Studies on the peripheral plasma sex steroids in a cow during the estrous cycle and in early pregnancy

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For the purpose of studying the correlation between the fertilization failure or early embryonic death and the anomaly of secretion of sex steroids in a cow, concerning the chemical measuring method and the radioimmunoassay method for measuring estrogen and gestagen in the blood of cow, the methods were established at first, and using these methods in combination with the hitherto used biological assay method, the aspects of estrogen and gestagen in the peripheral plasma in a cow during the estrous cycle and in early pregnancy were clarified. Secondly, an repeat breeding cow was autopsied in 15-28 days after artificial insemination, and the presence of embryo in the uterus or its growing state and the anomaly of sexual organs were investigated,and at the same time the aspects of estrogen and gestagen in the peripheral plasma before the artificial insemination up to the time of autopsy were pursued, and the fact that there is a remarkable difference in the secretion type of these sex steroids between an infertile cow and a fertile cow was made clear.

In the following is described of an outline of the results obtained.

1. Fluorometric determination of free plasma estrogens during the estrous cycle in the cow

The fluorescence method of Ittrich was applied to the determination of free estrogens in the peripheral blood of two Japanese beef cows during the estrous cycle.

The patterns of blood levels of estrone and estradiol were almost the same in tendency in the two cows during the estrous cycle. Estradiol was generally higher in level than estrone. Estriol was not detected at all. In the two cows,

total estrogen increased in the proestrous phase, presenting a sharp peak at a level of 35.3 and 99.8 ng/liter (estrone, 5.9 and 16.0 ng/liter ; estradiol, 29.4 and 83.8 ng/liter), respectively, prior to ovulation, and decreased rapidly to a minimum level, or 3.8 and 5.3 ng/liter (estrone,1.6 and 1.9 ng/liter; estradiol,2.2 and 3.4 ng/liter) after ovulation. In the luteal phase (6-8 days after ovulation), it reached a peak again at a level of 10.1 and 27.0 ng/liter (estrone, 2.4 and 3.4 ng/liter ; estradiol, 7.7 and 23.6 ng/liter). In short, two peaks appeared during one estrous cycle.

2. Fluorometric determination of plasma progesterone during the estrous cycle in the cow

The method of Heap for the determination (of progesterone) was modified by introducing thin layer chromatography (TLC) and improving some conditions for coloration.

TLC was applied to isolation and purification of progesterone and 20β -hydroxyprogesterone. Satisfactory results were obtained by developing a sample twice with n-hexane : ethylacetate (5:2) and once with benzene : ethylacetate (2:1).

The most intense and stable fluorescence was obtained when the color was developed with concentrate sulfuric acid : ethanol (3:2) at 60°C for 10 minutes. When determined by the spectrofluorometer, the maximum wavelength of this fluorescence presented peaks at 468 nm for excitation and 525 nm for emission. Under the same conditions as these the minimum amount of detection of 20β-hydroxyprogesterone was 2.5 ng.

The blood level of progesterone was determined by the modified method during the estrous cycle in four Japanese beef cows. Its minimum value was 0.2-0.8 ng/ml during the estrous

period, and its maximum value 2.6-6.2 ng/ml during the luteal phase (8 to 20 days after ovulation).

3. Radioimmunoassay of blood plasma estrogen and progesterone during estrous cycle and early pregnancy in the cow

Peripheral plasma estrogen and progesterone levels in the cow during the estrous cycle and early pregnancy were determined by radioimmunoassay.

In six cows showing normal estrous cycle, blood level of estrone and estradiol fluctuated with almost same patterns, but the concentration of estradiol was generally higher than that of estrone in three of six cows. Level of total estrogen formed a medium peak (9.0-10.5 ng/ml) during luteal stage of 3-12 days after ovulation, and a sharp peak (10.6-19.5 pg/ml) in proestrus, 3-1 days before next ovulation. In three other cows, no increase in total estrogen was found during luteal stage, but,they showed its sharp and high peak (12.7-20.0 pg/ml) 2-1 days before next ovulation. Plasma progesterone showed its minimum level (0.2-0.3 ng/ml) at estrus and maximum level (1.8-4.2 ng/ml) during luteal stage of 11-17 days after ovulation. It began to decrease rapidly 4 to 5 days before next ovulation.

In five cows of early pregnancy, within 32 days after insemination, plasma estradiol showed higher level than that of estrone. In two of them, concentration of total estrogen increaded (12.4 and 7.2 pg/ml) temporarily 5 days after insemination, but it maintained of low level (2.3-6.0 pg/ml) until about 12 days after insemination in three others. Thereafter, the level of total estrogen rose gradually in all of them and showed considerable high level (6.2-11.6 pg/ml) 27-28 days after insemination. Concentration of plasma progesterone in-

creased rapidly after the ovulation and showed higher level of 6.0 ± 1.7 ng/ml at 31-32 days of pregnancy than that in luteal stage of the estrous cycle.

4. Radioimmunoassay of blood plasma progesterone during estrous cycle in the cow

Radioimmunoassay method was compared to competitive protein assay and fluorometric assay method for the determination of plasma progesterone of the cow. Peripheral blood progesterone of the during estrous cycle was determined by radioimmunoassay.

High potent antiserum against progesterone-3-oxime-BSA (optimal dilution, 1:40,000) was prepared for radioimmunoassay. Calibratiin curve made by the antiserum showed clear lineality between 0 and 400 pg of progesterone, and percent binding of ^{3}H-progesterone was 70% or higher at 0 pg. The antiserum also showed highly specificity against progesterone, and no significant cross reaction was found against other various steroids except 5α-pregnanedione.

In radioimmunoassay method, values of progesterone determined on the crude samples which was extracted from blood samples with ether (direct method), were compared to those determined on the purified samples obtained by carring out column chromatography (chromatographic method). There was a good correspondence in the values of progesterone between direct and chromatographic methods.

No significant differences were found between the direct method of radioimmunoassay, competitive protein binding assay and fluorometric assay method, in values of progesterone, determined on the same plasma samples collected from the cow at various stages of the estrous cycle.

Concentrations of plasma progesterone, determined by the direct method of radioimmunoassay in 9 cows during estrous cycle,

showed minimum level (0.2-0.3 ng/ml) at estrus and maximum level (3.2-5.8 ng/ml) during luteal stage of 11 to 16 days after ovulation. The concentration was rapidly decreased about 4 to 6 days before the next ovulation.

5. Peripheral blood plasma sex steroids before and after insemination in repeat breeding cows

An experiment was carried out to investigate the relationships between the secretion pattern of sex hormones and infertility in cows.

Nine repeat breeding cows and two normal cows obtained from individual farms were inseminated experimentally. The animals, except one which was held under observation up to 70 days after insemination, were autopsied 15-28 days later, and their genital organs were examined. The levels of estrogen and gestagen in the peripheral blood plasma were assayed by the method of Sulman et al. and of Hooker & Forbes.

In one of nine repeat breeding cows, a dead fetus was found in the uterus, but not any other cows had fetus or any other conceptus. There was no evidence of bacterial infections in the uterus and oviduct of any of all these cows. Of two normal cows, one had a fetus developed normally in the uterus, and the other continued the normal pregnancy up to days after insemination. At estrus,estrogen levels in the fertile cows reached a maximum 2-3 days before ovulation. It decreased rapidly to a minimum immediately after ovulation. In the infertile cows, however, the time showing the maximum levels was later 1-2 days than that of the fertile cows. It appeared on the day of ovulation in four of eight cows. In the infertile cows, except one, the time exhibiting the minimum levels was also late, which appeared 1-3 days after ovulation, Estrogen levels observed in the luteal stage following insemination were classified to the two groups ; i.e. a high estrogen group, and a low

estrogen group. The normal pregnant cows were belonged to the later group and the cow having a dead fetus in the uterus to the former group. The gestagen levels showed a peak at around estrus in all three fertile and six of eight infertile cows, almost simultaneously with, or a little later than, the time of estrogen peak. In the luteal stage following insemination, the gestagen levels were relatively high in six infertile cows which formed a peak at estrus, but low in other two infertile cows. In two normal pregnant cows, the gestagen levels increased with advance of the pregnancy, and was maintained at a high level. However, it remained at a low level in the cow having a dead fetus in the uterus.

From these results, it may be postulated that when some abnormal secretions of estrogen and gestagen occurred before and after ovulation, fertlization failure or death of fertilized ova might be induced, and that when it occurred in the luteal stage following insemination, early embryonic death might be induced.