THE EFFECT OF ESTROGENIC HORMONE AND OVARIECTOMY ON THE NORMAL ANTIBODY CONTENT OF THE SERUM OF MATURE RABBITS*

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Evidence is accumulating that the ability of animals and man to produce antibodies is directly related to age,[2] the young individual having less antibody present in the serum and responding to a lesser degree to the introduction of antigen than does the older. While very few attempts have been made, thus far, to determine the rôle of the endocrine glands in this phenomenon, some evidence is at hand indicating that hormones may play an important part in determining the degree to which antibodies are normally present and the extent of reaction of an animal to injected antigens. Most attention has been focused on the hormones important in sexual maturation, and it has been shown, for instance, that during pregnancy, when there is a high level of gonadotropic and estrogenic substances, antibodies such as bactericidin, agglutinin, and others are present in larger amounts than during the non-gravid state. Ando and Nishimura[1] have found that pregnant guinea-pigs exhibit a decreased skin sensitivity to diphtheria toxin, while Jungeblut[8] and his co-workers have demonstrated an increase in the antiviricidal power of the serum against poliomyelitis in pregnant human beings. Pregnancy has not been found to increase resistance in all cases, however, as shown by the work of Torrance,[11] who noted that the gravid state increased the susceptibility of white mice to streptococcus infection, but did not affect the hemolytic activity of the blood serum of rabbits. Jackson[7] claimed that pregnancy increased the susceptibility of rabbits to anaphylactic shock.

Various hormone preparations have been administered in order to determine what effect these would have on the resistance of animals and on their ability to produce antibodies of various types. Cutler[4] found that partial hypophysectomy exerted no influence on the production or persistence of typhoid agglutinins, on hemagglutinins, or on hemolysins; continued ingestion or intraperitoneal injection of whole pituitary extract had no effect on the subsequent

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agglutinin titers for *E. typhi* as compared to those of normal animals. Although able to increase the rate of metabolism of the rabbits to whom she fed thyroid, Baumgartner[8] could not demonstrate any significant differences in the avidity of fresh antisera agglutinating *S. enteritidis* in groups of young, adult, and aged individuals. The same type of results were obtained by Dingle, Meyer, and Gustus,[5] who found that gonadotropic hormone obtained from pregnant mare serum, prolan, estrogenic hormone, or pituitary implants did not influence agglutinin production for *H. pertussis* in immature or infantile rats or rabbits. In contrast to the essentially negative data cited above, several investigators have reported positive findings as the result of hormone administration. Thus, Inamura[6] has claimed the inactivation of tetanus toxin by ovarian follicular fluid, and Jungeblut[9, 10] and his co-workers, while succeeding only in exceptional instances in protecting immature animals against intracerebral infection with poliomyelitis virus by the use of glandular anterior pituitary hormones, pregnancy urine, or pregnant mare serum, noted that the serum of animals treated with these substances frequently acquired the property of inactivating the virus in vitro. Inactivation of the poliomyelitis virus could be demonstrated by treatment with anterior pituitary-like principles; the cortical and medullary adrenal hormones brought about inactivation of diphtheria toxin in vitro.

Since, as has been stated, it appears that the antibody content of the serum increases with age, and there is strong inference that hormones may play a rôle, it was the purpose of the work here reported to determine whether or not changes in the amount of circulating antibody could be produced by the administration of hormones in mature animals in which the normal antibody content was already more or less established. Since the hormone which was being investigated was estrin, a series of ovariectomized animals was included in the study.

Materials and Methods

Sixteen mature rabbits, three of which were males, were used; eight of the females were ovariectomized following determination of the basal levels of agglutinin and hemolysin. The hormone was administered in the form of Theelin* in doses ranging from 20 to

* The Theelin used in these experiments was kindly supplied by Parke, Davis & Company, Detroit, Michigan.

1000 I.U. per day for varying periods. The controls were injected with sesame oil in quantities equal to the amount given with the hormone preparation.

A strain of *E. coli*, found to be agglutinable by the sera of all of the animals, was used and the agglutinin content of the various

TABLE 1.

Agglutinin Titers for E. coli in Rabbits, as Influenced by Ovariectomy and Administration of Estrin.

Week	Rabbit no.															
	1♂	11♂	12♂	3♀	4♀	5♀	20♀	21♀	22♀	24♀	25♀	26♀	27♀	28♀	29♀	31♀
1	1024	256	256	512	256	512	512	256	512	512	256	256	256	128	512	1024
2	1024	256	256	256	256	512	256	256	512	512	128	256	256	128	256	256
3	1024	256	256	512	128	256	256	512	512	256	256	256	256	128	256	128
4	1024	256	256	256	256	256	256	256	256	512	256	512	128	256	512	256
5	a	a	a	Ov.	Ov.	Ov.	Ov.	Ov.	Ov.	Ov.	Ov.	a	a	a	a	a
6	512	128	128	256	128	-	-	-	-	-	-	256	128	256	256	-
7	512	256	256	256	64	512	256	256	256	512	256	256	128	256	256	256
8	-	-	128	128	-	256	128	128	128	128	128	128	256	128	256	256
9	b512	b128	b512	64	128	256	-	-	64	128	-	b128	b128	b128	b256	b128
10	b512	b512	b512	128	256	128	64	256	64	128	64	b128	b128	b256	b128	b128
11	b2048	b512	b512	128	256	128	128	128	256	128	128	b128	b256	b128	b64	b128
12	2048	512	512	128	128	256	256	64	128	256	128	128	256	256	256	128
13	2048	512	1024	512	256	512	256	128	512	256	512	256	512	512	256	512
14	2048	1024	512	1024	256	256	256	256	256	512	256	512	512	512	256	512
15	2048	1024	512	1024	256	1024	128	256	256	256	512	256	512	512	512	512
16	2048	1024	256	1024	512	1024	256	512	1024	512	512	256	512	512	512	512
17	2048	256	512	1024	256	1024	512	256	512	1024	512	512	256	1024	1024	256
18	2048	512	512	1024	512	512	256	256	256	1024	512	512	256	512	512	256
19	2048	512	512	2048	512	1024	128	256	256	1024	512	256	256	256	512	512
20	1024	256	512	512	512	1024	256	512	512	512	512	256	1024	1024	256	256
21	t2048	x1024	x256	x1024	t512	x1024	x512	x512	x512	x1024	t512	x1024	x1024	t1024	t512	x512
22	t2048	x256	x512	x1024	t512	x1024	x512	x512	x512	x2048	t512	x1024	x512	t1024	t512	x512
23	t2048	x256	x256	x1024	t512	x 512	x512	x1024	x256	x1024	t256	x512	x256	t512	t512	x512
24	t2048	x512	x512	x2048	t512	x1024	x512	x512	x1024	x512	t256	x512	x256	t512	t512	x512
25	2048	256	256	512	256	512	256	512	512	1024	256	512	256	512	256	512
26	2048	512	256	512	256	512	512	512	256	512	512	512	256	256	256	512
27	2048	256	512	512	512	512	256	256	256	256	256	256	256	256	512	256
28	2048	512	256	512	256	512	512	512	256	512	256	256	256	256	256	256
29	2048	1024	512	512	256	512	512	512	512	512	512	512	256	512	512	256
30	2048	512	512	512	256	512	512	512	512	512	512	256	512	512	256	256
31	t2048	m512	m512	m1024	m256	t512	m256	m512	m512	m256	m256	t256	m512	t512	m512	m256
32	t2048	m1024	m512	1024	m512	t1024	m256	m512	m512	m512	m512	t256	m1024	t512	m512	m512
33	t2048	m1024	m512	512	m1024	t1024	m512	m512	m512	m512	m512	t256	m1024	t512	m1024	m1024
34	2048	1024	512	1024	512	1024	512	512	512	512	1024	512	1024	512	1024	1024
35	2048	Dead	2048	1024	512	1024	512	512	1024	512	1024	512	1024	512	1024	1024
36	2048		2048	1024	1024	1024	1024	1024	1024	512	1024	512	512	512	2048	1024
37	2048		2048	512	1024	1024	512	512	512	1024	512	512	256	512	1024	512
38	2048		1024	512	512	1024	1024	1024	512	512	512	512	256	512	512	512
39	2048		1024	1024	512	1024	1024	512	512	512	256	512	256	512	512	512
40	2048		512	1024	512	1024	512	512	512	512	256	256	512	512	512	512
41	2048		512	512	512	512	512	512	512	512	512	256	512	512	512	512
42	2048		256	512	512	512	256	512	512	512	256	256	256	512	512	256
43	2048		256	512	256	512	512	512	512	512	256	256	512	512	512	512
53	2048		256	256	128	512	256	256	256	512	256	256	256	256	256	256
54	2048		256	256	256	512	512	256	256	256	256	256	256	256	256	256

a—1000 I.U. estrin in one injection.
Ov.—ovaries removed.
b—2 injections of 500 I.U. estrin each.
x—3 injections of 20 I.U. estrin each.
t—sesame oil injected in same dose and same number of times as estrin.
m—3 injections of 1000 I.U. estrin each.

sera for this organism was determined throughout the experiment. At the same time the serum was tested for its content of hemolysin for sheep erythrocytes. After removal from the ear vein the blood

TABLE 2.

Hemolysin Titers for Sheep Erythrocytes in Rabbits, as Influenced by Ovariectomy and Administration of Estrin.

Week	Rabbit no.															
	1♂	11♂	12♂	3♀	4♀	5♀	20♀	21♀	22♀	24♀	25♀	26♀	27♀	28♀	29♀	31♀
1	48	48	24	48	48	48	24	0	48	48	48	24	12	48	12	48
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	48	48	24	48	48	48	24	6	48	48	48	48	24	24	12	24
4	48	12	6	48	6	24	12	6	6	24	24	24	12	24	12	24
5	a	a	a	Ov.	Ov.	Ov.	Ov.	Ov.	Ov.	Ov.	Ov.	a	a	a	a	a
6	-	-	24	-	-	-	-	-	-	-	-	12	12	24	12	24
7	192	48	48	96	6	24	12	24	96	24	12	12	12	24	12	24
8	192	96	12	96	24	24	24	24	192	24	48	12	12	24	12	96
9	b96	b24	b12	192	24	192	48	192	192	96	192	b6	b12	b48	b48	b48
10	b96	b24	b48	384	24	192	192	96	192	24	48	b24	b24	b12	b48	b192
11	b48	b48	b0	384	24	192	96	384	384	12	96	b6	b3	b12	b192	b384
12	384	48	192	384	24	384	96	192	192	96	96	12	96	24	384	384
13	384	0	192	384	24	384	382	48	384	96	96	6	12	96	192	96
14	384	384	96	384	192	384	384	192	384	192	192	96	192	192	384	192
15	384	96	6	384	96	384	384	192	384	96	192	48	192	192	192	192
16	384	96	96	384	96	384	192	48	192	48	96	24	24	48	192	96
17	768	96	96	384	96	192	384	192	192	96	192	48	48	96	192	48
18	384	96	12	384	96	384	384	192	384	96	192	48	96	192	192	24
19	768	192	12	384	96	384	384	192	384	192	192	192	96	192	384	48
20	384	192	48	192	96	192	384	192	192	96	24	48	48	96	384	48
21	t768	x96	x96	x192	t48	x384	x192	x48	x96	x48	t48	x192	x24	t96	t192	x24
22	t768	x192	x192	x384	t48	x384	x384	x96	x192	x96	t96	x96	x48	t96	t192	x96
23	t384	x192	x96	x384	t48	x384	x192	x96	x192	x192	t96	x192	x96	t96	t384	x96
24	t384	x384	x96	x384	t96	x384	x384	x192	x384	x192	t192	x384	x192	t96	t192	x384
25	768	192	96	384	96	384	768	192	384	192	192	192	96	96	192	192
26	768	96	48	384	96	384	384	192	192	192	96	192	96	48	96	96
27	768	192	48	192	96	384	384	192	192	192	192	192	96	24	24	96
28	384	96	12	384	24	384	384	96	192	192	48	48	48	48	48	48
29	384	96	-	192	48	384	192	48	192	96	96	24	48	48	48	48
30	384	96	6	192	96	384	384	96	192	192	48	48	24	48	48	48
31	t768	m48	m12	m192	m96	t384	m384	m96	m192	m192	m48	t24	m48	t48	m24	m0
32	t384	m96	m96	m192	m48	t192	m384	m192	m192	m96	m48	t12	m0	t24	m6	m24
33	t384	m192	m192	m384	m48	t384	m768	m96	m384	m96	m96	t6	m48	t24	m192	m24
34	768	384	192	192	192	384	1536	384	768	192	192	3	48	48	192	96
35	384	Dead	192	384	192	192	3072	384	768	96	192	24	192	12	96	96
36	768		192	384	96	384	1536	192	384	192	192	12	192	48	192	384
37	384		96	384	48	192	1536	96	192	96	96	6	96	24	96	192
38	384		96	48	12	192	384	48	96	24	48	6	96	24	192	96
39	384		48	384	6	192	384	96	192	192	48	6	48	12	96	192
40	384		48	192	384	96	192	192	192	192	96	12	48	12	48	384
41	384		24	192	12	96	192	192	96	96	48	24	24	24	24	96
42	384		24	96	24	96	192	192	48	48	24	12	12	48	24	48
43	384		24	96	12	48	96	96	48	48	12	12	6	12	24	24
53	384		12	48	6	48	48	24	24	48	12	24	12	24	12	24
54	384		12	48	12	48	24	6	48	48	24	12	24	24	12	24

Symbols as in Table 1.

was allowed to clot, the serum removed and heated at 56° C. for 20 minutes, and titrated for agglutinin and hemolysin, determinations being made weekly for 43 weeks and again at the end of 53 and 54 weeks following the start of the experiment.

Results

Titrations of the coli agglutinin and sheep hemolysin before treatment with estrin or ovariectomy showed these antibodies to be present in all of the animals, the latter showing some tendency to fluctuate, although the difference in titers was never more than two tubes. The results of the titrations for the entire experiment are presented in Tables 1 and 2.

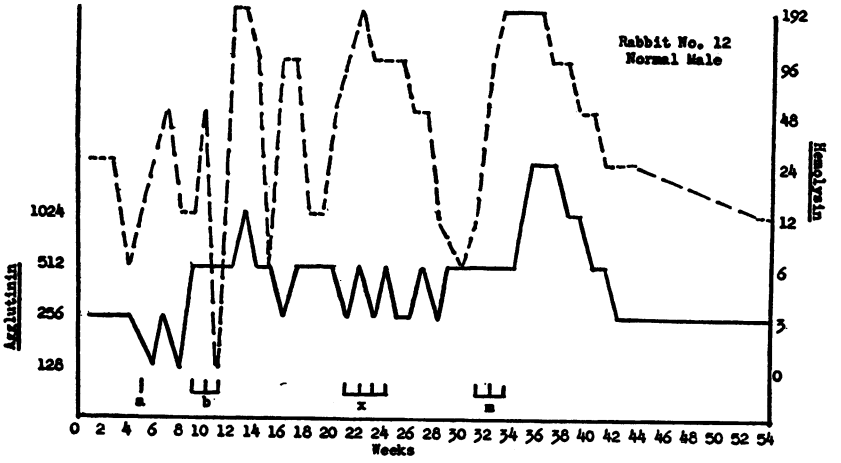
Normal Males:

The administration of one dose of 1000 I.U. of estrin was found to decrease the amount of circulating agglutinin for *E. coli* in the sera of all three of the rabbits, the degree of reduction in most cases being one dilution, followed by a rise in the agglutinin content to levels slightly higher than those of the period preceding treatment. The injection of 6 doses of hormone, each of 500 I.U. (3000 I.U. in all) over a period of two weeks led to an increase in the amount of coli agglutinin present in the serum, titers as high as four times those found previous to treatment being observed. Small doses of hormone (12 injections of 20 I.U. each over a period of four weeks) had very little effect on the agglutinin level, some fluctuation in the titers being evident, but not of greater degree than that to be expected as the result of experimental error. The administration of large doses of hormone (9000 I.U. of estrin in 9 doses of 1000 I.U. each in a period of 3 weeks) was followed by a marked increase in the quantity of coli agglutinin, this treatment giving the highest rise which had yet been observed. Following cessation of the hormone injections, the titer fell fairly rapidly and returned to levels found prior to the beginning of hormone treatment.

Titrations of the hemolysin for sheep erythrocytes revealed that this antibody was also influenced by the administration of estrogenic hormone, the degree of increase being larger than that found with the agglutinin. In every instance the administration of hormone was followed shortly by an increase in the quantity of circulating hemolysin. In contrast to the agglutinin, which was not affected by small doses of estrin, the hemolytic antibody was increased in amount following treatment with this dosage. At the end of the study the concentration of hemolysin was found to have returned to about the

same level as that present before treatment was given. Figure 1 illustrates the results obtained in the male animals.

FIG. 1. Agglutinin and Hemolysin Titrations in a Normal Male Rabbit, as Influenced by Administration of Estrin



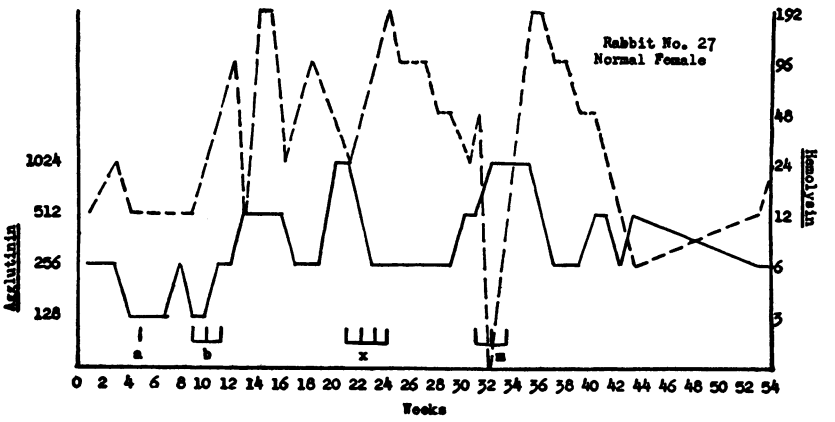
——Agglutinin titers (Values expressed on left of graph.)
- - - Hemolysin titers (Values expressed on right of graph.)
a—One injection of 1000 I.U. estrin.
b—6 injections, each of 500 I.U. estrin, in period of 3 weeks.
x—12 injections, each of 20 I.U. estrin, in period of 4 weeks.
m—9 injections, each of 1000 I.U. estrin, in period of 3 weeks.

Normal Females:

The results obtained in the treatment of normal female rabbits with estrogenic hormone were essentially the same as those found in the normal males. The administration of one dose of estrin (1000 I.U.) was followed by a decrease in coli agglutinin somewhat more marked than that observed in the male animals, the hemolysin not being affected to any great degree. As in the case of the males, small doses of hormone, while not altering the quantity of agglutinin present in the serum, did increase the concentration of hemolysin.

The greatest change was again obtained when the largest doses of hormone were administered. The results in this group are illustrated in Figure 2.

FIG. 2. Agglutinin and Hemolysin Titrations in a Normal Female Rabbit as Influenced by Administration of Estrin



Symbols as in Fig. 1.

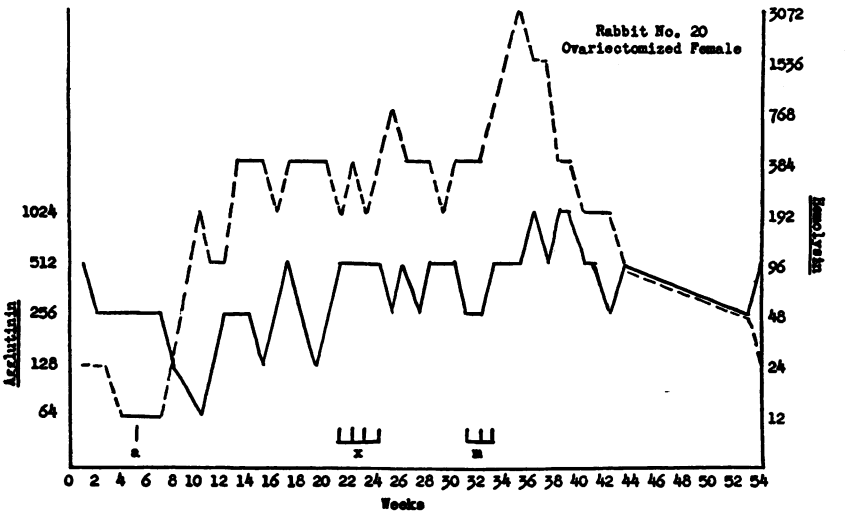
Ovariectomized Females:

Following ovariectomy, a marked decrease in the agglutinin content of the serum of the operated animals was observed, being followed by a gradual increase in the amount of antibody until a level was reached higher than that present before operation. The injection of small doses of estrin produced no change, while large amounts of hormone brought about a marked increase in the coli agglutinin. Following cessation of treatment, the titer of the sera of the various animals against *E. coli* returned gradually to a level equal to that found previous to operation.

Ovariectomy produced a marked increase in the amount of hemolysin, the titer in some instances being 32 times greater than that observed before operation. That this increase was not due to the trauma induced by the operative procedure seems likely, since the titer maintained a high level long after the animals had recovered. Small as well as large amounts of estrin further increased

the circulating hemolysin, the degree of increase being appreciably higher with the large than with the small doses. In most instances, at the end of the experiment, the hemolysin level had returned to approximately that found previous to operation. The results obtained are presented graphically in Figure 3.

FIG. 3. Agglutinin and Hemolysin Titrations in a Female Rabbit as Influenced by Ovariectomy and Administration of Estrin



——Agglutinin titers (Values expressed at left of graph.)
- - - Hemolysin titers (Values expressed at right of graph.)
a—ovaries removed.
x—12 injections, each of 20 I.U. estrin, in period of 4 weeks.
m—9 injections, each of 1000 I.U. estrin, in period of 3 weeks.

Control Animals:

In order to control the effect of injection itself, frequency of bleeding, and the menstruum in which the hormone was administered, several animals were injected with sesame oil in the same amount as that in which the estrin was given, and bled at the same intervals as the experimental rabbits. No change in either the hemolysin or agglutinin levels was observed as the result of the administration of this oil, the antibody levels either remaining unchanged

or falling slightly during periods when estrin was producing an increase in concentration of both the agglutinin and the hemolysin in the treated group. In one instance, however, Rabbit 5 showed an increase in coli agglutinin following injection of oil, the hemolysin level remaining constant for a short time and then falling. No explanation for this finding is at hand, but it should be noted that the animal in which it occurred had been ovariectomized and had a tendency to maintain a fairly high level of antibody without treatment of any kind. Tables 1 and 2 include the data obtained with the controls.

Discussion

The results obtained seem to indicate that estrogenic hormone possesses the ability to produce an increase in the amount of circulating antibody in male or female rabbits, the extent of increase depending directly on the amount of hormone administered. The increase in hemolysin and agglutinin content of animals following ovariectomy is of special interest since it indicates that the estrogenic hormone is not the only endocrine substance capable of producing a change in antibody level. Since castration may be assumed to be followed by the elaboration of large amounts of gonadotropic hormone by the pituitary gland, it would seem that this hormone is also capable of stimulating the production of antibody.

No information is available as to the mechanism responsible for the increase in antibody following treatment with estrin or after ovariectomy. It is, of course, not possible to ascertain from the data presented here whether the increase is due to the elaboration of new antibody, the introduction into the circulation of a greater amount of antibody from some depot, or is only an apparent change due to other alterations in the rabbit serum. Although it has been shown by Weinstein, Gardner, and Allen[12] that the administration of estrin to mice produces infection of the uterus with organisms normally present in the vagina, such an explanation for antibody changes can not hold in these experiments. Weinstein and Gardner[13] have obtained no evidence that estrin causes infection of the rabbit uterus with any demonstrable organism. On the basis of these results, it may be assumed that the increase in titer against *E. coli* following hormone treatment is not the result of infection of the internal genital organs with this bacterium.

Conclusions

1. Estrogenic hormone produces an increase in the amount of circulating agglutinin for *E. coli* and hemolysin for sheep erythrocytes in mature male and female rabbits, the degree of change being directly related to the amount of hormone administered.

2. Small doses of hormone, while capable of producing an increase in the concentration of hemolysin, are ineffective in provoking any change in agglutinin.

3. Ovariectomy in mature rabbits is followed by a decrease and subsequent increase to high levels in the agglutinin content of the serum, the hemolysin rising to levels much higher than those present previous to operation.

4. The action of ovariectomy in producing changes in the antibody content of the serum of rabbits may be related to gonadotropic hormone.

5. The mechanism of the change produced by the administration of hormone or the removal of the ovaries can not be explained at present, although there seems to be some evidence that it is not the result of infection of the uterus.

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