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著者 (英)	Ishii Mitsuo, Kobayashi Syuichi, Acosta Tomas J., Miki Wataru, Yamanoi Takahiro, Matsui Motozumi, Miyake Yoh-ichi, Miyamoto Akio
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—Research Note—

## Relationship between Peripartal Plasma Oxytocin and Prostaglandin F<sub>2α</sub> Metabolite and Placental Expulsion Time in Heavy Draft Mares

Mitsuo ISHII<sup>1)</sup>, Syuichi KOBAYASHI<sup>1)</sup>, Tomas J. ACOSTA<sup>1)</sup>, Wataru MIKI<sup>2)</sup>,  
Takahiro YAMANOI<sup>3)</sup>, Motozumi MATSUI<sup>1)</sup>, Yoh-Ichi MIYAKE<sup>1)</sup> and Akio MIYAMOTO<sup>1)</sup>

<sup>1)</sup>Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, <sup>2)</sup>Hokkaido NOSAI, Ebetsu 069-0867 and <sup>3)</sup>Hokkai Gakuen University, Faculty of Engineering, Sapporo 064-0926, Japan

**Abstract.** The aim of this study was to clarify the relationship between circulating oxytocin (OT) and PGF<sub>2α</sub> metabolite (PGFM) in mares at the third stage of labor and placental expulsion time in order to investigate a cause of retained placenta of which the incidence increase in a heavy draft mare. Blood was sampled every 5 min from foaling to expulsion of the placenta in 18 heavy draft mares to evaluate circulating OT and PGFM. The relationships between OT and PGFM concentration and recorded placental expulsion times were investigated. The results were as follows (1) The highest level of OT concentration was observed close to foaling in 15 mares. (2) The OT concentrations close to foaling were variable with a large difference from the lowest concentration, 22.1 pg/ml, to the highest concentration, 209.3 pg/ml. (3) The highest level of PGFM was observed close to foaling in 17 mares. (4) During the 60 min following foaling, the OT concentrations of the mares (n=11) that had a shorter placental expulsion time (i.e., <1 h), were significantly higher than those of the mares (n=7) that had a longer placental expulsion time (i.e., >1 h; P<0.05). Collectively, the OT concentration immediately after foaling is negatively related to the placental expulsion time. Deficiency of OT secretion at foaling have should be considered as one of the causes of retained placenta in heavy draft mares.

**Key words:** Heavy draft mare, Oxytocin, 15-keto-dihydro prostaglandin F<sub>2α</sub> (PGFM), Placental expulsion

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Recently, the heavy draft horses of Japan have increased in size and become one of the largest breeds in the world. Retained placenta is occurring more frequently in heavy draft mares due to corpulence with lack of exercise because they are not used for work. A prolonged retained placenta in a mare causes a puerperal infectious disease and laminitis, and it may also cause death. Several causes of retained placenta, such as uterine inertia, placentitis, chill stress and hormone imbalance, have been considered [10, 17, 20]. Extension of the placental expulsion time reduces the subsequent conception rate in mares [9]. Although the conception rate of foaling heat in mares whose placental expulsion time was less than 1 hour was 66%, the conception rate of mares in which the placental expulsion time exceeded 4 h fell to 51.7% [9]. Manual removal of the placenta led to a decrease in subsequent fertility [9]. The level of circulating oxytocin (OT) rises rapidly in the 2nd stage of delivery in mares [1, 8, 24]. Moreover, it is known that OT secretion helps discharge milk from the mammary glands [5, 21]. Prostaglandin F<sub>2α</sub> (PG) might play a role in contraction of the myometrium at delivery. The circulating PG concentration in mares is determined generally by measurement of 15-keto-dihydro PGF<sub>2α</sub> (PGFM) [2, 8, 16]. The PGFM concentration, which is PG and its metabolic product, rises during the last stage of pregnancy or at the time of delivery [2, 8, 16, 18, 20, 21]. According to detailed observations throughout delivery, the rise in PGFM concentration starts from the 1st stage of delivery, rises rapidly at the 2nd stage of delivery and peaks just before discharge of the foal [15] or simulta-

neously with discharge of the foal [8]. The action and release of OT and PG during delivery are mutually and closely related [3, 4, 6, 16, 21]. In heavy draft mares, no detailed hormonal data has been reported for during parturition. The aim of this study was to clarify the relationships between changes in the OT and PGFM concentrations and the placental expulsion time in heavy draft mares in order to investigate the cause of retained placenta.

### Materials and Methods

#### Animals

This study was conducted during two breeding seasons in Akancho in Eastern Hokkaido, Japan. It involved a total of 18 heavy draft mares comprised of mixed horse breeds belonging to the Breton, Percheron and Belgian Draught. The estimated average body weight of the nonpregnant mares was 800 kg; the animals varied from 3 to 16 years in age and were located on 9 farms. Foaling occurred from March to June and from 316 days to 352 days of pregnancy.

#### Blood sampling

Blood sampling began 20 min before foaling from 6 mares or immediately after foaling from the other 12 mares. Blood was collected every 5 min from immediately after foaling to 1 h after delivery of foals for all mares. When the placenta was retained for over 1 h after foaling, 5 min interval samplings were continued until expulsion of the placenta. Blood collected immediately after delivery of a foal was defined as sample at foaling. A catheter (14 gauge, 160 mm long, with a 2 m long tube) was inserted in most cases at the beginning of delivery, but was inserted in 6 mares as

early as 20 min before foaling. The catheter remained in the jugular vein during the experiment and was filled with 10 ml of physiologic saline in which 200 IU of heparin sodium was added after each sampling. The blood samplings were repeated carefully in order to cause as little stress as possible to the mare. After collection, the blood was placed in a chilled glass tube containing ethylenediaminetetraacetic acid (EDTA) and was centrifuged immediately. The harvested plasma was frozen at -20 C until assayed.

#### OT extraction

The plasma samples (5 ml) were diluted with 5 ml of distilled water, and the pH was adjusted to 2.5. All samples were then applied to a Sep-Pak C18 Cartridge (Waters, Millford, MA) as described previously [12]. The residue was evaporated and then dissolved in 200  $\mu$ l assay buffer [42 mM Na<sub>2</sub>HPO<sub>4</sub>, 8 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM NaCl, 4.8 mM EDTA, 0.05% bovine serum albumin (BSA), pH 7.5] for peptide EIA. Thus, the samples were concentrated 25-fold as a result of this process, which enabled us to determine peptide concentrations in EIA within the range of a standard curve. The recovery rate of OT that had been added to the plasma was 70%.

#### OT assay

The EIA for OT was previously described [14], and was based on the second antibody method using the biotin-streptavidin-peroxidase technique [13]. OT was labeled with D-biotinoyl- $\epsilon$ -aminocaproic-N-hydroxysuccinimide ester (biotin-7-NHS) using a commercial biotin labeling kit (Boehringer Mannheim Biochem., Mannheim, Germany) with a mol ratio of 1:2. For EIA, duplicates of 15  $\mu$ l standards or unknown samples in EIA buffer were incubated with 100  $\mu$ l polyclonal antibody for OT (1:1,000,000; donated by Dr. T Higuchi, Kochi University, Kochi, Japan) in a 96-well ELISA plate (F96 Maxisorp; Nunc, Roskilde, Denmark) at 4 C for 20 h prior to which the wells were coated with 50 mg of the secondary antibody (anti-rabbit IgG; Seikagaku, Tokyo, Japan). The plates were decanted, and biotinyl-peptide in 100  $\mu$ l EIA buffer (1:50,000) was added. The plates were further incubated for 2 h at 4 C, decanted and then 20 ng streptavidin-peroxidase (Sigma-Aldrich, St. Louis, MO, USA) in 100  $\mu$ l EIA buffer was added. After 15 min incubation at 4 C, the plates were decanted again and immediately washed 4 times with 0.05% Tween 80 at 300  $\mu$ l/well. The substrate reaction was induced by 0.025% 3,3',5,5'-tetramethylbenzidine (Wako Pure Chemical, Osaka, Japan), and stopped by 2 mol H<sub>2</sub>SO<sub>4</sub> [12]. The absorbance was measured at 450 nm with a plate reader (Bio-Rad, Hercules, CA, USA) until the absorbance of B0 reached 0.5-0.8. The standard curve for OT ranged from 1.9 to 2,000 pg/ml, and the ED50 of the assay was 80 pg/ml. The intra- and interassay CVs were 5.8 and 6.4%, respectively.

#### PGFM extraction

The plasma was extracted using diethyl ether after adjusting the pH of each plasma sample (2 ml) to 3.5 using HCl [12]. The residue was evaporated and then dissolved in 200  $\mu$ l assay buffer (40 mM PBS, 0.1% BSA, pH 7.2). The samples were concentrated 10-fold as a result of this process. The recovery rate of PGFM that had

been added to the plasma was 70%.

#### PGFM assay

PGFM concentrations were evaluated by a second antibody enzyme immunoassay reported previously [11]. The standard curve for PGFM ranged from 4 to 1,000 pg/ml, and the ED50 of the assay was 48 pg/ml. The intra- and interassay CVs were 7.7 and 13.0%, respectively.

#### Statistical analysis

Each placental expulsion time of the mares was recorded. The relationships between the level of circulating OT or PGFM concentration after delivery and placental expulsion time were analyzed statistically. The mares were divided into two groups depending on duration until placental expulsion (i.e., less than 1 h and over 1 h). To evaluate the effects of group (placental expulsion time) and sampling time after foaling on OT and PGFM concentrations, repeated measures ANOVA were performed. A significant interaction between group and sampling time was observed for the OT concentration ( $P < 0.05$ ). Therefore, each mean value was tested by differences in pairs, and differences in mean values were tested by t-distribution.

## Results

#### Foaling

All foalings were normal in appearance, and all foals survived. At all foalings, the owners of the mares provided minimal help (i.e., one person pulled the foal's legs) during delivery of the foals. All of the mares delivered at night time (2200-0230 h). The foals were comprised of 10 males and 8 females. The average body weight was 68.9 kg.

#### Placental expulsion

The mean placental expulsion time from foaling was 74.6 min (4-265 min). Eleven mares expelled the placenta within 1 h after foaling. Five mares expelled the placenta between 1 to 2 h after foaling. Two mares retained their placenta over 4 h after foaling.

#### Changes in plasma OT concentration

The highest OT concentrations were observed close to foaling in 15 of the 18 mares. In all 6 mares for which circulating OT concentrations were measured before foaling, the OT concentration rose before foaling. The highest mean OT concentration ( $81.7 \pm 90.0$  pg/ml, mean  $\pm$  SE; 4/6) was observed 5 min before expulsion of the foal. The plasma OT concentrations decreased gradually with time after foaling. At 1 h after foaling, the mean OT concentration decreased to about 1/3 ( $24.9 \pm 13.9$  pg/ml) of that recorded at foaling ( $70.3 \pm 31.7$  pg/ml; Fig. 1).

#### Changes in plasma PGFM concentration

The highest PGFM concentrations were observed close to foaling in 17 of the 18 mares. The highest average value for the PGFM concentration ( $32.9 \pm 16.0$  ng/ml) was recorded at foaling. This value decreased gradually after foaling, falling to 1/6 ( $5.6 \pm 7.0$  ng/ml) its original level at 1 h after foaling. Although the transition of

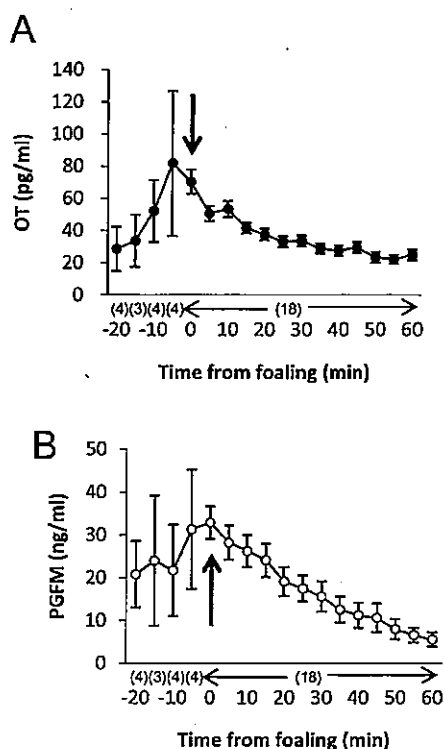


Fig. 1. Changes in plasma OT (A) and PGFM (B) concentrations. Numbers in parentheses under the x-axis indicate the number of the mares. The data are shown as the mean  $\pm$  SE. The arrows indicate the time of delivery of the foals.

the average value of PGFM was mostly in agreement with the motion of OT, the amplitude of the transition was smoother, and the highest level of PGFM was recorded 5 min after the peak of OT (Fig. 1).

#### Relationships between placental expulsion time and the plasma OT and PGFM concentrations

The OT concentrations close to foaling varied among the mares with a large difference from the lowest, 22.1 pg/ml, to the highest concentrations, 209.3 pg/ml (Fig. 2). During the 60 min after foaling, the OT concentrations of the 7 mares that had a longer placental expulsion time, i.e., over 1 h, were significantly lower than those of the 11 mares that had a shorter placental expulsion time of less than 1 h, as evidenced by repeated measures ANOVA ( $P < 0.05$ ; Fig. 3). Especially at foaling and 15 min after foaling, significant differences were observed between each mean value, as evidenced by testing with t-distribution ( $P < 0.05$ ; Fig. 3).

The PGFM concentrations immediately after foaling also varied among the mares with a large difference from the lowest, 2.3 ng/ml, to highest concentrations, 57.8 ng/ml (Fig. 2). There was no significant difference in the mean PGFM concentration between mares that had a shorter placental expulsion time of less than 1 h and mares that had a longer placental expulsion time of over 1 h (Fig. 3).

## Discussion

The present study clarified the relationships between circulating OT and PGFM in mares at the third stage of labor and the timing of placental expulsion in heavy draft mares. The plasma concentrations of OT and PGFM of all the mares decreased gradually after foaling. OT is considered the final hormone in the maternal cascade leading to parturition [19]. The OT concentration increases rapidly during the second stage of labor in mares [1, 8, 20]. In a report in which the OT in pituitary effluent collected from the intercavernous sinus was measured [21], the OT concentration increased markedly from 1 to 11 min after rupture of the chorioallantois, and OT secretion continued during delivery of the foal. In mares, elevation of the PGFM concentration has been reported to start from the first stage of labor increase rapidly at the second stage of labor, and reach a peak just before [8] or at expulsion of the foal [15]. In this study, however, the number of mares in which blood was sampled before foaling was limited. However, an increase in the circulating OT and PGFM concentrations close to foaling in the heavy draft mares was in agreement with previous findings in other breeds of horse [1, 8, 15, 20, 21].

OT is a neurohormone under the control of the central nervous system and therefore could be a factor in the mare's ability to determine the time of parturition accordingly to the presence of a favorable environment [7, 8, 19]. It is expected that the relationship between the peaks of the OT or PGFM concentrations and rupture of the membrane or delivery of the foal is influenced by the size of the foal or environmental stressors. The likelihood that the strength of labor pains or placental expulsion time also influences this relationship cannot be denied [7]. In the present study, preparation for blood collection, such as the placement of a catheter, began after labor pain began in most cases. We cannot claim that the blood collection method did not cause any stress on the mares. However, the hormonal changes (i.e., an increase in the circulating OT and PGFM concentrations) during foaling in the present research were mostly in agreement with previous studies [1, 8, 15, 20, 21].

In this study, although we could not collect a large number of blood samples before foaling, it seemed that large differences existed among the mares in the levels of circulating OT at foaling. Moreover, the differences were still detectable immediately after foaling. It can be argued that such individual differences could affect both the processes of foal delivery and placental expulsion. The hypothesis that the reduced OT plasma level is involved in the pathogenesis of retained placenta could also be supported by the excellent results obtained by treatment of mares with delayed placental expulsion with OT [20]. In this study, the relationship between the OT concentration during the 60 min after foaling and time of placental expulsion evidenced that the mean OT concentration of mares with placentas expelled over 1 h after foaling is significantly lower at foaling and 15 min later than that of mares with placental expulsion within 1 h after foaling. The present study suggests that one factor causing placental retention could be related to a low circulating OT concentration at foaling.

In conclusion, the OT concentration immediately after foaling is negatively related to the placental expulsion time. Placental expul-

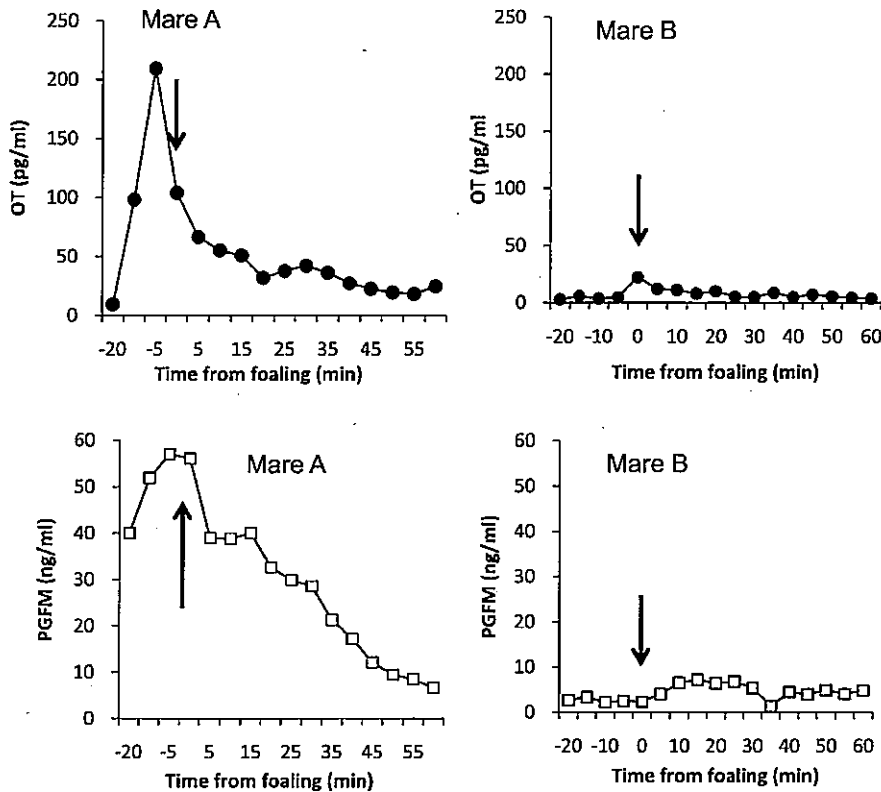


Fig. 2. Changes in the plasma OT and PGFM concentrations of two mares with different hormonal pattern. Mare A (left) shows high OT and PGFM concentrations close to foaling. Mare B (right) shows low OT and PGFM concentrations close to foaling. Mare B had a retained placenta over 4 h after foaling. The arrows indicate the time of delivery of the foals.

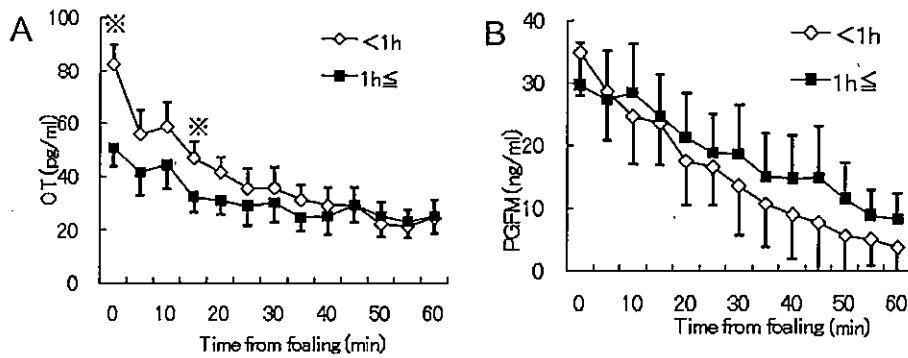


Fig. 3. Comparison of the mean OT (A) and PGFM (B) concentrations after foaling for placental expulsion times of less than 1 h ( $n=11$ ) and over 1 h ( $n=7$ ). The data are shown as the mean  $\pm$  SE. \*\*: The mean OT concentration was significantly different for a placental expulsion time of less than 1 h compared with over 1 h ( $P<0.05$ ).

sion time was extended in mares that had a low circulating OT concentration at the time of delivery. Deficiency of OT secretion at foaling could be considered one of the causes of retained placenta in heavy draft mares.

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