Changes of Plasma Concentrations of Steroid Hormones, Prostaglandin F₂₀-Metabolite and Pregnant Mare Serum Gonadotropin during Pregnancy in Thoroughbred Mares

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ABSTRACT. Plasma concentrations of estrogens, gestagens, cortisol (F), 13, 14-dihydro, 15-keto PGF₂₀ (PGFM) and pregnant mare serum gonadotropin (PMSG) in 10 Thoroughbred mares were measured for a 11-month pregnancy period. Estrone (E₁) and estradiol-17β (E₂) levels gradually increased as the pregnancy advanced, showing a peak around Month 8 and decreased thereafter. Progesterone (P) levels increased on Months 3 and 11, and 17α-OH-progesterone (17α-OHP) levels peaked on Month 3, whereas 20α-OH-progesterone (20α-OHP) levels increased sharply after Month 6. PGFM indicated peaks on Months 2 and 11. F and PMSG levels peaked on Months 2 and 3. From factor analysis, Month, E₁, E₂ and 20α-OHP were discriminated as Factor 1, increasing with the progress of pregnancy, PMSG, 17α-OHP and P as Factor 2, showing a relation with the secondary corpus luteum, and PGFM and F as Factor 3, affecting PGFM change on Month 2 by F. P also related to both Factors 1 and 3, showing an inverse relation against PGFM. In conclusion E₁, E₂ and 20α-OHP contained in Factor 1 were suggested to be important especially as parameters of placental function after Month 6.—KEY WORDS: PGFM, PMSG, pregnant mare, steroid hormone.


The variations of hormonal secretion in the mare during pregnancy differ from those observed in other domestic animals. In addition, a gonadotropin (PMSG) of placental origin appears at about Day 40 [1] and the secondary corpus luteum forms under PMSG secretion [2]. After Day 150, the pregnancy is maintained by hormones of placental origin in place of ovary. The habitual abortion is well known to often occur about Day 150 [15], but the cause of the hormonal changes with the problem remains unclarified. Unlike previous investigations where experiments made on pregnant mare were mainly carried out with 2 or 3 kinds of hormones [5, 6, 8, 17, 18], the present study determined 8 kinds of hormones in one single sample. We have attempted to clarify relationships among 8 hormones during the course of pregnancy.

MATERIALS AND METHODS

Horsses: Ten Thoroughbred mares, aged 6–10 years, from 3 racehorse stables in Hokkaido, Japan were used for the experiments.

Blood sample: The blood was collected every month after mating for a period of 11 months (between 8 and 9 a.m.). Blood samples were drawn from the jugular vein into siliconized glass tubes containing EDTA-2Na. They were put into an ice-box immediately after sampling, centrifuged at 3000 rpm for 15 min within 2 hrs, frozen and stored at −20°C until assay. Day 0 was taken as the last day of mating, and the following month was considered as the first pregnancy month or Month 1. The pregnant period of the mares used in this study was 338±8.5 (mean ± S.D.) days.

Estrone (E₁), estradiol-17β (E₂) and cortisol (F) determination: Plasma E₁, E₂ and F were determined according to RIA described by Makino [7]. Data on validity of antisera against E₁-6-CMO-BSA, E₂-6-CMO-BSA, F-21-succ-BSA was reported previously by Makino [7]. The coefficient of variations for intra- and inter-assay was 11.2% and 17.4% for E₁, 15.7% and 19.6% for E₂, 8.1% and 11.9% for F, respectively.

P, 17α-OH-progesterone (17α-OHP) and 20α-OH-progesterone (20α-OHP) determination: Assays on these hormones were performed according to RIA described by Den et al. [3]. Data on validity of antisera against P-3-CMO-BSA, 17α-OHP-3-CMO-BSA was reported previously [7, 23]. Cross-reactivity of antisera against 20α-OHP-3-CMO-BSA were 5.4% for 20β-OHP and 2.9% for P. The coefficients of variation for intra- and inter-assay were 8.7% and 12.6% for P, 11.1% and 15.7% for
17α-OHP, 10.2% and 14.5% for 20α-OHP, respectively.

PfGFM determination: PfGFM was determined according to RIA described by Satoh et al. [19]. Antisera against PfGFM-BSA was purchased from Institut Pasteur Production (Coquette, France). The cross-reactions against 13, 14-dihydro, 15-keto-PGF\(_{1α}\), 15-keto-PGF\(_{3α}\), and 15-keto-PGF\(_{2α}\) were 83%, 23% and 4.4%, respectively. The intra- and inter-assay coefficients of variation were 13.8% and 17.4%, respectively.

PMSG measurement: PMSG levels were determined by the following biological method. Standard solutions of PMSG 10–20 international units, were injected into 3–5 non-pregnant female rats. Six days later, the animals were sacrificed to order to ovary weight, and a reference curve was drawn. Sample plasma was diluted 5 to 10 times with physiological saline and injected into the rats in a similar manner as the standard. Six days later, the animals were sacrificed in order to measure the ovaries weight and the individual plasma PMSG levels were obtained from the reference curve.

Statistical evaluation: Data analysis was performed with the application program of SPSS and SAS. The means and standard deviation were calculated for each month. For the data on hormones, the analysis of variate was performed by Friedman’s Ranking Sum test, which shows the significant fluctuations throughout the observed period. Factor analysis was based on covariance where the varimax rotation for re-clarification of some factors (also known as common factors) was employed [9]. In discrimination of factor the highest score point in some factors is selected as the factor of the variate. A location of the variate in factor analysis is shown around horizontal and vertical axis. Each end point of axes is indicated as +1 or −1 as the highest coefficient of factor loading between the variate and the factor.

RESULTS

Variations of \(E_1\) and \(E_2\) levels: \(E_1\) showed a low level of 133.0±55.0 ng/ml on Month 3, increased to 210.0±40.0 ng/ml on Month 4 and peaked at 440–1400 ng/ml in all 10 mares on Month 7–9. \(E_2\) showed a low level of 25.0±16.0 ng/ml on Month 4 and ascended to a peak of 130–400 ng/ml on Month 8–10. During pregnancy, \(E_1\) and \(E_2\) indicated a monophasic plot and the variance ratio in the levels of pregnant month was statistically significant (p<0.01) by the Friedman’s Test.

Variations of \(P\), 17α-OHP and 20α-OHP levels: The change in \(P\) started at a low level of 8.36±3.62 ng/ml on Month 1 and the level increased to 12.81±4.07 ng/ml on Month 3. \(P\) level showed the highest at 15.89±5.18 ng/ml on Month 11, showing significant variations (p<0.05). Further, 17α-OHP levels showed 0.3±0.1 ng/ml on Month 1, peaked at 1.5±0.6 ng/ml on Month 3 and declined thereafter. From Month 1 to 5, 20α-OHP levels maintained at 5.5±3.9 ng/ml and increased sharply to 44.5±27.9 ng/ml on Month 6 to peak at 61.7±40.0 ng/ml on Month 11. From the above, 20α-OHP showed the highest level among the three geastegons.

Variations of PfGFM levels: Plasma PfGFM levels increased slightly yielding 159.0±75.0 ng/ml on Month 2. On Month 6, PfGFM levels started to increase and reached the highest level of 505.0±269.0 ng/ml on Month 11. PfGFM indicated a pattern of a biphasic plot with peaks on Month 2 and near terminal pregnancy.

Variations of PMSG levels: PMSG, during pregnancy, peaked at 70–290 i.u./ml on Month 2–3 and the levels decreased thereafter. The variance ratio in the levels of pregnant month was statistically significant (p<0.01).

Variations of \(F\) levels: On Month 1, \(F\) showed 30.19±9.41 ng/ml and increased sharply to 67.71±23.36 ng/ml in 8 of 10 mares, showing the highest level on Month 2. One of the two remaining mares showed the peak of 43.9 ng/ml on Month 4, while the other showed the peak of 96.5 ng/ml on Month 5. Individual differences were noticeable on Month 3 but they declined gradually thereafter. The variance ratio in the levels of pregnant month was found to be statistically significant (p<0.01) (Fig. 1).

Factor analysis of plasma hormone levels during pregnancy of Thoroughbred horses: The main 3 factors with eigen values exceeding 1 were discriminated. The ratio of contribution in each factor was 38.6, 18.5 and 15.1%, respectively, explaining 72.2% of total multiple R² of total variates (Fig. 2). Factor 1 indicated the highest contribution ratio and its variates such as Month, \(E_1\), \(E_2\) and 20α-OHP showed respective factor scores of 0.34143, 0.30680, 0.25580 and 0.22610. Factor 1 explained the variate increase with the progress of pregnancy, because Month showed the highest factor loading (0.92931). PMSG, 17α-OHP and \(P\), of which respective factor scores were 0.50419, 0.49420 and 0.30312, were
Fig. 1. Changes in plasma levels of E₁, E₂, P, 17α-OHP, 20α-OHP, F, PGFM and PMSG during pregnancy in the Thoroughbred mares. Ordinate values indicate the mean ± S.D. of the respective hormone in ng/ml or pg/ml of 10 horses.

contained in Factor 2. This explained the change of variates around Month 3. P was discriminated in Factor 2, but was also influenced by Factors 1 and 3. Furthermore, the factor loadings in Factor 3 indicated high values (0.42894 and 0.44013) by F and PGFM, and low value (-0.47421) by P, showing an inverse relationship. F and PGFM were contained in Factor 3 with factor scores of 0.62077 and 0.41014, respectively. Factor 3 explained especially the change of F levels around Month 3, because F showed the highest factor loading (0.93056) in Factor 3.

DISCUSSION

In this study, E₁ levels in blood peaked around Month 8 and manifested a monophasic plot with decreasing levels as pregnancy approached parturi- tion. The tendency was also observed in previous reports [11, 17], but the levels were lower [11] or higher [17] than our findings. E₂ levels showed comparatively lower on the whole, and varied almost in a similar pattern as compared to E₁, coinciding with previous findings [11, 12, 17]. Removal of the fetal gonads results in an immediate fall in maternal plasma concentrations of estrogens [14] and dehydroepiandrosterone derived from gonads is converted to estrone in equine placenta [13, 15].

Controversial reports [5, 12, 18, 20] on the estimation of P levels in blood of pregnant mares have been published. Our study showed the P levels to increase on Month 2–3 and Month 11 with values and pattern observed in previous findings [12]. The pattern of 17 α-OHP levels was in agreement with that observed in previous reports [5, 20]. The increases of 17 α-OHP and P on Month 2–4 coincided with high PMSG levels, showing parallel findings to previous studies [6, 8]. At this stage of pregnancy, the secondary corpora lutea detected until Month 5 [21] may synthesize P and 17 α-OHP.
In the present study plasma 20α-OHP levels in the pregnant mares increased sharply on Month 6 and further increased gradually until parturition, whereas previous report [20] indicated that the increase occurred abruptly insofar as the last month. Though the source from which 20α-OHP is derived is obscure, the presence of a high concentration of 20α-hydroxy-5α-progesterone-3-one (precursor of 20α-OHP) is found in the uterine artery and vein of Pony pregnant mares [10]; therefore it is suggested 20α-OHP to have derived from the placenta.

In factor analysis, Factor I containing E<sub>1</sub>, E<sub>2</sub> and 20α-OHP showed obvious variation accompanied by the progress in pregnancy, especially after Month 6. However, it was unknown whether secretion of E<sub>1</sub> and E<sub>2</sub> was affected by 20α-OHP. Although 20α-OHP inhibits 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in human placenta [16], there may be no effect of 20α-OHP on 3β-HSD activity in the mare since the influence of estrogen production by 20α-OHP was not clear in the pregnant mares. Factor 2 with PMSG and 17α-OHP indicated that PMSG relates closely with the development of the follicle whereas 17α-OHP relates with functions of the secondary corpus luteum formed thereafter [2]. Since the factor loading of P was indicated as about 0.43 in both Factors 1 and 2, P secretion in pregnant mare may be derived from the secondary corpus luteum with 17α-OHP in the early half-term of pregnancy and from the placenta with 20α-OHP in the later half-term, respectively. The interesting aspect, however, was that factor loadings in Factor 3 indicated a low value by P but high values by F and PGFM, showing an inverse relationship especially between P and PGFM. Therefore, the high PGFM stimulated by some factors such as F, may be a trigger to induce abortion or adverse influence on fetal development. In Factor 3, the reason for the transient increase of PGFM on Month 2 was not clear, suggesting that the increase of PGFM be influenced strongly by the F increase. PGFM strongly stimulates the activity of 20α-hydroxysteroid dehydrogenase [4], but the relationship between PGFM and 20α-OHP was not clear in this factor analysis.

In conclusion 20α-OHP, E<sub>1</sub> and E<sub>2</sub> contained in Factor 1 with the greatest ratio of contribution were suggested to be important especially as parameters of placental function in the pregnant mare after Month 6.

REFERENCES

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