Effects of Ovarian Hormones on Manifestation of Purulent Endometritis in Rat Uteruses Infected with *Escherichia coli*

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To assess the influence of hormones on uterine infections, *Escherichia coli* was infused into uterine lumens of ovariectomized or adrenoovariectomized rats receiving exogenous administration of various doses of ovarian hormones. Large numbers of *E. coli* were recovered from the rat uterine lumens, irrespective of hormonal influences. The number of leukocytes in the uterine flushings, representing the magnitude of purulent inflammation, differed significantly depending upon the hormonal regimen given to each host. Purulent endometritis was induced by *E. coli* in ovariectomized rats receiving progesterone or corn oil (hormone vehicle). Infections were asymptomatic in rats receiving estradiol, but promethazine-treated uterine horns were susceptible to infection. When progesterone was administered along with estradiol, purulent inflammation was caused by *E. coli*, but the number of leukocytes in the uterine lumens was significantly less than that obtained from the rats treated with progesterone or corn oil. These effects of ovarian hormones on uterine infections were observed in adrenoovariectomized rats as well as in ovariectomized rats. It is suggested that estradiol alters the nature of endometrial epithelium and prevent manifestation of purulent endometritis; progesterone antagonizes estradiol. Adrenal hormones appear not to participate in the pathogenesis of endometritis induced by *E. coli*.

In rabbits (9), sheep (10), and cattle (8), uteruses in the luteal phase were more vulnerable to infection than those in the follicular phase. In women, increased susceptibility to chlamydial infections (11), development of vaginal candidiasis (5, 22), and varying rates of the gonococcus isolation from cervical culture (17) were associated with the use of oral contraceptives or phases of the menstrual cycle. Although these reports suggest that ovarian hormones are implicated in the alteration of the course of genital infections, hormonal influence has only been demonstrated in limited laboratory models (3, 16, 24). A previous report has indicated that rat uteruses are another model for investigating the pathogenesis of uterine infection (21), in which *Escherichia coli* inoculated at diestrus or pseudopregnancy induced purulent endometritis but when inoculated at proestrus, endometritis caused asymptomatic infection. Although progesterone is a major ovarian hormone in pseudopregnancy, estrogenic effects are predominant at proestrus. Therefore, it was probable that these ovarian hormones were involved in the pathogenesis of uterine *E. coli* infection in rats.

Corticoids influence the course of experimental microbial infections in laboratory animals (6, 7, 13–15, 18, 20), and cortisol inhibits the physiological action of estrogens (23, 28).

The purpose of the present study was to clarify the relationship between hormones and uterine infections in rats. To estimate the influence of ovarian hormones, spayed rats were treated with the hormone and inoculated with *E. coli*. Adrenoovariectomized rats were used to examine whether adrenal glands play any part in the influence of ovarian hormones on uterine infections. The effects of estradiol on the uterine infection were also examined on the endometrial epithelium of the uterus which was chemically sloughed off. Hormonal influences on uterine infections were evaluated on the basis of the numbers of *E. coli* and leukocytes found in uterine contents and by histological observation.

**MATERIALS AND METHODS**

**Animals.** Virgin female Wistar rats, 7 to 8 weeks old, were obtained from the Shizuoka Agricultural Cooperative (Shizuoka, Japan). They were caged in groups of six, in a room at ca. 23°C with a lighting cycle of 7:00 a.m. to 7:00 p.m. Rats were preconditioned in the room for at least 2 weeks. Feed and water were available ad libitum. A total of 145 rats were used. Unless otherwise indicated, each group in an experiment consisted of five animals. Ovariectomy was performed through lateral incisions in rats under pentobarbital anesthesia. Adrenalectomy was conducted through dorsal incisions concurrently with the ovariectomy, and saline, instead of water, was available to these rats.

**Hormonal regimens.** Estradiol and progesterone were purchased from Sigma Chemical Co. (St. Louis, Mo.). At 2 weeks after ovariectomy, these hormones were administered subcutaneously in 0.1 ml of corn oil. Details in each experiment are described in the figure legends.

**Bacterial strain.** A strain of *E. coli* (O125, H1) isolated from the pus of a dog suffering from pyometra was used (21).

**Preparation of inoculum.** *E. coli* was grown on Tryptosoya agar (Nissui Pharmaceutical Corp., Ltd., Tokyo, Japan) at 37°C for 18 to 20 h and suspended in saline to contain ca. 10⁶ CFU per 0.02 ml.

**Uterine infection.** Uteruses were exposed surgically under the anesthesia. Both uterine horns of each animal were ligated at the cervical ends to prevent possible drainage of uterine contents. An *E. coli* suspension (10⁶ CFU) was inoculated into the lumen of one uterine horn through the uterine wall at the utero-tubal junction. The other horn was left uninoculated. At 24 h after injection, the rats were exsanguinated by cardiac puncture. The plasma was separated and stored for measurement of corticosterone levels.
Numbers of E. coli and leukocytes in the uterine contents. The inoculated horn was flushed with 1.0 ml of sterile saline solution into a test tube and dispersed by being pressed through syringe needles. A 10-fold dilution of the flushings was made serially in saline solution. A 0.1-ml sample was transferred to a petri dish and mixed with about 20 ml of melted Endo agar. The plates were incubated at 37°C, and the number of lactose-fermenting colonies was recorded to be E. coli, because no bacteria indigenous to uterine lumens of ovariectomized rats were detected. Leukocytes were counted by using a bacteria counting chamber (Erma Co., Tokyo, Japan) and expressed as the number of leukocytes in 0.02 μl of flushing. The noninoculated horn of the uterus was isolated, cleared of fat, and weighed with a torsion balance. Then, the uterine luminal fluid was eliminated, and the weight of the horn was measured again. The secretion index was calculated with the following formula:

\[ \text{Secretion index} = \frac{(B - A) \times 100}{A}, \]

where \( A \) represents the weight of uterine horn after the elimination of uterine luminal fluid and \( B \) is the weight of uterine horn before the elimination.

Protein binding assay. Corticosterone was measured by the protein binding method (32). Briefly, each of the plasma samples was extracted first with methylene chloride (5.0 ml). Then, the extract was dissolved in 25% ethanol (1.0 ml). This solution was partitioned by carbon tetrachloride (5.0 ml, twice), and then, the carbon tetrachloride layers were again reversibly partitioned with 50% methanol (5.0 ml). The methanol layer was extracted by methylene chloride (5.0 ml). Methylene chloride was transferred into a test tube and dried under nitrogen gas. To each tube, 1.0 ml of 0.05 M borate buffer solution containing 0.7% canine serum and [3H]corticosterone (New England Nuclear Corp., Boston, Mass.) was added (ca. 5,000 cpm/ml). After being agitated for a few seconds, the test tubes were placed in a 37°C bath for 5 min with constant agitation and cooled in an ice-water bath for 15 min. Dextran-coated Florisil (40 mg) was then added to each tube to separate the unbound [3H]corticosterone. The tubes were agitated vigorously for 60 s in the bath and kept there until the Florisil settled. A 0.5-ml portion of supernatant was transferred into vials containing 5 ml of scintillator solution, and radioactivity was counted in a Aloka liquid scintillation spectrometer. The concentration of corticosterone was read from the processed standard curve. The interassay coefficient of variation was 13% (\( n = 6 \)).

Histological study. Rats received estradiol (0.1 μg/day) or progesterone (1 mg/day) or both estradiol and progesterone regimens for 3 days. Preliminary dose-response experiments revealed that these doses of hormones are enough to affect the course of uterine infection with E. coli (See Fig. 2 and 4). One uterine horn was inoculated with viable E. coli, and the other horn was injected with the Formalin-killed organisms. At 24 h after inoculation, the rats were killed, and the entire uterus was fixed in 10% Formalin, sectioned, and stained with hematoxylin-eosin.

Promethazine-hydrochloride treatment. Five rats were injected with estradiol (0.1 μg/day) for 3 days. Before inoculation with E. coli, promethazine (1 mg in 0.02 ml of saline) was infused into the lumen of right uterine horn through the uterine wall near the utero-tubal junction. Promethazine, supplied by K. Tsuchiya (Takeda Chemical Industries Ltd., Osaka, Japan), causes massive sloughing of the epithelial layer of endometrium (30). At 30 min later, the horn was flushed with four 1-ml portions of saline; E. coli was then inoculated into the horn (promethazine plus E. coli). The left horn, as a control, received only saline flushings without the promethazone treatment and was inoculated with E. coli. The other group of five rats were also treated in a similar manner as described above, except that the rats were not inoculated.

RESULTS

Effects of ovarian hormones on uterine infection. A large number of E. coli organisms were recovered from the uterine horn of each rat (Fig. 1). In rats receiving progesterone or corn oil, large numbers of leukocytes were observed in the uterine contents. Few leukocytes were recovered from uterine lumens of rats injected with estradiol alone. When progesterone and estradiol were administered simultaneously, the number of leukocytes found in the lumen was

FIG. 1. Influence of ovarian hormones on the number of E. coli and leukocytes recovered from the uterine lumen and influence of the hormones on the secretion index. Ovariectomized rats were injected daily with 1.0 μg of estradiol (E) or progesterone (P) or both with 0.1 ml of corn oil. Control rats (C) were administered with 0.1 ml of corn oil alone. On the last day of the 3-day treatment, E. coli was inoculated into the uterus. Animals were killed 24 h after inoculation. The bars represent the mean of five animals per group, and the vertical lines on the bars indicate the standard deviation.
and a increased, on estradiol (Fig. 2). The effects of different doses of estradiol on the number of E. coli and leukocytes recovered from the uterine lumen and effects on the secretion index. Estradiol (0.001 to 1.0 µg) in corn oil was administered daily for 3 days. On the last day of the 3-day treatment, E. coli was inoculated into the uterine horn. Animals were killed 24 h after inoculation. Each bar represents the mean ± standard deviation of five rats in each group.

greater than that of the rats treated with estradiol alone (P < 0.001), but still fewer than that in rats administered progesterone or corn oil alone (P < 0.02).

Five animals receiving each of the four kinds of hormonal regimens were inoculated with Formalin-killed E. coli. Leukocytes were hardly found, irrespective of hormonal regimens; no indigenous bacteria which formed colonies were detected (data not shown).

Effects of different doses of estradiol on uterine infection. As the amount of estradiol increased, the number of leukocytes decreased, and the volume of uterine luminal fluid secreted increased (Fig. 2). A 0.1-µg amount or more of estradiol prevented purulent inflammation. Mean concentrations of corticosterone in each group ranged from 177 to 245 ng per ml of plasma, and there was no correlation between the dose of estradiol and the level of corticosterone.

Time course of the effect of estradiol treatment on uterine infection. As the number of estradiol treatment increased, the secretion index increased, and the number of leukocytes decreased (Fig. 3).

Effects of different doses of progesterone administered with estradiol on uterine infection. When the dose of progesterone increased, a progressive increase in number of leukocytes and a decreasing volume of uterine luminal fluid was observed (Fig. 4). A 1.0-mg amount or more of progesterone abolished the effect of estradiol. Mean concentrations of corticosterone in each group ranged from 162 to 210 ng per ml of plasma. There was no correlation between the dose of progesterone and the level of corticosterone.

Time course of the effect of progesterone treatment on uterine infection. When progesterone was given for all 3 days, a large number of leukocytes were observed (Fig. 5).

Histological findings. No histopathological changes were observed in uteruses of the estradiol-treated rats after inoculation with viable E. coli (Fig. 6A). When progesterone was administered concurrently with estradiol, leukocytic exudate was found in the uterine lumens (Fig. 6B). In groups receiving progesterone or corn oil, induction of purulent endometritis was apparent (Fig. 6C). When Formalin-killed E. coli was injected, no inflammatory changes occurred, regardless of hormonal treatments (Fig. 6D and E).

Effect of ovarian hormones on uterine infection in adrenoovariectomized rats. Adrenoovariectomized rats received estradiol (0.1 µg/day) with or without progesterone (1.0 mg/day) for 3 days. On the last day of a 3-day treatment, E. coli was inoculated into the uterine lumen. Few leukocytes (1.8 ± 1.8/0.02 µl of flushing; n = 7) were recovered from uterine horns of rats injected with estradiol alone. When
progesterone was administered along with estradiol, significantly large numbers of leukocytes (97.0 ± 51.0/0.02 μl of flushing; n = 8) were detected. Complete removal of adrenal was confirmed by the protein-binding assay. Results indicated no corticosterone in the plasma which was collected at autopsy.

Effects of pretreatment with promethazine-hydrochloride on uterine infection. Despite treatment with estradiol, E. coli caused purulent inflammation in promethazine-treated horns (Fig. 7). A large number of leukocytes and E. coli organisms were recovered from the lumen. Few leukocytes were found in the control horn. Promethazine-treatment alone did not increase the number of leukocytes (PZ).

**DISCUSSION**

The results of present study show that estradiol prevents the manifestation of purulent endometritis, and progesterone antagonizes the effects of estradiol. Formation and accumulation of uterine luminal fluid caused by estrogens have been well established (1, 12, 19, 25); thus, the secretion index was introduced as an indicator of the physiological response of uteruses to estradiol. In spite of uterine infection with E. coli, estradiol prevented purulent endometritis at doses that are known to induce an accumulation of uterine luminal fluid as indicated by the increased secretion index. Progesterone injected along with estradiol antagonized the dose-dependently inhibitory effect of estradiol on the manifestation of purulent endometritis. Progesterone antagonizes the physiological effects of estrogens (31); therefore progesterone may permit E. coli to cause purulent inflammation by inhibiting the effects of estradiol.

Significant changes in corticosterone levels were not induced by the administration of estradiol or progesterone in ovariectomized rats. In adrenoovariectomized rats as well as in ovariectomized rats, estradiol prevented E. coli from causing purulent endometritis. It seems evident that adrenal glands are not involved in the effect of ovarian hormones on the course of uterine infections.

Because E. coli injected into the uterus at proestrus-estrus caused no histopathological changes (21), it is likely that estrogens are responsible for the prevention of endometritis during proestrus-estrus.  

![Diagram](http://iai.asm.org/)

**FIG. 4.** Effect of estradiol and progesterone on the number of E. coli and leukocytes recovered from the uterine lumen and effect of the hormones on the secretion index. Ovariectomized rats were injected with estradiol (0.1 μg/day) and different doses (0.125 to 2.0 mg/day) of progesterone for 3 days. On the last day of the 3-day treatment, E. coli was inoculated. Each bar represents the mean ± standard deviation of five rats in each group.

![Diagram](http://iai.asm.org/)

**FIG. 5.** Time course of the effect of progesterone administered along with estradiol. Ovariectomized rats were injected with estradiol (0.1 μg/day) for 3 days. Progesterone (1.0 mg/day) was administered along with estradiol on all 3 days (3E + 3P), on the last 2 days (3E + 2P), or on the last day (3E + 1P) of a 3-day estradiol treatment. On the last day of the 3-day treatment, E. coli was inoculated. Each bar represents the mean ± standard deviation of five rats in each group.
FIG. 6. Histological changes 24 h after the inoculation with viable (A, B, and C) or Formalin-killed (D and E) E. coli. Ovariectomized rats were injected daily with 0.1 μg of estradiol in 0.1 ml of corn oil (A). In the other groups (B and D), progesterone (1.0 mg/day) was administered along with estradiol. Controls received only corn oil (C and E). On the last day of a 3-day treatment, E. coli was inoculated. (A) The uterine horn is filled and dilated with uterine luminal fluid. No inflammatory changes are observed. (B) Viable E. coli caused purulent inflammation. Cellular debris are seen in the uterine lumen. (C) Inoculation of viable E. coli resulted in purulent endometritis. Pus in the lumen dilated the uterine horn. (D) and (E) No histological changes are seen. Hematoxylin-eosin staining. Magnification, ×20.
The mechanisms by which uterine infection does not bring about endometritis under the influence of estradiol still remain to be elucidated. Because Roth et al. (26) observed that even suprapharmacological doses of estradiol in vivo failed to affect neutrophil function and total and differential leukocyte counts, it seems unlikely that estradiol suppresses acute inflammation of rat uteruses through systemic host responses such as leukocytopenia or neutropenia. The finding that purulent inflammation was observed in estradiol-treated rats when E. coli was infused into the promethazine-treated horn of the uterus suggests that suppression of purulent endometritis by estradiol must be a localized effect in uteruses. Asymptomatic infection in estradiol-treated rats may be accounted for by the lack of susceptibility of the uterine epithelium to infection by E. coli.

Bacterial adherence to mucosal surface is known to be an important process in the pathogenesis (2, 4, 27, 29). Recently, Nishikawa examined the endometrial mucosa of the uterus inoculated with E. coli by scanning electron microscopy. Adherence of E. coli to the epithelium of ovariectomized rats was observed, but not on the mucosa of estradiol-treated rats (21a). It seems that estradiol modifies the susceptibility of the endometrial epithelium for E. coli adherence that may lead to purulent endometritis, because estradiol increases the cell division (15a) and modifies the morphology (5a) and surface nature of carbohydrates (20a) of endometrial epithelial cells.

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LITERATURE CITED
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