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HISTOPATHOLOGICAL OBSERVATIONS ON THE GENITAL ORGANS OF REPEAT BREEDING HEIFERS

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As one of the important problems of dairy cattle breeding, it must not be overlooked that not a few heifers, as well as older cows, have been disposed of as sterile, without successful results in spite of various treatments. It is very interesting and valuable for practitioners to make a comparative investigation on the clinical histories of these cases and the autoptical findings after slaughter, in order to find out the real causes of the infertility or to discuss the effects of the therapeutic treatments prescribed.

This report deals with an outline of the histopathological observations on the genitalia of 7 infertile heifers of which the clinical histories were relatively well-known.

MATERIALS AND METHODS

Materials used in this experiment were genital organs taken from the 7 heifers. These heifers had been sent to the abattoir at Sapporo from several farms at one village in the middle part of Hokkaido. They were all undersized and found to have small ovaries even at the time when they reached puberty, so that in most of them it was difficult to palpate per rectum any ripe follicle in the ovaries until they were about 20 months of age. From about 18 months of age, some hormonal therapies mainly with P.M.S. preparations were prescribed, and then they were bred 4~10 times naturally or artificially, without success in conception, till at the time when they became 25~36 months old. So, they were finally decided to be disposed of because of economic reasons. Their breeding times and ages at slaughter are shown in detail in the table. After slaughter, the genital organs of each were taken from these 7 heifers and examined macroscopically. Materials for bacteriological examination were taken from the uteri and cultured on blood agar medium for 48 hours at 37°C. The post-mortem tuboinsufflation test was carried out by means of the present authors' method^{3,5)}. Specimens for histological examination were taken from the ovaries, oviducts, uterine horns, uterine body, cervix and vagina. After having been fixed with 10 per cent formol solution, these specimens were embedded in paraffin. Sections were made from them and stained with hematoxylin-eosin solution for microscopical examination.

TABLE *Materials Used and Results Obtained*

CASE NO.	AGE AT SLAUGHTER (months)	BREEDING TIMES	MAIN MACROSCOPICAL FINDINGS	POST-MORTEM TUBO- INSUFFLATION TEST		HISTOPATHOLOGICAL DIAGNOSIS
				Pressure added (mmHg)	Patency	
419	28	2	Bilateral ovaro- tubal adhesions	{ R* — L** 60~ 0	very good good	<i>Mesovaritis et mesosalpingitis eosinophila subacuta</i>
420	30	7	No remarkable changes	{ R 120~ 0 L 80~ 0	good good	No remarkable changes
421	36	7	"	{ R 90~ 30 L 90~ 20	good good	<i>Endometritis catarrhalis chronica</i> <i>Mesovaritis eosinophila subacuta</i>
422	31	10	Unilateral ovaro- tubal adhesions	{ R 220~ 120 L 200	poor impassable	Slight degree of mesovaritis and mesosalpingitis Cystic follicle
423	27	4	Double cervix	{ R 90~ 25 L 170~ 90	good poor	Slight degree of uterine catarrh
424	25	4	Bilateral ovaro- tubal adhesions	{ R 230~ 150 L 160~ 10	poor good	<i>Peritonitis*** eosinophila chronica</i> Slight degree of <i>Endometritis catarrhalis chronica</i>
425	27	6	No remarkable changes	{ R 130~ 10 L 120~ 20	good good	Slight degree of <i>Endometritis catarrhalis superficialis chronica</i> Abnormal corpus luteum Cystic follicle

Notes: * right oviduct.

** left oviduct.

*** meaning multiple serositis including mesovaritis, mesosalpingitis and perimetritis.

RESULTS

The results obtained are outlined in the table.

Macroscopically, each ovary was nearly normal in shape and size. In all the cases, except No. 425, a large corpus luteum, ranging from 0.6 to 1.5 cm in diameter, was noted in either side of the ovaries. One or 2 follicles about 1.0 cm in size, and a number of follicles of middle or small sizes were also present in all the cases. Fine bilateral ovaro-tubal adhesions were noted in Nos. 419 and 424, and unilateral ones in No. 422. No macroscopical changes were observed in the oviducts of the other 4 cases. The uterus and vagina were almost normal in all the cases, except No. 423 which showed double cervix. The results of the post-mortem tuboinsufflation test are shown in the table. In No. 422, the left oviduct was completely impassable for air even at the high pressure of 200 mmHg, and the opposite oviduct also showed poor patency. In the other 2 cases (Nos. 423 and 424), each unilateral oviduct had poor patency.

The bacteriological examination of the uterine contents in all the cases revealed no microorganisms.

Histologically, subacute or chronic inflammations in the mesovarium and mesosalpinx were noted in 4 cases (Nos. 419, 421, 422 and 424), being characterized by a marked cellular infiltration of eosinophilic leucocytes and lymphocytes, and by a slight degree of histiocytic proliferation. In 3 of these 4 cases, ovaro-tubal adhesions were observed macroscopically. On the other hand, a comparatively slight degree of chronic catarrhal endometritis was noted in 3 cases (Nos. 421, 424 and 425). In another one case (No. 423) a slight tendency of catarrh in the endometrium was found. In 2 cases (Nos. 422 and 425), cystic degenerated follicles 1.4 and 1.0 cm in diameter were noted respectively. The wall of these follicles was thin due to degeneration of the *Membrana granulosa*. The most interesting finding was in the right ovary of No. 425: An abnormal corpus luteum, which was small in size, located in the ovarian medulla. But histologically, it showed an early stage of the development; the *Theca interna* penetrated like branches of a tree and the luteal cells were relatively fresh. In addition, marked infiltration of eosinophilic leucocytes was observed in the corpus luteum.

DISCUSSION

1. That chronic catarrhal changes in the uterine mucosa, though relatively slight, were found in 4 out of 7 animals, would suggest that uterine infection might play an important role causing infertility in these repeat breeding heifers. As has been described by many workers, such pathological changes of the uterus may be the principal cause of early death of the fertilized ovum, failures in its implantation into the endometrium, or death of the embryo during the early stage of pregnancy.

2. It is noteworthy that in most of the 4 cases, which were diagnosed histologically as having subacute or chronic mesovaritis, mesosalpingitis, perimetritis, etc., ovaro-tubal adhesions were observed macroscopically, and that the

result of the post-mortem tuboinsufflation test showed impassable for air or poor patency of the oviduct. FUJIMOTO also reported 6 cases of perimetritis (6.9%) of 87 cows slaughtered because of sterility. The majority of investigators, including MOBERG, KOIKE, DAWSON, and others have suggested that adhesions between the oviducts and the neighboring tissues may be concerned with fertilization directly or indirectly. The causes of serositis in these genital organs should mainly be attributed to various artificial factors involving therapeutic performances per rectum, such as manual expression of a retained corpus luteum, breaking a cystic follicle or careless rectal examinations, rather than infectious factors.

3. The significance of a marked cellular infiltration of eosinophilic leucocytes in these inflammatory areas or in the abnormal corpus luteum could not yet be explained clearly. MARUYAMA et al. noted that in the normal bovine endometrium eosinophilic leucocytes increase in number at the estrous phase of the cycle and decrease at the luteal phase. According to SIMON and McNUTT, a highly eosinophilic cellular infiltration in the endometrium was present in 16 cases of 109 repeat breeders, but they did not consider it as a direct cause of infertility. On the other hand, SUGAWA and HOMMA noted comparatively severe eosinophilic cellular infiltration in the endometrium of myxometra cases in the cow. FUJIMOTO also reported a case of *Endometritis catarrhalis eosinophila* in his series of sterile cases mentioned above.

4. Clinically, the case with the abnormal corpus luteum had an abnormal long estrous period continuing more than 5 days without ovulation. This clinical observation would suggest that the abnormal corpus luteum may bring about to some extent a hormonal unbalance causing an abnormal estrus and subsequent infertility.

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EXPLANATION OF PLATES

PLATE I.

- Fig. 1. Case No. 419: Dorsal view of internal genital organs with urinary bladder (scale: cm). [Right ovary is 3.1×2.1×1.8 cm in size, with a degenerating corpus luteum (0.6 cm in diameter), 3 follicles of middle size (each 0.6 cm) and many small follicles. Left ovary is 3.3×1.9×2.0 cm in size, with a relatively fresh corpus luteum (0.8 cm), 2 large follicles (1.0 and 0.8 cm, respectively) and many other follicles of middle or small size. These findings may indicate that both ovaries were physiologically active before slaughter. Many fine adhesions between ovary and mesosalpinx are observed bilaterally, but in this picture they are not clearly illustrated. Uterine horns are symmetrical and almost normal in size. But they yield a slightly softer sensation to the touch. General view of the other 6 cases resembles that of this case in most respects.
- Fig. 2. Case No. 423: Figure shows double cervical openings (scale: cm); a pair of completely separated cervical canals measured 5.5 cm in length.
- Fig. 3. Case No. 425: × 35: Abnormal corpus luteum in right ovary, of which the cavity is divided by the complicated, branch-formed tissues developed from *Theca interna*.
- Fig. 4. Case No. 425: × 100: More enlarged picture of a part of abnormal corpus luteum, showing *Theca interna* tissues penetrating into luteal cavity associated with abundant capillaries. A number of eosinophilic leucocytes infiltrate in whole area of corpus luteum, especially around capillaries.

PLATE II.

- Fig. 5. Case No. 425: $\times 35$: A part of wall of cystic degenerated follicle in left ovary. *Membrana granulosa* is thinned and desquamated. *Theca interna* is also thinned and degenerated, while *Theca externa* is thickened and highly vascularized.
- Fig. 6. Case No. 419: $\times 100$: Mesovaritis in left side. Marked eosinophilic and lymphocytic cellular infiltration with a slight degree of histiocytic proliferation.
- Fig. 7. Case No. 424: $\times 35$: Mesosalpingitis in left side. Slight degree of eosinophilic cellular infiltration in mesosalpinx is observed.
- Fig. 8. Case No. 421: $\times 35$: In right portion of picture markedly distended cystic uterine glands with thinned, degenerated epithelia are seen. Cellular infiltration mainly by lymphocytes is noted around capillaries in left portion of picture.
- Fig. 9. Case No. 421: $\times 35$: At central portion of picture, there are two lymphocytic cellular foci in deep layer of uterine *Tunica propria*.
- Fig. 10. Case No. 421: $\times 400$: Higher magnification of one of cellular foci shown in fig. 9. It consists mainly of a number of lymphocytes.



