

Plasma oxytocin concentrations during late pregnancy and parturition in the dog

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Abstract

While oxytocin is widely used in the treatment of dystocia in dogs, there is little information about its secretion before and during normal unassisted whelping. We therefore measured plasma oxytocin concentrations during late pregnancy and the expulsive stage of parturition. Blood samples were collected from eight dogs at 3-min intervals during a 42-min period between the 2nd and 14th day before whelping and during parturition after the birth of 1–3 pups. The litters consisted of 5–15 pups and the progression of the expulsive stage was linear and nearly parallel in the eight bitches. The overall mean (\pm S.D.) plasma oxytocin concentration during late pregnancy was 3.6 ± 2.1 pg/ml. Mean values in individual dogs ranged from 1.2 to 7.4 pg/ml, but the intra-animal variation was rather small. During the expulsive stage the overall mean (\pm S.D.) plasma oxytocin concentration was 12.9 ± 13.9 pg/ml, with mean values in individual dogs ranging from 3.5 to 46 pg/ml. The mean area under the oxytocin curve for parturient dogs was significantly higher ($P < 0.05$) than for pregnant dogs. During the expulsive stage, the peak plasma oxytocin level in individual dogs ranged between 10 and 117 pg/ml. In six of the eight dogs a pup was born during blood collection and in five of these animals the plasma oxytocin concentration increased temporarily during periods of abdominal straining and expulsion. However, straining efforts and expulsion were not consistently associated with a rise in the circulating oxytocin level. It is concluded that in the dog plasma oxytocin levels are higher and more variable during the expulsive stage of parturition than during late pregnancy. Interrelationships between the secretion pattern of oxytocin, the level of uterine contractility, and the progress of fetal expulsion in dogs need further exploration.

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1. Introduction

Studies in polytocous species have shown a relationship between the pattern of oxytocin secretion during normal parturition and the process of fetal expulsion. In

the rat, there is not only an increase in the basal plasma oxytocin level during the expulsive phase, but also an increase in the circulating oxytocin concentration after a few cycles of abdominal straining followed by expulsion of a fetus [1]. In the rabbit, the plasma oxytocin concentration increases within 40–120 s prior to the onset of the expulsive phase and peak concentrations coincide with the delivery of the first or second fetus. These peak concentrations are significantly related to the speed of delivery [2].

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Although experiments with oxytocin knock-out mice demonstrated that oxytocin is not essential for parturition in this species [3], more recent experiments in mice [4] clearly indicated that oxytocin neuron activity and secretion contribute to the speed of delivery. In sows, significant fluctuations in peripheral plasma oxytocin concentration during the expulsive stage were shown to be superimposed on an increased baseline secretion. These peaks in oxytocin secretion were not related to straining movements prior to the expulsion of a piglet. Pooling of data from different sows at the time of fetal expulsion revealed a small but significant elevation during the minute following expulsion [5].

Synthetic oxytocin is commonly used in veterinary practice for the treatment of apparent uterine inertia in the bitch [6], but there are few published data on plasma oxytocin concentration during pregnancy and spontaneous whelping. Hoffmann et al. [7] reported that the plasma oxytocin concentration in two parturient dogs only increased several hours after luteolysis and the first signs of the onset of whelping. A more recent study [8] reported consistently higher plasma oxytocin levels during the expulsive stage than during gestation. Information on oxytocin release based on very frequent sampling during whelping is still lacking in dogs. Due to the relatively long time required for complete expulsion of a litter (5–10 h) and the volume of plasma needed for each measurement of oxytocin, it is not possible to characterize the oxytocin secretion pattern during the entire expulsive stage without inducing hypovolemia. Given this restriction, the present study was undertaken to investigate the plasma profile of oxytocin in eight bitches, both during late pregnancy and during the expulsive stage of parturition, by means of frequent blood sampling.

2. Materials and methods

2.1. Animals

Eight adult bitches of four different breeds were used for this experiment: two Beagles, two Foxhounds, two Great Danes and two Labrador retrievers. The ages of the Labrador retrievers were unknown, but because age was not expected to influence oxytocin release, we decided to include these two dogs. The ages of the other dogs varied from 2 to 7 years. All but one had whelped at least once. Except for one Foxhound, the single day of breeding was known and pregnancies had been confirmed by ultrasonography. The dogs were fed a standard commercial dog food and water was available *ad libitum*. The Foxhounds were owned by the Royal

Dutch Hunting Society and were used for hunting and housed in a pack. They remained at their home kennel during pregnancy but were housed individually a few days before the expected day of parturition. The Beagles and Great Danes were born and raised at the Department of Clinical Sciences of Companion Animals at Utrecht University and were housed in indoor–outdoor runs. During the last week of pregnancy, they were moved to a separate kennel and housed individually. The Labrador retrievers were privately owned breeding bitches kept in indoor–outdoor runs. Foxhounds and Labradors were brought to the University Clinic for the last week of pregnancy and parturition.

2.2. Experimental protocol

The following protocol was approved by the Committee for the use of animals in research and education (DEC) of the Faculty of Veterinary Medicine of Utrecht University. During the presumed last 10 days of pregnancy, body temperature was measured three times daily. During the first sampling session, blood samples of 3 ml were collected by jugular vein puncture from the Beagles and via a temporary catheter in the jugular vein of the Foxhounds or the cephalic vein of the Great Danes and Labrador retrievers. Samples were collected at 3-min intervals for 42 min from seven dogs between the 2nd and 8th day before parturition, but in the Foxhound in which the date of breeding had to be estimated, this pregnancy sampling proved to be on the 14th day before whelping (Table 1). During a second bleeding session, blood samples were collected during the expulsive stage of parturition, with the dogs in their whelping cages. When parturition was imminent, as indicated by a sudden drop in body temperature and/or restlessness and nest-building behavior ($n = 4$), or when at least one pup had been expelled ($n = 4$), a catheter was inserted in the jugular vein of the Beagles and in the cephalic vein of the other dogs. Following the birth of one, two, or three pups, blood samples were collected as soon as the bitch again made definite abdominal straining efforts. Samples were then collected at 3-min intervals for 42 min, except for a temporary interruption of some 33 min in dog 6, because straining had stopped after the 3rd blood sample; bleeding was resumed again in this bitch as soon as she again started with abdominal straining.

The catheter was then removed and parturition was observed until expulsion of pups was completed. None of the bitches received synthetic oxytocin. The blood samples were collected in ice-chilled tubes containing

Table 1
Breed, time of blood sampling, litter size, and parturition

Dog	Breed	First sampling (days before parturition)	Second sampling (after/during pup number)	Litter size (alive/dead)	Duration of expulsive stage (min)
1	Foxhound	14	1/2 and 3	8/0	635
2	Foxhound	7	1/2	5/0	364
3	Beagle	5	2/3	4/2	300
4	Beagle	8	2/	9/1	500
5	Great Dane	8	3/4	14/1	532 (pup 4–15)
6	Great Dane	8	2/3	7/0	573 (pup 3–7)
7	Labrador	2	2/	8/0	253 (pup 3–8)
8	Labrador	4	3/4	9/0	622 (pup 3–9)

EDTA and were stored in ice until centrifugation at $2000 \times g$ (within 45 min). The plasma samples were stored at -20°C until assayed, as soon as parturition had ended.

2.3. Extraction and radioimmunoassay of oxytocin

Plasma oxytocin concentration was determined by a specific radioimmunoassay after ethanol extraction. The extraction was performed by the addition of 3 ml of chilled (4°C) ethanol to 0.5 ml plasma, followed by rotation for 30 min at 4°C and then centrifuged for 30 min at $5500 \times g$, also at 4°C . The supernatant was decanted and evaporated to dryness using a Speedvac vacuum concentrator. The residue was dissolved in 125 μl assay buffer consisting of 63 mM sodium phosphate (pH 7.2), 13 mM Na_2EDTA , 0.02% (w/v) sodium azide, Trasylol (20,000 units/100 ml; to prevent proteolytic degradation of oxytocin), 0.1% (v/v) Triton X-100, and 1% (w/v) BSA (RIA grade). The extraction recovery was determined by the addition of known amounts of synthetic oxytocin to pooled plasma from a male dog and subsequent measurement in the radioimmunoassay. The recovery was to $96 \pm 10.4\%$ (mean \pm S.D., $n = 4$).

Oxytocin in the extracts was measured by radioimmunoassay using the THF-3 oxytocin antiserum kindly donated by Dr. Higuchi [1]. The cross-reactivity of this antiserum was $<0.0004\%$ for arginine vasopressin, $<0.0005\%$ for arginine-vasotocin, $<0.0017\%$ for MSH-release inhibiting factor, and $<0.0001\%$ for TRH, LHRH, angiotensin II, LH, FSH, prolactin, and TSH. Oxytocin was radioiodinated using the Iodogen method. In short, polypropylene tubes were coated with 50 μg Iodogen in 50 μl dichloromethane and evaporated to dryness. The iodination reaction was started by addition of 3 μl of ^{125}I (0.3 μCi) to 20 μl phosphate buffer (0.5 mM, pH 7.4), and 10 μl of the peptide (10 $\mu\text{g}/\text{ml}$ in 10 mM

HCl). After 10 min, 50 μl TFA was added and the labeled oxytocin was purified on a Seppak column that had been conditioned with methanol and rinsed with water. The column was eluted with 2×1 ml 0.1% TFA and 2×1 ml of increasing concentrations of methanol (25%, 50%, 75%, and 99.9%). The ^{125}I -oxytocin was eluted in 75% methanol and stored at -20°C until used in the assay.

The assay was performed by the addition of 50 μl synthetic oxytocin (Peninsula Laboratories, Merseyside, UK) in a concentration range of 5–1000 pg/ml or plasma extract to 100 μl assay buffer and 50 μl antiserum in a final dilution of 1:600,000. After the tubes were incubated at 4°C for 24 h, 50 μl ^{125}I -oxytocin (10,000 cpm) was added to each tube and they were incubated at 4°C for an additional 48 h. Antibody-bound oxytocin was precipitated by the addition of 50 μl anti-rabbit second antibody-coated cellulose (Sac-Cel; IDS Ltd., Boldon, Tyne and Wear, UK). The sensitivity of the assay, calculated as the lowest measurable concentration two standard deviations above the zero standard (buffer control), was 1.06 ± 0.33 pg/ml (mean \pm S.E.M., $n = 4$). Serial dilutions of a pooled plasma sample with a high endogenous oxytocin level appeared to be parallel to the standard curve. The intra-assay and interassay coefficients of variation were 6.9% and 12.8%, respectively, at a concentration of 30 pg oxytocin/ml.

2.4. Data analysis

Descriptive analyses were performed for plasma oxytocin concentrations in each dog during pregnancy and parturition and overall mean values (\pm S.D.) were calculated. The area under the curve (AUC) above the zero level was calculated for each of the plasma oxytocin profiles. The AUCs during pregnancy and the expulsive stage were compared by the Wilcoxon signed rank test. $P \leq 0.05$ was considered significant.

3. Results

The litters consisted of 5–15 pups. The duration of expulsion of the entire litter ranged from 300 to 635 min in four bitches. In the other four bitches intervals between pups were only recorded after the third or fourth pup had already been born (Table 1). Fig. 1 shows the cumulative birth intervals of the subsequent pups for each dog. Apart from differences in litter size, progression of the expulsive stage was linear and nearly parallel in the eight bitches. The mean of all recorded birth intervals was 75 min ($n = 51$) and the range was 6–212 min (nine intervals were unknown/not recorded). The duration of 82% of the birth intervals lasted less than 2 h.

Blood samples were collected during parturition after 1, 2, or 3 pups had been born. In six bitches it was possible to collect a blood sample at about the time of expulsion of a pup (Table 1). Individual oxytocin values in the eight bitches are shown in Fig. 2. In one of the Great Danes the scheduled 42-min sampling period was interrupted for 33 min and sampling was resumed near the time of expulsion of the third pup. The mean and AUC values of plasma oxytocin concentrations for each of the two series of samples are given in Table 2.

Circulating oxytocin concentrations were lower and fluctuated within narrower limits during late pregnancy

than during whelping (as reflected by the smaller S.D. of the overall means), except for bitch 7, in which there was a single peak value of 15.8 pg/ml 2 days before whelping, and for bitches 4 and 7, that did not expel a pup during sampling. The difference of the AUC values between late pregnancy and the expulsive stage was significant ($P = 0.017$) when the data of all eight bitches were included. Peak values in the six bitches that delivered a pup during sampling ranged from 10.4 to 117 pg/ml. In five of them (nos. 1–4 and 8, Fig. 2) plasma oxytocin concentrations increased around the time of expulsion of a pup, but single peak values were also found in the period following expulsion of a pup, at some 90 and 120 min before the birth of the next one (no. 8, Fig. 2). In bitch no. 4, 15 blood samples were collected while there was no visible abdominal straining or expulsion of a pup and the mean plasma oxytocin in these samples was similar to those eight days before whelping (Table 2).

4. Discussion

To the best of our knowledge, this study was the first one to examine profiles of plasma oxytocin concentrations during late pregnancy and the expulsive stage of spontaneous parturition in dogs, using frequent blood sampling. Experiments in mice [4], rats [9], and pigs [10] have shown inhibiting effects of environmental disturbances on the progression of the expulsive stage that are at least partially caused by inhibition of oxytocin secretion. Although the intervals between births in the present study were somewhat greater than reported by others [11], the progression of the expulsive stage did not appear to be affected by our experimental approach, for it was linear and nearly parallel in the eight bitches.

Our data demonstrate that during late pregnancy the plasma oxytocin concentration is low, close to the lowest detectable level, and fluctuates within narrow limits in individual bitches, although the mean concentration differs among bitches. During the expulsive stage of parturition, the concentration is elevated and much more variable in individual bitches. In five of them, the oxytocin level increased temporarily around the time of expulsion of a pup, but similar elevations were also observed in the absence of expulsive efforts and, in contrast, were sometimes absent during a prolonged period of straining.

Apart from differences in absolute values, probably attributable to differences between the RIA methods, our findings during late gestation are in agreement with findings in dogs reported by Olsson et al. [8] and with the relatively low circulating oxytocin levels observed during pregnancy in other species [2,4,5,12]. However,

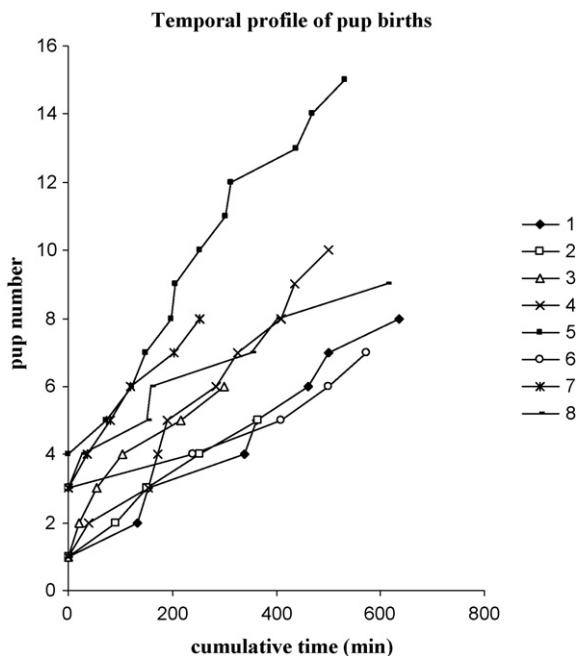


Fig. 1. Cumulative birth intervals of pups in eight whelping dogs. Note that in four bitches, observations started only after the birth of the third or fourth pup.

the profiles reported by Olsson et al. [8] suggest that plasma oxytocin is consistently elevated throughout the expulsive stage, in contrast to the pulsatile pattern found in the present study and reported in other polytocous species [5,13,14]. Uterine electromyographic recordings during whelping [15] have demonstrated prolonged persistence of myometrial electrical activity (for several

minutes) during the expulsion of a pup, presumably associated with the Ferguson reflex. The temporary increase in plasma oxytocin around the time of expulsion of a pup, as found in the present study, is in agreement with this presumption. But, as in the pig [5], the temporal relation between oxytocin secretion and expulsion appears to be much more complex, for in dog 8 there

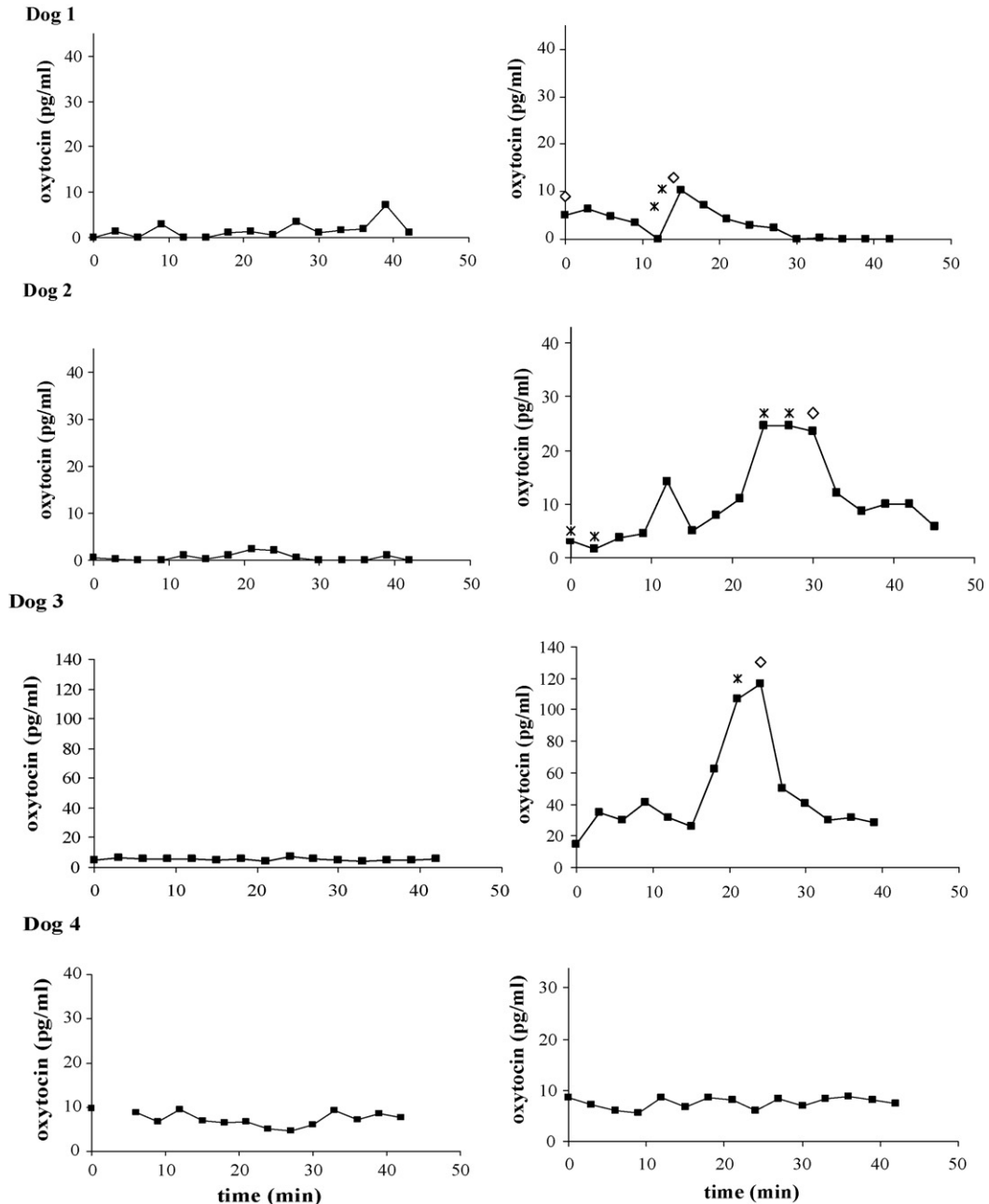
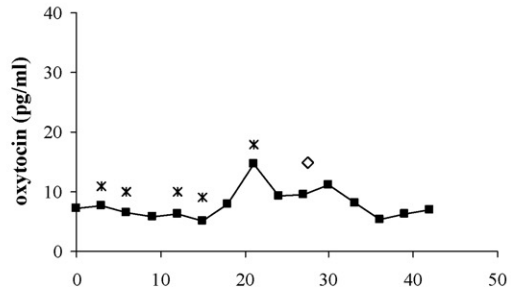
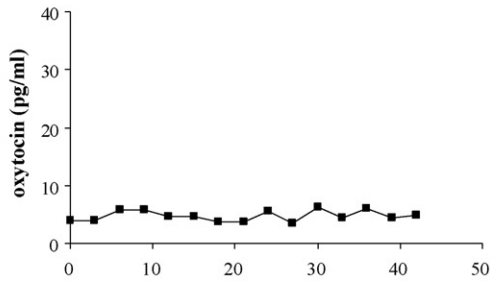
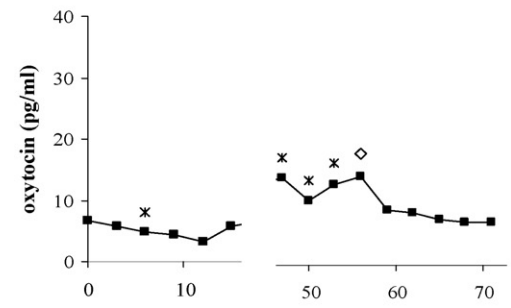
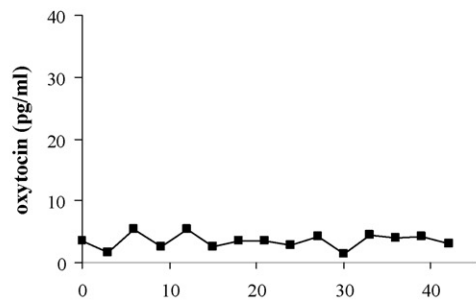


Fig. 2. Plasma oxytocin profiles in eight bitches during late pregnancy (left) and the expulsive stage of parturition (right). Samples were collected at 3-min intervals (with an interruption of 33 min in dog 6. Asterisks (*) indicate visible abdominal straining. Rhomboid symbols (◊) denote the birth of a pup (compare Table 1). Note that the scale of the y-axis of the profiles of dog 3 is different from that in the other dogs.

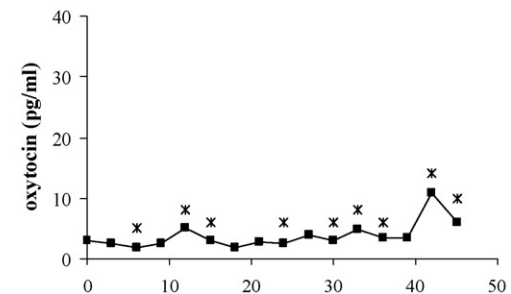
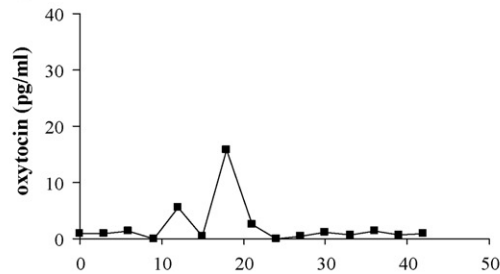
Dog 5



Dog 6



Dog 7



Dog 8

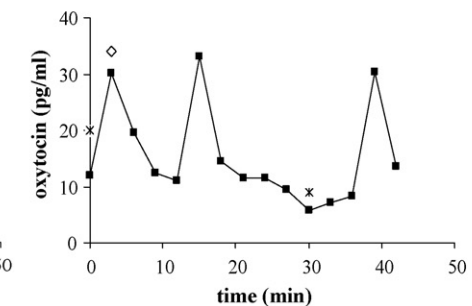
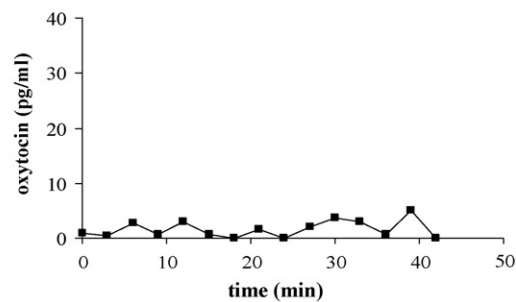


Fig. 2. (Continued).

were also oxytocin peaks in the absence of straining and expulsion. Other stimuli, such as suckling by pups already born might have caused such elevations. The exact interrelations between myometrial activity, entrance of pups into the pelvic canal, onset and frequency of abdominal straining, the actual moment

of expulsion, suckling during the expulsive stage, and the pattern of oxytocin release still deserve a more detailed investigation.

Other factors could have influenced the pattern of oxytocin release in the present study. We used four different breeds of dogs and breed differences might

Table 2

Plasma oxytocin concentration (pg/ml) and area under the curve (AUC; pg/ml* 42 min) in the eight bitches during late pregnancy and the expulsive stage of parturition

Dog		Late pregnancy		Expulsive stage of parturition	
No.	Breed	Mean \pm S.D.	AUC	Mean \pm S.D.	AUC
1	Foxhound	1.9 \pm 1.6	1.3	3.5 \pm 2.8	2.5
2	Foxhound	1.2 \pm 0.4	0.9	10.6 \pm 7.6	7.8
3	Beagle	5.3 \pm 0.9	3.7	46.0 \pm 30.2	31.1
4	Beagle	7.4 \pm 1.6	5.2	7.5 \pm 1.1	5.2
5	Great Dane	4.8 \pm 0.9	3.4	7.9 \pm 2.5	5.6
6	Great Dane	3.5 \pm 1.2	2.5	8.4 \pm 3.9	6.0
7	Labrador	2.5 \pm 3.9	1.8	3.8 \pm 2.2	2.9
8	Labrador	2.0 \pm 1.3	1.5	15.3 \pm 8.8	10.9
Overall		3.6 \pm 2.1	2.5 \pm 1.5	12.9 \pm 13.9	9.0 \pm 9.3

contribute to between-animal variability as much as litter size or the timing and site of blood collection. There was no obvious relation between mean values during pregnancy or parturition and the site of sampling. The protocol was dictated by the volume of blood that could be withdrawn without influencing physiological processes and the presumptive short half-life of oxytocin. The few published data on the half-life of oxytocin in canine plasma are inconsistent. Robinson [16] reported the half-life to be 4.3 ± 1.5 min, but others [17] considered it to be much shorter on the basis very brief peaks in samples collected at intervals of 20–30 s around the time of feeding of dogs. Our sampling protocol did not allow characterization of oxytocin secretion during the entire expulsive stage in dogs, but data from Olsson et al. [8] suggest that plasma oxytocin concentration in dogs only begins to increase with the onset of abdominal straining, i.e., many hours after the onset of uterine contractions [15]. Thus other uterotonic agents, such as prostaglandins, might be responsible for the early increase and maintenance of uterine contractions, a possibility consistent with the rather early rise in the circulating prostaglandin concentration that has been reported in parturient dogs [8,18–20].

In conclusion, this study demonstrates that plasma oxytocin concentration in the bitch is low and varies within rather narrow limits during late gestation. It is significantly elevated during the expulsive stage of parturition and there are temporary elevations, although not exclusively, around the time of expulsion.

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