Gross and Microscopic Features of the Cervix in the Non-Pregnant Rabbit

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Abstract Structure of the rabbit cervix in estrus was examined macroscopically and microscopically, as a part of physiological and morphological studies of the rabbit cervix. Two cervical canals surrounded by mucosa and musculature were separated completely by a thick median septum. Each canal was provided with the external and internal ora. The cervical mucosa was characterized by the formation of longitudinal folds with many complex crypts. The mucous membrane was lined with a single layer of high columnar epithelial cells composed of two cell types, ciliated and non-ciliated secretory cells. The ciliated cells were predominant in the internal os rather than in the mid-cervix and the external os. Macroscopic examination revealed a distinct transition of the mucosa from uterus to cervix at the internal os, although the transition was histologically gradual. Both inner circular and outer longitudinal muscular layers were well developed in the uterus, and their muscular bundles were arranged uniformly and regularly. At the proximal end of the cervix, the uterine musculature became loose and the muscular bundles became slender, decreased in number, and dispersed to the vaginal wall. The rabbit cervix contained much fibrous connective tissue as compared with the uterine horn.

Studies on the transport of eggs through the oviduct have been carried out with the emphasis on ciliary activity, flow of secretions, contraction of oviductal smooth muscles, function of the ampullary-isthmic junction and function of the uterotubal junction. These physiological activities are regulated by ovarian hormones after ovulation¹⁻³⁾. However, only a limited amount of information is available on the distribution pattern of eggs in the uterus. BÖVING⁴⁾ reported that intra-uterine rabbit blastocysts were arranged at random from 3 to 5 days *post coitum* (*p.c.*) and evenly spaced by 7 days *p.c.* He also described that no blastocyst was seen to have travelled the full length of the cornu before about 5 days *p.c.* On the contrary, TSUTSUMI and HAFEZ⁵⁾ demonstrated that some distal blastocysts appeared very close to the internal os of the cervix at 78 hours *p.c.* and that even spacing occurred at 114 to 120 hours *p.c.* This observation indicates that some intrauterine blastocysts can be transported more rapidly through the whole uterine lumen within several hours, because eggs require about 72 hours to be transported into the uterus.

The rate of egg transport through the reproductive tract can be greatly altered by ovariectomy or administration of certain agents such as ovarian hormones and prostaglandins⁶⁻¹⁵⁾. Some investigators described that uncollected eggs were considered to have been expelled into the vagina, when reduced numbers of eggs were recovered from oviducts and uteri after various treatments. Indeed, the eggs were detected from vaginas of estrogen- or prostaglandin F₂α-treated

Anatomical Features of the Rabbit Cervix

rabbits at autopsy^{10,12,16-20}) and by the vaginal washing method²¹⁻²³). TSUTSUMI and TAKEDA²⁴) observed that some of the intrauterine eggs were expelled into the vagina through the cervix at an early period of pseudopregnancy.

These recent reports have raised anew the question of whether the cervix plays a role in the transport of intrauterine eggs during early pregnancy, although most of the attention has been paid to the sperm transport through the cervix and to cervical changes during late pregnancy and at labor. In the present investigation, as a part of physiological and morphological studies on the rabbit cervix, structure of cervixes from non-pregnant (estrous) rabbits was examined macroscopically and microscopically. The investigation was divided into three parts; (1) macroscopic observations, (2) histological observations and (3) observations of muscular orientation.

Materials and Methods

1. *Macroscopic observations*

Ten adult female rabbits (Japanese White) were deeply anesthetized by an intravenous injection of sodium pentobarbital (Somnopentyl: Pitman-Moore, Inc.). Their uteri and cervixes were excised together with parts of the vaginas after laparotomy. The excised specimens were ligated at the middle part of the vagina and pinned onto a paraffin board. Bouin's fixative was lightly instilled into the vaginal lumen with care of avoiding excessive distension of the lumen and then the specimens were immersed into the same fixative. After fixation, the specimens were washed with 70% ethanol and were dissected, and then the surface of the mucosa was observed under 70% ethanol with the aid of a dissecting microscope.

The reproductive tracts from 29 rabbits, which had been cleared by ORSINI's method²⁵) and used for other experiments, were observed additionally. Parts of cervixes of these cleared tracts stored in benzyl benzoate were returned to benzol and were passed through 100% ethanol twice, stored in 70% ethanol, and subjected to observation.

2. *Histological observations*

The cervixes and uteri were collected from ten deeply anesthetized female rabbits. No corpus luteum was found in the ovaries of these animals. The specimens were fixed in Bouin's or Carnoy's fixative through procedures similar to those in experiment 1. After 2-hour fixation, these specimens were dissected as follows: The rabbit uterus is duplex in type, provided with paired cervixes, so that one cervix of each pair was cut transversely in three segments, lower, middle and upper; the contralateral cervix was cut in longitudinal slices. Pieces of the distal end of uterine horn (about 1 cm upwards from the proximal end of the cervix) were cut longitudinally or transversely. These specimens were fixed again, dehydrated, embedded in paraffin and sectioned in 5-10 μ m thickness. Sections were stained with hematoxylin and eosin (H. and E.), Gomori's trichrome stain or azan for differential staining of connective tissue and musculature.

3. *Observations of muscular orientation*

Observation of muscular orientation was carried out on specimens from 16 female rabbits by the maceration method of KREILKAMP and BOYDEN²⁶). Some preliminary experiments were also made to determine good macerative conditions for the rabbit cervix.

The animals were dispatched by an overdose of Somnopentyl, and the uteri were immediately excised together with the attached portion of the vagina. The dissected specimens were immersed into two kinds of solutions in various concentrations for 24 to 48 hours; nitric acid solution in 15, 20 and 25%, and fuming nitric acid solution in 5, 10 and 15%. After maceration, the specimens were placed in water to remove the macerative solution. With the aid of a dissecting

microscope, the interior or the exterior surface was stripped carefully from the specimen. Since the fine muscular bundles became too soft and fragile to separate from the connective tissue by this macerative method, the macerated specimens were fixed in Bouin's fixative to harden such bundles *in situ* for 24 hours and preserved in 70% ethanol before complete dissection. Although the muscular bundles and connective tissues of these specimens became harder in the fixative, the tissues recovered their softness gradually during the washing process in 70% ethanol. Easier stripping of muscular bundles was attained as a result of such treatments.

Results

1. Macroscopic observations

In the rabbit uterus, two cervical canals were completely separated by a septum, each possessing an independent external os (Fig. 3). The dome-shaped vaginal portions of the cervixes projected into the vaginal lumen, and the space between the cervical projections and the vaginal wall was defined as fornix (Fig. 4). Although there was little difference between the length of dorsal lips of the cervical projections and that of ventral lips, the ventral gap of the vaginal fornix was deeper than the dorsal.

The mucous membrane of each cervix was composed of longitudinal folds (Figs. 1 and 5) leading to the lips of intravaginal cervical projection which formed the rosette-like external os (Fig. 4). The internal os was clearly distinguished from uterine mucosa by the formation of these cervical longitudinal folds (Figs. 7 and 8), but showing no typical rosette-like structure (Fig. 6). It seemed that closing of the internal os by mucosal folds was looser than that of the external os (Figs. 4 and 6). As the longitudinal folds were branching and/or fusing, the number of folds in cross section varied in different regions; 7-9 at the external os and almost 6 at the internal os. The longitudinal folds in the cervix were longer at the mesometrial side (1.70 ± 0.04 cm) than those at the anti-mesometrial side (1.35 ± 0.03 cm).

In contrast, the uterine mucosa was fundamentally characterized by the formation of continuous papillae-like folds which were arranged in parallel and consisted of 6 primary mucosal folds. Occasionally, however, the arrangement of the folds was irregular in some parts of the uterine horn. The surface of the cervical mucosa branched complicatedly, forming cervical crypts (Fig. 9), and the openings of the uterine glands were seen to be situated regularly on the surface of the uterine mucosa.

2. Histological observations

Generally, the uterine mucosa was constructed with 6 primary mucosal folds in cross section, making the form of uterine lumen stellate (Fig. 10). The endometrial epithelium was a simple layer of cuboidal or columnar ciliated cells and non-ciliated cells. The non-ciliated cells were common, and the ciliated cells were seen either solitary or in clusters. The nuclei of the epithelial cells were relatively large, ovoid, and located centrally or near the cellular base. The uterine glands were not well developed, located mainly near the surface of the folds (Fig. 10). There was no clear distinction between glandular and luminal epithelia of the uterus. Leucocytes were found numerous both in the stroma and in the surface epithelium. The endometrial stroma was highly cellular and consisted of wavy collagenous fibers intersecting complicatedly with each other.

The cervical mucosa was characterized by the formation of longitudinal folds which were arranged in parallel from the external os to the internal os. The mucosa branched complicatedly to form fine crypts (Fig. 11). In the cross sections, the cervical mucosa was provided with "fern

Fig. 1. Diagrammatic illustration of the gross morphology of the rabbit cervix cut open to show the inner surface of the mucosa. The right side of the diagram is mesometrial and the left side is anti-mesometrial. Note the cervical longitudinal folds (CF) with many crypts, differentiated from the uterine (U) and the vaginal mucosa (V). The folds are branching and/or fusing, constructing the cervical ora at the both ends. The caudal cervical portion projects into the vaginal lumen to form the vaginal fornix (VF). The external os (EO) shows typical rosette-like structure *in situ* surrounded by the longitudinal folds, while the internal os (IO) is somewhat irregular in shape because of the various lengths of the folds.

Fig. 2. Diagrammatic illustration of the muscular orientation of the rabbit uterus and cervix (dorsal view). A part of the vaginal wall at the vaginal fornix has been turned over to this side, showing the surface of the vaginal inner muscular layer and the circular muscular layer of the caudal cervical portion. In the vaginal portion in this figure, the dorsal lips of the cervical mucosa have been removed to show the circular muscular layer. A: Longitudinal muscular layer. B: Area joined externally with both uterine horns. C: Transitional area from the uterine longitudinal muscular layer to the vaginal muscular layer. D: Vascular layer in uterus. E: Circular muscular layer in uterus. F: Circular muscular layer in cervical portion. G: Circular muscular bundles at the caudal cervical portion. H: Circular muscular layer in vagina. I: Transitional area from cervix to vagina.

leaf-like folds which protruded centripetally into the lumen (Fig. 11). The axial core of each fold was supported by connective tissue and blood vessels. The folds were lined with a layer of tall columnar epithelial cells, which were taller than those in the uterus (20.0 μ m and 14.2 μ m in average, respectively).

Two cell types, ciliated and non-ciliated, were observed in the cervical epithelium similarly to those in the uterine epithelium. The non-ciliated cells were slender in comparison with the ciliated cells. The nuclei of the non-ciliated cells were also slender and those of the ciliated cells were round or oval. The non-ciliated cells were secretory in type, because of storing a large amount of mucin in the supranuclear region, whereas the ciliated cells seemed devoid of mucus. Ordinarily, the nuclei of the non-ciliated secretory cells were located in the basal region of the cell body, but those of the ciliated cells were located centrally. The proportion of ciliated cells varied in different parts of the cervix, with approximately 63% in the external os, 57% in the mid-cervix, and 77% in the internal os.

Although the height of the cervical epithelial cells differed from that of the endometrial epithelial cells, transition of the epithelium from the uterine horn to the cervix was not clear in general. However, based on the features of the folds and the existence of the uterine glands in the stroma, the transition from the uterus to the cervix seemed to occur at the beginning of the cervical longitudinal folds or in the vicinity. In some sections, the transition was clear-cut and the cervical folds were located closely to the uterine folds. In other sections, however, the beginning area of the cervical longitudinal folds showed the characteristics of both uterine and cervical folds (Fig. 12).

The muscular layer was very thick in the uterine horn and the muscular bundles were arranged regularly (Fig. 14). In some part of the cervix, the muscular layer decreased abruptly in thickness and the musculature became loose (Fig. 15). In longitudinal sections, the cervical longitudinal muscular bundles became slender and ended at the outer side of the beginning of the vaginal wall and never appeared to connect with the vaginal musculature (Fig. 13). The circular

Explanation of Figures

- Fig. 3.* General view of the ventral half of cervixes.
Fig. 4. External ora project into the vaginal lumen. The vagina is cut away partially to expose the ora. Note the rosette-like appearance.
Fig. 5. Cervix and uterine horn cut open shows the longitudinal folds in the cervix and continuous papillae-like folds in the uterus arranged in parallel rows. Note the distinct morphology in the internal os.
Fig. 6. Profile of internal os of the cervix. Uterine horn is removed to expose the cervical folds.
Fig. 7. Uterine and cervical folds in a vertical and longitudinal section.
Fig. 8. Internal os region of the cervix (arrow) in the cleared specimen. Note the morphological change between cervical (C) and uterine folds (U).
Fig. 9. Surface of the cervical folds. Note the complex cervical crypts.
Fig. 10. Cross section of the uterus (H. and E., $\times 20$).
Fig. 11. Cross section of the cervix (H. and E., $\times 20$).
Fig. 12. Area of internal os in longitudinal section. Note the transitional fold (arrow) (Azan, $\times 20$).
Fig. 13. Muscular bundles of the cervix have disappeared abruptly (arrow) at the beginning of the vaginal wall (Azan, $\times 20$).
Fig. 14. Longitudinal section of the uterine wall shows well developed musculature (H. and E., $\times 20$).
Fig. 15. Musculature of the cervix in longitudinal section. Slender muscular bundles, abundant connective tissues and well developed cervical crypts were noted (H. and E., $\times 20$).

Anatomical Features of the Rabbit Cervix

Anatomical Features of the Rabbit Cervix

bundles of the cervix also became slender and sparse and decreased in thickness with an increase of connective tissue in comparison with those of the uterus. Some isolated small muscular bundles appeared in the connective tissue between two muscular coats. These isolated and slender muscle bundles seemed to run in various directions. The musculature of the cervix contained much fibrous connective tissue as compared with that of the uterus. The connective tissue was constructed mainly with dense and tightly woven bundles of interlacing collagen fibers.

3. Muscular orientation

Some trials were conducted to obtain good macerative conditions for the rabbit cervix. Favorable results were obtained by using 25% nitric acid or 15% fuming nitric acid solution after 24-hour maceration, and the separation of muscular bundles from each other seemed to be easier in 15% fuming nitric acid solution. Therefore, maceration was made mainly using 15% fuming nitric acid solution.

A diagrammatic illustration of the orientation of muscular bundles in the rabbit uterus and cervix is given in Fig. 2. The mucous membrane was surrounded by a muscular coat, in which two layers were distinguishable according to the direction and disposition of their muscular bundles; namely, the inner circular and the outer longitudinal layer.

The muscular bundles of the outer layer ran in parallel to the long axis of the uterus (Fig. 2, A), except in the area joined externally with both uterine horns where the bundles crossed over to the contralateral horn (Fig. 2, B). The outer muscular bundles of the uterine horns were arranged uniformly and regularly. This outer layer was thinner at the anti-mesometrial side than at the mesometrial side where the outer layer consisted of thicker bundles. Approaching the vagina through the cervix, however, the uterine longitudinal outer musculature became loose and diminished in thickness. The muscular bundles decreased gradually in number and dispersed in the transitional area to the outer side of the vaginal wall (Fig. 2, C). No connection of the longitudinal bundles from the uterine to the vaginal layer was recognized.

Beneath the longitudinal muscular layer, a vascular layer (Fig. 2, D) was found to be developed variously in different positions. This layer was predominant in the peri- and mesometrial sides rather than in the anti-mesometrial side. Therefore, a distinct border between the circular and the longitudinal muscular layers at the peri- and mesometrial sides was recognized macroscopically. At the anti-mesometrial side, on the other hand, the vascular layer was not so clear because it was very thin at this side. There were no bridges of the muscle bundles between the outer and the inner muscular layers in the uterine horn. The inner layer (Fig. 2, E) was well developed and arranged regularly around the long axis to make a tube of rings through the entire uterine horn.

At the cervix, the inner muscular bundles turned markedly slender, arranged sparsely (Fig. 2, F). The amount of connective tissue among them increased remarkably. At the area joined externally with both uterine horns, the inner circular layers of both horns were completely separated from each other by a thick median septum and no bridges of bundles were recognized between them. It was found that some slender muscular bundles originated from the uterine outer muscular layer were elongated into the median septum. These elongated muscle bundles ran longitudinally or obliquely within the dense connective tissue of the septum. The amount of connective tissue in the vascular layer of the cervix was larger than in that of the uterus.

The musculature of the vaginal wall was composed of two main layers, thin outer and thick inner. The outer layer was principally longitudinal and its muscular bundles were arranged very sparsely, crossing over one another. These bundles were slightly woven, more slender than those

of the uterine horn. The inner layer was well characterized by the facts that its interior surface was composed of interlacing fine muscular bundles and that its exterior surface consisted of densely arranged circular fine bundles (Fig. 2, H).

At the vaginal portions of cervix, a pair of cylindrical projections was found to consist of cervical circular muscular bundles (Fig. 2, G). A few of the circular cervical bundles stretched their short branches onto the inner surface of the vaginal wall at the transitional area (Fig. 2, I), although incorporation between the cervical circular bundles and the vaginal musculature was not observed.

Discussion

The correct "timing" of the entry of eggs into the uterus is vitally important for their further survival, and if premature, the eggs are quickly transported through the uterus and expelled into the vagina¹¹⁾. TSUTSUMI and TAKEDA²⁴⁾ found that some of the intrauterine eggs in pseudo-pregnant rabbits were expelled through the cervix into the vagina after 90 hours *p. c.* They considered that some early maternal recognition of the fertilized eggs must be established prior to 90 hours *p. c.* for the persistence of pregnancy, because some mechanisms in the cervix may be involved in interrupting egg transport. However, the mechanism has not been elucidated yet. According to ADAMS' finding¹¹⁾ that the eggs were no longer expelled into the vagina when the uteri had atrophied by castration, some of the blocking mechanisms in the cervix might depend on the anatomical character of the cervix. However, very little information is available on the anatomy of the cervix in relation to the egg transport through the canal.

Our description of the gross anatomy of rabbit cervix was in parallel with the observations by HAFEZ²⁷⁾. The surface morphology of the cervical epithelium of rabbits has been studied scanning-electron-microscopically^{28,29)}. Results of the present histological observations of the endometrium in estrous rabbits were well in agreement with the findings of previous investigators^{30,31)}. The present observations of the cervical mucosa were also in harmony with observations by TSUTSUMI³⁰⁾ and TYLER³²⁾. The proportion of the ciliated cells in the cervical epithelium varied from region to region in such a manner in that they were predominant in the internal os rather than in the mid-cervix and the external os. This tendency was in agreement with that described by HAFEZ and KANAGAWA³³⁾ and KANAGAWA *et al.*²⁹⁾. Although histological observations by many investigators showed no clear transition of the mucosa from the uterus to the cervix in the rabbit (see review by HAFEZ²⁷⁾), our macroscopic observation revealed a clear transition; morphological features of the cervical folds were markedly different from those of the uterine and vaginal folds, as described in the results. The transition, histologically judging from the presence of the uterine glands, seemed to occur around the beginning of the cervical longitudinal folds at the internal os.

There are no observations on the rabbit cervical musculature, as far as we know, except for the description by SAJI³⁴⁾. Our observations of the muscular orientation were well line with his description and were extended more in detail. In the uterus, both inner circular and outer longitudinal muscular layer were well developed, and the muscular bundles were arranged uniformly and regularly. At the transitional portion from the uterus to vagina, the uterine muscular bundles became slender and diminished in size, and the number of muscular bundles decreased gradually. Small muscular bundles running in various directions between the inner and outer coats were found sparsely in the area of the cervix. In the present study, however, the musculature of the cervix was speculated not to act as a sphincter in estrous rabbits on the basis of the architectural pattern of the musculature; strong muscular bundles of the uterus disappeared at the

Anatomical Features of the Rabbit Cervix

proximal end of the cervix and slender muscular bundles were found in the cervix.

The cervical epithelium and musculature may be considered to have some distinct functions relating to the physiological state of ovarian steroids, since it has been demonstrated that eggs are recovered from the vagina in a short time after ovulation in those rabbits which have been treated with estrogens or prostaglandin $F_{2\alpha}^{21-23}$. The rhythmical beat of cervical cilia towards the vagina creates a directional flow of the surface epithelium⁸⁵. Therefore, it seems that intrauterine eggs transported asynchronously to the distal end of the uterine horns are expelled easily into the vaginal lumen with secretory mucus through the cervical canal. Further studies are needed to know the function of the cervix in the early pregnant stage.

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家兎に卵巣ホルモンやプロスタグランジンを処理すると、受精卵が子宮頸を通過して腔に排出されてくることから、子宮頸内の卵子通過の機構について新たな問題が提起されている。本研究では家兎子宮頸の生理・形態学的研究の一環として、非妊娠子宮頸を肉眼的、組織学的に観察し、また筋束の走向も検討した。2本の子宮頸は厚い中隔により完全に分離され、それぞれ腔内に突出して明瞭な外子宮口を形成している。家兎では内子宮口は不明瞭とされていたにも拘らず、肉眼的にはその縦走ヒダにより、子宮粘膜の乳頭状ヒダの連続とは明らかに区別され、かつ縦走ヒダが取り囲んでつぼまり、内子宮口

と称するにふさわしい構造を呈していた。しかし、組織学的には粘膜上皮の移行は突然ではなく、内子宮口部位で行われていた。なお粘膜上皮は単層の高円柱状細胞より成り、線毛細胞ならびに非線毛分泌細胞が認められた。線毛細胞の割合は、子宮頸中央や外子宮口よりも内子宮口で高い傾向が認められた。子宮では内輪走筋および外縦走筋ともよく発達し、その筋束の配列は規則的であった。しかし、頸部ではそれらの筋束の配列は乱れ、筋束は細くなり、数も減じて腔壁の近位端に放散し、腔壁へは連続していなかった。家兎子宮頸は子宮角に比べ、結合組織が豊富であった。