Changes of Estrogens in Milk of Thoroughbred Mares During Foal Estrus

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Summary. Levels of three kinds of estrogens, estrone, estradiol-17 β and estriol, in milk from 5 Thoroughbred mares during foal estrus were measured by radioimmunoassay. Estradiol-17 β and estriol levels in milk have no relevant importance in predicting conceptionable estrus. Only estrone concentration in milk showed a relation to the conceptionable estrus cycle in thoroughbred mares. This is probably the first report that deals with estrone concentration in milk during foal estrus and the measurement of estrone could be a reliable guide to know the estrus to be capable of conception in postpartum mares.

KEY WORDS: MARE, MILK, ESTROGEN, FOAL ESTRUS.

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Plasma levels of estradiol in mares of different breeds have been measured near estrus (Norden *et al.*, 1976) and before ovulation (Pattison *et al.*, 1974). Further, progesterone concentration in milk and blood plasma from Quarter Horse mare during foal estrus have been determined (Gunter *et al.*, 1980).

However, no papers have been published on estrogen concentration changes in milk of Thoroughbred mares. Therefore the objectives of this study were to compare daily changes in concentrations in three kinds of estrogens, estrone (E1), estradiol-17 β (E2) and estriol (E3), in milk from Thoroughbred mares during foal estrus, and to determine the relationships between the levels of estrogens and conceptional ability in that estrus.

Materials and Methods

Milk samples were obtained daily from 5 lactating Thoroughbred mares by manual milking from the onset of foal estrus to 30 days afterward. Mares were checked daily for estrus with a stallion during estrus. Ovaries were examined by rectal palpation in estrus mare to detect the

estimated time of ovulation. Pregnancy was also detected by rectal palpation of cervix, uterus and ovaries. Milk collected was immediately centrifuged 3000 rpm for 10 min. and milk plasma was stored in a polyethylene tube at -20°C until assayed.

Each milk plasma sample of 500 μl was pipetted into a test tube and 4 ml of diethyl-ether was added. After mechanical shaking for 3 min., further centrifugation at 3000 rpm for 10 min. was done and the supernatant was placed into an other test tube. This process was repeated twice and the solvent extracts were obtained. The extracts were then washed with 1 ml of distilled water and subjected to mechanical shaking for 2 min.. After shaking, they were centrifuged at 3000 rpm for 10 min. The supernatant, placed in a test tube, was immersed in a waterbath (37° – 38°C) and a continuous flow of nitrogen evaporated the sample to dryness. Dried residue was dissolved in 100 μl of benzene : methanol (85 : 15 v/v) solvent twice before it was separated by the Sephadex LH-20 gel column chromatography. Three fractions of estrogens obtained from the purification methods described

Fig. 1. Changes of estrone, estradiol-17 β and estriol concentration in milk of mare case 1 during foal estrus.

above were measured by the Dextran-Charcoal method of radioimmunoassay (RIA) according to Makino (1972).

Antisera against E1-, E2- and E3-6-CMO-BSA were purchased from the Teikoku Hormone Manufacturing Co. (Tokyo Japan). The Cross-reactions of E1 antisera with E2 and E3 were 2% and 1.6%. Cross-reactions of E2 antisera with E1 and E2 were 3% and 2%. These of E3 antisera with E1 and E2 were 0.01% and 0.5%, respectively. The coefficient of variations for intra- and inter-assays was 10% and 16% for E1: 15% and 19% for E2: 12% and 18% for E3, respectively.

Results

The variations of concentration in three estrogens in milk daily obtained from each mare were presented in Figs. 1~5.

Case 1: The mare showed the onset of foal heat on day 7 postpartum and she was bred on day 5 of the foal heat and conceived on that mating. E1 concentration in milk maintained more than 30 pg/ml for 5 days from the onset of estrus (5 days before breeding). A peak of 50 pg/ml was seen on day 2 of the estrus (3 days before breeding) and then the level of E1 declined with time. On day 7 of the estrus (day 1 after breeding), E1 level was less than 20 pg/ml. During the foal estrus, E2 level was less than 10 pg/ml without significant changes. On day 7

after breeding it increased to 22.5 pg/ml. On day 9 after breeding, it showed a peak of 32.5 pg/ml, and thereafter it declined with time. E3 level never exceeded 20 pg/ml before and after breeding (Fig. 1).

Case 2: The mare showed the onset of estrus on day 14 postpartum. She was bred on day 6 of the foal estrus and conceived on that mating. E1 level showed more than 60 pg/ml for 2 days (3 days before breeding), then it declined to 40 pg/ml on day 5 of the estrus (on breeding day). After the breeding, the level maintained less than 30 pg/ml. E2 level during estrus fluctuated between 20 pg/ml and 40 pg/ml. Thereafter, it subsequently increased and decreased with 1 or 2 days interval. E3 level never exceeded 15 pg/ml throughout the experiment (Fig. 2).

Case 3: The mare showed the onset of estrus on day 2 postpartum (8 days before breeding). This mare did not conceive on the first breeding on day 8 of the foal estrus. The second estrus came on 14 days after the first breeding. She was mated again on day 21 after the first breeding and conceived on the second breeding. E1 level remained less than 20 pg/ml during the first estrus. However, 7 days before the second estrus, it increased to 40.9 pg/ml and maintained at this level throughout the 7 days. E2 level was 28.5 pg/ml on day of the onset of foal estrus, subsequently declining with time. It was 17.7 pg/ml on the final day of estrus. E3 level was less than 10 pg/ml throughout the first and sec-

Fig. 2. Changes of estrone, estradiol-17 β and estriol concentration in milk of mare case 2 during foal estrus.

Fig. 3. Changes of estrone, estradiol-17 β and estriol concentration in milk of mare case 3 during foal estrus.

Fig. 4. Changes of estrone, estradiol-17 β and estriol concentration in milk of mare case 4 during foal estrus.

ond estrus (Fig. 3).

Case 4: The mare was bred on day 11 postpartum, but she failed to conceive. E1 level was low, approximately 20 pg/ml or less during the foal estrus. After breeding, it maintained at 20 pg/ml for 4 days, and decreased to less than 10 pg/ml throughout the experiment. E2 level was 26.6 pg/ml on the breeding day and then it decreased with time. Six days after breeding it increased again to 42 pg/ml. Thereafter, it subsequently increased and decreased. E3 level fluctuated between 5 pg/ml and 10 pg/ml throughout the experiment (Fig. 4).

Case 5: The first estrus commenced on day 9 postpartum, but breeding was skipped because of detection of two mature follicles by rectal palpation. E1 level was higher than 30 pg/ml for 3 days before the onset of estrus, subsequently it decreased. During estrus, it increased again to 40 pg/ml for 3 days and subsequently declined.

Fig. 5. Changes of estrone, estradiol-17 β and estriol concentration in milk of mare case 5 during foal estrus.

E2 level was 15.8 pg/ml on day 2 and decreased to less than 1 pg/ml on day 5 of the estrus, without any increase again thereafter. E3 level decreased to 5.1 pg/ml on day 2 postpartum, remaining to be low thereafter (Fig. 5).

Discussion

The length of estrous period of the mare averages 7 days and ovulation occurs one and half

days before termination of estrus (Sato and Hoshi, 1939). Further, the optimum breeding time is penultimate to ovulation day, totaling 3 days.

There are few reports on sexual steroids in milk of the mare during estrus. Estradiol level in jugular peripheral blood plasma peaks at 62 ± 26 (mean $\pm$ SE) pg/ml on the onset of foal estrus and decreases to 21 ± 5.3 pg/ml on ovulation (Norden *et al.*, 1978). On the other hand, estradiol increases to a peak in mongrel mares, 1 to 3 days before ovulation (Pattison *et al.*, 1972). Furthermore, the progesterone levels in blood and milk during the foal heat of Quarter Horse mares averaged less than 2 ng/ml in milk and 1 ng/ml in blood plasma (Gunter *et al.*, 1980).

In the present study, only fluctuation of E1 concentration in milk showed a close relation to the estrous cycle in 5 Thoroughbred mares. No relevant and significant levels during the estrous cycle were obtained on E2 and E3 concentration in the milk. Therefore, it may be possible to assume that these two estrogens, E2 and E3, have no relevant importance in predicting capability of conception at estrus of mares.

Mares of case 1 and 2 conceived on the first breeding in foal estrus. Estrone concentrations in milk of these mares during foal estrus were over 40 pg/ml and maintained over 30 pg/ml during the following 5 days after the onset of estrus. A mare of case 3 did not conceive on the first breeding during foal estrus but she conceived on the second breeding of the subsequent estrus. The peak concentration of E1 in milk during foal estrus was more than 60 pg/ml and maintained over 30 pg/ml during the following 3 days, but in the second estrus a peak of over 40 pg/ml was observed and maintained at this level for the following 7 days. As a result, conception occurred on the second breeding.

These results suggest that E1 concentration in milk is reliable in predicting capability of conception at foal estrus. It is assumed that a higher surge of E1 concentration in milk more than 30 pg/ml at least for 5 days, with a peak of over 40 pg/ml, may be necessary for good conception, whereas in the case of lower E1 level during foal estrus conception will be unsuccessful.

One breeding (case 5 mare) was terminated in spite of its E1 concentration peaked more than 40 pg/ml, because 2 follicles were detected by

rectal examination. El surge length of this mare was short (only 3 days).

Multiple ovulation was observed in 38% of mares during a estrous period (Arthur, 1958; Osborne, 1966). Furthermore, other authors (Jeffcot and Whitwell, 1973; Platt, 1973) recommend that breeding should be skipped when more than 2 mature follicles are detected in the ovary during estrus, because 65% of multiple pregnancies in the mare would abort or undergo immature delivery.

It is difficult to define the optimum breeding period of mares in foal estrus, because the estrous length is short and the cycle is irregular. Today, detection of estrus in mares still relies on testing by the stallion and rectal examination. Furthermore, these methods do not assure the estrus detected to be conceivable.

This is probably the first report that deals with El concentration in milk during the estrus and the measurement of El may give a good guide to know the estrus to be capable of conception.

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分娩後初回発情期のサラブレッド雌馬の乳汁中エストロジェンの変化

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分娩後初回発情期のサラブレッド雌馬 5 頭より乳汁を採取し、乳汁中のエストロン、エストラジオール 17 β およびエストリオールの 3 分画を RIA 法で測定した。

初回発情期の乳汁中エストラジオール 17 β とエストリ

オールの推移はその発情における受胎の可能性を予測するための指標として適切でなかったが、エストロンはその指標となりうる可能性が示唆された。