Relationship between Plasma Oxytocin and Placental Retention Time Immediately after Foaling in Heavy Draft Mares

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The aims of this study were to determine the relationship (1) between oxytocin release during parturition especially during the third stage of labor and placental retention time, and (2) between hand-milking or suckling and oxytocin release after foaling. Blood was sampled every 5 min from foaling to expulsion of the placenta in 9 heavy draft mares to evaluate circulating oxytocin. The relationship between the oxytocin concentration and recorded placental retention, hand milking and suckling times were investigated. Results: (1) High peak of oxytocin concentration observed around foaling in 7 mares. (2) Peaks of oxytocin were observed after expulsion of the placenta and suckling or after hand-milking about 2 hr after foaling in 2 mares. (3) The mean oxytocin concentration in mares which, had a shorter placental retention time (i.e.<1 hr) was significantly lower than that of mares which had a longer placental time (i.e.>1 hr). (4) A significant negative correlation was observed between oxytocin concentrations immediately after foaling and placental retention times. (5) Oxytocin increased distinctly after hand-milking and suckling in 2 mares. The response was most extensive in the mare, which had the lowest oxytocin concentration immediately after foaling and the longest placental retention time. In conclusion, the oxytocin concentration immediately after foaling is negatively related to the placental retention time. Low circulating oxytocin immediately after foaling may result in placental retention.

Key words: *mare, oxytocin, retained placenta, suckling*

Retention of the placenta after parturition can be a significant problem in foaling mares. Retention of the placenta in the mare has been thought to be caused by various factors such as uterine inertia, endometritis, low temperature stress and hormonal imbalance [7, 11, 13]. A long placental retention time decreases reproductive efficiency [6]. Mares with a placental retention time of less than 1 hr or longer than 4 hr had pregnancy rates of 66% and 51.7% respectively, when bred on their foal heat. Manual removal of a retained placenta was subsequently associated with reduced reproductive performance in those mares. Oxytocin concentrations are well known to increase during the second stage of labor in mares [1, 3, 15]. Various methods of oxytocin treatment for retained placenta, i.e. 30–100 IU of oxytocin in physiological saline administered intravenously [4, 14], 20–120 IU of oxytocin administered repeatedly subcutaneously or intramuscularly [5, 8, 13, 16], were effective. High pregnancy rates were observed in oxytocin treated mares after foaling [6]. Mares with a placental retention time of less than 4 hr and which had received oxytocin treatment, had a pregnancy rate at foaling heat of greater than 70% [6]. Moreover, the involvement of oxytocin in milk let down is well

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documented [2, 15], Therefore, the aims of this study were to determine the relationship (1) between oxytocin release during parturition especially during the third stage of labor and placental retention time, and (2) between hand-milking or suckling and oxytocin release after foaling.

Materials and Methods

Animals

This study was conducted in Eastern Hokkaido, Japan. It involved a total of 9 heavy draft mares which were mixed breed belonged to Breton, Percheron or Belgian Draft horse breeds, and the estimated average body weights of the mares were 800 kg, varying from 3 to 16 years in age and located on 7 farms. The foaling occurred from March to June, and from 316 days to 352 days of pregnancy. All of the foaling were normal to all appearances and all foals lived. The owners of the horses helped a little at the expulsion of the foal.

Blood sampling

Blood samples were collected from 45 to 20 min before foaling in 4 mares or from immediately after foaling of other 5 mares to 2 hr after expulsion of the placenta in all mares. Blood was collected every 5 min from immediately after foaling to 1 hr after foaling in all mares. When the placenta was retained, 5 min interval samplings were continued until expulsion of the placenta, and blood was sampled at 1 hr and 2 hr after that. The 14 gauge catheter 160 mm long with a 2 m length of tube was kept in the jugular vein during the experiment and filled up with 10 m*l* of ringer's solution in which 200 IU of heparin sodium was added after every sampling. After the solution was pumped up and dumped, the next blood was pumped up and the tube was filled up with the solution again. The blood samplings were repeated carefully with as little stress as possible for the mare. After collection, the blood was placed in a chilled glass tube containing ethylenediaminetetraacetic acid (EDTA). The blood was centrifuged immediately after collection. The harvested plasma was frozen until assayed.

Oxytocin extraction

The plasma samples (5 m*l*) were diluted with 5 m*l* of distilled water, and the pH was adjusted to 2.5. All samples were then applied to a Sep-Pak C_{18} Cartridge (Waters, Millford, MA) as described previously [9]. The residue was evaporated and then dissolved in 200 μl assay buffer (42 mM Na_2HPO_4, 8 mM KH_2PO_4, 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 oxytocin that had been added to the plasma was 70%.

Oxytocin determination

The EIA for oxytocin was previously described [9], and was based on the second antibody method along with the biotin-streptavidin-peroxidase technique [10]. Oxytocin was labeled with D-biotinoyl-e-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 μl standards or unknown samples in EIA buffer were incubated with 100 μl polyclonal antibody for oxytocin (1: 1 000 000; donated by Dr T. Higuchi, Kochi University of Medicine) in the 96 wells ELISA plate (F96 Maxisorp, Nunc, Roskilde, Denmark) at 4 for 20 hr. The wells were previously coated with 50 mg of the second antibody (anti rabbit IgG, Seikagaku Co. Tokyo, Japan). The plates were decanted and biotinyl-peptide in 100 m*l* EIA buffer (1: 50 000) was added. Plates were further incubated for 2 hr at 4°C, decanted and added 20 ng streptavidin-peroxidase (Sigma, St. Louis, MO, USA) in 100 μl EIA buffer. After 15 min incubation at 4°C, the plates were decanted again, and immediately washed 4 times with 300 μl/well 0.05% tween 80. The substrate reaction was induced with 0.025% 3,3',5,5'-tetramethylbenzidine (Wako Chemicals Co. Osaka, Japan), and stopped with 2 mol H_2SO_4 [10]. The absorbance was found to be at 450 nm with a plate reader (Bio-Rad Lab. CA, USA), and the obtained absorbance of B0 was 0.5–0.8. The standard curve for oxytocin ranged from 1.9 to 2000 pg/m*l*. The intra- and interassay CVs were 5.8% and 6.4%.

Hand-milking and suckling

All suckling and hand-milking was recorded. Each hand-milking was done by taking about 200 m*l* of milk within the time interval from 1 to 5 min. One mare was not stimulated with any hand-milking or suckling during the experiment (No. 4; Fig. 1). Four mares received a stimulus only by hand-milking (No. 3, 5, 7, 9; Fig. 1). Four mares experienced a stimulus with

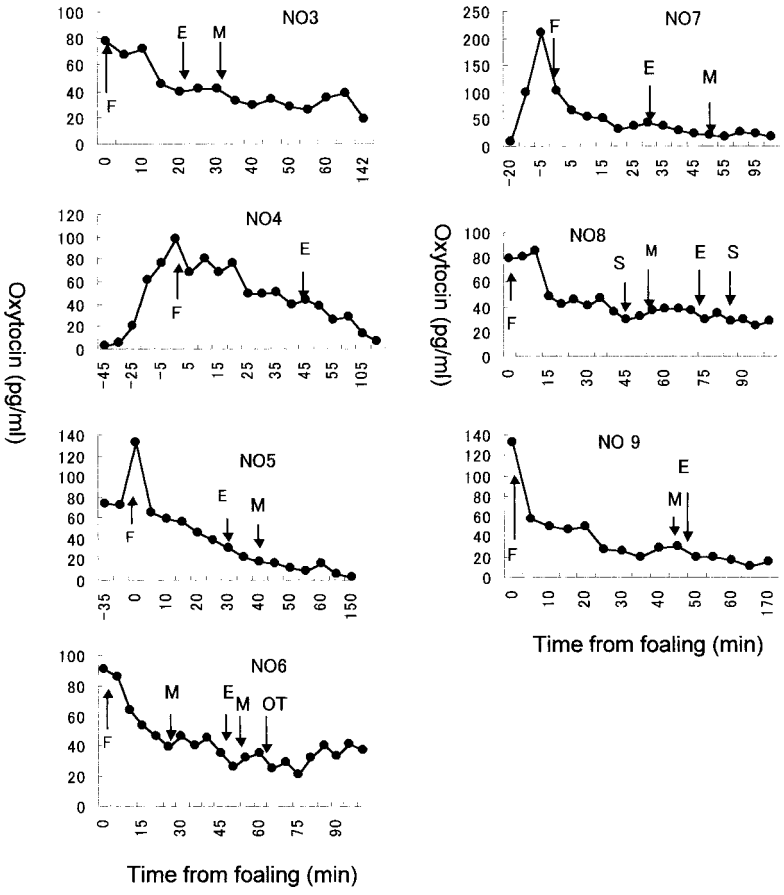

Fig. 1. Oxytocin concentrations in 7 mares with the peak of oxytocin around foaling. F: foaling. M: hand-milking. S: suckling. E: expulsion of the placenta. OT: Oxytocin 50 IU intramuscular administration.

suckling and hand-milking (No. 6, 8: Fig. 1; No. 1, 2: Fig. 2).

Oxytocin injection

One mare that did not expel the placenta within 3 hr after foaling was given two doses of oxytocin (50 IU) intramuscularly at 1 hr intervals (No. 2; Fig. 2). Another mare received oxytocin (50 IU) at 1 hr after foaling after expulsion of the placenta (No. 6; Fig. 1).

Statistical analysis

Each placental retention time of the mares was recorded. The mares were divided into two groups depending on the length of placental retention time (i.e. less than 1 hr and over 1 hr), and the oxytocin concentrations of the two groups were compared at specific times. Each mean value was tested by the difference between pairs. Difference in the mean value was tested by *t*-distribution. The correlations between the oxytocin concentration immediately after foaling and placental retention time were analyzed statistically. The effects of suckling or of hand-milking on oxytocin release were investigated. Hand-milking and suckling after oxytocin injection were excluded in judging the effect of hand-milking or suckling on the oxytocin concentration. The effect of the stimulus was confirmed when the oxytocin concentration exceeded the 11.6% range (2 × intraassay CVs) of the oxytocin concentration just before the stimulus to 10 min after the hand-milking stimulus or the period 5 min before to 10 min after suckling of the teat.

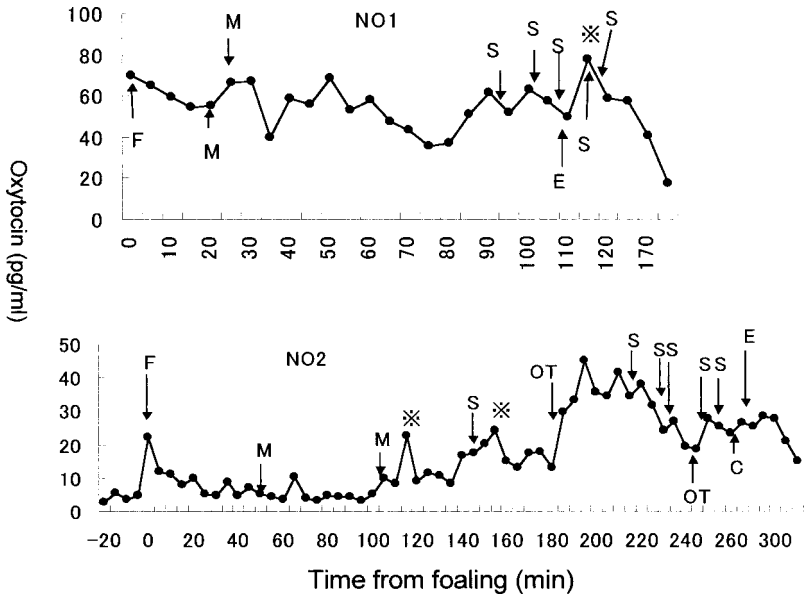

Fig. 2. Oxytocin concentrations in 2 mares with other peaks of oxytocin after parturition not only at foaling. F: foaling. M: hand-milking. S: suckling. E: expulsion of the placenta. C: colic. OT: Oxytocin 50 IU intramuscular administration. ※: oxytocin concentration exceeded the value at foaling.

Results

Change in the oxytocin concentration

A high peak of the circulating oxytocin concentration was observed around or near the foaling in 7 mares (mean: 117 pg/m*l*) (No. 3–9; Fig. 1). The plasma concentrations of oxytocin decreased gradually with the passage of time after foaling in the 7 mares (No. 3–9; Fig. 1). The oxytocin concentrations were less than half the peak value at 40 min after foaling. The oxytocin concentration declined continuously after expulsion of the placenta (No. 3–9; Fig. 1). In 2 other mares, not only a peak of oxytocin at foaling was found but also higher peaks of oxytocin were observed about 2 hr after foaling (No. 1, 2; Fig. 2). One of them had an oxytocin peak after expulsion of the placenta and suckling at the same time (No. 1; Fig. 2), and another mare had two peaks of oxytocin after hand-milking and suckling (No. 2; Fig. 2).

Relationship between placental retention time and oxytocin concentration

The oxytocin concentration immediately after foaling in the mare that had retained the placenta was noticeably lower than that in the other 8 mares (Retained placenta: mean of the other 8 mares; 22.1: 98.2 pg/m*l*; Fig. 3). The mean oxytocin concentration of the mares that had a longer placental retention time over 1 hr (56.9 pg/m*l*), was significantly lower than that of those with a placental retention time of less than 1 hr (106.2 pg/m*l*) ($p<0.05$; Fig. 4). A significant negative correlation was observed between the placental retention time and oxytocin concentration immediately after foaling ($r=-0.8193$) ($p<0.01$; Fig. 5).

Effect of hand-milking or suckling on the oxytocin concentration

Six of 8 mares showed no distinct increase oxytocin concentration (No. 3, 5–9; Fig. 1) after suckling (No. 8, Fig. 1) or hand-milking stimulus (No. 3, 5–9; Fig. 1), whereas one mare (No. 4; Fig. 1) received no stimulus by suckling or hand-milking. The rest, two mares, revealed on increase in the oxytocin concentration after suckling (No. 1, 2; Fig. 2) or hand-milking (No. 2; Fig. 2). The response was most pronounced in mare No. 2, which had the lowest oxytocin concentration immediately after foaling (22.1 pg/m*l*) and the longest placental retention time (269 min) (Fig. 2). The oxytocin concentration after hand-milking and suckling in the mare exceeded the peak value at foaling (No. 2; Fig. 2).

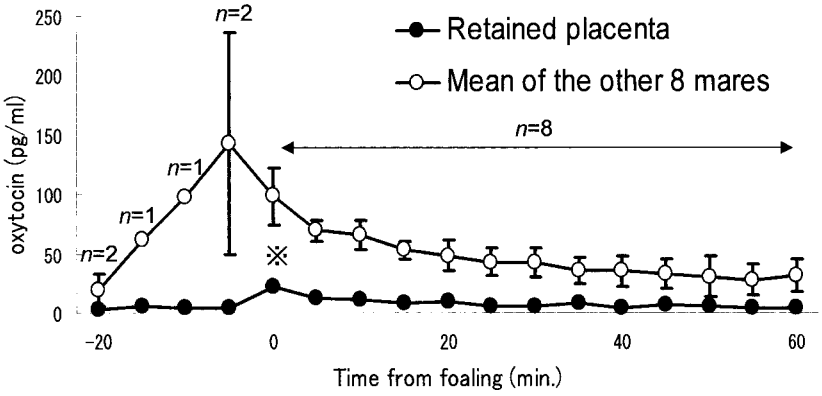

Fig. 3. Comparison of oxytocin concentrations in the mare with a retained placenta and the other 8 mares. Mean data indicate mean ± SD. ※: Oxytocin concentration at foaling in the mare that retained her placenta was noticeably lower than in the other mares.

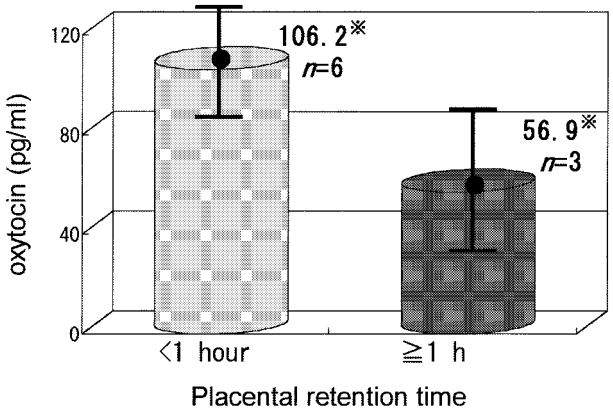

Fig. 4. Comparison of oxytocin concentration at foaling for placental retention time less than 1 hr and over 1 hr. Data indicate the mean ± SD. ※: The mean oxytocin concentration was significantly different for a placental retention time of less than 1 hr and over 1 hr ($p<0.05$).

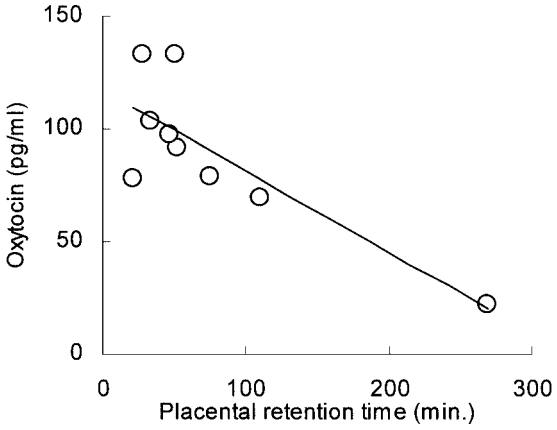

Fig. 5. Relationship between the oxytocin concentration at foaling and the placental retention time of the mare. A significant negative correlation was observed between placental retention time and the oxytocin concentration immediately after foaling ($r=-0.8193$) ($p<0.01$).

Oxytocin treatment

The circulating oxytocin concentration increased rapidly after intramuscular administration of oxytocin (50 IU) and the level of oxytocin exceeded the peak at foaling in the mare with a retained placenta (No. 2; Fig. 2). However the oxytocin concentration did not change noticeably after the second oxytocin injection, and the retained placenta was expelled 30 min after the second injection (No. 2; Fig. 2). On the other hand, the oxytocin concentration did not increase after intramuscular administration of oxytocin (50IU) after expulsion of the placenta in the mare (No. 6; Fig. 1), but the level of circulating oxytocin in the mare remained constant for over 30 min after oxytocin administration, not decreasing to the base level (No. 6; Fig. 1).

Discussion

In the present study, the peak circulating oxytocin concentration was observed around or near foaling.

The plasma concentrations of oxytocin of all mares decreased gradually after foaling. Oxytocin is considered the final hormone in the maternal cascade leading to parturition [12]. Oxytocin concentrations increase rapidly during second stage of labor in mares [1, 3, 13]. In a report on measuring the oxytocin in pituitary effluent which was collected from the intercavernal sinus [15], the oxytocin concentration increased markedly from 1 min to 11 min after rupture of the chorioallantois, and oxytocin secretion continued during delivery of the foal. In this study, however, the number of mares that were sampled before foaling were few, on the increase in the circulating oxytocin concentration around foaling agreed with previous investigations.

In this study, circulating oxytocin concentrations at foaling were found to be extremely dispersed in each mare. Oxytocin at foaling of a mare that had a retained placenta was significantly lower than in other mares. And, the mean of oxytocin concentration in the mares that had a longer placental retention time over 1 hr was significantly lower than for less than 1 hr. Moreover, a significant negative correlation was observed between placental retention time and the oxytocin concentration immediately after foaling. Excellent results were obtained by treating the retained placenta with oxytocin, suggesting a possible deficiency or lack of this hormone in the circulation [15]. The present study revealed that one factor in the cause of retained placenta is lower circulation of oxytocin, but a retained placenta may not be caused by only one factor. Further investigation should be done.

In 2 of 8 mares, which excluded a mare that was not receiving any stimulus by suckling and hand-milking, not only did a peak of oxytocin occur at foaling but also higher peaks of oxytocin were observed about 2 hr after foaling. The oxytocin concentrations increased reflexively after suckling or hand-milking in 2 mares. Whereas the oxytocin reflex theory of milk ejection was investigated in the horse [2], a report on the collection of pituitary effluent concluded that suckling is not significantly related to oxytocin release in mares [15], but in the same report a significant effect of suckling on oxytocin release was detected in 2 of 9 mares. In this study, the oxytocin concentration rose after suckling in 2 of 3 mares that received stimulus by suckling before expulsion of the placenta or oxytocin injection. The ratio of oxytocin reflexive mares caused by suckling was different from the previous study. The response was most extensive in the mare which had the lowest oxytocin concentration immediately after foaling and the longest placental retention time. Probably this result is related to the idea that the pituitary may have only a limited oxytocin stock during parturition. Namely, mares in which oxytocin concentrations at foaling were low, may have a large amount of retained oxytocin in the pituitary, therefore, and could have a release of oxytocin caused by suckling or hand-milking.

Injected oxytocin increased the oxytocin concentration after foaling in the mare that had a retained placenta and the lowest oxytocin concentration. The retained placenta was expelled after the second oxytocin administration. These results support those of previous studies that provided various effective oxytocin treatments for retained placenta in the horse [4–6, 8, 13, 14, 16]. Oxytocin treatments were effective not only in expelling the placenta but also in increase fertility at foal heat [6]. The most effective method for oxytocin treatment should be established.

Conclusion

The oxytocin concentration immediately after foaling is negatively related to the placental retention time. Low levels of circulating oxytocin immediately after foaling may result in placental retention.

Acknowledgments

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