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## Microscopic anatomy of the reproductive organs of female Philippine native pig (*Sus scrofa*)

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### Abstract

The study described and compared the histological characteristics of the reproductive organs of female Philippine native pigs (PNPs). About 5 cm of each segment of the female reproductive tract (ovary, oviduct, uterine horn, cervix, vagina and vulva) were collected, fixed in 10% formalin and sent to the Department of Pathology, Providence Hospital, Quezon City for slide processing. Photomicroscope was used in the examination of the cross and longitudinal sections of the prepared slides under scanner, LPO (40x) and HPO (100x). Results showed that the number and size of the functional structures of the organs examined increased with aging of the animal. The oviduct epithelium was thickest in infundibulum and diminished towards the uterus where the isthmus has the thinnest epithelium. No notable differences on the epithelial linings of the vulva and vagina of the female PNPs were observed. These findings provide additional information about the structure and organization of the reproductive organs and systems of PNPs which could be useful for the development of technologies for the improvement of its reproduction. Also, this is the first study to provide detailed histological descriptions of the PNPs reproductive organs.

**Keywords:** Native pig, reproductive organs, microscopic anatomy

### 1. Introduction

Philippine native pigs (PNPs; *Sus scrofa*) are black or black with a white belly similar to Berkshire which is also black often with white hair on their legs, faces and tips of the tails. They have fairly small ears and eyes and an elongated snout, terminating in an even, movable disc with the nostrils in the middle. Their teeth are well-developed, with the huge lower and upper canines and upwardly projecting tusks in the male pigs. As wild pigs, they mostly feed on the leaves, roots, and tubers of grasses and other vegetation by making use of their movable snouts to dig the ground for their food. PNPs population are commonly raised in subsistence pig farms and are composed of several genetic groups, including Visayan and Ilocos breeds and Berkjala, Diani, Kaman, Koronadel and Libtong breeds that were all developed from a breed known as the Jalajala, which is now thought to be extinct<sup>[14]</sup>. In rural areas, PNPs are usually kept either for home consumption or raised for slaughter for a special occasion.

This breed is extremely prolific and could show the signals of sexual maturity as early as 4-5 months. The female produces an average of two litters per year. They have a low nourish conversion ratio. The juvenile pigs are capable of attaining a weight of 132 lbs (60 kg) within 6-8 months after birth. They may be found alone, in pairs at the time of the breeding season, or in groups, containing 7 to 12 pigs, which consists of a male pig, several female pigs and juvenile pigs. Even though they are most active during the night, they also move during daytime<sup>[4]</sup>. They are known for their ability to grow and reproduce even under adverse conditions and are more resistant to parasites and common diseases compared to the standard or imported breeds. They are preferred for production by small-scale farmers because of their low input requirements in terms of housing and feeding. Despite these advantages over commercial breeds of pigs however, information regarding its reproductive system remained insufficient if not nil, hence this study on the microscopic characteristics of its reproductive organs. Results of this study will serve as a baseline information to better understand the reproductive functions of the female PNPs. A basic understanding of the microscopic anatomy and physiological function of the female pig reproductive system can aid potential producers in anticipating and troubleshooting reproductive problems, and in facilitating decisions which impact performance of the breeding herd.

## 2. Materials and Methods

### 2.1 Experimental animals

Female PNPs (n=9) from Micor's Farm, Cayambanan, Urdaneta, Pangasinan were used in the study. The animals were divided into age groups (eg., Group 1= 6 month old; Group 2 = 9 month old; Group 3 =  $\geq 1$  year old). After slaughtering, the reproductive organs were removed and collected from the lower abdominal and pelvic cavity using a sharp butcher's knife and rinsed with physiologic saline or clean water. The female genitalia, including the ovaries, oviduct, uterus, cervix, vagina and vulva were collected, identified and individually stored in polystyrene boxes with ice and were transported to the Histology Laboratory of the CLSU-College of Veterinary Science and Medicine, Central Luzon State University.

### 2.2 Tissue sample collection

Each organ (ovaries, oviducts, uterine horns, cervix, vagina and vulva) was dissected from the rest of the reproductive tract and cleared of any debris or adjacent structure. Thereafter, Matchbox-sized tissue samples from each organ were collected and fixed in 10% neutral buffered formalin, sent to the Providence Hospital, Quezon City, where the samples were processed and stained using hematoxylin and eosin (H&E) protocol.

### 2.3 Microscopic examination

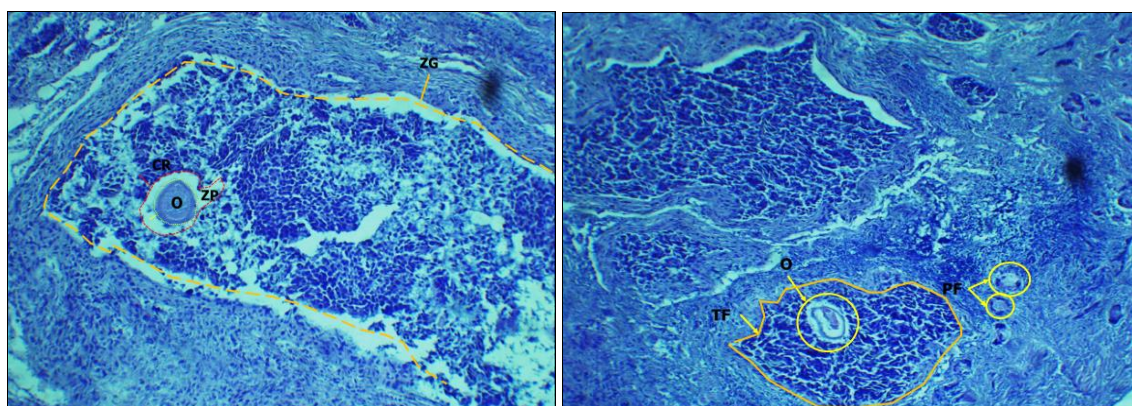
Microscopic examination of the structures of the ovary, oviduct, uterus, cervix, vagina and vulva was done at the Histology Laboratory of the College of Veterinary Science and Medicine, Central Luzon State University. A photomicroscope was used in the examination of the prepared slides. Each slide was examined first under the scanner (10x)

to observe its overall histological appearance. All the tunics, laminae, glands and other visible structures of each organ were carefully examined under LPO (40x) and HPO (100x).

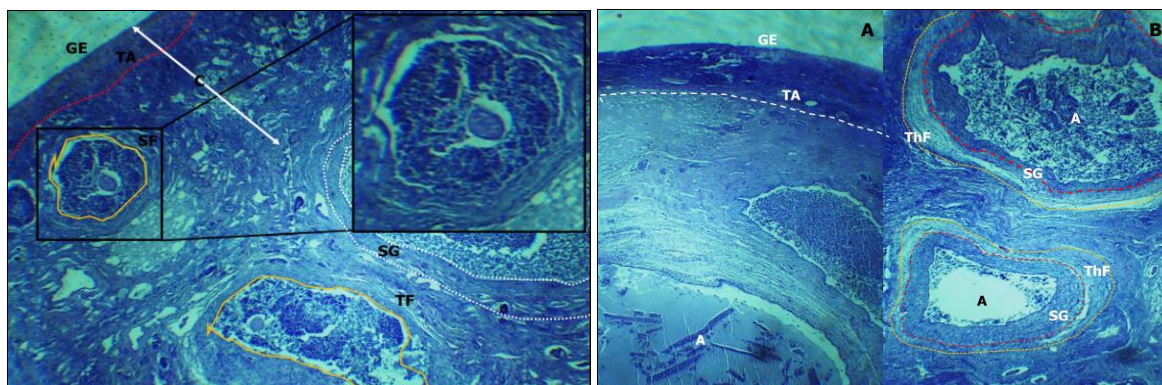
## 3. Results and Discussion

The microscopic structural characteristics of the reproductive tract of female PNPs were described according to the type of epithelial lining, the layers present, and other functional structures.

**Ovary:** Composed of an outer cortex which houses the follicles and an inner medulla which contains blood vessels, connective tissues, lymphatics, and nerves. The organ is covered by a single layer of cuboidal cells called germinal epithelium. Beneath this is a dense connective tissue layer called the tunica albuginea. Tissue section of the ovary from Group 1 PNPs showed several primary follicles and secondary follicles with only few tertiary follicles. The primordial follicles which represent the earliest stage in follicular development is surrounded by a single layer of squamous cells. Like the primordial follicles, primary follicles were situated in the peripheral regions of the cortex. These follicles were characterized by the proliferation of the cuboidal follicular cells as seen in collared peccary (*Tayassu tajacu*), a wild pig from north-western part of Peruvian amazon [11]. Secondary follicles have a monolayered to multilayered granulosa cells around the oocytes whereas tertiary follicles possess the characteristic fluid-filled cavity called antrum lined by layers of granulosa cells called the stratum granulosum. Their primary oocytes are eccentrically positioned within the follicles and reside in a mound of granulosa cells known as the cumulus oophorus (Fig. 1-2).



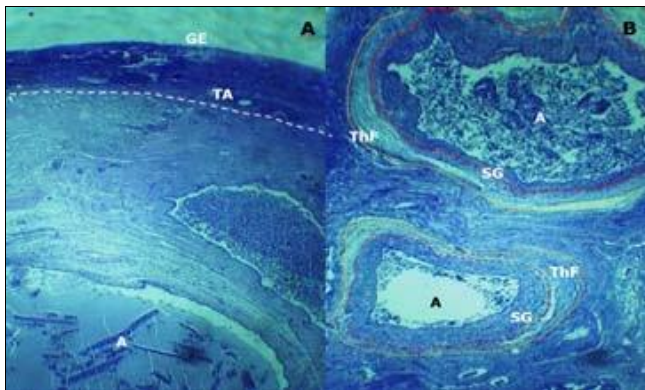
**Fig 1:** Ovary from Group 1 PNPs showing primary oocyte (O), secondary follicles (SF) and tertiary follicles (TF). 40x.



**Fig 2:** Ovary from Group 2 PNPs lined by germinal epithelium (GE) and tunica albuginea (TA) showing the cortex with secondary (SF) and tertiary follicles (TF) with the oocyte (O) and stratum granulosum (SG). 40x.

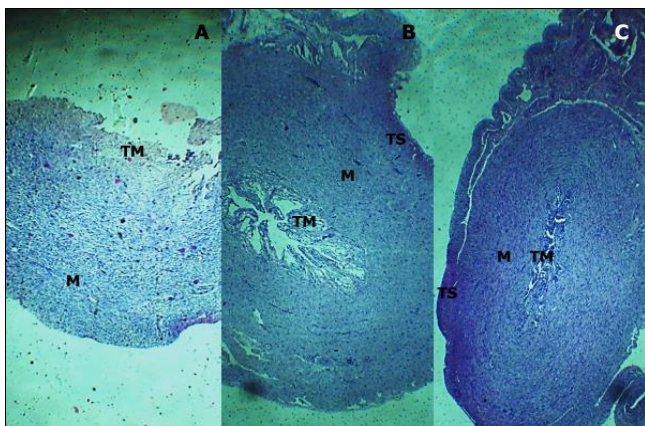


Several follicles of primary, secondary and tertiary type were observed in the ovary of Group 3 PNPs (Fig. 3). However, some of the antral follicles present contain no visible oocytes. The theca externa was difficult to differentiate due to its continuity with the stroma. The same observations were also noted in collared peccary [11].



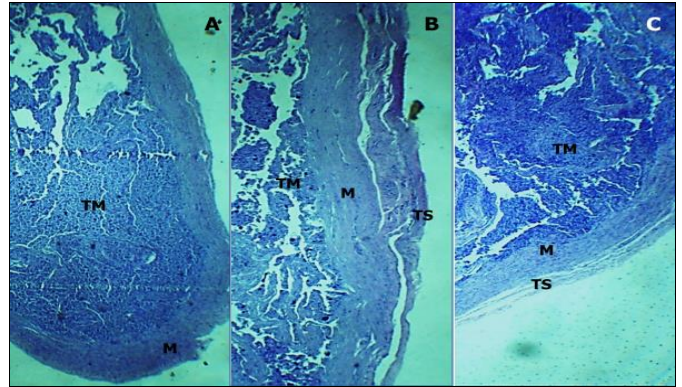
**Fig 3:** Ovary from Group 3 PNPs covered by germinal epithelium (GE) and tunica albuginea (TA) showing several antral follicles (A) characterized by large antrum and lined by theca folliculi (ThF) and stratum granulosum (SG). 40x.

**Oviduct:** The oviduct is divided to 3 regions: the isthmus which is located between the uterus and ampulla, ampulla which is the connection between isthmus and infundibulum and the infundibulum which is connected to the ovary. Its wall consisted of three layers: tunica mucosa, muscularis and serosa. The tunica mucosa-submucosa of the oviduct possessed primary, secondary and tertiary longitudinal folds. In the isthmus, the folds were much shorter and less branched. Whereas in the ampulla, the longitudinal folds were larger than those of the infundibulum with longer and slender folds, as earlier described [5]. The isthmus of the oviduct from Group 1-3 showed a thicker tunica muscularis than the ampulla and infundibulum. A prominent inner muscle layer blends with the uterine circular muscle (Fig. 4).



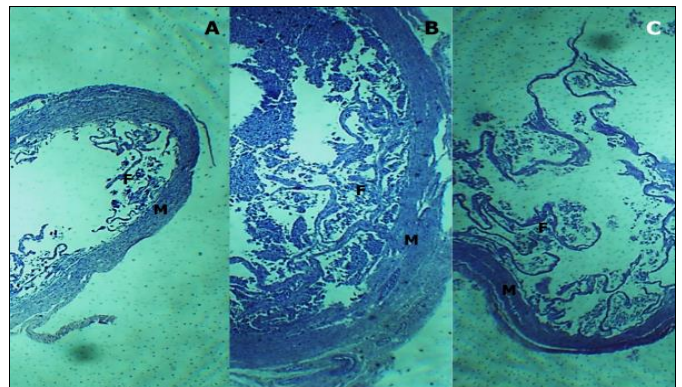
**Fig 4:** Isthmus of the oviduct of Group 1 (A), Group 2 (B), and Group 2 (C) PNPs showing the tunica mucosa (TM), thick tunica muscularis (M) and tunica serosa (TS). 40x.

There are no morphological differences that can be observed in the ampulla tissue sections between Group 1 to 3. The epithelial lining is simple columnar or pseudo stratified columnar and the tunica muscularis is thin compared with the isthmus (Fig. 5).



**Fig. 5.** Ampulla of the oviduct of 6-month (A), 9-month (B), and 12-month (C) old PNP showing tunica mucosa (TM), thin tunica muscularis (M) and tunica serosa (TS). 40x.

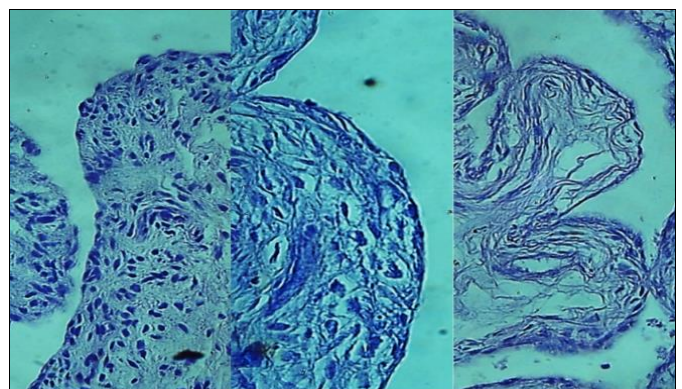
Similarly, the infundibulum of PNPs showed no morphological difference among age groups examined showing the thin muscularis and the longitudinal folds (Fig. 6).



**Fig 6:** Infundibulum from a section of oviduct of Group 1 (A), Group 2 (B), and Group 3 (C) PNPs showing the thin tunica muscularis (M) and the longitudinal folds (F). 40x.

**Uterus:** The uterus of PNPs is a bicornuate type similar with that of the bitch and queen [1, 5]. The wall of uterus consists of three layers: endometrium, myometrium and perimetrium. The endometrium is comprised by the fused mucosa and submucosa.

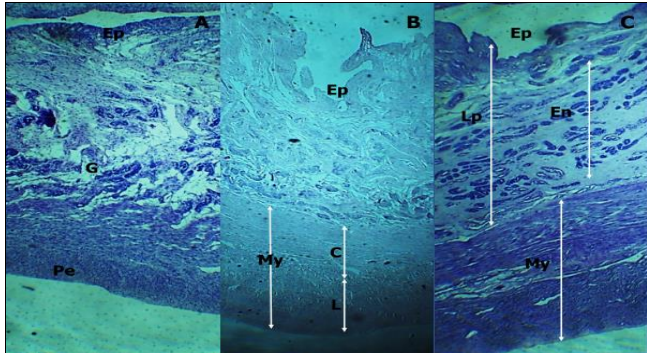
However, in PNPs, the tunica mucosa is lined with stratified squamous epithelium (Fig. 7) rather than pseudo stratified or stratified columnar as previously reported [13, 15].



**Fig 7:** Stratified squamous epithelium of the uterus of Group 1 (A), Group 2 (B), and Group 3 (C) PNPs. 400x.



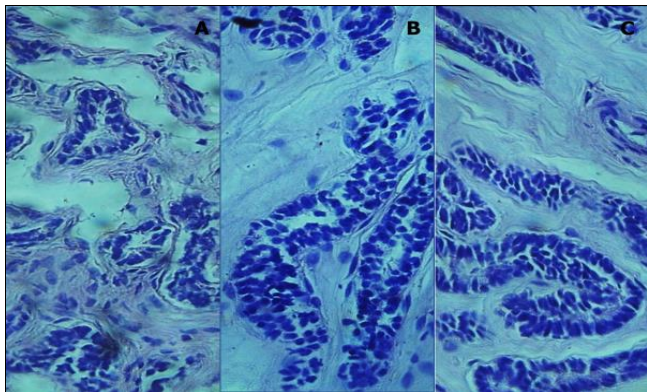
No changes were noted in the lamina propria which consists of loose connective tissue and blends with the underlying tunica submucosa. Also, no lamina muscularis mucosae were found in the entire female reproductive tract (Fig. 8). The stratum vasculare which separates the muscle layers in other domestic species was found indistinct similar to commercial breeds [3].



**Fig 8:** Layers of the uterus of Group 1 (A), Group 2 (B), and Group 3 (C) PNP pigs showing the surface epithelium (Ep), lamina propria (Lp), endometrium (En), myometrium (My), circular layer (C), longitudinal layer (L), perimetrium (Pe), and endometrial glands (G). 40x.

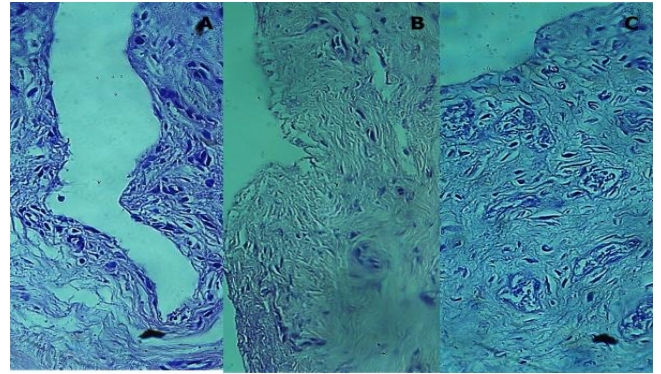
The uterine glands of PNP pigs are coiled glands located in the lamina propria-tunica submucosa of the uterus (Fig. 9). The epithelial lining of the endometrial gland was pseudostratified [3]. The glands penetrate the whole endometrium with increasing age and continued developing as the endometrium became thicker [7].

Apparently, the rising estrogen levels stimulate the growth and branching of the glands, but coiling and copious secretion from the glands generally do not occur until progesterone stimulation occurs [6].



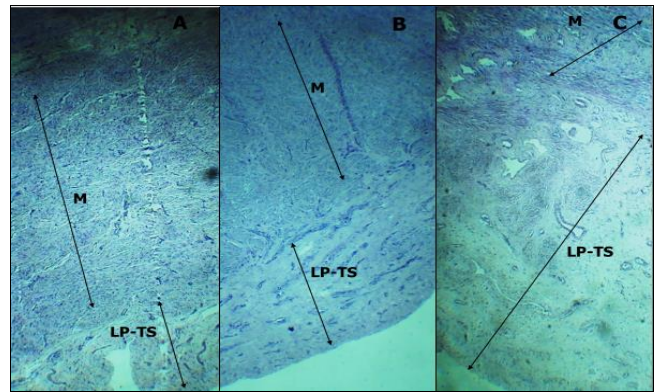
**Fig 9:** Uterine glands of Group 1 (A), Group 2 (B), and Group 3 (C) PNP pigs showing increased coiling with increasing age. 400x.

**Cervix:** Histological examination of the cervix of Group 1-3 PNP pigs showed no morphological differences. The organ is lined with stratified squamous epithelium (Fig. 10). This finding supported the observation that more than 90% of the cervix may have a vaginal type of mucosa as the organ undergoes cyclic alterations [6]. Whereas, others have reported that the epithelial layer of the cervix of gilt is simple columnar [12] and/or changes between simple columnar, pseudostratified and stratified squamous epithelium, which is primarily columnar in diestrus and primarily stratified in estrus [9].



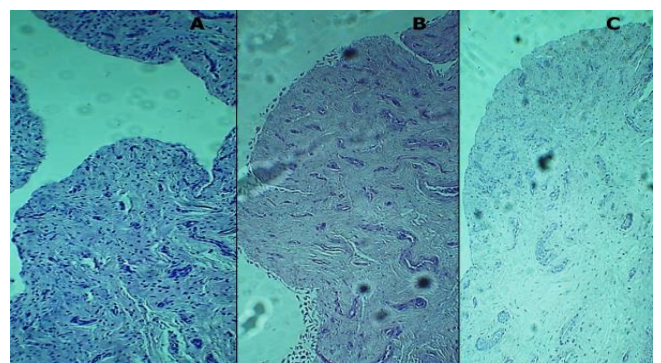
**Fig 10:** Stratified squamous epithelium lining of the cervix of Group 1 (A), Group 2 (B), and Group 3 (C) PNP pigs. 400x.

The propria-submucosa of the cervix consists of dense irregular connective tissue (Fig. 11). This becomes edematous and assumes a loose areolar structure during estrus. The tunica muscularis consists of inner circular and outer longitudinal smooth muscle layers. Thickening and infolding of circular layer or prominences occurs in the region of the circular folds in sows [6].



**Fig 11:** Layers of the cervix of Group 1 (A), Group 2 (B), and Group 3 (C) PNP pigs showing lamina propria-tunica submucosa (LP-TS) and tunica muscularis (M). 40x.

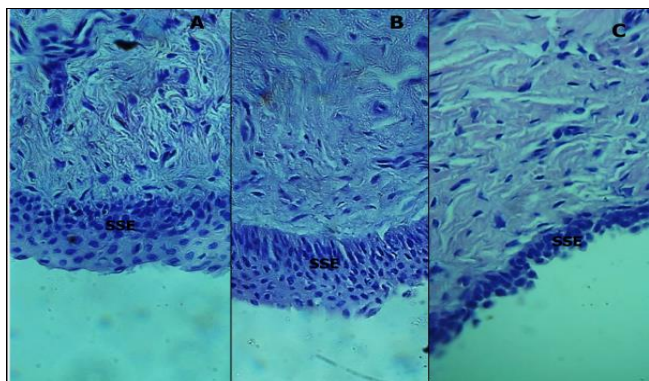
Cervix presents mounds or cushions called pulvini which enlarge in the increasing age (Fig. 12). These structures interdigitate with each other to close the cervical canal ensuring a tight coupling of the penis. The histological findings were also observed in collared peccary [11] and in domestic pigs [10]. The enlargement of the pulvini was confirmed in a separate study on the morphology of the cervix [17].



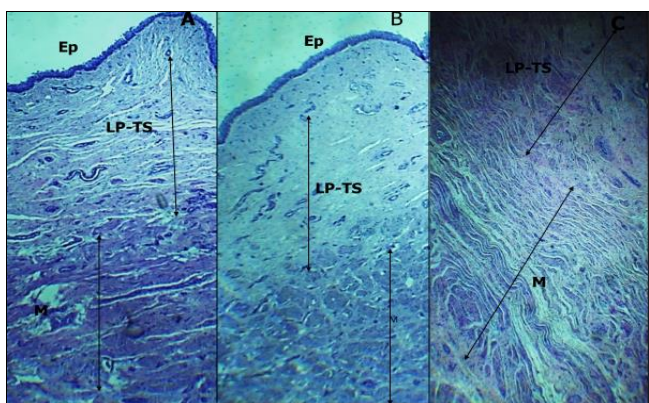
**Fig 12:** Cervical pulvini of Group 1 (A), Group 2 (B), and Group 3 (C) PNP pigs. 100x.



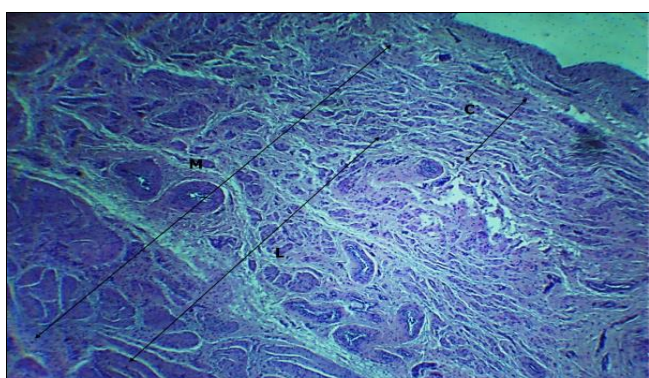
**Vagina:** the vagina regardless of age difference was characterized by non-keratinized stratified squamous epithelial lining (Fig. 13). The wall of the vagina composed of four layers: mucosa, submucosa, muscularis and adventitia. The lamina propria-tunica submucosa consists of dense connective tissues (Fig. 14). The muscularis consists of irregularly arranged circular and longitudinal bundles of smooth muscle cells (Fig. 15). Overall, these findings were similar to other reports on mammals [3, 6, 10, 16].



**Fig 13:** Stratified squamous non-keratinized epithelial lining of the vagina of Group 1 (A), Group 2 (B), and Group 3 (C) PNPs. 100x.



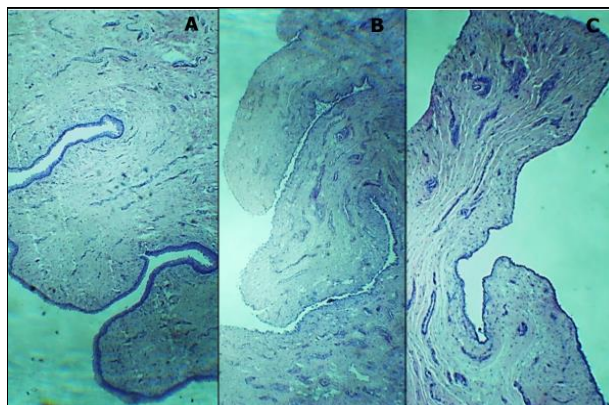
**Fig 14:** Epithelial layers of the vagina of Group 1 (A), Group 2 (B), and Group 3 (C) PNPs showing the surface epithelium (Ep), lamina propria-tunica submucosa (LP-TS), muscularis (M), and blood vessels. 40x.



**Fig 15:** Tunica muscularis layer (M) of the vagina of Group 3 PNPs composed of inner circular (C), and outer longitudinal (L). 40x.

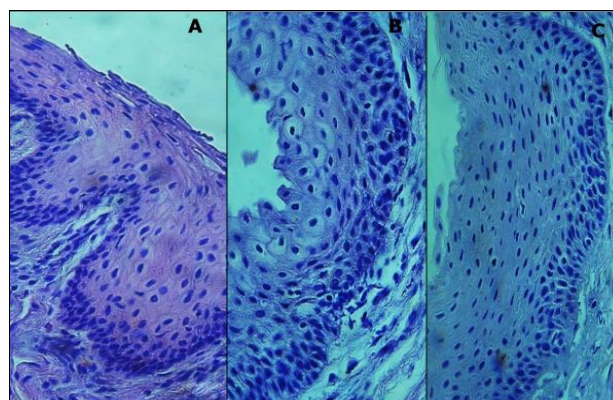
The lamina epithelialis forms the longitudinal folds called rugae which appear to increase in length with advancing age (Fig. 16). These findings were similar to the

vagina of women [8, 16] and that these foldings were not considered to be pathologic [2].

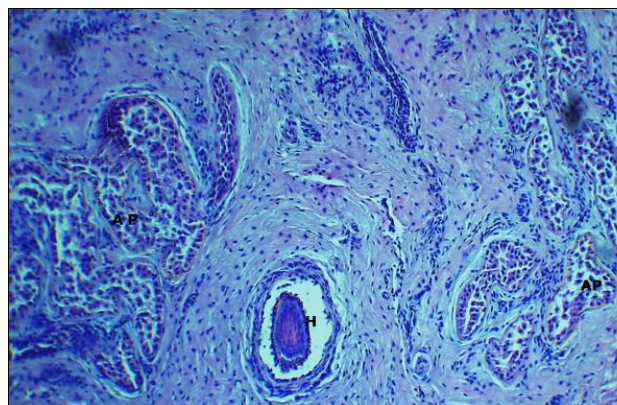


**Fig 16:** Vaginal fold of Group 1 (A), Group 2 (B), and Group 3 (C) PNPs. 40x, H&E

**Vulva:** Histological examination of the vulva showed no morphological differences between Group 1 to 3. The organ is lined with stratified squamous epithelium (Fig. 17) and possess a structure similar to external skin. It is well-endowed with hair follicles, apocrine glands, and blood vessels which can become congested during estrus (Fig. 18). These findings were in support of previous results on other mammals [3]. The vulva also contains layers of striated muscle (possibly the constructor vulvae) and subcutaneous adipose tissue (Fig. 19).

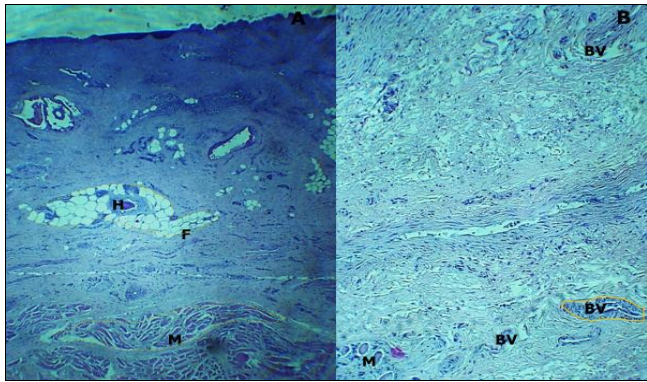


**Fig 17:** Stratified squamous epithelium of the vulva of Group 1 (A), Group 2 (B), and Group 3 (C) PNPs. 100x.



**Fig 18:** Vulva of Group 1 PNPs showing presence of hair follicles (H), apocrine glands (AP) and blood vessels (BV) embedded in the connective tissues. 40x.





**Fig 19:** Section of the vulva of Group 3 PNPs (A) showing stratified epithelium (E), subcutaneous adipose tissue (F) and muscle bundles (M) and of Group 2 PNPs (B) showing the numerous blood vessels (BV) and muscles at the bottom of the image. 40x.

#### 4. Conclusion

Overall, the results of this study provide additional information about the structure and organization of the reproductive organs and systems of PNPs. Morphological differences were evident in the oviduct, vagina and vulva between Group 1 to 3. However, some notable features in relation to aging were noted including the increasing number of endometrial glands in the uterus, increasing length of the vaginal folds, enlargement of the pulvini in the cervix, and increasing number of mature follicles. These observations were in agreement with other reports related to the morphological morphometry of pig [2, 13, 17]. It is suggested that the histological appearance as well as measurements of the organs studied be characterized during various reproductive processes to determine the needed measures for the development of technologies for its reproduction.

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