Fertility following cervicectomy and utero-vaginal anastomosis in the rabbit*

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Summary. The role of the cervix in maintenance of pregnancy was investigated following unilateral resection of the cervix and utero-vaginal anastomosis in 23 rabbits. Fertility and morphological studies were made to compare the operated and contralateral intact uterine horns. In 5 animals autopsied 2 days post coitum, 52.6% of the eggs ovulated from the ovaries on the operated side were fertilized compared with 92.9% on the intact side. At laparotomies or autopsies performed 9 days post coitum (18 does), 93 of 104 (89.3%) ova released from the ovaries on the intact side and 55 of 106 (52.1%) ova on the operated side became implanted. There was no marked difference in the spacing and size of implants between the operated and intact uterine horns, but distribution of implants in the operated horns had shifted slightly toward the vagina compared to the controls. When allowed to go to term, pregnant does delivered young normally from the intact uteri; but in the operated uteri dystocia was noted frequently. Morphological observations revealed no abnormality in the endometrium of either uterine horn following operation, but there was occasional inflammatory reaction in the newly formed vaginal portion. The present findings indicate that the rabbit cervix does not always play a significant role in holding embryos or fetuses in the uterine cavity once fertilization has been accomplished.


Introduction

Preimplantation loss of eggs is known to occur even under normal conditions in the rabbit⁴, one cause of which is the expulsion of eggs from the uterus into the vagina, as revealed by TSUTSUMI and co-workers⁵. In order to evaluate the functional contribution of the cervical portion in preventing discharge of preimplantation embryos, investigations of morphological and physiological aspects of the rabbit cervix have been undertaken by SUZUKI and co-workers⁶-¹². Furthermore, our preceding report⁶ described the effect of expanding the cervical canal by installing intact or fenestrated polyethylene tubing (complete or partial exclusion of cervical influence) on fertility. Another approach to study the functional roles of the distinct region of the cervix would be to remove it and make an utero-vaginal anastomosis surgically.

The present investigation was designed to develop such a technique in the rabbit and to examine fertility following cervicectomy. The present paper reports also the results of a detailed morphological observation on the reconstructed region.

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Materials and Methods

Animals

Twenty-three virgin female Japanese White rabbits, 5-7 months old and weighing 2.6-3.9 kg, were used. All of the animals were reared with food and water ad libitum, which were withheld the day preceding surgery. They were subjected to unilateral surgical resection of the cervix, followed immediately by utero-vaginal anastomosis.

Surgical Procedures

Anesthesia was induced with an intravenous (iv) injection of sodium pentobarbital (Somnopenthyl, Pitman-Moore Inc.). The distal part of the reproductive tract was exteriorized through a midventral incision and supported on saline-moistened gauze pads. The larger blood vessels distributed in the area of the cervix were ligated with 6-0 silk or gut sutures. The smaller blood vessels were electrocoagulated using a Mizuho electrosurgical unit (GT-S4U-SM, Mizuho Ikakogyo, Japan) during surgery.

Cervicectomy and reconstructive procedures are diagrammed schematically in Fig. 1. The two uterine horns and cervices were divided at the septum by cutting the musculature of cervix to be removed, from the beginning of uterine jointing portion to transitional portion to the vaginal wall, with electrocoagulation if necessary. The uterine part adjacent to the cervix to be removed was then transected and dissected free of the mesometrium. Subsequently, the cervix attached to the uterine portion was removed by hollowing out circumferentially from the vaginal wall at the portion of vaginal fornix.

![Fig. 1](image-url)  
Schematic illustration of procedure of cervicectomy and utero-vaginal anastomosis in the rabbit. a; Dissection of the cervix. b; Restoration of uterine horn and vagina. A part of the vaginal wall has been removed to show the vaginal lumen in b.

![Fig. 2](image-url)  
Schematic diagram of suturing procedure at the transected end of the uterine horn. S; suture. M; myometrium. E; endometrium.
The lumen of the transected uterine horn was then reduced in diameter by one of the suturing methods shown in Fig. 2. Then, a utero-vaginal anastomosis was performed with interrupted 6-0 sutures. Care was taken to place the sutures through the myometrium, avoiding the endometrium, and to position and maintain a new external ostium protruding into the vaginal lumen for approximately 5 mm (Fig. 1b). The contralateral unoperated tract remained intact to serve as a control.

Fertility Study

Four to 6 weeks after surgery, matings with bucks of proven fertility were made twice, followed by an iv injection of 20 IU of human chorionic gonadotropin. Five does were sacrificed 2 days post coitum (p.c.), in order to examine the effect of removal of the cervix on achievement of fertilization. The remaining 18 does were laparotomized or autopsied 9 days p.c., and the number of corpora lutea and implantation sites was noted. The location and size of the uterine swellings were also recorded.

Morphological Study

From 2 does killed 2 days p.c. and 5 does killed 9 days p.c., the reproductive tracts were excised for morphological evaluation using light (LM) and scanning electron (SEM) microscopy. Tissues for LM and SEM were subjected to the procedure reported previously. SEM examination was performed using a JEOL JSM-200 scanning electron microscope at 15 kV.

Results

Table 1. Effect of cervicectomy on fertilization in rabbits 2 days p.c.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Intact side</th>
<th>Operated side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of corpora lutea</td>
<td>No. of eggs</td>
</tr>
<tr>
<td></td>
<td>Recovered</td>
<td>Fertilized</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
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<td>6</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Totalb)</td>
<td>28</td>
<td>27</td>
</tr>
</tbody>
</table>

| Fertilization ratec) | 92.9% | 52.6% |

a) Fertilized eggs were at the 8–16 cell stages.

b) Data from the operated side of no. 3 doe were excluded, because no eggs were recovered.

c) Ratio of number of fertilized eggs to corpora lutea count.
the intact side, 93 implants were identified with a mean of 5.2 ± 0.4 implants per horn. The mean ratio of implants to corpora lutea count on the intact side was 89.3%.

On the operated side, however, only 12 of the 18 uterine horns became pregnant. Of 106 ova released from ovaries on this side, 55 succeeded in implanting with a mean of 3.0 ± 0.6 implants per horn. The mean implantation rate was 52.1 ± 10.1%. The differences in the mean number and rate of implantation between intact and operated sides were significant (P < 0.02 and P < 0.01, respectively).

Although there was no marked differences in the spacing and size of the uterine swelling between the intact and operated horns, the median location of implantation sites in the operated horns had shifted slightly toward the vagina compared to that in the control horns (56.0 ± 1.5% vs. 51.3 ± 1.0% of cornual length from the utero-tubal junction, P < 0.02; data in the operated side were from 10 horns except for 2 having only one implant). Several implants were even located near the vaginal end of the uterine horn, adjacent to the anastomosis area.

When 9 females were permitted to go to term after laparotomy, normal parturition occurred on the intact side, but dystocia occurred on the operated side in 4 does because of insufficient dilation at the anastomosis site of uterine horns.

**Morphological Study**

Reconstructive surgery did not result in occlusion of the new external ostium, consisting of uterine tissue only. LM examination, as well as gross inspection, demonstrated a propensity of the new vaginal portion of the uterus to hypertrophy by at least 30 days following surgery. The external surface of the new vaginal portion of the uterus was found to be covered with endometrial tissue, which became everted and connected to the vaginal mucosal tissue around the anastomosis area (Figs. 3 & 4). The subepithelial stroma in the new vaginal portion of the uterus contained much connective tissue and many leukocytes, showing productive and exudative inflammation. Leukocytes had frequently invaded the uterine epithelium in the external surface of the new vaginal portion. Transition of epithelium from the uterus to the vagina could be identified as a small zone (Fig. 4). The uterine muscular layer had clearly ended in the mucosa of the newly formed vaginal portion of uterus.
and was separated from the vaginal muscular layer.

SEM showed uterine mucosal tissue overlying the vaginal epithelium in a serrated fashion (Figs. 6 & 7), like an invasion of pseudopodia. On examination at a higher magnification, the uterine luminal epithelium in the operated area showed a normal distribution of non-ciliated and occasional ciliated cells, and did not differ from contralateral control tissue.

In the specimens from rabbits 9 days p.c., uterine epithelial cells had been converted into the multinucleated cells, even in the area facing the vaginal lumen (Fig. 5).

**Discussion**

Morphological examination demonstrated no marked difference in the endometrium of the operated horn from that of the control uterine horn, or from that in intact does. However, the uterine tissue facing the vaginal lumen on the operated side displayed an inflammatory reaction of various degrees, which may have caused considerable variation in the rates of fertilization and implantation on this side. Resection of the cervix was shown to reduce fertilization rate from a mean of 92.9% in the intact horns to 52.6% in the reconstructed horns, and implantation rates from 89.3% to 52.1%. It is noteworthy that the implantation rate was essentially identical to the fertilization rate. This fact suggests that any failures in implantation were due to failures in fertilization. Alternatively, maintenance of pregnancy in the rabbit is not affected by removal of the cervix if fertilization is accomplished.

Previous studies by Suzuki et al. suggested that expulsion of preimplantation embryos is dependent upon ciliary action of the cervical epithelium rather than a hydrostatic pressure generated by gentle uterine motility. Therefore, it is reasonable to speculate that embryos could not be discharged from the uterus into the vagina in the rabbits following cervicectomy, because there would be no such ciliary action. The present results may support the hypothesis that intrauterine eggs which have been transported rapidly or asynchronously to distal ends of the uterine horns are expelled easily into the vagina, floating in a directional stream of secretory fluid caused by the rhythmic beat of cervical cilia towards the vagina.

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**References**


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子宮頸を切除し子宮・膣吻合術を施した家兔の受胎能について

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片側子宮頸を切除了のも子宮・膣吻合術を施した23羽の家児で、受精能および妊娠維持に対する子宮頸の役割を検討した。交配後2日目に屠殺した5羽では、処理子宮頸側（対照側）の受精率92.9％に対し、子宮頸摘出側（吻合側）では排卵数の52.6％が受精していた。交配後9日目の再御手術または屠殺による検査では、対照側の排卵数104個中93個（89.3％）が、また吻合側の106個中55個（52.1％）が着床していた。子宮角における着床部位の間隔や、その大きさには吻合側と対照側間の著差はなかったが、吻合側で全体として着床位置が仮側にやや片寄っている傾向がみられた。分娩までの経過をみると、対照側子宮角では正常に分娩したが、吻合側ではしばしば難産を認めた。両側子宮角の内膜には形態学的異常はみられなかったが、吻合側の子宮腔部に時折炎症性の反応を認めた。以上の結果、卵が正常に受精すれば、着床し、分娩に至るものもあるので、家児子宮頸は子宮内の胚や胎児の保持に必ずしも重要な役割を果していないものと推測された。

Explanation of Figures
(The bar in each figure shows 100 μm.)

Plate I

Fig. 3. LM appearance of an anastomosis site 30 days following surgery (2 days p.c.). Arrows point to the site of utero-vaginal anastomosis. E; endometrium in the vaginal lumen. V; vaginal epithelium. H. E.

Fig. 4. Higher magnification of the anastomosis area in Fig. 3. Transition of epithelial cells has occurred within a small zone. H. E.

Fig. 5. LM appearance of a new vaginal portion of uterus from a rabbit 9 days p.c. Conversion of endometrial cells into multinucleated cells is evident. H. E.

Plate II

Fig. 6. Low-magnification SEM view of an anastomosis site 30 days following surgery (2 days p.c.). Arrows point to the site of utero (U)-vaginal (V) anastomosis. Remarkable distortion is not seen.

Fig. 7. Higher magnification of an area in Fig. 6. Considerable regeneration of endometrium (E) is evident, looking like overlying vaginal epithelium (V).