Abstract

Study on Neutrophilic Migration to Uterine Cervical Mucus of Estrus Sows

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Sows and gilts of reproductive age repeat the 2~3 days of estrus in 21-day cycle throughout the year; they are so called the polyestrus animal.

Each animal species exhibits the different signs during estrus. One of the characteristic changes displayed by sows and gilts during estrus is the neutrophils' appearance to mucus secretion of uterine cervix (cervical mucus). This phenomenon has been known, clinically; however, its biological significance and the mechanism were hardly elucidated. Generally, neutrophilic migration to local tissue is considered the sign of inflammation. It is interesting that similar phenomenon occurs in estrus, which is a transient physiological phenomenon, and furthermore, it is only seen in cervical mucus of sows and gilts. Therefore, we conducted the series of experiments for the purpose of clarifying the endocrinological mechanism of this neutrophilic migration.

Chapter 1

The Neutrophilic Count in Cervical Mucus and External Signs in the Reproductive Cycle

First, the fluctuations of neutrophilic count in cervical mucus (NCCM) and external signs in reproductive cycle were analyzed. Neutrophilic counts from cervial mucus were taken twice, in morning and afternoon, every day throughout the period of estrus, pregnancy, farrowing, and lactation, on two sows with normal cycle of estrus. Cervical mucus was collected by cotton swab inserted in 1cm-diameter vinyl chloride tubing; the measured size cotton swab was pressed against the mucus wall area of second ruga in cervix. Cotton swab was washed in 1 mL phosphate buffer solution, and the resulting cell-suspension fluid was subjected to cell count. On the other hand, as the external signs of estrus, observation were made

and recorded on the appearance of male receptivity, and hyperemia and swelling of vulva. As the result, except for the several days immediately after farrowing, the period when numerous neutrophils appear in cervical mucus was identified to be within one week prior to and within one week from the estrus, the day of the external signs were conspicuous. Evidently during this period, the number of neutrophil count in cervical mucus was 2.5 to 15 times the level of other period. The possibility, which the neutrophilic count increase by the effect of estrogen, is suggested from this finding.

Chapter 2
The Neutrophilic Count in Cervical Mucus and External Signs and Their Relationship to Ovulation Time

While in the estrus, external signs become conspicuous, and at the same time, follicles are maturing and ovulation occurs in ovary. I further analyzed which part of these stages is the most closely related to the increase of NCCM. For this investigation, 7 sows without any clinical abnormality in reproductive organs and displaying normal estrus cycle were used. Daily records of external signs and neutrophils counts were entered for the 4 sows among the above, for 9 days before and after the starts of estrus, which include two starting days, or 14 days. For remaining 3 sows, their ovulation was identified, and in order to estimate the ovulation timing, they were bred and subjected to laparotomy operation. After the observation was made on ovary, ova were collected by perfusion of physiological saline solution in oviduct and uterus area. Simultaneously, these 3 sows' blood progesterone and estrogen levels and white blood cell count in peripheral blood were measured.

The total of 8 estrus was observed in aforementioned 4 sows. As the result, the increase in NCCM occur from the day before the start of estrus to the next day of the start, when the external signs are the most notable. Duration of time the increase was observed was within 24 to 36 hours. The finding within the individual had shown that there is a case (1 sow in 4) shows the increase of neutrophils at the same timing and the same level in first and the second estrus, and a case (1 sow in 4) shows at the different timing and the different level. Though in the latter case, the difference in

timing remained within 24 hours.

The three sows subjected for identification of ovulation were mated either once or twice within 36 hours after standing heat. One sow was laparotomically incised after 28 hours, and 5 embryos at 3 to 4-cell stage were identified. No ovulation was identified in one saw by exploratory incision after 15 hours and 25 hours from mating; however, when sacrificed after approximately 30 hours, 8 embryos at 2- cell stage were collected. In the remaining sow, one ovum was collected from the exploratory incision after 38 hours; however, the fertilization was not identifiable about this ovum. In general, fertilized swine ova need 14~19 hours to reach 2-cell stage embryo, and 24 hours, to 4-cell stage. Presumably, from this information, the ovulation timing of subject sows, identified with fertilized ova, were speculated 28 and 38 hours each after the start of estrus. These time period almost overlap or extremely close to the peak hours of neutrophils count in cervical mucus.

The blood estrogen level of the above three sows began to increase 2~3 days before the start of estrus, and decreased as the sow approaches to the start of estrus or estimated ovulation date. On the other hand, progesterone level displayed the low-grade increase in the day before the start of estrus towards estimated ovulation date. When the time period the neutrophils count in cervical mucus was at the peak, estrogen level was decreased, and progesterone level begins slight increase. Clear correlation was not identified between the neutrophils count in cervical mucus and white blood cell count in circulation.

From above results, it is concluded that the increase of the neutrophils count in cervical mucus is limited to the short period of time in estrus, and at the same time, the increase is closely related to ovulation and blood estrogen/progesterone level.

Chapter 3

The External Signs and Neutrophilic Count in Cervical Mucus of Ovariectomised Sows with Induced Pseudo-Estrus

According to the result of Chapter 2, the effect or estrogen and progesterone (P) administrations were analyzed. As estrogen, estrone (E_1),

estradiol-17 β (E_2), and estriol (E_3) were used. Administration program was as in following table.

Subject Sow	E_1 for 3 Days	$E_2$for 3 Days	E_3 for 3 Days	E_2 Single Adm.	P for 3 Days	E_2+P	E_2 Single Adm.
#68	①	③					
#69	②	④					
#70			⑤		⑩		
#71			⑥		⑪		
#154				⑦		⑫	
#138				⑧		⑬	⑮
#164				⑨		⑭	
#108	Natural (Non-induced) estrus sow						

①② Administrations of 3 consecutive days in the order of 1.0, 2.0, 1.0 mg

③④ Administrations of 3 consecutive days in the order of 2.0, 4.0, 2.0 mg

⑤⑥ Administrations of 3 consecutive days in the order of 1.0, 2.0, 1.0 mg

⑦ 0.2 mg ⑧ 0.5 mg ⑨ 1.0 mg

⑩⑪ 40 mg administration for 3 consecutive days

⑫ 60 mg administration of P on 4 and 1 day before and 2, 5, and 8 day after 1.0 mg E_2 administration

⑬ 140 mg administration of P on 4 and 1 day before and 2, 5, and 8 day after 0.8 mg E_2 administration

⑭ 100 mg administration of P on 4 and 1 day before and 2, 5, and 8 day after 0.8 mg E_2 administration

⑮ 1 mg administered for the purpose of comparison with #108

In ① and ②, external signs appeared, and neutrophilic count in cervical mucus increased as well. However, the levels of increase were extremely low. In ③ and ④, the external signs were retained, and NCCM increased 8 ~ 10 folds, compared to the level before E administrations. However, transition pattern as observed in natural estrus was not identified, for observations such as multiple peaks and high value after the disappearance of external signs were made. The external signs also

appeared in ⑤ and ⑥; however, the transition pattern differed from natural estrus, such as the mild increase of NCCM and biphasic peaks, were observed. In cases of ⑦, ⑧, and ⑨, which the amount of E_2 administration was reduced, external signs were observed in each individual, and the increase of NCCM was observed in dose-dependant manner. Moreover, their levels and transition pattern closely resembles that of natural estrus. The external pattern did not appear in ⑩ and ⑪, throughout the observation period, and the characteristic change was not observed in NCCM. In ⑫, ⑬, and ⑭, mild hyperemia and swelling of vulva were observed, but no standing heat was observed. The level of increase observed in ⑫'s NCCM was low compared to ⑨, which administered E_2 dose was the same; however, the transition pattern closely resembles that of natural estrus. As the amount P's administrations increase, the characteristic transition pattern was lost and the amounts of fluctuations were diminished.

In case of naturally occurred estrus (in #108), NCCM exhibited the characteristic peak in the next day the estrus started, and it was the 7-fold increase. Accompanying with this rise, blood E_2 level reached the maximum of 109 pg/mL; and on the next day, sharply decreased to approximately 10 pg/mL. In ⑮, the external signs appeared on 3 days after E_2 administration. Blood E_2 concentration suddenly increased 2 days after the administration (to 317 pg/mL), and this level was maintained for 3 days. NCCM increased 7-fold, with the appearance of external signs. Even afterward, the high level of blood E_2 was maintained, and the cyclical rise and fall was repeated in every other day throughout the period.

From above results, the increase in NCCM, which is characteristically observed during the estrus, requires estrogen, especially the E_2. And progesterone was shown to have inhibitory effect.

Chapter 4

The Morphological Change of Cervical Mucus Tissue in Sows with Naturally Occurred Estrus and Induced Pseudo-Estrus

In the previous chapters of Chapter 1 through 3, we have clarified the followings. The time periods when the great numbers of neutrophils appear in cervical mucous membrane closely relates to the ovulation time, and

corresponds to the time when the estrogen level decrease and progesterone level began mild increase. And the increase of NCCM similar to the natural estrus occurred in ovariectomised sow with induced pseudo-estrus by administration of estrogen. Progesterone was shown to inhibit this phenomenon. Subsequently, histological analysis was conducted to investigate what type of change in tissue yield the migration of neutrophils.

One sow with natural estrus and 7 ovariectomised sows were subjected to experiment. For 3 of ovariectomised sow, one dose of 1.0 mg, 0.8 mg, and 1.0 mg was administered to each. The tissue samples from second spirette wall area in cervix were obtained by using endometrial biopsy apparatus, immediately before and immediately after the administration; also on day 2, 3, 5, 7, and 9 from the administration. Other 3 sows were allocated as E_1, E_2, and E_3 administration group, and all of them received three consecutive intramuscular injection of 1.0 mg in the first time, 2.0 mg the second, and 1.0 mg the third. The remaining 1 sow was administered 40 mg of P for three consecutive times. The cervical tissue of the last 4 sows were collected on 4 days prior to administration, on the day of administration, and 2, 3, and 6 days after the administration. The biopsies from the sow with natural estrus were conducted on 4 days prior to the predicted date the estrus start, on the day the estrus started, and 2, 3 and 4 days after the start.

The external signs and the transition of NCCM were mostly the same as results described in Chapter 3. In the E_2 single administration group, proliferation of mucosal epithelium cell in cervical area, infiltration of deformed neutrophils into epithelial cell layer, lamina propira mucosae, and submucosal layer was identified from 2 days after the administration, and the submucosal vessels were dilated. Epithelial cell proliferation began to decelerate after the peak observed on day 5. However, 2 sows among the 1.0 mg-administered sows continued to display dilation of vessels in lamina propira mucosae and submucosal layer up to day 9, and infiltration of deformed neutrophils and white blood cells in the vessels were clearly identified.

Proliferation of mucosal epithelium layer in cervical area of sows, administered E_2 for 3 consecutive days, became marked on 2 days after the end of administration, and the level of proliferation exceeded that of sow with natural estrus in same period. In sows administered E_1 or E_3, the maximum proliferation of mucosal epithelium layer in cervical area was

observed 1 day later. On the other hand, such proliferation was not observed in sows administered P. Similar tendency has been identified in the cases of vessel dilation in lamina propira mucosae and submucosal layer, and deformed neutrophils infiltration. The cells of interior vessel wall was at first cylindrical or spindle shaped. However, as the estrus or pseudo-estrus progress, such observation as the cells became thin as squamous cells and the dilated vessel lumen became filled with white blood cells were observed.

As described in Chapter 3, the major cause of estrus in these cases was, again, identified to be the estrogen, and especially, the E_2. Moreover, it is speculated that the neutrophils in cervical mucus migrated from the dilated and thinly changed cell-lined vessels. However, at this moment, whether the estrogen directly causes these histological changes or whether there is any unidentified mediating factor presents are unknown.

Chapter 5
The Effect of Indomethacin and Granulocyte Colony Stimulating Factor on Neutrophilic Migration to Cervical Membrane

From the series of experimental results described in Chapter 2, 3, and 4, ovulation time, blood level of estrogen (E), and progesterone (P, hereafter) were identified to have close relationship with the change in NCCM. Based on these results, following experiment was planned. Prostaglandin (PG) is recognized as the substance relates to inhibition of ovulation, and PG is strongly related to inflammatory events. The change in neutrophilic count and external signs were observed after administering Indomethacin (Ind), which is PG synthesis inhibitor agent. Moreover, endocrinological and macroscopic confirmation of ovary were conducted. Furthermore, the verification is conducted on the inhibition by concomitant administrations of E_2 and Ind on the promoting effect of E_2 to increase NCCM. Also, verification on the effect of concomitant administration of E_2 and granulocyte colony stimulating factor (G-CSF) on the neutrophils of cervical mucus was conducted.

For the experiment 1, 6 normal pigs of normally repeating estrus cycle were used. As Ind group, 4 of them were administered Ind (720 mg per day,

3 mg/kg), which has inhibitory effect on ovulation. The 7-day administration started on 6, 3, and 1 day before the start of estrus. Ind non-administration group was fed flour. Ovariectimised sows were subjected to the experiment 2. E_2 was administered by intramuscular injection, and Ind was administered for 16 days starting 2 days before E_2 administration.