Effect of Ovariectomy in the Early First Half of the Diestrus on Induction or Maintenance of Canine Deciduoma

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ABSTRACT. The effect of ovariectomy in the early first half of the diestrus was examined on the induction or maintenance of suture-induced canine deciduoma. Ovariectomy immediately, or some days, after the insertion of suture had no effect on the induction or maintenance of deciduoma. Even when ovariectomy was performed within 4 days before insertion, deciduoma could be induced in spite of there being no ovary. However, when ovariectomy was done 4 or more days before suture insertion, the rate of deciduoma was decreased or no deciduoma was induced. These results indicate that the influence of the ovary on the endometrium may persist for at least 4 days after ovariectomy. Ovariectomy after the suture insertion had few effects. It is suggested that canine uterine glands in the early first half of the diestrus maintain a certain degree of self-proliferative ability even after ovariectomy, and thus canine deciduoma is not as dependent on the ovary that of the rodentia. — **KEY WORDS:** canine deciduoma, maintenance, ovariectomy.

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In the rodentia, generally, deciduoma can be induced under functional corpora lutea [9]. After ovariectomy, however, it is degenerated and cannot be maintained [6].

The canine physiological decidual reaction in pregnancy is characterized by the proliferation and dilatation of the uterine glands [1–3]. This glandular proliferation in the process of placentation needs the ovaries; i. e. ovarian hormones are indispensable for the maintenance of pregnancy [12]. Furthermore, it is recognized that canine glandular proliferation and dilatation and/or cystic glandular hyperplasia in the endometrium can be reproduced by the administration of the ovarian hormones [7, 13]. Canine deciduoma is also represented histologically by glandular proliferation and dilatation including cystic glandular hyperplasia reflecting the normal histology of the placenta [10].

The present study was designed to clarify the influence of the ovariectomy in the early first half of the diestrus on the induction or maintenance of the canine deciduoma.

Experimental animals: Forty-five mongrel bitches in estrus were used. These bitches were clinically healthy and 8 to 12 kg in body weight. They were daily checked for their sexual behavior and their vaginal smears were taken to determine the first day of diestrus (Day 1). Day 1 was determined by the refusal behavior of male dogs and vaginal cytology [8], and was used as the starting point for the following treatments. Before treatment, the bitches were laparotomized and both ovarian tubes were ligated, to prevent pregnancy, under general anesthesia with xylazine hydrochloride and ketamine hydrochloride. These bitches were divided into 9 groups each of which consisted of 5 bitches for the experimental procedures described below. In all bitches, the left horn was used for the test and the right horn was left intact as the control. At the end of the test (12 days after insertion of the silk suture), the bitches were laparotomized again and their uteri were removed. All experimental surgeries were performed under the standard aseptic techniques and a daily injection of an antibiotic

(aminophenyl acetamido penicillanic acid 10 mg/kg/day) was used to prevent bacterial infection until the experiment was terminated.

Experimental groups: [Group 1] a silk suture was inserted into the left horn at Day 12, and ovariectomy was not performed (positive control). [Group 2] silk suture insertion was performed at Day 12, and ovariectomy was done at Day 18 (6 days after insertion). [Group 3] ovariectomy and silk suture insertion at Day 12 were performed immediately after ovariectomy. [Group 4] ovariectomy was performed at Day 8, and silk suture was inserted at Day 12 (4 days after ovariectomy). [Group 5] ovariectomy was performed at Day 5, and silk suture was inserted at Day 12 (7 days after ovariectomy). [Group 6] ovariectomy was performed at Day 1, and silk suture was inserted at Day 12 (11 days after ovariectomy). [Group 7] ovariectomy was performed at Day 5, and silk suture was inserted at Day 9 (4 days after ovariectomy). [Group 8] ovariectomy was performed at Day 3, and silk suture was inserted at Day 7 (4 days after ovariectomy). [Group 9] ovariectomy was performed at Day 1, and silk suture was inserted at Day 5 (4 days after ovariectomy).

Ovariectomy: A second or third laparotomy was performed under the same method of general anesthesia as that of the first operation, and then both ovaries with a piece of cranial uterine horn (about 1 cm long) were removed by the routine surgical method. The removed piece of uterine horn was reserved as the control for the uterine condition at the time of ovariectomy.

Insertion of silk suture: A laparotomy was performed under general anesthesia and a surgical suture (0.6 mm in diameter) was inserted into the uterine lumen through the uterine wall at the point of cranial one-third of the left uterine horn, and the head of the suture was pulled out at the caudal part of the left uterine horn closed to the uterine body. An intraluminal suture was placed loosely in the lumen and the ends of the suture were not tied in order to prevent tension trauma (linear sawing) to the uterus. Three

to five cm ends were left on each end of the suture. The cranial one-third of the left horn and the right horn were left intact and used as a control.

Macroscopic and microscopic observations: The bitches underwent routine hysterectomy at 12 days after inserting the silk suture. The uteri and any contents in the uterine lumina were examined for gross lesions. The uteri were fixed in 10% buffered formalin solution immediately after macroscopic observation. Some pieces of tissues from the uteri and the ovaries were taken and sliced from each organ, embedded in paraffin and sectioned for histological preparations. They were stained with hematoxylin and eosin (H-E) plus periodic acid Schiff (PAS), and Azan-Mallory Heidenhain stains. From the results of the microscopical test, a judgment for positive reaction was given if a stronger degree of glandular proliferation and dilatation and/or cystic endometrial hyperplasia (CEH) [4, 10] could be observed at the tested portions than that of the control parts (intact right uterine horn and the cranial one third of the left horn). The degree of the histological changes was classified into severe (++++), marked (+++), moderate (++) , weak (+), slight ($\pm$) and negative (-) in order of the magnitude of the histological reaction.

Induction rate of decidualoma: The results are shown in Table 1. The induction rate of decidualoma was kept at a high level (100%) in the groups ovariectomized after insertion of the silk suture (Group 2–3). The decidualoma induction rate of the groups which underwent ovariectomy before insertion decreased gradually according to the duration from ovariectomy to insertion (Group 4–6). However, when the insertion interval was within 4 days (Group 7–9), the decidualoma induction rate was maintained at a high level (80–100%), although the ovariectomy at the earliest stage of diestrus (Day 1) decreased the decidualoma induction rate even though the interval to insertion was within 4 days (Group 9).

Macroscopic findings of the uterus: In the left horn of all groups, the region which was in contact with the suture was hypertrophic when compared with the control. Especially in groups 1, 2 and 3, the appearances of the uteri contained a suture formed segments which looked like a rosary. Among these groups, hyperplastic parts in each left horn

were almost the same diameter in spite of the difference of the stage at hysterectomy. However, the bitches in which decidualoma could not be induced (almost all bitches in group 6 and only one bitch in each of groups 5 and 9) showed no hyperplasia of the uterus. In each of these dogs, a long straight narrow appearance as can usually be seen in the normal uterus at the middle diestrus was shown, however the uterus was a little more hyperplastic than the normal. Marked differences were seen in the endometrial mucosa among groups. In groups from 1 to 4, 7 and 8, these endometria showed marked hyperplasia and had velvet-like appearances with some folds. Some of them held many small vesicles on the folds. On the other hand, in some bitches of groups 5 and 6, the endometrium was slightly edematous with a little hyperplasia. In all groups, the muscle layer was unaffected.

Microscopical findings of the uterus: The results of the histological observation are shown in Table 2. The groups ovariectomized after insertion of the suture (Groups 2 and 3) showed a tree branch-like hyperplasia of the superficial endometrium with cystic glandular hyperplasia in the basal layer. This finding was similar to the so-called “Swiss cheese endometrium” which was the condition of the positive control (Group 1). On the other hand, the groups ovariectomized before insertion (Group 4–9) showed weaker proliferation of the glands than that of the former groups. Of the latter groups, however, the bitches ovariectomized within 4 days before insertion (Group 4 and Group 7–9) showed stronger reactions in the proliferation and dilatation of the glands than these of each of the control parts of the endometrium. However, these reactions of the glands in the stimulated endometrium did not expand over the layer corresponding to that in the control endometrium where the differentiation of the glands was interrupted by the ovariectomy. The groups ovariectomized before 7 days or more prior to insertion (Groups 5 and 6) showed the weakest reaction of the glands of all groups and had few differences with each control endometrium.

Microscopical findings of the ovaries: The ovaries in groups 6, 8 and 9 contained some early developing corpora lutea which had small cavities in the center surrounded by newly formed luteal cells. The other groups had some

Table 1. Induction of decidualoma by inserting silk suture before and after ovariectomy.

Group	Ovariectomy at diestral day	Insertion of silk suture at diestral day	Days before or after ovariectomy	Induction rate of decidualoma Positive/No. of bitches (%)
1	NO	12	NO	5/5 (100)
2	18	12	+ 6	5/5 (100)
3	12	12	0	5/5 (100)
4	8	12	- 4	5/5 (100)
5	5	12	- 7	2/5 (40)
6	1	12	-11	0/5 (0)
7	5	9	- 4	5/5 (100)
8	3	7	- 4	5/5 (100)
9	1	5	- 4	4/5 (80)

NO: No ovariectomy, Day 1: The first day of diestrus, -: Before, +: After.

Table 2. Histological findings in each group

Histological findings / Group		1	2	3	4	5	6	7	8	9
Functional layer	Glandular proliferation	+++	+++	++++	+	+~±	-	+	+	+
	Glandular dilatation	+++	+++	++	+	+~±	-	+	+	+
	Degree of glandular secretions	+++	++	++	+	+	±	++	+	+
Stroma	Edema	+	±	±	±	±	±	-	-	±
Basal layer	Glandular proliferation	+++	+++	+++	++	+~±	-	++	+	-
	Glandular dilatation	++	++	+++	++	+~±	-	++	+	-
	Degree of secretions	++	+	+	+	±	-	±	±	-
Degree of cystic endometrial hyperplasia		+++	+++	+++	++	+~±	-	++	±	-

++++: Severe, +++: Marked, ++: Moderate, +: Weak, ±: Slight, -: Negative.

mature corpora lutea in each ovary, which had mature luteal cells with generally no cavity in the center of the tissue.

In these experiments, as the Table 1 shows, the decidual reaction did not regress in the groups 2 and 3 which underwent ovariectomy after stimulation, that is, once the decidual reaction was induced, no ovary was needed for maintenance or advance of the decidual reaction. When the interval from ovariectomy to stimulation is within 4 days, as shown in the groups 4, 7, 8 and 9, the decidual reaction was maintained at a high rate. However, as the results from groups 5 and 6 show, when the interval became more than 4 days, the reaction gradually decreased and at last disappeared. That is, the uterine receptivity for stimulation remains at least for 4 days, even when the ovaries were removed. From the results of group 9, however, even if the interval was within 4 days, the reaction seemed to regress when the ovariectomy was performed at the early stage of corpora lutea in the diestrus (e.g., day 1). These results show that the uterine receptivity for stimulation may be maintained for a certain time after ovariectomy, though, it may vary with the time of ovariectomy and interval from ovariectomy to stimulation, i.e., the uterine receptivity may have a period of validity.

Histological examination revealed that the control uterine endometrium in the right horn was also influenced by ovariectomy. That is, the histological changes especially in the glandular differentiation at hysterectomy were different among the groups, however, the individual findings showed no advance from the conditions seen at the time of ovariectomy. These results may show that ovariectomy may arrest the advance of the growth and differentiation of the uterine glands to the same level as just before ovariectomy. The condition may be maintained until hysterectomy, if the interval from ovariectomy is not so long. Therefore, the endometrial histology, especially in the uterine glandular differentiation in the control horn, may reflect the degree of the ovarian activity at the time of ovariectomy.

In the groups which were ovariectomized before the suture insertion (Group 4–9), generally the stimulated endometrium brought about a stronger endometrial hyperplasia with glandular dilatation than that of the control.

However, histologically their glandular hyperplastic region could not exceed beyond the layer of the control endometrium which showed the interruption of the growth of the glands by ovariectomy. These depth of the histological reaction in the stimulated endometrium may also be limited by ovariectomy as well as the control endometrium. Whereas, in the bitches which received ovariectomy after insertion of the suture (Groups 2 and 3), the control uterine glands fully differentiated, and also the stimulated endometrium reacted in the whole layer and brought about the so-called “Swiss cheese endometrium”. That is, the degree of the reaction in the endometrium may depend on the function of the corpora lutea at the time of ovariectomy, especially in the bitches which underwent ovariectomy before insertion of the suture.

The condition of the corpora lutea at Day 1 showed histologically a mass of tissue containing newly formed luteal cells even though small cavities remained in the center of the tissue. From the reports of canine blood progesterone levels [5, 11], it was estimated that the progesterone level at Day 1 was low (about a half of the peak value). These findings indicate the corpora lutea in this stage might be immature and inadequate in function compared with that of the implantational phase. It is therefore supposed that the ovary at Day 1 may not be able to provide sufficient endometrial receptivity for stimulus throughout the whole endometrium, and so the ovary at Day 1 may not be able to induce a typical decidual reaction, the so-called “Swiss cheese endometrium”, as was seen throughout the endometrium at the implantational phase (at about Day 12) [8]. The ovaries at Day 5 had some corpora lutea which histologically was composed of compact masses with newly formed luteal cells but few central cavities. Judging from the histology and blood progesterone level [5, 11], the ovaries may be more functional than at Day 1. At Day 5, the control endometrium in the right horn showed enough differentiation of the uterine glands in the whole endometrium. Therefore, the endometrium at Day 5 may have enough receptivity for stimulus, and the uterine glands in the left horn could react to the stimulus fully throughout the whole endometrium. At Day 12, the ovary may have

full function and the control endometrium may have the ability to differentiate the glands fully. The stimulated endometrium at this stage could demonstrate entire uterine glandular differentiation and may show the cystic endometrial hyperplasia in the whole endometrium, so-called "Swiss cheese endometrium".

In conclusion, it is suggested by this study that, if once a decidual reaction was started by artificial stimulation, ovariectomy had little effect on maintenance of the deciduoma. Whereas, if the stimulation was done after ovariectomy, whether and to what degree canine deciduoma can be induced or not may depend on the length or strength of the residual capacity of the endometrial reactivity after ovariectomy. Furthermore, length or strength of the reactivity may vary with the functional condition of the corpora lutea at the time of ovariectomy. Further studies on the hormonal background may help to clarify this.

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