PATHOLOGICAL STUDIES ON STERILITY IN DAIRY COWS

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INTRODUCTION

Recent remarkable improvements made in the artificial insemination of domestic animals has brought extended knowledge in the physiology and pathology of reproduction. However, reproductive failures in domestic animals, more specifically, infertility of cows have shown, in recent years, a tendency of increase in Japan. Especially diseases caused by non-contagious factors are abundant. The causes of infertility are attributed to the hormonal disturbances, malnutrition and lesions in the sexual organs, as reported by many workers.

In Japan, YAMAUCHI and co-workers[23~26] are investigating follicular cyst from the clinico-endocrinological approach and OCHI with his co-workers[14,15] are making bacteriological studies of the flora found in the vaginae and uteri of so-called infertile cows comparing with healthy cows. Hitherto many reports on prevention and therapy of so-called infertile cows have been made, but there are few on the etiology of infertility.

Can the sterility in cows really be attributed to the ovarian cyst and endometritis as morphological changes ?

The present study was made to ascertain the actual condition of the sterility of dairy cows from the pathological point of view. Investigations were made on 87 cases (A group) and 286 control cases (B group).

MATERIALS AND METHODS

Investigated cases with a total of 373 consist of A (87 cases) and B groups (286 cases). The A group consists of cows released by the Hokkaido Agricultural Mutual Relief Cooperative Associations due to sterility in 1955 and 1956. They had considerably detailed clinical data. B group consists of slaughtered cows collected at the Sapporo Slaughter House in 1946 to 1949.

After macroscopical observations, materials were collected from each portion of the female reproductive organs. They were fixed in 10% formalin solution and paraffin sections were prepared. Sections were stained with hematoxylin-eosin, and sometimes WEIGERT stain for elastic fiber and VAN GIESON stain for collagen fiber were applied.

RESULTS

The following results were obtained:

1. Statistics

Statistical results are shown in tables 1~8.

TABLE 1. *Number of Cases by Age in A Group*

AGE (Years)	4	5	6	7	8	9	10	11	12	13	14	15	Unknown
NO. OF CASES	6	8	9	11	5	8	12	8	7	6	5	1	1

TABLE 2. *Clinical Diagnosis in A Group*

PORTION	CLINICAL DIAGNOSIS	NO. OF CASES	(%)
Ovary	Ovarian cyst	42	(48.3)
	Ovarian dysfunction or hypofunction	9	(10.3)
	Ovarian atrophy	2	(2.3)
	Small cystic degeneration	1	(1.1)
Oviduct	Salpingitis	8	(9.2)
	Hydrosalpinx	1	(1.1)
	Stenosis	1	(1.1)
Uterus	Endometritis (Metritis)	55	(63.2)
	Tumor or swelling	2	(2.3)
	Extrauterine abscess	2	(2.3)
	Prolapse	1	(1.1)
	Atonia	1	(1.1)
	Atrophy of the broad ligament	1	(1.1)
Cervix	Cervicitis	3	(3.4)
Vagina	Colpitis	3	(3.4)
	Prolapse	3	(3.4)
	Atresia	1	(1.1)
Others	Adhesion	5	(5.7)
	Dystocia	3	(3.4)
	Retained afterbirth	1	(1.1)
	Sterility	1	(1.1)
	Senility	1	(1.1)
	Ocular neoplasm	1	(1.1)
	Mastitis	2	(2.3)

Those encountered in high frequency are endometrits (63.2%), ovarian cyst (48.3%), pathological conditions in the oviducts (11.5%), ovarian dysfunction or hypofunction (10.3%) and adhesions (5.7%) in order.

TABLE 3. *Pathological Diagnosis in A Group*

PORTION	PATHOLOGICAL DIAGNOSIS	NO. OF CASES	(%)
Ovary	Ovarian cyst	35	(40.2)
	Small cystic degeneration	23	(26.4)
	Lutein cyst	5	(5.7)
	Follicular hematoma	1	(1.1)
Oviduct	Hydrosalpinx	8	(9.2)
	Salpingitis chronica	3	(3.4)
Uterus	Endometritis		
	Endometritis catarrhalis chronica	39	(44.8)
	Endometritis purulenta subacuta	1	(1.1)
	Pyometra	1	(1.1)
	Endometritis catarrhalis eosinophilica	1	(1.1)
	Hyperplasia glandularis cystica endometrii	6	(6.9)
	Perimetritis		
	Perimetritis purulenta circumscripta with abscess	1	(1.1)
	Perimetritis fibrosa adhaesiva	2	(2.3)
	Perimetritis chronica	2	(2.3)
	Pelveoperitonitis chronica	1	(1.1)
Cervix	*Cervicitis catarrhalis chronica*	21	(24.1)
Vagina	*Colpitis catarrhalis chronica*	32	(36.9)
Others	Squamous cell carcinoma associated	1	(1.1)

Pathological changes which showed high frequency in the sexual organs are: *Endometritis cat. chr.* (44.8%), ovarian cyst (40.2%), *colpitis cat. chr.* (36.9%), ovarian small cystic degeneration (26.4%), *cervicitis cat. chr.* (24.1%), hydrosalpinx (9.2%), perimetritis (6.9%) and *hyperplasia glandularis cystica endometrii* (6.9%) in order.

TABLE 4. *Pathological Diagnosis in B Group*

PORTION	PATHOLOGICAL DIAGNOSIS	NO. OF CASES	(%)
Ovary	Ovarian cyst	53	(18.5)
	Small cystic degeneration	23	(8.0)
	Lutein cyst	13	(4.5)
	Follicular hematoma	7	(2.4)
	Adenoma	1	(0.3)
Oviduct	Hydrosalpinx	1	(1.4)
	Salpingitis catarrhalis chronica	1	(1.4)
	Cysts in serosa	5	(7.1)
Uterus	Endometritis		
	Endometritis catarrhalis chronica	25	(8.7)
	Endometritis purulenta chronica	3	(1.0)
	Endometritis eosinophilica	2	(0.7)
	Pyometra	1	(0.3)
	Hyperplasia glandularis cystica endometrii	15	(5.2)
	Leiomyoma uteri	2	(0.7)
	Periuterine abscess	2	(0.7)
	Perimetritis adhaesiva chronica	1	(0.3)
	Hypoplasia uteri (Stenosis)	1	(0.3)
Cervix	*Cervicitis catarrhalis chronica*	12	(7.8)
	Cervicitis purulenta chronica	2	(1.3)
	Cerivicitis catarrhalis acuta	1	(0.7)
	Cyst	2	(1.3)
	Cystadenoma	1	(0.7)
	Cervix duplex	2	(1.3)
Vagina	*Colpitis catarrhalis chronica*	28	(36.8)
	Colpitis purulenta chronica	2	(2.6)
	Colpitis catarrhalis acuta	3	(3.9)
	Cyst	9	(11.8)
	Cystadenoma	3	(3.9)
	Septum	2	(2.6)
	Hypoplasia vulvae	1	(1.1)

Investigated cases totaled as follows: Oviduct in 70 cases, cervix 153 cases, vagina 76 cases and the others 286 cases respectively.

In B group, colpitis (43.4%), ovarian cyst (18.5%), cyst in the vagina (11.8%), endometritis (10.8%), cervicitis (9.8%), ovarian small cystic degeneration (8.0%), cysts in serosa

(7.1%), *hyperplasia glandularis cystica endometrii* (5.2%), lutein cyst (4.5%) and cystadenoma in the vagina (3.9%) are observed in high frequency. As special lesions, the author observed benign neoplasm, such as ovarian adenoma, *leiomyoma uteri*, cystadenoma in the cervix and vagina. As other abnormalities, *hypoplasia uteri*, *hypoplasia vulvae*, septum formation in the vagina and *cervix duplex* may be mentioned.

TABLE 5. *Relation between Ovarian Cyst and Symptom*
(A Group)

SYMPTOM	NO. OF CASES
Anestrus	10
Nymphomania	16
Abnormal estrus	3
Normal estrus	1
Unknown	5
Total	35

TABLE 6. *Relation between Ovarian Cyst and Endometritis*
(A Group)

NO. OF CASES	OVARIAN CYST	ENDOMETRITIS AND *HYPERPLASIA GLANDULARIS CYSTICA ENDOMETRII*
24	+	+
11	+	−
24	−	+
Total	35	48

TABLE 7. *Relation between Clinical Ovarian Dysfunction or Hypofunction and the Pathological Changes*
(A Group)

CHANGES	NO. OF CASES
Small cystic degeneration	7
Numerous atrophic follicles	2
Total	9

TABLE 8. *Indications of Small Cystic Degeneration of the Ovary in Each Case* (A Group)

CASE NO.	AUTOPSY NO.	SYMPTOM	OTHER LESIONS EXCEPT SMALL CYSTIC DEGENERATION
1	E. 1712	Anestrus	None
2	E. 1829	"	"
3	E. 1835	"	*Endometritis cat. chr.*, Cervicitis
4	E. 1868	"	Ovarian cyst
5	E. 1869	"	None
6	E. 1972	"	Hydrosalpinx, *Endomet. cat. chr.*
7	E. 1996	"	None
8	E. 1999	"	"
9	E. 2007	"	*Endomet. cat. chr.*, Perimetritis
10	E. 1830	Weak estrus	None
11	E. 1842	"	"
12	E. 1879	"	Lutein cyst, *Endomet. cat. chr.*, Perimetritis, Cervicitis
13	E. 1901	"	None
14	E. 1973	"	Follicular hematoma, Hydrosalpinx
15	E. 1992	"	Ovarian cyst, *Hyperplasia glanduralis cystica endometrii*, Cervicitis, Colpitis
16	E. 1890	Abnormal estrus	None
17	E. 1905	"	Hydrosalpinx, *Endomet. cat. chr.*, Colpitis
18	E. 1911	"	None
19	E. 1960	"	"
20	E. 1648	Normal estrus	*Endomet. cat, chr.*
21	E. 1711	"	Perimetritis
22	E. 1713	Unknown	Cervicitis
23	E. 1981	"	Lutein cyst

2. Pathological changes

The important pathological changes in the sexual organs may be described as follows:

OVARY

1) Ovarian cysts (Follicular cysts) Macroscopically, they occupy the periphery, the centrum or sometimes almost all of the ovary. They are single or multiple in one or both ovaries. In size, they are normally as large as somewhere between that of small graafian follicles and the fist. The cysts contain a clear, serous fluid which presents gelatinous milky white color in the formalin fixation. Microscopically, the ovum is absent.

The *zona granulosa* disappears entirely, the *theca folliculi interna* becomes fibrous and the graafian follicles lose their original form. In some cases the granulosa cells shrink and are represented by a layer of flat cells which lines the inner wall of the cyst.

2) Small cystic degeneration of the ovary Such degeneration is observed mainly in the cortex of the ovary. It varies in both size and number of occurrences. It is usually smaller than a soybean and appears as small cysts. It contains a clear, serous fluid and sometimes forms in groups of cysts on the surface of the ovary. There is no evidence of ovum. The inner surface of the cysts often is covered with cylindrical or flat epithelia. In most cases, they disappear entirely by pressure atrophy and become fibrous.

3) Lutein cysts The center of the *corpora lutea* becomes a cyst which contains serous fluid and is frequently accompanied by hemorrhage. The cysts wall is bounded by thick connective tissues. The cysts are sometimes so large that lutein tissues are recognized only in the periphery of the ovary.

4) The other lesions observed were follicular hematoma and adenoma.

FALLOPIAN TUBES

1) Hydrosalapinx Macroscopically, the tubes swell and fluctuate by palpation. They contain a large amount of clear fluid. Microscopically, the glands are extensively dilated. The tubes show a mesh or cluster appearance. The glandular epithelia which line the inner wall are flattened.

2) *Salpingitis chronica* The epithelia proliferate in most cases and the part of them are flattened. The capillaries are distended with blood. There is infiltration with plasma cells and lymphocytes in the mucosa.

3) Cyst in serosa They are noted on the fimbriae of the oviduct which range in size from that of red beans to small finger tips. They contain serous fluid. The cyst is covered with cubic epithelia. The cysts are surrounded by fibrous connective tissues.

UTERUS

1) *Endometritis catarrhalis chronica* The mucosal epithelia are atrophic and degenerated, and sometimes desquamated. But most of them are covered with a single layer of cylindrical epithelia. The mucosa is hyperemic and has cellular infiltration with mainly lymphocyte, then plasma cells, leucocytes and sometimes mast cells. Especially these changes are conspicuous in the periglandular and subepithelial tissues with inflammatory edema. Sometimes cellular foci also appear in the *T. propria*. Hemosiderosis is also noted.

2) *Hyperplasia glandularis cystica endometrii* Macroscopically, there are multiple cysts which vary from pin head to pea in size in the surface of the endometrium. These changes are observed conspicuously in the pericaruncular regions containing clear, serous fluid. Microscopically, these changes are revealed as a hyperplastic glandular dilatation. The glandular epithelia consist of cylindrical or cubic or flat cells. The interestitial tissues are edematous and sometimes fibrous.

3) Other changes *Endometritis purulenta subacuta*, pyometra, *endometritis eosinophilica* and perimetritis, etc. are recognized. Especially perimetritis is important change clinico-pathologically.

CERVIX

1) *Cervicitis catarrahlis chronica* Macroscopically, the mucous membrane is swollen

and congested, and there are secreta with erosion sometimes. Microscopically, the proliferation of the cells is followed by desquamation and an exudate accumulates. Subepithelial cellular infiltration is conspicuous and the capillaries are distended with blood.

2) Other changes Cysts, cystadenoma and cervicitis are observed.

VAGINA

1) *Colpitis catarrhalis chronica* Colpitis is the same as that for inflammation of other mucous surfaces. Sometimes erosions are recognized.

2) Other changes *Colpitis purulenta* and cystadenoma are noted.

DISCUSSION

The author as reported above studied A group mainly, and B group as control cases, from the pathological point of view. As indicated in table 1 among those cows abolished on account of sterility, many are considerably old cows. This fact shows that the causes of sterility can not be sought only in organic lesions. In A group, almost all cases had experienced parturition. The number of months from the day of last delivery to the day of slaughter, was between 3 months and 52 months showing an average of 17.2 months.

The author examined each of the organs pathologically. In ovaries, ovarian cysts, small cystic degeneration and lutein cysts attracted attention. On the cystic ovarian degeneration, hitherto there have been published many reports; it is regarded as an important factor in sterility.

There are 2 forms of clinical symptoms such as nymphomania and anestrus. YAMAUCHI et al.[24] stated that 9 cases (75%) were of nymphomania and 3 cases (25%) were of anestrus in his 12 cases of hormonal experiment cows. ROBERTS described 195 cases (73%) as nymphomania and 70 cases (26.4%) as anestrus in 265 ovarian cyst cows. GARM stated that cystic ovarian degeneration in cows is not an independent condition, but is only a part of the picture in two multiglandular syndromes, namely, nymphomania and adrenal virilism. The clinical symptoms vary greatly, and many of the cows do not show continuous heat. Nymphomania, therefore, is not a suitable name for the disease. On the basis of the clinical symptoms, GARM has classificated nymphomania in 4 groups: namely, group I, cows with permanent heat or periods of intense heat at frequent intervals, group II, cows with regular heat intervals, group III, cows with long heat intervals and mild symptoms of heat, and group IV, cows showing no symptoms of heat. The author understands that as the symptoms may vary from time to time individually, it is unreasonable that the cases of ovarian cysts should be divided into 2 groups of symptoms. YAMAUCHI et al.[24] divided this symptom of "nymphomania" into 2 groups definitely by the estrogen contents and histological findings. Author's cases showed these 2 types of symptoms and

abnormal estrus which could not be classified into either (Table 6). The relationship between symptoms and ovarian lesions was not always constant.

Hitherto, many workers have attached importance to the relationship between cystic ovarian degeneration and endometritis. Some authors considered that endometritis is the primary change and the ovarian cysts secondary because of either an ascending or a toxic influence of the uterine contents. GARM concluded that bacterial infections and inflammations of the genitalia are not the primary causes for cystic ovarian degeneration. In GARM's nymphomania, group I, sometimes the uterus and the cervix are very large and the uterine mucosa is often the seat of cystic glandular hyperplasia. He discovered endometritis in only 3 among 62 cases examined. QUEISSER recognized an abnormal endometrium in 13 out of 14 cases in ovarian cyst cows and considered it as an advanced picture of *endometritis glandularis cystica*. FEDRIGO observed the same changes in sheep and considered them as hormonal in origin. WOOLDRIDGE also described the same lesions in cat. The present author's cystic glandular hyperplasia of the endometrium is identical to that of these changes, but in some cases the hyperplasia took the form of retention cysts. As indicated in table 6, the relation between ovarian cyst and endometritis is acceptable to some extent, but there is no conclusive evidence that endometritis takes the form of primary lesions nor that it is closely connected with ovarian cyst. The author experienced a large number of cases of *endometritis catarrhalis chronica*, but there are many connected problems which need further studies in the future.

Most authors obtain the impression of the existence of endometritis based on only clinical observations and are apt to emphasize the relation between ovarian cyst and endometritis. It seems that enlargement and atony of the uterus by estrogenic influence serve to emphasize the impression of endometritis in the cystic ovarian degeneration. In the present investigation, there are no changes histopathologically in 22 (40%) out of 55 endometritis cases which were clinically diagnosed.

As for lutein cyst, JOEST concluded as follows based upon WILLIAMS and KRUPSKI's reported results. There are 2 types histogenetically. The first type which develops from the *corpus luteum* arising after ovulation, did not disturb ovulation or estrus. The second type which develops from a follicle incapable of ovulation, was named a follicular cyst with the *corpus luteum* tissue. This type restrains ovulation and did not show nymphomania.

KAWATA et al., WILLIAMS and AZIZUDDIN reported that cystic degeneration of the *corpus luteum* was often associated with hydrosalpinx or salpingitis. They emphasized it as a factor in sterility.

The most interesting finding in ovaries is small cystic degeneration (A group 26.4%, B group 8.0%). Infertile cows show a high frequency of it. Such changes

caused by such degeneration are detected in more than 70% out of cases clinically diagnosed as ovarian dysfunction or hypofunction. So the author studied 23 cases of these changes in A group. As indicated in table 8, except these changes, 11 cases showed no other remarkable changes which may be considered as the causes for sterility. Except each 2 cases of ovarian cyst and lutein cyst, most of them have no influence upon sterility in the ovary. RUDOLF recognized these changes in cattle and horses and reported that they disturb the follicle formation and development in some conditions. VATTI also recognized same changes in calf and regarded causal genesis as early activities in the anterior lobus of hypophysis. The author considered that these changes restrained the development of immature follicle and disturbed the estrous cycle as a factor of sterility.

Pathologic alterations of the oviduct were found with high incidence in A group (12.6%) and are considered as important factors for sterility. According to LOMBARD et al., GILMAN studied the oviducts of 86 cows, the majority of which were sterile, and presented case histories on 8 animals. KÜTTEL found pathologic oviducts in 40 cases (18.9%) out of 211 cattle. ROWSON reported severe, bilateral adhesions of the oviduct in 13.3% of 300 cows examined. CEMBROWICZ found hydrosalpinx (1.5%), cysts on the inside of the oviduct (1.3%) and adhesions (2.5%) in 138 cases examined. LOMBARD et al. studied the oviduct of 154 repeat-breeding cows pathologically and found pathologic oviducts in high incidence. MOBERG studied 1,662 cases of normal slaughtered cows and 516 cases of clinical infertile cows. Disease conditions in the oviducts and ovarian bursae were observed by him in 15.5% and 4.8% respectively. The majority of authors agree that this group of conditions is much more common than is often assumed. There are many factors for infertility, but in recent years, enucleation of the *corpus luteum*, uterine washing and hormonal treatment are regarded as important factors.

On the frequency of endometritis in slaughtered cows, the author experienced 10.8% in B group. LÁSZLÓ examined the uteri from 300 slaughtered cattle. Three percentage were affected with chronic suppurative endometritis, 3.1% had chronic non-suppurative type, 3.6% had tuberculous endometritis and 1.6% showed pigmentation (hematogenous) of the mucosa, which was considered to be the remains of previous estrous bleeding. Although tuberculous endometritis was not recognized, there are a large number of occurrences of chronic catarrhal endometritis in the present author's cases.

With regard to the pathological changes in cervix and vagina, most of the practitioners do not consider it serious clinically, however, many cases were observed histopathologically.

As for the causes of sterility, the author studied organic diseases of sexual organs. There is need to study sterility from the general point of view.

Furthermore, there are many problems concerned with the causes of sterility, such as malnutrition, hormonal disturbances, partial symptom of general disease and hereditary constitutional disposition, etc. HIRT and SZÉKY studied ovary, uterus, hypohysis and adrenal gland in infertile cows pathologically. GARM was made clinical, bacteriological, pathological, chemical and endocrinological observations in ovarian cystic degeneration. He classified into 2 types the multiglandular syndromes, namely, nymphomania and adrenal virilism. He considered that both of these diseases are connected with a weak endocrine constitution which is most likely hereditary. In the adrenal virilism, the sexual disturbances are connected with a hyperproduction of androgenic substances in the adrenals.

The author considered, that in order to ascertain the causes for sterility, observations clinical, pathological, bacteriological, biochemical, endocrinological and hereditary must be made covering a wide field.

SUMMARY

Investigative results are as follows:

1. In order to ascertain the actual condition of the sterility in dairy cows, the author investigated a total of 373 cases histopathologically which were classified into 2 groups A (87 cases) and B (286 cases). A group consists of infertile cows which have considerably detailed clinical history. B group consists of slaughtered cows.

2. In A group, *endometritis catarrhalis chronica* (44.8%), ovarian cyst (40.2%), *colpitis catarrhalis chronica* (36.9%), ovarian small cystic degeneration (26.4%), *cervicitis cat. chr.* (24.1%), hydrosalpinx (9.2%), perimetritis (6.9%), *hyperplasia glandularis cystica endometrii* (6.9%), etc. had high frequency.

3. In B group, colpitis (43.4%), ovarian cyst (18.5%), cyst formation in vagina (11.8%), endometritis (10.8%), cervicitis (9.8%), ovarian small cystic degeneration (8.0%), cyst in perioviduct (7.1%), *hyperplasia cystica glandularis endometrii* (5.2%), lutein cyst (4.5%) and cystadenoma in vagina (3.9%), etc. were frequently observed.

4. The most interesting finding in ovaries is small cystic degeneration. This change is more observed in infertile cows than in healthy cows. It is considered that change disturbs the estrous cycle as a factor of sterility.

5. Pathologic alterations of the oviducts are considered as an important factor of sterility; they show high frequency. Perimetritis which is considered as an adhesion (clinically) is an important factor clinico-phthologically.

6. Cystic glandular hyperplasia of the uterine mucosa are considered to have a connection with cystic ovarian degeneration. However, on the other hand, it

is necessary to study *endometritis catarrhalis chronica* in the future.

7. Colpitis and cervicitis have unexpectedly high frequency.

8. The author considered that in order to ascertain the true causes of sterility clinical, pathological, bacteriological, biochemical, endocrinological and hereditary observations must be made from a wide view point in the future. Especially there must be studies of malnutrition, hormonal disturbances, partial symptoms of general diseases and disposition in an attempt to give an explanation to the cause of the non-contagious common diseases.

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EXPLANATION OF PLATE

Fig. 1. Small cystic degeneration of the ovary. H.-E. stain. × 30.

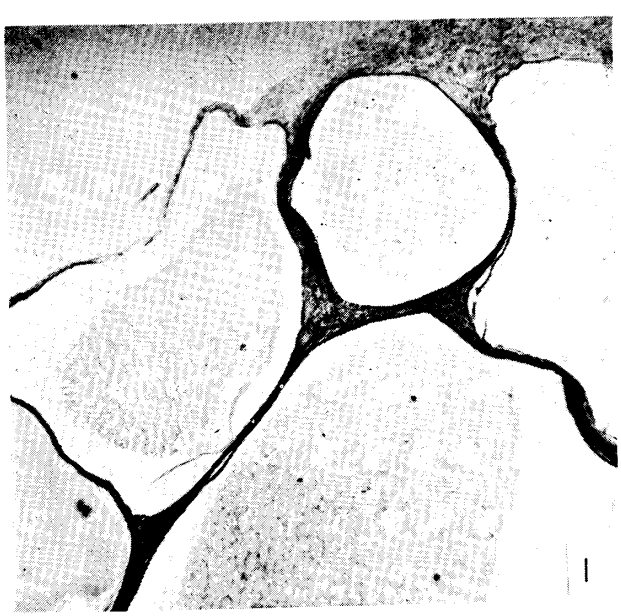
Fig. 2. Hydrosalpinx. H.-E. stain. × 30.

Fig. 3. Cystic glandular hyperplasia of the uterine mucosa showing a large cyst. H.-E. stain × 30.

Fig. 4. Retention cysts in the uterine mucosa. H.-E. stain. × 30.

Fig. 5. *Cervicitis catarrhalis chronica.* H.-E. stain. × 50.

Fig. 6. *Colpitis catarrhalis chronica* with cysts. H.-E. stain. × 30.



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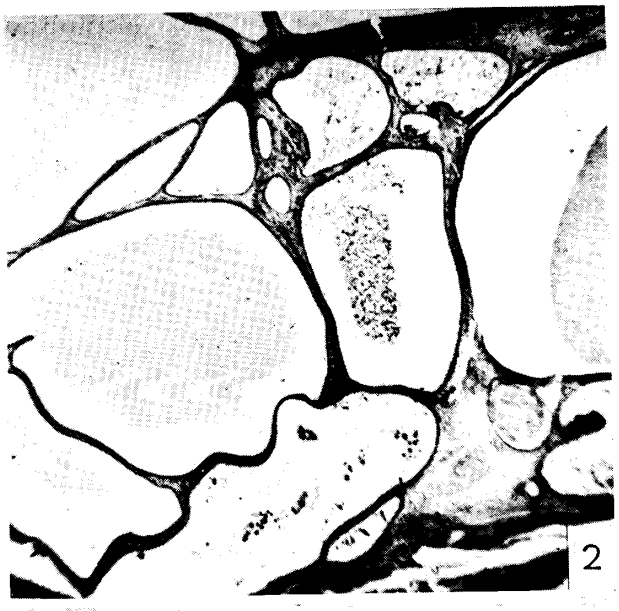

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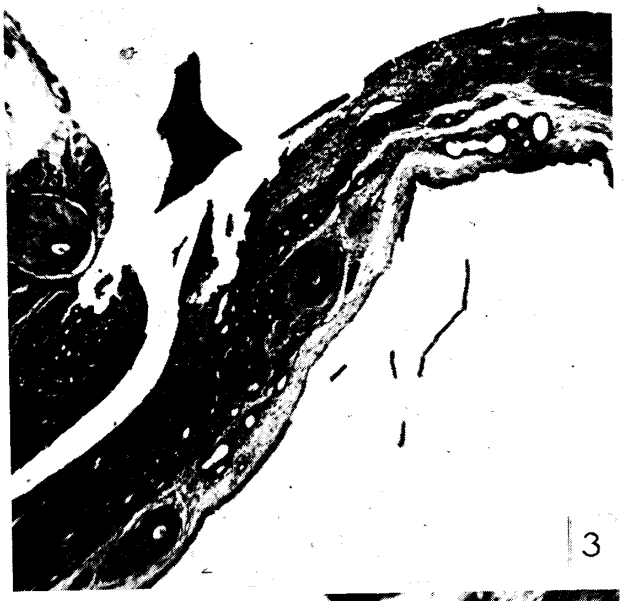

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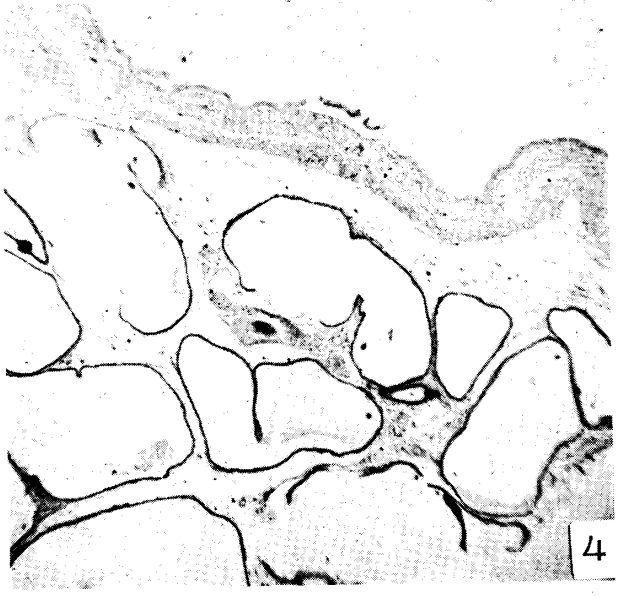

4