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Research Article

Histomorphogenic Changes during Uterine Development of the Nigerian Indigenous Breed of Dog: A Post-Natal Study

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Abstract

Demand for dog is on an increase because they are used as watchdog, hunting and its breeding serves as a source of income to both rural and urban dwellers. As demand is increases, breeders often mate dogs at premature age thereby causing several complications from conception to birth.

This study was aimed at considering the post-natal development of the uterus from histomorphological aspects and to correlate features to attainment of puberty. Hysterectomy was carried out to secure the uterus from Day 2, Week 4, Week 8, Week 12 and Week 20. This developmental study attempted highlighting some silent microanatomical parameters, including the circular muscle, longitudinal muscle, myometrial glands, blood vessels and epithelial features of the uterus in the Nigerian indigenous bitch. The inner circular muscle and the germinal epithelium were the first microstructures to be observed on day 2 and week 4 of postnatal development respectively. Within these range of development, little or no histological variations occurred. Simple columnar epithelium and longitudinal smooth muscle were seen on the twelfth week. The inner circular muscle and outer longitudinal muscles made up the fundamental component of the smooth muscle. The significant of this study is to indicate histological changes that takes place within the uterus and this finding show some corresponding relationship that exist between age and uterine development, an indicator for determining puberty.

Keywords: *Dog; Histomorphogenic; Indigenous; Uterus*

Introduction

Globally, there are more than 400 million pure bred, mongrel or feral dogs, domestication of this species started at least 15,000 years ago, but some authors have suggested that it dates back to even more than 40,000 years ago [3]. The ancestor of the domestic dog is nowadays considered to be the wolf [16]. It is a unique situation that both the ancestor, the wolf and the domesticated descendant, the dog, are still present around the world, which gives them a very important place in the fields of interest for scientific research [5].

The Nigerian indigenous breed of dog is often referred to as Mongrel (*Canis familiaris*). The total population of dogs in Nigeria as at 1992 is 4.5 million [4].

Puberty in the bitch appears, in most breeds, not to depend on day length, an exception being the Basenji which usually cycles only in the autumn [7]. Puberty seems to be related to size and weight, in that it occurs when the bitch has reached around 85% of the adult weight, and consequently, bitches of the smaller breeds in general have their first oestrus at an earlier age than those of the larger breeds [6]. Most bitches, thus, reach puberty at between 6 - 15 months of age, but some, especially of the large breeds reach puberty at 18 - 20 months of age [20].

Gestation length in the dog is on an average it lasts 62 - 64 days [8]. The range of the gestation length seems to be from 56 to 72 days, and it depends on many factors [9].

Population of dogs is drastically thinning out, as a result of high consumption of its meat use as guard dogs and for hunting which has cause it increase demand [15]. This demand has cause many breeder to mate premature bitch to cover the gap in demand. This has led to numerous complications; as a result, this study was designed to serve as guide to correlate some histological changes that takes place in the uterus to age of dog.

Currently, there are little or no information describing the female reproductive organs, precisely the uterus of the Nigerian indigenous breed of dog at post-natal development. To effectively monitor attainment of sexual maturity and for efficient breeding initiation in pre-pubertal bitch, developmental knowledge on the histomorphological index of the uterus is required.

Despite general anatomical descriptions for some species of dogs exist, no documentations is available to define the histomorphogenic changes that occurs in the uterus of the Nigerian Indigenous dogs. Information obtain here will serve as baseline data for Nigerian indigenous breed of dog.

Materials and Methods

Experimental animals

Five (5) female Nigerian indigenous breed of puppies were purchased from a private dog breeder in Makurdi, Benue State. Puppies were kept in a well ventilated and disinfected kernel in the Veterinary Teaching Hospital, University of Agriculture, Makurdi. They were fed with cooked meat, rice and water *ad libitum.*

Materials for hysterectomy

Five (5) puppies at different ages; 2 days old, Week 1, Week 4, Week 12 and Week 20 were used for this study. Others include; gloves, ruler, scalpel blade, cotton wool, syringes and needle, suture materials (chromic catgut, nylon), atropine, Xylazine, Ketamine, 10% formalin, normal saline, complete surgical kit, sample bottles, gauze, Chlorhexidine (disinfectant), Charmil$^{(R)}$ antibiotic spray were used in the study.

Procedure for hysterectomy

Hysterectomy or spaying was carried on each of the puppies. The dogs were fasted for 12 hours prior to the surgery. For each puppy, pre-surgical, surgical and post- operative guidelines were followed. With the puppy on dorsal recumbency, hairs on the ventral abdominal region was shaved from the xiphoid cartilage to the pelvic inlet, using a clipper. The skin was scrubbed with Dettol soap and chlorhexidine to disinfect the area shave. Preanesthetic administration were made; Atropine and xylazine at 1 mg/kg intramuscular and 0.05 mg/kg intramuscular respectively. Later, xylazine at dose rate of 12mg/kg was administered.

A sterile drape was placed over the surgical site. Using scalpel after desensitization of the area, a 5 cm midline incision along the linea alba was made from the xiphoid cartilage to the pelvic brim; cutting through the skin, subcutaneous fat fascia, rectus abdominis muscle, extra-peritoneal fat and the peritoneum. The abdominal contents were exteriorised and the reproductive organs were located using the bladder as the landmark. The major blood vessels (utero-ovarian artery and the posterior uterine artery) supplying the

ovaries and the uterus were ligated using chromic catgut and cut off to free it from the body. The fascia and rectus abdominis muscles were then closed in two layers of sutures using chromic catgut. The outer layer of skin was closed with non-absorbable sutures (nylon); these sutures were to be removed in 10 days.

Postoperative medication to relieve pain was administered (Piroxicam 0.3 mg/kg Intramuscular injection), antibiotic was administered to prevent secondary bacterial infection (Penstrep 1 ml/10 kg 3/7 Intramuscular), Charmil(R) antibiotic spray was also used.

Histological techniques

Both right and left ovary were obtained and placed in a label sample bottle containing 10% buffered formalin. The samples were thereafter transferred into a container with increasing serial concentration of alcohol (70%, 80%, 95% and 100%) with an interval of 24 hours for each stage of dehydration. Tissues were again cleared in xylene for 2 hours before infiltrating with molten paraffin wax at 50°C and blocked in paraffin according to standard procedures [11] and labeled.

Transverse sections were made, at the thickness of 7 µm, using Jung rotary microtome (Model 42339, Berlin, Germany) and labeled. The sections were mounted on glass slides and allowed to dry, deparaffinized, stained, dehydrated and cover slipped using diphenylphthalate propylene xylene as mountant. Photomicrograph of sections were taken using digital eyepiece (Scopetek DCM500, Resolution: 14M pixels, attached to a trinocular light microscope (OLYMPUS- XSZ107BN, Hamburg, Germany).

Results

The epithelial layer of the uterus at day 2 of postnatal development was observed to be made up of epithelial layer which is the germinal centre of this organ spreading toward the submucosa. The germinal cells occupy mainly the epithelial layer, submucosal layer and are consistence with cells of the smooth muscle. The circular muscle was formed at this stage of development and this might be liken to early deposition of connective tissue within the stoma of the uterus prior to birth. There were little or significant histological changes within day 2 to the fourth week (Plate 1).

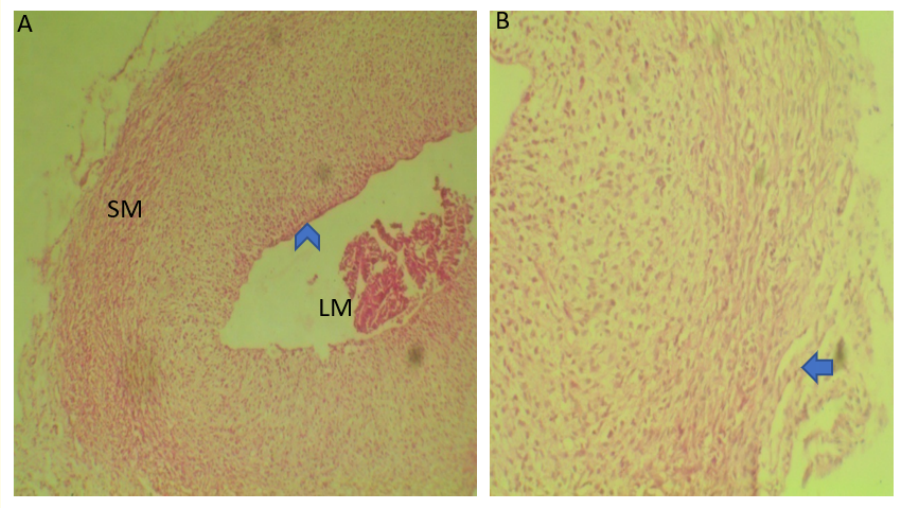

Plate 1: *Uterus at Day 2 indicating; A: SM; circular smooth muscle, Arrowhead; Epithelium, LM; Lumen, X40, H&E. B: Arrow; Formation of longitudinal muscle. X100, H&E.*

The fourth week of development was characterized by formation of simple tubular glands that were concentrated within the submucosa layer. At this week, smooth muscles, of the circular type was fully developed and the longitudinal type was yet at its formative stage. The epithelium and blood vessels were not prominent at this stage of uterine development (Plate 2).

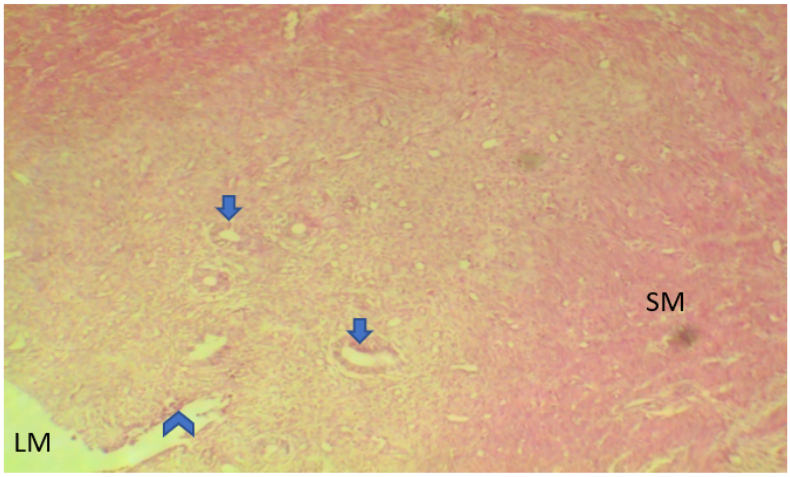

Plate 2: *Indicating the uterus at Week 4; LM: Lumen, Arrowhead: Developing epithelium, Arrow; Glands, SM; Longitudinal muscle, H&E X100.*

At week 12, the epithelium was of the simple columnar type with each having its nuclear basally. Development of the circular, longitudinal and oblique smooth muscles were established. Transverse section of the uterine tissue reveals circular muscle appearing like a transected skeletal muscle fascicule with blood vessels appearing within the longitudinal muscles (Plate 3).

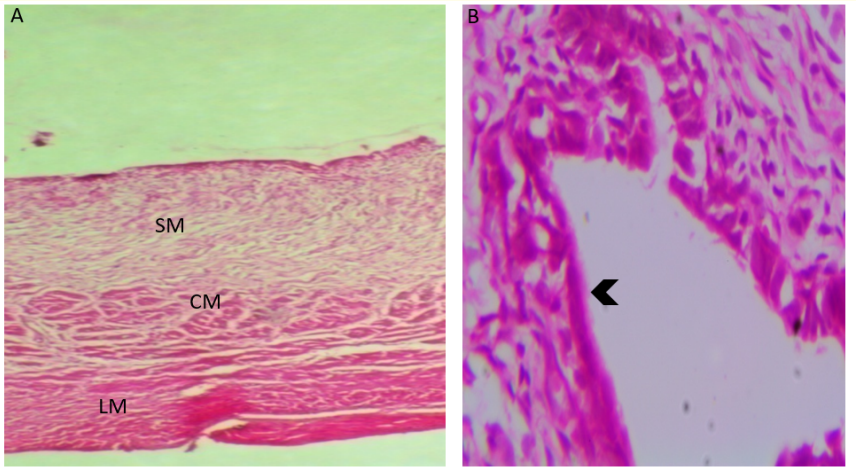

Plate 3: *The uterus Week 12 showing; A: SM; Submucosal layer, CM: Circular muscle, LM: Longitudinal muscle. H&E, X 40. B: Arrowhead; simple columnar epithelium. H&E, X 100.*

The twentieth week had must of the features being formed, including; submucosal glands which were seen to be invaded by tubular glands, smooth muscles, blood vessels within the submucosal layer and the epithelium. Predominant histological features of the uterus were formed at this stage of development (Plate 4).

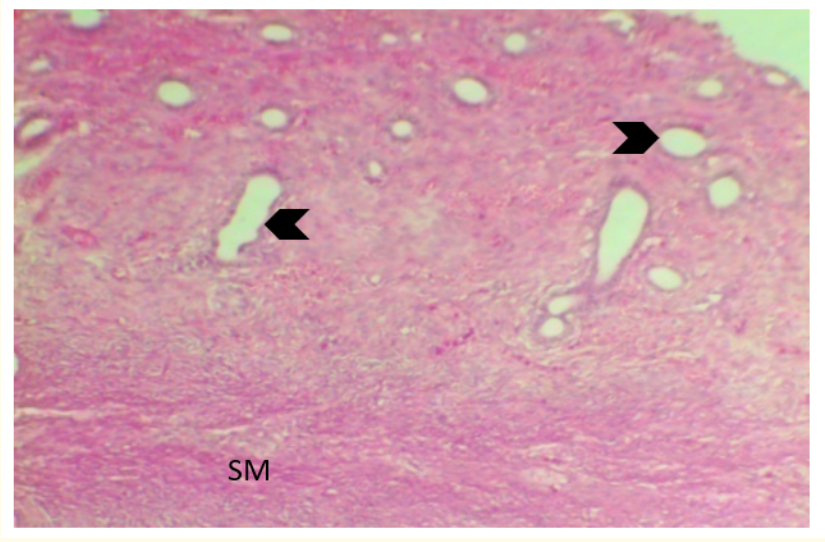

Plate 4: *Uterus at Week 20 showing; arrowhead; simple tubular glands, SM; smooth muscle. H&E X10.*

Discussion

The uterus is a unique organ in that, during pregnancy, it undergoes profound, largely reversible, changes made through secretions and muscular contraction. This study was able to define some outstanding features of the uterus with respect to the epithelium, glands and smooth muscles after birth.

Immediately after birth, postnatal development of the uterus is an indication that uterine formation has taken place at prenatal stage having germinal epithelium, the submucosal layer and circular muscle being the first features to be establish at postnatal stage of day 2 development. This study in agreement with the statement that must of the mammalian reproductive tract are been form prior to birth, but subsequent development of individual part vary with age of the animal [1]. The endometrial epithelium is simple columnar type and this epithelium is similar to those of the queen and mare but it is pseudostrafied columnar in the sow and ruminants [10]. The epithelium serves, as germinal center where basic cells within the organ are formed and this tend to vary with reports from development of some organs where germinal epithelium during oogenesis is cortical [17], while development of germinal epithelium is cortical in the cerebrum of the helmeted guinea fowl [19]. This proof that various organs has it germinal epithelium either in the cortical, medullary or the mucosa layer.

Development of the simple tubular glands was evident within the submucosa, with longitudinal muscle at formative stage from week 4. Apart from secretion of the hormone, glands of the submucosal contribute to the volume of fluids within the uterus and primarily, they aids in keeping the endometrium moist, rid of infection [12].

Microanatomical structures at twelfth week of uterine development included evidence of simple columnar epithelium, distinct components of the smooth muscle; the circular and longitudinal muscles. Primarily, splanchnic organs are surrounded with smooth muscles and

these structures forming at different stages of ontogeny and is consistent in the bitch, mare and queen [14] with the endometrial surfaces of the aforementioned species maintaining a simple columnar epithelium unlike that of the sow [2].

Glands of the endometrium were of simple tubular type that occupy the submucosa and the smooth muscles were microstructures formed at week 4 and 12 of development. The thick inner circular muscle and thin outer longitudinal muscle were the myometrium and the width expand with the age of the bitch. This agree with the fact that, myometrium is important during parturition and helps during contraction of the uterus following action of actin and myosin [13].

Conclusion

Silent but interested interplay of both physiological and pathological condition takes place within the uterus. Some of these interplay include, pregnancy, contraction, secretions and neoplasm. Understanding development of these structures not seen with unaided eyes has to be probe to prefer some relevant answer to some pathophysiological conditions surrounding conception in our indigenous bitch. Study at postnatal stage from Day 2 to Week 20 outline these important developmental features; the inner circular layer was the first form of smooth muscle to be form. Simple tubular glands develop at the fourth week. At week 12, simple cuboidal epithelium and longitudinal muscle were form. These histological evidences suggests that the uterus of Nigerian indigenous breed of dog at week 20 has develop basic histological features, which are indicative stage of attending puberty.

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Conflict of Interest

No conflict of interest exists.

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