horn uterus, which connects with a thin piece of oviduct and a separated ovary (Fig. 1C-K).

The general histology of reproductive organs in tree shrew.

H&E staining was performed on the transverse sections of oviduct (Fig. 2A-J) and ovary (Fig. 2L-P). Here, we could see that there are many finger-like mucosal folds in the lumen of oviduct, and the oviduct wall consists of mucosa, muscularis and serosa from luminal to apical side (Fig. 2A-C, E-G). Mucosa is formed by a ciliated epithelium on lamina propria in both segments of tube and fimbriate oviduct (Fig. 2D-H). The ovary is separated into an outer cortex and an inner medulla (Fig. 2L). The differential developing ovarian follicles including primordial follicle (Fig. 2M), primary follicle (Fig. 2N), secondary follicle (Fig. 2O) and Graafian follicle (Fig. 2P) are embedded in cortex connective tissue. In the mature follicles, the oocyte is surrounded by zona pellucida and granulosa cells which form the corona radiate, and the oocyte is ultimately suspended in the cumulus oophorus at Graafian follicle (Fig. 2M-P).

Like the oviduct, the walls of uterus and vagina are composed of endometrium (mucosal layer), myometrium (fibromuscular layer) and perimetrium (serosa) (Fig. 3A-O). Less finger-like mucosal folds were found in the uterus horn than in the uterine body, cervix or vagina (Fig. 3B-L). However, much stronger Masson staining was presented in the connective tissues of vagina and cervix than in uterus horn and body (Fig. 3C-M).

The endometrium, uterine gland and myometrium of uterus and vagina in tree shrew.

In the uterine horn and body, the endometrium is composed of consists a simple columnar epithelium (ciliated cells and goblet cells) and an underlying stroma (connective tissue) (Fig. 4A-E, B-F, C-G). Meanwhile, many simple tubular uterine glands are formed through the mucosa's invagination into stroma (Fig. 4A-E, B-F) (Fig. 5). Correspondingly, there are many finger-like mucosal folds in the endometrium of cervix and vagina, instead of many uterine glands (Fig. 4I-M, J-N). The epithelium is full of ciliated and goblet cells, and more fibromuscular components in stroma, which is also reflected by the stronger Masson staining (Fig. 4S-T) in comparison to the uterine horn and body (Fig. 4Q-R).

Again, we could find stronger Masson staining in myometrium of the cervix and vagina than in uterine horn and body (Fig. 6A-J) but more arteries (spiral artery) could be found (as indicated by arrows, fig. 6) in uterine horn and body than in cervix and vagina.

The histological characters of endometrium in immature and mature tree shrew.

In the endometrium of anestrus, most of the endometrium is full of well-arranged ciliated columnar epithelial cells (Fig. 7A-B), the majority of blood vessels are veins (Fig. 7C) and round tube-like endometrial glands are predominant (Fig. 7D-E). During estrus, the endometrium becomes thicker (Fig. 7F); the ciliated cells in endometrium are reduced; the number of arteries in the endometrium increased (Fig. 7H); the endometrial glands are presented as long-tubules (Fig. 7I) and more secretions could be found in those glands (Fig. 7J).

Compared to mature endometrium, we could not observe the clear boundaries between the three layers of the endometrium in immature tree shrew (Fig. 7K), Their epithelial cells arrange closely (Fig. 7L); very few ciliated cells were observed on the surface of the endometrial epithelium (Fig. 7M) and the majority of blood vessels are vein (Fig. 7N).

Tree shrew shares the histological characters of uterine wall with mice.

In order to compare the characters of uterine wall between tree shrew and mouse, we carefully observed the histology at uterine horn from tree shrew and mouse. Here, we can see that both of them could be divided into three layers as described above although more finger-like mucosal folds were observed in C57 mouse in this segment (Fig. 8A-D). In addition, few ciliated cells and uterine glands could be seen in C57 uterine endometrium compared to tree shrew (Fig. 8F-I). However, endometrial stroma are rich of blood vessels in both mice and animals; and both have similar arrangements of muscularis (Fig. 8G-J).

Discussion:

The reproductive system has attracted much attention given that it is an intricate and complex system. Its functionallity includes not only the reproductive organs but also the endocrine organs. It is because of its complex nature that the exact pathological mechanisms of infertility are still not fully understood. To elucidate the various pathways of pathogenesis in the human reproductive system, it is necessary to establish a suitable experimental animal model that is physiologically analagous to humans. In this study, we displayed the morphological features of the female reproductive system of the tree shrew, from the outward appearance to submicroscopic structure. Firstly, the tree shrew possesses a pair of double horn-like uterus, in a "Y" shape, connecting with oviducts and ovaries respectively at the ends. Obviously, there are abundant blood vessels distributing to the surface of the horns (Fig. 1J) and ventral side of the body (Fig. 1F). The oviduct is composed of tube and fimbriae portions, and its histology consists of three layers including serosa, muscularis and mucosa (Fig. 2A-E). The function of the oviduct is to collect oocytes and transport them or zygotes to the uterus (Coy et al. 2012; Ezzati et al. 2014; Hu et al. 2014). The follicle development in tree shrew is similar to human being, and progresses from immature follicles to mature follicles via primordial follicles, primary follicles, secondary follicles and Graafian follicles (Fig. 2L-P). This suggests that that tree shrew could be a good animal model for addressing the mechanisms of many reproductive system diseases such as premature ovarian failure (POF), eccyesis and other diseases of adnexa uteri.

Under the light microscope, the histological structures of horn, body, cervix and vagina of trees shrew uterus are generally similar to human being, in which the wall comprises endometrium myometrium and perimetrium. Endometrium undergoes cyclic proliferation, come-off and repair if there is no pregnancy during the female fertile window (Hawkins and Matzuk 2008; Kawano et al. 2014). As shown in figure 4, the endometrium epithelium is comprised of different types of epithelial cells including goblet cells and ciliated epithelium, which are again similar with those in the endometrium of humans. Under the epithelium, there is basement membrane underlying basal cells in the segments of horn and body. The endometrium of the cervix and vagina is constituted only with epithelium including ciliated epithelium and goblet cells (Fig. 4I-M). Masson staining demonstrated that the stroma is composed of stromal cells, in which fibroblasts and fibrocytes are presented differently in horn and body because of their different functions (Fig. 4Q-S). Accumulating evidence has shown that uterine glands and their secretions have considerable biological roles in pregnancy. However, an obvious knowledge gap remains regarding how human uterine glands support early pregnancy as well as the impacts of deficient glandular function on successful pregnancy and complications (Spencer 2014). Hence, a suitable experimental animal is required to fill in the gap. In tree shrew endometrium, there are abundant “tube like” glands under the surface epithelium, which consist of ciliated epithelium and goblet cells secreting mucus into lumen (Fig. 5). Uterine glands are essentially for normal pregnancy. These glands only exist in endometrium of the horn and body (Fig. 4B-N) and would develop and secrete (Fig. 7D-I). Horn of uterus is the standard site for embryo implantation during pregnancy in tree shrew (Zhang et al. 2013). In the endometrium of fetal (11 day) female tree shrew, we could not identify the glands and differential endometrium which just present unclear layers (Fig. 7K-N). Hence, we speculate that uterus of tree shrew matures after birth, which provides a chance to study the development of the uterus and the mechanisms of uterine diseases. Myometrium of uterus plays an important role during pregnancy and giving birth. As shown in Fig. 6, the myometrium of tree shrew contain two layers of smooth muscles with transverse and longitudinal smooth muscle bundles respectively. In addition, there are many arteries and veins in the middle of myofibers which are the branches of blood vessels on the uterine surface.

Female reproductive organs go through different developmental periods from childhood to adolescence. In the tree shrew, many small arteries in the endometrium, and small glands in anestrus develop into long tube glands in estrus (Fig. 7H-J), which is similar those in secretory phase of human uterus (Dixon et al. 2014; Manohar et al. 2014; Spencer 2014). It also displays that tree shrew could be a suitable non-monkey primate animal model for the elucidation of biological functions of uterine glands during the reproductive cycle and pregnancy (Hawkins and Matzuk 2008; Spencer 2014).

In sum, our current study reveals that: 1) tree shrew uterus contains four different segments, which are horn, body, cervix and vagina; 2) endometrium of tree shrew is comprised of surface epithelium, stroma cells and uterine glands; 3) myometrium of tree shrew consists of two layers of smooth muscle with perpendicular directions; 4) in estrus, endometrium presents significant changes, such as development of small arteries and uterine glands; 5) uterus of tree shrew undergoes development from immature to mature after birth in a similar manner to humans. The similarity with humans makes tree shrew a very suitable primate experimental animal model in studies of life sciences; 6) there is no doubt that tree shrew could be good option as a primate animal model on the study of reproductive system.

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Competing interests

The authors declare no competing financial interests.

References

Cao J, Yang EB, Su JJ, Li Y, Chow P (2003) The tree shrews: adjuncts and alternatives to primates as models for biomedical research J Med Primatol 32:123-130

Cobb M (2002) The new encyclopedia of mammals Tls-the Times Literary Supplement:27-27

Coy P, Garcia-Vazquez FA, Visconti PE, Aviles M (2012) Roles of the oviduct in mammalian fertilization Reproduction 144:649-660 doi:10.1530/REP-12-0279

Dixon D et al. (2014) Nonproliferative and proliferative lesions of the rat and mouse female reproductive system Journal of toxicologic pathology 27:1S-107S doi:10.1293/tox.27.1S

Emerson D (2002) The encyclopedia of mammals, 3 volumes. Library Journal 127:136-136

Ezzati M, Djahanbakhch O, Arian S, Carr BR (2014) Tubal transport of gametes and embryos: a review of physiology and pathophysiology Journal of assisted reproduction and genetics 31:1337-1347 doi:10.1007/s10815-014-0309-x

Fan Y et al. (2013) Genome of the Chinese tree shrew Nat Commun 4:1426 doi:10.1038/ncomms2416

Fan Y, Yu D, Yao YG (2014) Tree shrew database (TreeshrewDB): a genomic knowledge base for the Chinese tree shrew Scientific reports 4:7145 doi:10.1038/srep07145

Fang H et al. (2016) High activity of the stress promoter contributes to susceptibility to stress in the tree shrew Scientific reports 6:24905 doi:10.1038/srep24905

Feng Y et al. (2015) Identification and Characterization of Liver MicroRNAs of the Chinese Tree Shrew via Deep Sequencing Hepat Mon 15:e29053 doi:10.5812/hepatmon.29053

Glebe D, Urban S (2007) Viral and cellular determinants involved in hepadnaviral entry World J Gastroenterol 13:22-38

Hawkins SM, Matzuk MM (2008) The menstrual cycle: basic biology Annals of the New York Academy of Sciences 1135:10-18 doi:10.1196/annals.1429.018

Hu J et al. (2014) The regulation of nitric oxide synthase isoform expression in mouse and human fallopian tubes: potential insights for ectopic pregnancy International journal of molecular sciences 16:49-67 doi:10.3390/ijms16010049

Kawano N et al. (2014) Absence of CD9 reduces endometrial VEGF secretion and impairs uterine repair after parturition Scientific reports 4:4701 doi:10.1038/srep04701

Lazarous DF, Shou M, Unger EF (1992) Combined bromodeoxyuridine immunohistochemistry and Masson trichrome staining: facilitated detection of cell proliferation in viable vs. infarcted myocardium Biotechnic & histochemistry : official publication of the Biological Stain Commission 67:253-255

Li Y et al. (2011) [Progress on establishment of tree shrew (Tupaia) chronic infection with HBV in vivo] Dongwuxue Yanjiu 32:104-108 doi:10.3724/SP.J.1141.2011.01104

Li Y et al. (2014) Excess ROS induced by AAPH causes myocardial hypertrophy in the developing chick embryo International journal of cardiology 176:62-73 doi:10.1016/j.ijcard.2014.06.044

Manohar M et al. (2014) Alteration in endometrial proteins during early- and mid-secretory phases of the cycle in women with unexplained infertility PloS one 9:e111687 doi:10.1371/journal.pone.0111687

Nair J, Topka M, Khani A, Isenschmid M, Rainer G (2014) Tree shrews (Tupaia belangeri) exhibit novelty preference in the novel location memory task with 24-h retention periods Front Psychol 5:303 doi:10.3389/fpsyg.2014.00303

Pan XH et al. (2016) Development of a tree shrew metabolic syndrome model and use of umbilical cord mesenchymal stem cell transplantation for treatment Cytotechnology 68:2449-2467 doi:10.1007/s10616-016-9966-1

Spencer TE (2014) Biological roles of uterine glands in pregnancy Seminars in reproductive medicine 32:346-357 doi:10.1055/s-0034-1376354

Wang J et al. (2013) Basal physiological parameters in domesticated tree shrews (Tupaia belangeri chinensis) Dongwuxue Yanjiu 34:E69-74 doi:10.3724/SP.J.1141.2013.E02E69

Wu X et al. (2013) Relationships between body weight, fasting blood glucose concentration, sex and age in tree shrews (Tupaia belangeri chinensis) J Anim Physiol Anim Nutr (Berl) 97:1179-1188 doi:10.1111/jpn.12036

Ye L, He M, Huang Y, Zhao G, Lei Y, Zhou Y, Chen X (2016) Tree shrew as a new animal model for the study of lung cancer Oncol Lett 11:2091-2095 doi:10.3892/ol.2016.4156

Yu D, Wu Y, Xu L, Fan Y, Peng L, Xu M, Yao YG (2016) Identification and characterization of toll-like receptors (TLRs) in the Chinese tree shrew (Tupaia belangeri chinensis) Dev Comp Immunol 60:127-138 doi:10.1016/j.dci.2016.02.025

Zappi E, Lombardo W (1984) Combined Fontana-Masson/Perls' staining The American Journal of dermatopathology 6 Suppl:143-145

Zhang L et al. (2016) Tree shrew (Tupaia belangeri chinensis), a novel non-obese animal model of non-alcoholic fatty liver disease Biol Open 5:1545-1552 doi:10.1242/bio.020875

Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, Armant DR (2013) Physiological and molecular determinants of embryo implantation Molecular aspects of medicine 34:939-980 doi:10.1016/j.mam.2012.12.011

Figure legends

Figure 1. The morphological characters of female reproductive organs in tree shrew.

A-L: The representative dorsal (A), ventral (B), left lateral (G) and right lateral (H) view of uterus, oviduct and ovary of 12-week tree shrew. **C-K**: The upper portions of A and H respectively. **D-L**: The lower portions of A and H respectively. **E-F**: The schematic diagrams for front (E) and lateral (F) view of uterus, oviduct and ovary of tree shrew. Scale bars = 1 mm in A-B&G-H and 2 mm in C-F&I-L.

Figure 2. The histological features of oviduct and ovary in tree shrew.

A-E: Representative images of H&E stained transverse sections of tube (A) and fimbriae (E) segment of oviduct. **B-C**: The higher magnification images focus on mucosa (B) and muscularis (C) of tube region of oviduct respectively. **D**: The higher magnificent image focus on epithelium of tube region of oviduct. **F-G**: The higher magnification images focus on mucosa (F) and muscularis (G) of fimbriate region and oviduct respectively. **H**: The higher magnification image focus on epithelium of fimbriae region of oviduct. **I-J**: The schematic diagrams for whole uterus horn, oviduct and ovary (I) and oviduct histology (J) of tree shrew. Scale bars = 400μm in L; 100μm in A-E, P; 50μm in B-C, F-G, M-N; 20μm in D-H.

Figure 3. The histological features of uterus and vagina in tree shrew.

A-J: Representative images of H&E stained transverse sections at level of horn (A), body (D), cervix (J) of uterus and vagina (I). **B-L**: The higher magnification images focusing on the wall of uterus or vagina from A-J respectively. **C-M**: Representative images of Masson stained transverse sections at level of horn (C), body (F), cervix (I) of uterus and vagina (M). **N-O**: The schematic diagrams for the levels of transverse sections at uterus horn, body and cervix (N) and vagina (O) of tree shrew. Scale bars = 500μm in A-J and 250μm in B-L, C-M.

Figure 4. The histological features of uterus and vagina endometrium in tree shrew.

A-M: Representative images of H&E stained transverse sections focus on endometrium at level of horn (A), body (E), cervix (I) of uterus and vagina (M). **B-N**: The higher magnification images focus on endometrial surface epithelium from A-M respectively. **C-O, D-P**: The higher magnification images were taken from the sites indicated by dotted squares in B-N respectively. **Q-T**: Representative images of Masson stained transverse sections focus on endometrium at level of horn (Q), body (R), cervix (S) of uterus and vagina (T). Scale bars = 100μm in A-M, Q-T; 50μm in Q-N; 10μm in C-O, D-P.

Figure 5. The histological features of endometrial glands in tree shrew.

A-B: Representative images of H&E stained transverse sections focus on endometrium at level of uterus horn (A) and body (C). **B-D**: The higher magnification images were taken from the sites indicated by dotted squares in A-C respectively. Scale bars = 50μm in A, C; 20μm in B, D.

Figure 6. The histological features of myometrium in tree shrew.

A-J: Representative images of Masson stained transverse sections focus on partial myometrium and perimetrium at level of horn (A), body (D), cervix (G) of uterus and vagina (J). **B-K**: The higher magnification images focus on myometrium from A-J respectively. **C-L**: Representative images of H&E stained transverse sections focus on myometrium at level of horn (C), body (F), cervix (I) of uterus and vagina (L). Abbreviation: T, transverse smooth muscle bundle; L, longitudinal smooth muscle bundle.

Scale bars = 100μm in all.

Figure 7. The histological features of uterus walls at different developmental stage of tree shrew.

A-K: Representative images of H&E stained transverse sections focus on uterus walls of mature tree shrews in anestrus (A), estrus (F) and immature tree shrews (K). **B-E, G-J**: The higher magnification images focus on endometrium sites indicated by dotted squares in A-F respectively. **E-J**: The higher magnificent images focus on endometrial glands in E-J respectively. **K**: The H&E stained uterus wall of immature tree shrew. **L-N**: The higher magnification images focus on endometrium sites indicated by dotted squares in K. N: The higher magnification images focus on stroma of uterus wall indicated by dotted squares in K. Scale bars = 100μm in A-F; 50μm in C-H, D-I, K; 10μm in B-G, L-N.

Figure 8. The comparison of uterus walls at uterus horn between mice and tree shrews.

A-B: Representative images of H&E stained transverse sections focus on uterus walls of tree shrew (A) and C57 mouse (B). **C-D**: The higher magnification images focus on endometrium in A-B respectively. **E-H, F-I**: The higher magnification images focus on endometrial surfaces (E-H) and endometrial vessels (F-I) indicated by dotted squares in C-D respectively. **G-J**: The higher magnification images focus on myometrium and perimetrium. Scale bars = 200μm in A; 100μm in B, C, G; 50μm in D, J; 20μm in F-I; 10μm in E-H.