# Evaluation of Ovarian Function from the Crystallization Phenomenon of Uterocervical Mucus in Cattle

## - With Special Reference to Diagnosis and Treatment of Ovarian Dysfunction -

Hidéo Harada

# Evaluation of Ovarian Function from the Crystallization Phenomenon of Uterocervical Mucus in Cattle - With Special Reference to Diagnosis and Treatment of Ovarian Dysfunction -

Hideo Harada

## Summary

Recent introduction of biochemical assay techniques such as radioimmunoassay (RIA) and enzyme immunoassay (EIA) into the sphere of animal reproduction has immensely contributed to quantification of hormones in blood and milk and consequently to determination of the kinetics of sex hormones in vivo. However, because many of sex hormones actually manifest their actions with great subtlety due to their interactions, scrupulous care is needed for the assessment of these actions. Reponses to hormones are also known to considerably differ among individual animals. Establishment of a simple and objective method for the diagnosis of ovarian function which can be readily applied on the field has been strongly desired from the standpoint of veterinary clinicians.

The properties of uterocervical mucus has been regarded to well reflect the vicissitudes of estrogen (E) and progesterone (P) in the follicular and luteal periods. A crystallization phenomenon in a normal sexual cycle is characterized by specific patterns. The patterns are characteristic of

each phase of the ovarian cycle. In ovarian dysfunction, however, such patterns remain mostly obscure.

The pattern of mucus crystallization varies with delicate quantitative fluctuations of these hormones not only in the estrous and diestrous periods but also during the transitional periods from P-dominant to E-dominant and from E-dominant to P-dominant phases. In this study, changes in crystallization patterns in reflection of subtle quantitative fluctuations of E and P were investigated in detail. The results verified that the crystallization of uterocervical mucus can be effectively used for the diagnosis of ovarian function in cows.

1. Preparation of mucus specimens

Mucus of a rice grain size was smeared on a slide-glass and spread to approximately 2 $cm^2$ in area. It was then air-dried at temperature below 35°C for more than 10 minutes.

2. Examination in immature cows

The specimens from immature cows below 6.5 months of age, in which the ovarian activity appeared to be in a stationary state, showed a sign of positive crystallization which suggested the beginning of very slight endocrinological activity. The pattern of crystallization was found to sensitively reflect fluctuations of even extremely small amounts of E and P.

3. Examination in adult cows

In adult cows with recurring normal sexual cycles, external signs, ovarian findings and mucous properties are all known to correlate mutually. This experiment especially confirmed that the fern-like crystallization in the center of the specimen not only expressed the degree of crystallization (+) - (+++) but also demonstrated differences in size, shape, thickness and even arrangement. In addition, the crystallization patterns expressed characteristic findings in response to the fluctuations of E and P balance, such as the appearance of granular cells (leukocytes), formation of air bubbles, formation of nebular or layered margins at the peripheral area of crystal formation and the presence or absence of mucous masses.

On the basis of the above confirmation, the mucous findings in normal adult cows were divided into 6 categories by the differences in E and P balance. Another type obtained in immature cows (E- and P-deficient) was added to make the total of 7 types (types I - VII). These 7 types were used as a measure for estimation of the E and P levels to reversely assess the hormone secretory function of the ovary from the mucous findings.

4. Examination in cows with experimentally induced hormone abnormality

Dyshormonism of E and P was artificially produced by

administration of $E_2$ at the functional stage of the corpus luteum in cows with recurring normal sexual cycles. This produced the same mucous findings as obtained in cows with ovarian dysfunction. This result was helpful in confirming the nonphysiological kinetics of the ovary.

5. Examination in pasturing cows with ovarian dysfunction

Crystallization patterns of the uterocervical mucus were examined in cows in which correct diagnosis was precluded due to unknown sexual cycle. It was found that patterns which represented neither the luteal nor estrous stage but consisted of a mixture of these stages and also irregular patterns which, instead of strand-like or fern-like crystallization, showed a mottled, stelliform or asteroid shape of crystallization could be diagnosed as ovarian dysfunction.

On applying the 7 types of crystallization patterns to ovarian dysfunctions, they corresponded to the following.

Type I: P-dominant. In normal cows, this type is seen at the functional stage of the corpus luteum. The crystallization of mucus is(-). The formations of air bubbles and mucous masses are found. When viewed from the pathological aspect, this type is detected when a follicular cyst coexists with the persistent corpus luteum or corpus luteum of pregnancy.

Type II:Though P-dominant, E is also slightly involved. In normal cows, this type is seen at the initial phase of luteal regression. The crystallization of mucus is (+).

The major portion of the specimen represents the luteal phase. Small and irregular crystal formations are observed in some areas. The crystallization is surrounded by granular cells (leukocytes). Pathologically, this type is detected in cows with follicular hypoplasia or during continuous estrus.

Type III: E is somewhat more dominant than P. In normal cows, this type is seen immediately prior to the onset of estrus. The crystallization of mucus is (++). Small fern-like and irregular crystal formations are present over a wide area. Nebular or layered margins are formed around the crystal formations. Pathologically, this type is detected in cows with coninued estrus and hypoplasia of corpus luteum.

Type IV: This type represents E-dominance. It is seen during the estrous stage in normal cows. The crystallization of mucus is (+++). This is as a typical fern-like crystallization. Regular crystal formations are closely arranged even in the peripheral areas of the specimen. In the proestrus, crystal formations are rather small in size. However, in the middle and late stages, they grow larger. Pathologically, this type is rarely detected in the nymphomaniac cow with follicular cyst.

Type V: Although somewhat feebly, only E is active. In normal cows, it is seen immediately after ovulation. The crystallization of mucus is (++). Usually, the pattern begins to rapidly shrink into regression from the pattern of

the estrous stage. When the mucus during the estrous stage contains a large amount of watery mucus, the crystal formations become rough in arrangement and closely resemble a pattern seen in a moisture-absorbed specimen. This type is detected during the estrus in cows with cystic corpus luteum.

Type VI: E and P are active at low levels. In normal cows, this type is seen on the 4th - 5th day after ovulation. The crystallization of mucus is (+) - (++). In the center of the specimen, thick curvilinear irregular crystals are formed. The peripheral area represents the luteal phase and shows the appearance of granular cells. Pathologically, this type is detected during the treatment of follicular cyst or in cows with lutein cyst.

Type VII: This type appears when both E and P are recessive. The crystallization of mucus is (-) - (+). In normal cows, such a hormone balance does not exist and is seen in only heifers. Pathologically, this type is detected in cows with anestrous follicular cyst and ovarian quiescence.

In nymphomaniac cows with follicular cyst, the composition of mucus varies in accordance with the functional levels of follicles. Therefore, the mucus manifests diversified patterns of crystallization. The general profile of the specimen lacks congruity and is characterized by a partial commixture of various types.

If the prognosis of follicular cystoma is evaluated on

the basis of the mucous findings, regression of crystallization in the specimen could be judged as a reflection of uneventful prognosis. When it was succeeded by type I, it could be judged as a sign of complete luteinization.

As described above, the assessment of the patterns of mucous crystallization in cows with ovarian dysfunction was compared with other simultaneously obtained results, namely, the clinical findings and ovarian findings by rectal examination. On the basis of the duplicate diagnoses, the pathology was evaluated and appropriate treatment was prescribed. This produced highly effective treatment results.

6. Crystallization pattern during estrus and gestation

The relation between the crystallization pattern during estrus and gestation was investigated. Not only in normal cows but also even in cows after healing of ovarian dysfunction, the estrus preceded by formation of the functional corpus luteum displayed distinct external signs of estrus and the mucus formed large and regular-shaped fern-like crystals. In cows which were inseminated at this stage and which had the corpus luteum enough to secrete a certain amount of P until the 7th day after ovulation, the pregnancy rate amounted to 72% (46/64).

7. Administration of LH-RH-EA and APG, and mucous properties

Administration of LH-RH-EA or APG in cows with func-

tional immatured follicles responsive to hormones was found to not only stimualte the growth of follicles but also improve the properties of mucus remarkably. This indicated that the responsiveness of the ovary to drugs could be assessed from the properties of mucus.

8. Mucous findings in pregnant cows

The mucus from pregnant cows disclosed no findings specific to pregnancy. In the initial period of pregnancy, the crystallization pattern was type I. From approximately 90 days after pregnancy, a thick-lined irregular crystallization pattern appeared partially in the specimen. However, at any stage of pregnancy, the specimen had a general appearance of P-dominance. This suggested that the mucous findings are very helpful as one of the supplementary test methods for the early diagnosis of pregnancy.

9. pH in uterocervical mucus

In normal estrous cows, mucous pH was 7.0 - 7.2 in nulliparity and 6.4 - 6.8 in multiparity. At the luteal phase, it was 6.6 - 6.8 in nulliparity and 6.8 - 7.0 in multiparity

In cows with anestrous follicular cyst, luteal cyst and ovarian quiescence , pH exceeded 6.8 on an averge at the time of initial diagnosis. In the cow with continuous estrus, it was also above 6.8. It ranged over a wide range (6.2 - 7.2) in the cow with nymphomaniac follicular cyst. In the cow with purulent endometritis mucous pH increased to above 7.4. No especial correlation

was found between pH of the uterocervical mucus and the crystallization phenomenon.

10. Serum concentrations of $E_2$ and P in cows with hypovarianism

The serum concentrations of $E_2$ and P in cows with ovarian hypoplasia were determined by RIA. Although both $E_2$ and P were very low (below 10 pg/ml and 1 ng/ml respectively), some of the mucus specimens reflected the luteal phase and some others showed crystal formations. This result indicated that hormonal actions at the E and P target sites does not necessarily correspond to the serum concentrations of E and P.

The results obtained in this study confirmed that the detailed analysis of uterocervical mucous properties (especially crystallization) was a very helpful means for estimating the endocrinological function of the ovary. Comparison of the mucous findings with the ovarian findings obtained by rectal examination enabled not only morphological but also functional diagnoses. In planning of treatment methods, it was considered to provide a reliable guideline to determine the course of ovarian activity (whether to induce luteinization or follicle formation). Furthermore, it seemed to permit correct and early evaluation of the response and prognosis of the treated ovary and to contri-

bute to the accurate and objective diagnosis and treatment of reproductive disorders in cattle.