Changes of PGFM and Sex Steroid Hormones in Blood of Horses Pre-, During and Post-Parturition

Kaoru TAKAGI, Toshitada HIGASHINO,
Shigehisa TSUMAGARI, Shigeo OHBA,
Shinichi NAMBA, Kazumasa MORIKI,
Shosuke SATOH and Masatoshi TAKEISHI

*Department of Veterinary Medicine, College
of Agriculture and Veterinary Medicine, Nihon
University, Fujisawa, Kanagawa 252, Japan*

Abstract. Investigations on the pre-parturition, immediately after (during) parturition and post-parturition blood levels of 13, 14-dihydro-15-keto-PGF₂ α (PGFM) and sex-steroid hormones of 5 thoroughbred mares were carried out for a period of 12 months (May, 1984—April, 1985). PGFM and hormonal levels were determined with radioimmunoassays. PGFM and cortisol concentrations on pre-parturition day (prePD) 1 and 0 were much higher than on prePD 2, and returned to pre-parturition basal values on following parturition, respectively. Progesterone, estrone and estradiol-17 β gradually decreased from prePD 7 to parturition day. Estradiol-17 β was consistently low compared with estrone. In view of the above findings, PGF₂ α probably influences parturition in pregnant mares.

Key words: Parturition, PGFM, Steroid hormone, pregnant mares.

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Controversial and different theories such as the estrogen-progesterone theory, prostaglandin theory, oxytocin theory and embryonic cortisol theory are recent thoughts that focus on the phenomenon that involves endogenous hormonal secretions in triggering parturition. In equine research, the progesterone concentration in the blood of breeders from term-pregnancy to parturition and suckling is 4.4 ± 1.1 ng/ml on pre-parturition day (prePD) 5, and decreases to 0.5 ng/ml on prePD 1–3 [1]. Moreover, estrogen and progesterone in the peripheral blood of hybrid horses manifest a marked decrease 48 h before parturition [2]. Further, when the prostaglandin F₂ α (PGF₂ α) content in uterine venous blood was determined, a sharp increase was seen during parturition. However, such a finding in thoroughbred mares has yet to be reported [3].

This study was carried out to investigate the changes of 13, 14-dihydro-15-keto-PGF₂ α (PGFM) and sex-steroid hormonal levels in peripheral blood of Thoroughbred mares pre-parturition, immediately after (during) parturition and post-parturition. Hormonal levels were determined with radioimmunoassays.

Materials and Methods

Horses

Five thoroughbred mares, aged 6–10 years, from 2 race stables in Japan (Hokkaido) were used for the experiments.

Blood sampling

Monthly collections (between 08:00–09:00 h) of blood for a period of 14 days (prePD 7 ~

post-parturition days (postPD) 6) from 5 pregnant horses were carried out. To study the changes in hormonal levels in relation parturition, samples of blood from the jugular vein were taken from 5 pregnant horses throughout prePD 3 to 7, during parturition and postPD 3 to 6. The blood samples were subjected to centrifugation (3,000 rpm, 10 min), and the supernatant was then placed in polyethylene containers and stored at -20°C until the assay.

Data recording

The last day of mating was termed as day 0, and the next day was considered as pregnancy day (PD) 1. In the case of a parturition horse, prePD 1 was read as -1 , parturition day as day 0 and the following days were $+1$, $+2$ and so on.

Estrone, estradiol- 17β and cortisol determinations: Plasma estrone, estradiol- 17β and cortisol levels were determined with the RIA described by Makino [4]. Plasma was extracted with ethyl ether and the dried extract was subjected to Sephadex LH-20 chromatography for estrone and estradiol- 17β , and cortisol determinations. Antisera to estrone-6-CMO-BSA, estradiol- 17β -6-CMO-BSA and F-21-succ-BSA were purchased from the Teikoku Hormone Manufacturing Co. (Tokyo, Japan). The cross-reactivity values for estrone antisera against estradiol- 17β and estriol were 2% and 1.6%, respectively; those of estradiol- 17β antisera to estrone and estriol were 3% and 2%, respectively. In the case of cortisol antisera, the respective values were 92.3% and 28.1% for 17α -OHP and cortisone respectively. The coefficients of variation for intra- and inter-assay were 11.2% and 17.4% for estrone; 15.7% and 19.6% for estradiol- 17β ; 8.1% and 11.9% for cortisol, respectively.

Progesterone determination: Assays on these hormones were performed with the RIA described by Den *et al.* [5]. The antiserum to Progesterone-3-CMO-BSA was purchased from the Teikoku Hormone Manufacturing Co. (Tokyo, Japan). Cross-reactivity values for 5 α -pregnanedione and 20α -OH progesterone (20α -OHP) were 42.6% and 7%, respectively. Those of 17α -OH progesterone (17α -OHP) antisera against progesterone and 20α -OHP were 7.9% and 3.2%, respectively. However, those of 20α -OHP antisera against 20β -OHP and progesterone were 5.4% and 2.9%, respectively. The coefficients of variation for intra- and inter-assay

were 8.7% and 12.6% for progesterone; 11.1% and 15.7% for 17α -OHP; 10.2% and 14.5% for 20α -OHP, respectively.

PGFM determination: PGFM was determined with the RIA described by Satoh *et al.* [6]. The Antiserum against PGFM-BSA was purchased from Institute Pasteur Production (Coquette, French). Cross-reactions with 13, 14-dihydro, 15-keto-PGF $_{1\alpha}$, and 15-keto-PGF $_{2\alpha}$ were 83%, 23% and 4.4%, respectively. The intra- and inter-assay coefficients of variation were 13.8% and 17.4%, respectively.

Results

Changes in the hormone concentrations in the blood of horse No. 1: Estrone concentrations were 0.79–0.98 ng/ml on prePD 7–2, decreased to 0.48 ng/ml on prePD 1, and then maintained at 0.34–0.52 ng/ml thereafter. Estradiol- 17β concentrations were approximately 0.18 ng/ml during pre-parturition and dropped lower post-parturition. Progesterone was high at 45 ng/ml on prePD 7–2, decreased to 26.8 ng/ml on parturition, and dropped steeply to 3.3 ng/ml on postPD 1. Cortisol was about 50 ng/ml on prePD 2, increased to 88.5 ng/ml during parturition but decreased to 29.0 ng/ml post-parturition. PGFM reached a low level of 0.48 ng/ml till prePD 2, and then increased sharply from prePD 1 to register a peak value of 20.37 ng/ml on parturition (Fig. 1).

Changes in the hormone concentrations in the blood of horse No. 2: Estrone declined gradually from 0.82 ng/ml on prePD 4 to 0.42 ng/ml on prePD 2. Similarly, estradiol- 17β was maintained at 0.11 ng/ml and declined to 0.04 ng/ml near parturition. Progesterone was more than 40 ng/ml till prePD 1, but declined to less than 21 ng/ml on parturition. Cortisol reached 55 ng/ml on prePD 1, peaked with a value of 121.0 ng/ml on parturition, only to decrease below the pre-parturition level the next day. PGFM increased sharply on prePD 1, peaked at 15.25 ng/ml parturition and declined abruptly on post-parturition (Fig. 2).

Changes in the hormone concentrations in the blood of horse No. 3: Estrone was at a low of 0.49 ng/ml when first determined, followed by a further decrease thereafter. Estradiol- 17β varied around 0.06 ng/ml. Similarly to estrone, progesterone was low in initial determinations, decreased from 19.8 ng/ml on parturition to 4.5 ng/ml on

Fig. 1. Changes of PGFM, Cortisol, Progesterone, Estrone and Estradiol-17 β in blood of horse No. 1 pre- during and postparturition.

Fig. 2. Changes of PGFM, Cortisol, Progesterone, Estrone and Estradiol-17 β in blood of horse No. 2 pre- during and postparturition.

postPD 1. Cortisol, after reaching the peak level of 120.7 ng/ml on parturition, decreased to 43.4 ng/ml on postPD 3. PGFM maintained a high level of 12.90 ng/ml on parturition only to decline to 0.05 ng/ml on postPD 2 (Fig. 3).

Changes in the hormonal concentrations in the blood of horse No. 4: Estrone decreased from 0.39 ng/ml on prePD 4 to 0.21 ng/ml post-parturition. Estradiol-17 β hardly changed around 0.15 ng/ml. Progesterone registered a pre-parturition level of 38.8 ng/ml, decreased to 18.9 ng/ml on parturition and dropped further to 3.7 ng/ml on postPD 1. Cortisol peaked at 128.9 ng/ml on parturition from a pre-parturition level of 59.7 ng/ml and decreased to 36.0 ng/ml on postPD 6. PGFM was low on prePD 4, increased sharply on prePD 1, peaked at 18.21 ng/ml on parturition but decreased to 3.98 ng/ml on postPD 1. The concentrations decreased further to 0.46 ng/ml on postparturition. (Fig. 4).

Changes in the hormonal concentrations in the

blood of horse No. 5: Estrone levels registered 0.42 ng/ml on pre-parturition and lowered to 0.05–0.27 ng/ml on parturition. Estradiol-17 β levels approximated to 0.13 ng/ml on parturition, and declined to a low value of about 0.03 ng/ml on post-parturition. Progesterone registered a pre-parturition level of 45 ng/ml, decreased to 16.5 ng/ml on parturition and varied around 1–9 ng/ml on postPD1. Cortisol levels increased to 83.8 ng/ml from 55 ng/ml on prePD 1, peaked at 116.8 ng/ml on parturition but only to decrease to 42.2 ng/ml on postPD 1. PGFM increased sharply on prePD 2 to peak at 9.11 ng/ml on parturition, the level declined sharply on postPD 1 (Fig. 5).

The blood PGFM concentrations in the 5 thoroughbred mares were low until a few days prior to parturition. However, this PGFM concentrations increased from prePD 1 to peak at 15.17 ± 4.42 ng/ml on parturition. PGFM decreased gradually after parturition, and recovered to its preparturition level of 0.06 ± 0.02 on postPD 2 (Fig.

Fig. 3. Changes of PGFM, Cortisol, Progesterone, Estrone and Estradiol-17 β in blood of horse No. 3 pre- during and postparturition.

Fig. 4. Changes of PGFM, Cortisol, Progesterone, Estrone and Estradiol-17 β in blood of horse No. 4 pre- during and postparturition.

6).

On prePD 2, the cortisol concentration showed gradual increases and quickly attained the peak level on parturition. However, this hormone declined suddenly on postPD 1 to eventually reach the prePD level (Fig. 6).

Progesterone was high on prePD 2, but declined to 20.5 ± 3.8 ng/ml on parturition. This was followed by further decreases to register at 4.3 ± 1.6 ng/ml on postPD 1 (Fig. 6).

Estrone already showed a declining trend at the start of the experiments. The readings indicated a high value of 0.69 ± 0.24 ng/ml on prePD 2, declined to 0.45 ± 0.14 ng/ml on prePD 1 and dropped to 0.37 ± 0.10 ng/ml on parturition. The level low thereafter.

Estradiol-17 β varied around 0.15 ng/ml until prePD 1, decreased on parturition and decreased to 56.9 ± 32.4 pg/ml on postPD 1 (1/3 of prePD level). However, the level dropped thereafter (Fig. 6). A comparison of estrone with estradiol-17 β ,

showed the former approximately 3 to 5 times as high as the latter on the whole.

Discussion

Liggins *et al.* [7] experimented extensively on the mechanism of parturition in sheep extensively. The important role played by the adrenal cortex in parturition induction has been demonstrated. During pregnancy, endogeneous secretory functions of the hypothalamus-hypophysis-adrenal axis are enhanced, and a large quantity of cortisol is released from the adrenal cortex. Cortisol acts on the embryo placenta, enhances estrogen secretion and inhibits progesterone release. In addition, estrogen acts on the mother's placenta to trigger PGF $_2\alpha$ biosynthesis. Biosynthesized PGF $_2\alpha$ in the placenta is transported to uterine muscles and transits swiftly into the uterine venous bloodstream.

Fig. 5. Changes of PGFM, Cortisol, Progesterone, Estrone and Estradiol-17 β in blood of horse No. 5 pre- during and postparturition.

Fig. 6. Changes of PGFM, Cortisol, Progesterone, Estrone and Estradiol-17 β in blood of horse No. 6 pre- during and postparturition.

When PGFM concentrations in the uterine venous blood were investigated 30 min pre-parturition, during parturition and 30 min post-parturition, Lovell *et al.* [3] found them to increase suddenly on parturition. In our experiments, PGFM showed a sudden increase on prePD 1 and registered the peak on parturition, suggesting a close relationship between PGFM and parturition. PGFM in the blood increased prior to that of cortisol pre-parturition and its post-parturition decreased was delayed slightly, suggesting that PGFM induces cortisol release in certain ways not yet explained.

In parturition induction in horses by PG, with the use of a single sc bolus administration of 12.5 or 2.5 mg PGF₂ α to 40- to 150-day pregnant ponies, resulted in about half of them having miscarriages 3–9 days after treatment, regardless of the pregnancy term [8]. Moreover, in ponies of 80- to 300-day pregnancy term, repeated single bolus sc administrations of 2.5 mg PGF₂ α on a

continuous basis at 12-h intervals induce miscarriages at a mean of 38.0 h after the first treatment for a mean of 3.7 administrations in all animals. As such, PGF₂ α is applicable in parturition induction in horses, and is known to be related to parturition induction in horses.

Progesterone in the blood determined in the present study registered a high value of more than 40 ng/ml on prePD 7, and was further increased at 11-month pregnancy term. Progesterone began to decrease on prePD 1 to record half the peak value on parturition and dropped sharply on postPD 1.

Estrone in the jugular venous blood around parturition in thoroughbred mares registered a high value of 35 times the level of estradiol-17 β , but declined on parturition. Moreover, estradiol-17 β showed a decreasing tendency on parturition initiation. Apart from the hitherto unpublished estrone concentrations, the estradiol-17 β changes coincided well with those reported previously [9].

Cortisol concentrations in rabbits [10], cows [11]

and pigs [12] have been documented to increase on parturition. Lovell *et al.* [3] have reported no changes in 11β -hydroxycorticosteroids from prePD 45 to postPD 15 in horses. However, corticosteroid has been observed to register a significant increase from 12 hrs prior to parturition [13]. In our present study, sharp corticosteroid increases were recorded from prePD 1 to the time of parturition, confirming previous findings [13]. Moreover, successful parturitions were induced with repeated administrations with 100 mg

dexamethasone in pregnant mares from pregnancy day 321 to 324 [14, 15]. As such, corticosteroid hormones are probably the factors that influence parturition.

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