Hormonal changes in spontaneous and aglépristone-induced parturition in dogs

M. Baan a,1, M.A.M. Taverne b, J. de Gier a, H.S. Kooistra a, H. Kindahl c, S.J. Dieleman b, A.C. Okkens a,*

a Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, P.O. Box 80154, NL-3508 TD Utrecht, The Netherlands
b Department of Farm Animal Health, Fetal and Perinatal Biology Section, Faculty of Veterinary Medicine, Utrecht University, Marburglaan 2, P.O. Box 80154, NL-3508 TD Utrecht, The Netherlands
c Department of Clinical Sciences, Division of Reproduction, SLU-Sveriges lantbruksuniversitet, P.O. Box 7054, SE-750 07 Uppsala, Sweden

Received 10 July 2007; received in revised form 10 October 2007; accepted 10 October 2007

Abstract

To increase our understanding of the endocrine changes associated with parturition in dogs, plasma concentrations of progesterone (P4), 15-ketodihydroprogaglandin F2α (PGFM), estradiol-17β (E2β), cortisol, ACTH, prolactin (PRL), LH, and FSH were measured in six spontaneously whelping bitches and in six bitches in which parturition was induced with the progesterone-receptor blocker aglépristone on day 58 of pregnancy.

Expulsion of pups in the induced group took place in the presence of P4 concentrations that were still elevated. PGFM concentrations increased before parturition in both groups, but levels were lower in the induced bitches. PGFM levels reached a maximum in both groups during parturition and quickly decreased in the spontaneously whelping group after parturition, but remained elevated in the induced group. In both groups, cortisol concentrations reached similar maximum levels during the last 30 h before the onset of expulsion. During the 3 days postpartum, cortisol concentrations were higher in the induced group. The highly variable ACTH concentrations did not differ significantly throughout the study within or between groups. In both groups, E2β concentrations decreased and PRL concentrations increased between the late gestational period and the 30-h period before parturition. Concentrations of both LH (spontaneously whelping group) and FSH (both groups) decreased between late gestation and the postpartum period. The results of this study illustrate the hormonal changes around parturition in the bitch, and reveal that aglépristone-induced parturition is associated with still incomplete luteolysis, an altered PGFM profile, and elevated postpartum cortisol concentrations as compared with spontaneously whelping dogs.

© 2008 Published by Elsevier Inc.

Keywords: Progesterone; Prostaglandin F2α; Cortisol; Estradiol; Gonadotropins; ACTH; Prolactin

1. Introduction

Progesterone (P4) is necessary for maintaining pregnancy in mammals [1]. In the dog the corpora lutea are the sole source of P4 during gestation [2,3]. Ovarian P4 production is independent of luteotropic support from the pituitary during the first half of gestation in this species [4,5]. Maintenance of the
corpora lutea during the second half of the luteal phase or pregnancy is mainly a function of prolactin (PRL) and possibly gonadotropic hormones [6–10].

Parturition in the dog takes place after a pregnancy with an average length of 61.4 days, when bitches are mated once on the guidance of the preovulatory increase of the plasma P4 concentration [11]. During the last 1–2 days prior to whelping, the plasma P4 concentration decreases rapidly [12–14], while the plasma 15-ketodihydroprostaglandin-F2α (PGFM) level begins to increase [15,16]. The plasma PGFM level reflects the peripherally active prostaglandin F2α (PGF2α) concentration, as PGF2α itself has a short half-life and is rapidly converted to PGFM. As a result of the decrease in P4 and the rise in PGF2α concentrations, myometrial activity gradually increases [14], which leads to the onset of whelping.

It remains unclear which signal(s) trigger(s) these hormonal changes associated with whelping in the dog. In sheep and goats, a fetal corticoid signal is the trigger for parturition [17,18]. The subsequently rising estrogen levels enhance prostaglandin production during gestation, which in turn increases uterine myometrial contraction activity in sheep. In dogs, data on fetal hormone secretion are not available, and circulating estrogen concentrations in the bitch decrease rather than increase towards parturition [12,14,19]. In addition, the circulating estrogens seem to be of ovarian rather than placental origin in dogs [19,20].

Although it remains to be elucidated which factors result in pre-partum luteolysis in the dog, the sharp decline of the plasma P4 concentration before whelping appears to be essential for a successful parturition [14,15,21]. Progesterone-receptor blockers such as aglépristone and mifepristone are competitive antagonists of the progesterone receptor, and also have affinity for the glucocorticoid receptor [22,23]. The anti-progesterone effect of these drugs has been used for the induction of abortion or whelping [14,24–27].

The aim of this study was to increase our knowledge of the endocrine changes associated with parturition in the dog. In addition, we compared the hormonal changes in spontaneously whelping bitches with those in bitches in which parturition was induced on day 58 of pregnancy with aglépristone.

2. Materials and methods

2.1. Animals

Animal data and maintenance procedures, the methods to determine optimal mating time, and management of parturition were as previously described [27]. The study protocol was approved by the committee for the use of animals in research and education (DEC) of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Blood samples were collected, from six spontaneously whelping dogs and six dogs in which parturition was induced with the progesterone-receptor blocker aglépristone, from day 54 of pregnancy until 4 days after parturition. Frequency and times of blood sampling are indicated in Table 1. A total of 16 blood samples were taken during the expulsion phase, 6 in the spontaneously whelping group and 10 in the induced group. Thirteen of these samples were collected within 30 min after the birth of a pup (n = 9) or after vaginal exploration (n = 4).

On day 58 of pregnancy, bitches assigned to the induced group were treated at 10:00 and at 19:00 h with a subcutaneous dose of 15 mg aglépristone (Virbac BV, The Netherlands) per kg late-pregnancy body weight.

Table 1
Blood sampling protocol and hormone determinations of bitches in the spontaneously whelping and in the induced group

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Hormone determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 54–56 of pregnancy</td>
<td>08:00</td>
<td>PGFM, Cort, ACTH, LH, FSH, P4, E2β</td>
</tr>
<tr>
<td></td>
<td>19:00</td>
<td>PGFM, Cort, LH, FSH, PRL</td>
</tr>
<tr>
<td>Day 57 of pregnancy–1st day after expulsion of the last pup</td>
<td>01:00</td>
<td>PGFM, Cort, LH, FSH, PRL</td>
</tr>
<tr>
<td></td>
<td>08:00</td>
<td>PGFM, Cort, P4, E2β</td>
</tr>
<tr>
<td></td>
<td>13:00</td>
<td>PGFM, Cort, ACTH, LH, FSH, PRL</td>
</tr>
<tr>
<td></td>
<td>19:00</td>
<td>PGFM, Cort, P4</td>
</tr>
<tr>
<td>2nd, 3rd and 4th day after expulsion of the last pup</td>
<td>08:00</td>
<td>PGFM, Cort, P4, E2β</td>
</tr>
<tr>
<td></td>
<td>19:00</td>
<td>PGFM, Cort, ACTH, LH, FSH, PRL</td>
</tr>
</tbody>
</table>

PGFM, prostaglandin F2α metabolite; Cort, cortisol; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; progesterone, P4; E2β, estradiol-17β.
2.2. Hormone determinations

Plasma P4 concentrations were measured by a previously validated ³H-RIA using extraction with hexane [28, 29]. The intra-assay and interassay coefficients of variation (CVs) were 11% and 14%, respectively. The sensitivity was 0.13 nmol/L. Possible interference with the assay by aglépristone was investigated as follows. Aglépristone was administered subcutaneously, twice with a 9-h interval (t = 0 and t = 9 h) at a dose of 15 mg/kg body weight to five intact female beagle bitches during anestruś. Blood samples for determination of the plasma P4 concentration were collected prior to aglépristone administration (t = −46 h, −22 h and 0 h), and twice daily thereafter until 60 h after the first aglépristone dose. The mean (±S.E.M.) P4 concentration before aglépristone administration was 0.8 ± 0.3 nmol/L. The mean P4 concentration 60 h after the first dose of aglépristone administration was 1.0 ± 0.6 nmol/L. The general linear model for repeated measures showed that the P4 concentrations did not change significantly (P = 0.22) after aglépristone administration in any of the bitches.

Plasma PGFM concentrations were measured by RIA, as described previously [30, 31]. The intra-assay CVs were between 3.4% and 7.6% at different ranges of the standard curve. The interassay CV was 14%. The sensitivity was 300 pmol/L.

Plasma E₂β concentrations were measured by a solid phase ¹²⁵I-RIA (Coat-a-Count TKE; DPC, Los Angeles, USA) according to the manufacturer’s instructions, with modifications as described previously [32] and validated for the dog [33]. The intra-assay and interassay CVs were 14% and 11.8%, respectively. The sensitivity was 7 pmol/L.

Plasma cortisol concentrations were measured by RIA according to the manufacturer’s instructions (Coat-a-Count TKC; DPC, Los Angeles, USA). The intra-assay and interassay CVs were 4.3% and 5.2%, respectively. The sensitivity was 5.5 nmol/L.

Plasma adrenocorticotropic hormone (ACTH) concentrations were measured by RIA according to the manufacturer’s instructions (ACTH 65T Kit Nichols Institute Diagnostics, San Juan Capistrano, USA). The intra-assay and interassay CVs were 3.0% and 7.8%, respectively. The sensitivity was 1.0 ng/L.

Plasma PRL concentrations were determined by a homologous canine IRMA (AHCOO4; Biocode SA, Liège, Belgium) as described previously [34]. The intra-assay and interassay CVs for values above 0.5 µg/L were 2.3% and 10.5%, respectively. The sensitivity was 0.3 µg/L.

Plasma follicle stimulating hormone (FSH) concentrations were determined by a homologous canine IRMA (AHCOO4; Biocode SA, Liège, Belgium) as described previously [34]. The intra-assay and the interassay CVs for values above 1.60 µg/L were 3.5% and 15.1%, respectively. The sensitivity was set at the value of the lowest standard, 1.5 µg/L.

2.3. Data analysis

Statistical analysis was performed using SPSS® for Windows, version 11.0.1 (SPSS Inc., Chicago, USA) and SAS/STAT® (SAS Institute Inc., Cary, USA).

In order to compare mean hormone concentrations between the two groups, the complete sampling period was divided into five time intervals. Period 1: from day 54 until 10:00 h on day 58 of gestation (‘late gestation’); period 2: the 30-h period before expulsion of the first pup (‘before parturition’); period 3: from the expulsion of the first pup until the expulsion of the last pup (‘during parturition’); period 4: 0–24 h after the expulsion of the last pup (‘the day after parturition’); period 5: 24–72 h after the expulsion of the last pup (‘the 2nd and 3rd day after parturition’). For each bitch, a mean hormone concentration was calculated for each of these periods. This mean value was entered into the statistical analysis. It should be noted that the dogs in the induced group received the first dose of aglépristone at the end of period 1.

Because blood samples were not available for each dog during parturition (period 3), an ANOVA for Repeated Measures was performed in each group for the periods 1, 2, 4, and 5 only. To apply this ANOVA, data that were found not to be normally distributed were either log-transformed (ln; P4 concentrations in the induced group) or reciprocally transformed (1/x; PGFM concentrations in the induced group). Mean hormone concentrations in both group were compared with Student’s t-test, with Bonferroni correction.

The concentrations of P4, cortisol, and PGFM during the expulsion phase (period 3) were calculated by taking the mean of the average concentration measured for each bitch during the expulsion phase (n = 6 in the spontaneously whelping group, and n = 5 in the induced group; one dog was not sampled during the expulsion phase). For period 3, mean concentrations of P4, cortisol and PGFM were compared with Student’s t-test.

Data are expressed as mean ± S.E.M. A P-value ≤0.05 was considered significant.
3. Results

The mean gestation length of the spontaneously whelping bitches (62.2 ± 0.5 days) was significantly longer \((P = 0.001)\) than that of the bitches in the induced group (59.5 ± 0.2 days). Length of expulsion phase, mean inter-pup interval, and mean litter size did not differ between both groups. The aglépristone treatment had no effect on the duration of the interval to the next ovulation. The interestrous interval, i.e., from the ovulation in the cycle in which the bitch was mated until the ovulation of the following cycle was 211 ± 15 days in the induced group and 213 ± 8 days in the spontaneously whelping group (for further details see [27]).

Mean P4 and PGFM concentrations did not differ significantly between both groups in the period between days 54 and 58 of pregnancy, but significant changes were found after day 58. Fig. 1A and B illustrates P4 and PGFM concentrations for a single bitch and mean values for the whole group of spontaneously whelping animals, respectively. P4 concentrations decreased to basal levels before expulsion of the first pup, at which time PGFM concentrations were increasing to reach a peak value just after expulsion of the first pup (Fig. 1A). Mean P4 concentration in the spontaneously whelping group decreased significantly \((P < 0.01)\) in the 30-h period before parturition, whereas the mean PGFM concentration increased significantly \((P < 0.01)\) during that same period (Fig. 1B). On the 1st day after parturition, both mean P4 and PGFM concentrations had decreased significantly again.

Fig. 1C and D shows P4 and PGFM concentrations for a single bitch and mean values for the whole group of the induced whelping bitches, respectively. The P4 concentration also dropped to basal levels in this group, but this occurred only after – not before – the expulsion of pups. The PGFM concentration started to increase before parturition, but peak concentration remained lower than in the spontaneously whelping bitch (cf. Fig. 1A and C). Overall, in the bitches from the induced group the mean PGFM concentrations increased significantly \((P < 0.05)\) between late gestation and the 30-h period before parturition and the day after parturition.
parturition. PGFM concentrations then dropped during the 2nd and the 3rd day after parturition, but this decline was not significant (Fig. 1D). Despite the increase in the pre-partum period, PGFM concentrations were significantly lower ($P = 0.01$) than in the spontaneously whelping group during that period. On the 2nd and 3rd day after parturition, PGFM concentrations in the induced group were significantly higher ($P < 0.01$) compared with those in the spontaneously whelping group.

In both groups, the mean E2β concentration decreased significantly between the late gestational period and the 30-h period before parturition ($P < 0.05$ and $P < 0.01$ for the spontaneously whelping and the induced group, respectively; Fig. 2). No further decrease was observed during the postpartum periods. For each of the four periods, mean E2β concentrations were similar in both groups.

In both groups, cortisol concentrations increased significantly ($P < 0.05$) between late gestation and the 30-h period before parturition (Fig. 3A). In the spontaneously whelping group, cortisol concentrations in the postpartum periods were not significantly different from values during late gestation. In the induced group, cortisol concentrations decreased significantly ($P < 0.05$) on the 1st day after parturition, compared with the 30-h period before parturition, but the levels were still significantly higher ($P < 0.05$) than those during late gestation. During the 2nd and 3rd day after parturition cortisol concentrations were again similar to those in late gestation. During the two time periods before expulsion of pups, cortisol concentrations did not differ significantly between both groups. During the postpartum periods, cortisol concentrations were significantly higher ($P < 0.01$) in the bitches from the induced group.

There was a large intra-individual variation in ACTH concentrations, especially in the last 30 h before the start of parturition (Fig. 3B). ACTH concentrations did not change significantly throughout the study in bitches from both groups during the first three periods. During the 2nd and 3rd day after parturition, however, ACTH concentrations in the induced group had decreased significantly ($P < 0.05$) compared with the values in the

---

Fig. 2. Mean (±S.E.M.) plasma concentrations of estradiol-17-β in the bitches from the spontaneously whelping (white bars) and the induced group (black bars) in late gestation, before parturition, the day after parturition, and the 2nd and 3rd day after parturition. Different letters (A, B and X, Y) denote significant differences within the spontaneously whelping and induced group, respectively. The first dose of aglupristone was given to the bitches in the induced group at the end of the late gestational period.

Fig. 3. Mean (±S.E.M.) plasma concentrations of cortisol (A) and adrenocorticotropic hormone (B) in the bitches from the spontaneously whelping (white bars) and the induced group (black bars) in late gestation, before parturition, the day after parturition, and the 2nd and 3rd day after parturition. Different letters (A, B and X–Z) denote significant differences within the spontaneously whelping and induced group, respectively. Significant differences between groups within one period are indicated with an asterisk. The first dose of aglupristone was given to the bitches in the induced group at the end of the late gestational period.
30-h period before parturition. There was no difference in mean ACTH concentrations between the two groups in any of the periods.

The PRL concentrations in the bitches from the spontaneously whelping group increased significantly ($P < 0.05$) between late gestation and the 30-h period before parturition, while those of the induced group only tended to increase ($P = 0.057$) (Fig. 4). There was no difference in PRL concentrations between the two groups in any of the periods.

The LH concentrations in bitches from the spontaneously whelping group decreased significantly ($P < 0.01$) between late gestation and the 30-h period before parturition (Fig. 5). After parturition, LH concentrations remained significantly lower ($P = 0.05$) as compared with the values in the late gestational period. Within the induced group, there was a large variation in LH concentrations between days 54 and 58 of gestation, and there were no significant differences between LH concentrations in any of the periods. LH concentrations did not differ significantly between the two groups in any of the periods. In both groups, FSH concentrations gradually and significantly decreased from late gestation until the 2nd and 3rd day after parturition. During the prepartum periods, FSH concentrations did not differ significantly between the two groups, but they tended to be higher ($P = 0.026$, with Bonferroni correction $\alpha \leq 0.01$) in the spontaneously whelping group on the day after parturition (Fig. 6).

The LH concentrations in bitches from the spontaneously whelping group decreased significantly ($P < 0.01$) between late gestation and the 30-h period before parturition (Fig. 5). After parturition, LH concentrations remained significantly lower ($P = 0.05$) as compared with the values in the late gestational period. Within the induced group, there was a large variation in LH concentrations between days 54 and 58 of gestation, and there were no significant differences between LH concentrations in any of the periods. LH concentrations did not differ significantly between the two groups in any of the periods. In both groups, FSH concentrations gradually and significantly decreased from late gestation until the 2nd and 3rd day after parturition. During the prepartum periods, FSH concentrations did not differ between the two groups, but they tended to be higher ($P = 0.026$, with Bonferroni correction $\alpha \leq 0.01$) in the spontaneously whelping group on the day after parturition (Fig. 6).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Spontaneously whelping group $(n = 6)$</th>
<th>Induced whelping group $(n = 5)$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4 (nmol/L)</td>
<td>5.9 ± 1.2</td>
<td>22.1 ± 4.9</td>
<td>0.036</td>
</tr>
<tr>
<td>Cortisol (µg/L)</td>
<td>123 ± 29</td>
<td>141 ± 11</td>
<td>0.575</td>
</tr>
<tr>
<td>PGFM (nmol/L)</td>
<td>47 ± 12</td>
<td>23 ± 4</td>
<td>0.121</td>
</tr>
</tbody>
</table>
The P4 concentrations in the samples taken during the expulsion phase were significantly higher in the induced group \((n = 5)\) than in the spontaneously whelping group \((n = 6, \text{ Table 2})\). Cortisol and PGFM concentrations did not differ significantly between both groups in this period.

4. Discussion

In the spontaneously whelping dogs, the pre-partum decline to basal levels of the P4 concentration coincided with a clear increase of the PGFM concentration, as has been reported before [15]. Plasma P4 concentrations in the induced group, however, reached basal levels only after parturition, indicating that luteolysis was not complete at the time of expulsion of the first pup. In line with the important role that PGF\(_2\alpha\) plays in prepartum luteolysis, a significant, albeit still modest, increase in circulating PGFM concentrations before parturition was noted in the induced whelping dogs, as was also seen in another study [35]. The corpora lutea, however, remained functional as reflected by the relatively high P4 concentrations. The level of PGF\(_2\alpha\) production before parturition in the induced group might have been too low to cause complete luteolysis, or the luteolysis was incomplete because of a lower sensitivity of the corpora lutea to PGF\(_2\alpha\) at the time of aglépristone administration, i.e., on day 58 of pregnancy. Furthermore, because maximum plasma aglépristone levels are only reached after approximately 2.5 days (unpublished data, provided by the manufacturer), only part of the total number of P4 receptors will be blocked initially and circulating P4 around the time of parturition can still exert its activity at the receptor level, which may have resulted in repression of PGF\(_2\alpha\) secretion. In addition, P4 may have a stimulating paracrine/autocrine effect on its own production within the luteal cells [36,37].

After parturition, the mean PGFM concentration in the spontaneously whelping group very quickly returned to basal values, contrary to the PGFM concentrations in the induced group. We speculate that the elevated postpartum PGFM concentrations in the induced group are associated with the completion of the luteolytic process after parturition.

In agreement with previous reports [12,14,19], a significant decrease in estradiol-17-\(\beta\) concentrations was observed prior to parturition in both groups. Apparently, aglépristone treatment does not affect the plasma profile of this hormone around the time of parturition. The physiological significance of the decreasing E2\(\beta\) concentrations before parturition in the dog is not known. It also remains to be investigated if the ante-partum decrease in gonadotropic hormone concentrations are associated with the pre-partum estradiol decrease.

In line with previous studies [38–41], cortisol concentrations increased significantly before parturition in both groups. After parturition, cortisol concentrations in the induced group were significantly higher than in the spontaneously whelping group, possibly due to partial blocking of glucocorticoid receptors by aglépristone. Blocking of pituitary glucocorticoid receptors results in an increased ACTH release and a subsequently elevated cortisol secretion [42,43]. The blood sampling frequency used in the present study does not allow strong statements about hormones with highly fluctuating plasma concentrations such as cortisol and ACTH [44]. Elevated postpartum cortisol concentrations in the induced group may also have been caused by a sustained postpartum PGF\(_2\alpha\) production as reflected in the elevated PGFM concentrations. However, there was no clinical evidence for an elevated stress response in the induced bitches, as postpartum behavior, the number of postnatal losses and the weight-increase of the puppies were similar to those in the spontaneously whelping dogs [27].

In the spontaneously whelping group, a significant increase in PRL concentrations was observed before parturition reflecting the modulating effect of P4 on PRL secretion [26,45–47]. In the induced group, the PRL concentrations before parturition only tended to increase, possibly due to the less abrupt decrease in P4. PRL plays an important role in mammogenesis and lactogenesis [48,49]. Because in both groups the puppies grew steadily [27], it appears that aglépristone does not affect mammary function around the time of parturition, which is important for pup survival and growth after birth.

Both the concentrations of FSH (both groups) and LH (spontaneously whelping group) decreased between late gestation and the postpartum period. This decline in circulating gonadotropin levels may be due to the increase in PRL secretion [50–52], but an influence of the declining E2\(\beta\) concentration, which has been shown to have an effect on the secretion of gonadotropic hormones in other periods of the estrous cycle, may also play a role [53].

In conclusion, the results of this study have further expanded our knowledge on the hormonal changes around parturition in the bitch. After comparison of hormonal patterns between the two groups, it may be concluded that aglépristone-induced parturition is associated with a still incomplete luteolysis, an altered...
plasma PGM profile, and elevated postpartum cortisol concentrations.

**Acknowledgements**

Part of this study was financially supported by Virbac Nederland B.V., Barneveld, The Netherlands. The authors thank Mrs. D.M. Blankenstein, Mrs. C.H.Y. Oei, Mrs. J. Wolfswinkel and Mrs. A. Slob for the plasma hormone determinations. We thank Dr W.E. van den Brom and Mr. J.C.M. Vernooij for their help with the statistical analyses.

**References**


Induction of parturition in the bitch with the progesterone-receptor blocker aglépristone

M. Baan\textsuperscript{a}, M.A.M. Taverne\textsuperscript{b}, H.S. Kooistra\textsuperscript{a}, J. de Gier\textsuperscript{a}, S.J. Dieleman\textsuperscript{b}, A.C. Okkens\textsuperscript{a,*}

\textsuperscript{a}Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 8, P.O. Box 80154, NL-3508 TD Utrecht, The Netherlands
\textsuperscript{b}Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Received 4 June 2004; received in revised form 3 September 2004; accepted 17 September 2004

Abstract

The triggering mechanism for parturition in the bitch remains unclear. Consequently, the development of drugs to successfully induce parturition in the dog has been difficult. The aim of this study was to evaluate the efficacy of the progesterone-receptor blocker aglépristone for the induction of parturition in beagle bitches. The course of parturition was therefore investigated in six parturitions induced by aglépristone and in six spontaneous parturitions. In addition, data were collected on pup survival and growth rates. Aglépristone was administered twice with a 9 h interval on day 58 of pregnancy. If parturition did not proceed a standard intervention protocol was applied.

Expulsion of the first pup occurred between 32 and 56 h after the first treatment with aglépristone, at which time the plasma progesterone concentration was still elevated. Accordingly, the gestation length of the bitches in the induced group (59.5 ± 0.2 days) was significantly shorter than that of the spontaneously whelping bitches (62.2 ± 0.5 days). The expulsion phase length, the inter-pup interval, the number of puppies born dead, and the number of clinical interventions needed during parturition did not significantly differ between the spontaneously whelping and the induced group. Pup survival and mean birth weights in the two groups did not differ significantly and aglépristone treatment had no significant influence on the growth rates.

* Corresponding author. Tel.: +31 302531684; fax: +31 302518126.
E-mail address: a.c.schaefers-okkens@vet.uu.nl (A.C. Okkens).
The results of this study show that aglépristone is an effective drug which can be used safely for the induction of parturition in the dog. © 2004 Elsevier Inc. All rights reserved.

Keywords: Progesterone; Length of expulsion phase; Pup survival; Pregnancy; Dystocia

1. Introduction

Progesterone is necessary for maintaining pregnancy [1]. In the dog, the corpora lutea are the sole source of progesterone during gestation [1,2]. Plasma progesterone concentrations decrease significantly prior to the onset of parturition while at the same time myometrial activity increases [3]. The decline of plasma progesterone concentrations may be considered essential for normal whelping. Inhibition of the decline in progesterone concentration by implanting medroxy-progesterone acetate (MPA) resulted in failed parturitions [4]. Although the MPA-treated bitches were visibly distressed and fluids were discharged from the vagina, no puppies were expelled. The treated animals either died with puppies in utero or a caesarean section had to be performed.

Parturition in the dog takes place after a pregnancy with an average length of 61.4 days, measured from the single day of mating which was determined on the basis of the plasma progesterone pattern [5]. It is not clear what triggers the onset of whelping in the dog. In ruminants, elevated levels of fetal cortisol trigger parturition via increased estrogen production at the expense of progesterone production in the placenta. The elevated levels of estrogen stimulate the production of prostaglandin F$_{2\alpha}$ resulting in increased myometrial activity and softening of the cervix [6]. In dogs, data on hormone concentrations in fetal blood are not available, and circulating estrogen concentrations in the bitch seem to decrease instead of increase towards parturition [3,7–9].

Since the triggering mechanism for parturition remains unclear, it has been difficult to select or develop drugs that are useful for the induction of parturition in the dog. Ideally, this drug should induce whelping with a high efficiency and within a predictable, short time frame after treatment. In addition, treatment should be safe for the bitch and her puppies, i.e. it should induce a normal parturition without side effects.

In the bitch, prolactin is an important luteotropic factor during the second half of gestation. The dopamine agonists cabergoline and bromocriptine induce abortion in this period by suppressing the release of prolactin [10–13]. However, dopamine agonists are not useful for the induction of whelping since an effect may be expected only after several days. In addition, the time between the start of dopamine agonist treatment and the onset of parturition is quite unpredictable. Furthermore, treatment with prolactin secretion inhibitors would reduce or even abolish lactation after parturition. Prostaglandin F$_{2\alpha}$ and its synthetic analogues also induce regression of the corpora lutea resulting in the termination of pregnancy. Just as dopamine agonists, prostaglandins must be administered for several days before luteolysis takes place in the bitch. In addition, treatment with prostaglandins is often accompanied by side effects such as tachypnoe, salivation, vomiting, and diarrhea [14–17], and there may be an increased risk of an abnormal parturition process [18].
Progesterone-receptor blockers such as aglépristone (RU 46534) and mifepristone (RU 38486) are competitive antagonists of the progesterone receptor [19,20]. Because of their anti-progesterone effect, these drugs have been widely investigated for their use as abortifacient agents [21–25]. In addition, progesterone-receptor blockers may be useful for the induction of whelping as well. Studies with the progesterone-receptor blocker mifepristone have shown variable results. Some researchers could only induce an incomplete parturition, which did not proceed beyond the stage of dilatation of the cervix [26]. In contrast, van der Weyden et al. [3] reported a normal course of parturition in five bitches treated with this drug. Fieni et al. [27] induced parturition with a single treatment with the progesterone-receptor blocker aglépristone, followed by a standard additional treatment with alfaprostol (a prostaglandin F2α analogue) or oxytocin. Riesenbeck et al. [28] described a single case of prolonged pregnancy that was successfully terminated by treatment with aglépristone in combination with prostaglandin F2α.

The aim of this study was to evaluate the efficacy and safety of the progesterone-receptor blocker aglépristone for the induction of parturition in the bitch. To this extent, the course of parturition, i.e. the length of the expulsion phase, the mean inter-pup interval and the number of clinical interventions, were investigated, and data were collected on pup survival during parturition, postnatal survival and growth rates.

2. Materials and methods

2.1. Animals

Eleven healthy beagle bitches, with ages ranging from 1.5 to 5 years and body weights ranging from 9.8 to 17.3 kg, were used in this study. All dogs had been born and raised at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine in Utrecht, The Netherlands, and were accustomed to procedures such as routine clinical examinations and jugular venipuncture. All dogs were examined three times weekly for the presence of swelling of the vulva and a serosanguineous vaginal discharge, which were considered to signify the onset of pro-estrus. Plasma concentrations of progesterone were determined thrice weekly during (pro)estrus with a rapid 125I-RIA and the bitches were mated once with one of three male dogs based on the peri-ovulatory rise of progesterone values as described previously by Okkens et al. [5]. The day of mating was considered to be day 0 of gestation. Between days 23 and 28 of gestation, pregnancy was confirmed by ultrasonography in all dogs (Aloka S 50-500, 3.5-MHz probe, Biomedic Nederland, Almere, The Netherlands).

Until the sixth week of pregnancy, the bitches were housed in pairs in indoor–outdoor runs, fed a commercially available dry dog food once daily, and given water ad libitum. Starting 3 weeks before the expected parturition, the pregnant bitches were housed individually and were fed three times daily. From day 53 of pregnancy, bitches were housed in a separate kennel equipped with a whelping basket and a heating lamp.

On day 53 of pregnancy, a radiographic diagnosis of pregnancy was made and the number of fetuses was established. In order to create homogenous experimental groups, bitches were assigned to the spontaneously whelping (n = 6) or to the induced group (n = 6)
on the basis of the number of fetuses, their age, and their parity. Six bitches were primiparous (three in each group), three others gave birth for the second time (one in the induced group and two in the spontaneously whelping group) and one bitch for the third time (induced group). One of the bitches was used twice, once in the induced group (her first pregnancy) and 7 months later in the spontaneously whelping group. Because of differences in parity, litter size and siring male, this bitch was not treated as her own control, and the results were thus statistically treated as independent data (Nos. 3 and 11). The resulting spontaneously whelping and induced group were similar with respect to litter-size, age and parity. The three siring male dogs were also evenly distributed over the two groups.

2.2. Experimental protocol

The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Starting on day 54 of pregnancy, a general physical examination of the bitches was performed daily. Rectal temperatures were measured at 01:00, 08:00, 13:00 and 19:00 h.

From day 54 of pregnancy until 4 days after parturition, blood samples were collected by jugular venipuncture 2–4 times a day in EDTA-coated tubes. Within 5 min after collection they were centrifuged at 1500 \( \times g \) and 4 \( ^\circ C \), and plasma was stored at \(-20\) \( ^\circ C \) until analysis. Blood samples were taken at 08:00 and 19:00 h on days 54, 55 and 56 of pregnancy, at 01:00, 08:00, 13:00 and 19:00 h on day 57 of gestation and on each of the following days up to and including the first complete day after the day on which the last puppy was born. On the second, third and fourth day after the day of expulsion of the last pup, blood samples were taken twice daily at 08:00 and 19:00 h. All samples taken at 08:00 h and those taken at 19:00 h from day 57 until the first day after parturition were selected for determination of the plasma progesterone concentration. An analysis of various hormones was performed in all blood samples, however, not all of the results were used in this study, but in related studies.

The packed cell volume (PCV) was determined every 2–3 days, starting on day 54 of gestation until the last day of sampling. PCV values declined significantly from an average of 34% at 54–55 days of pregnancy to a nadir of 30% between 62 and 64 days \((P < 0.01)\). There was no difference in PCV between the spontaneously whelping and the induced group. The volume of blood samples was not adjusted to match this decline in PCV.

On day 58 of pregnancy, bitches assigned to the induced group were weighed, and examined ultrasonographically to assess the presence of any dead fetuses before the start of treatment. Subsequently, at 10:00 and at 19:00 h these bitches were treated with aglépristone (30 mg/ml dissolved in oily solvent; Alizine\( ^{\text{TM}} \), Virbac, Carros, France) at a dose of 15 mg/kg late-pregnancy body weight sc. Each dose was distributed over two injection sites: one high up in the neck and the other more caudally between the shoulder blades. The injection sites were massaged for 1 min following treatment. At 31 h after the first treatment with aglépristone, vaginoscopy was performed to check for cervical dilatation. In addition, the fetuses were monitored by means of transabdominal ultrasonography to assess the presence of any deceased fetuses.
2.3. Management of parturition and puppies

The dogs of both groups were regularly observed for parturient behavior via a camera connected to a monitor in a separate room. Parturition was constantly monitored, but bitches were not disturbed. However, if parturition did not proceed, a standard intervention protocol was applied. When bitches had made straining movements regularly and intensely for 45 min prior to whelping of the first puppy or for 30 min prior to any subsequent puppy without any externally visible progress, digital vaginal exploration was performed. When bitches had shown only non-intense, abdominal straining efforts for 1.5–2 h, digital vaginal exploration was also performed. During vaginal exploration, additional straining was evoked by massaging the dorsal vaginal wall. When a puppy was felt inside the birth canal gentle manual traction was exerted on the pup to aid the birth. When no pup was present in the birth canal, a second digital vaginal exploration took place after 30 min. When, at that time, a puppy had advanced towards or had entered the birth canal, the bitch was left again for 30 min, after which, if no pup had been expelled, the bitch was treated with 2 IU oxytocin sc (Intervet Nederland B.V., Boxmeer, The Netherlands). However, if the pup had not advanced in caudal direction upon the second exploration, bitches were treated with 2 IU oxytocin sc without delay. This procedure was repeated after 45 min if no pup had been expelled. Forty-five minutes thereafter, a course of 2 IU oxytocin treatments was started, given every 1.5–2 h until the birth of the next puppy.

After parturition, the puppies were weighed daily until the age of 31 days. Tube feeding with milk replacement (Denkadog Doggylac, IPP, Apeldoorn, The Netherlands) was started 4–6 times a day when puppies lost weight or had not shown a weight gain of 10% above their birth weight within 48 h after parturition. Puppies were de-wormed at 2 weeks with Vitaminethe (niclosamide and oxibendazol, Virbac Laboratories, Carros, France), and at 4 and 6 weeks of age with Drontal dog (praziquantel, pyrantel embonate and febantel, Bayer B.V. Division Animal Health, Mijdrecht, The Netherlands). The puppies were vaccinated for distemper and canine parvovirus at 6 weeks of age (Nobivac Puppy DP, Mycofarm, De Bilt, The Netherlands). At 7 weeks, the puppies were adopted. Puppies which were born dead or died after parturition were pathologically examined.

2.4. Progesterone determinations

Plasma concentrations of progesterone in the samples taken around the time of parturition were measured by a previously validated 3H-RIA using extraction with hexane [29,30]. The intra-assay and inter-assay coefficients of variation were 11% and 14%, respectively. The limit of quantitation was 0.13 nmol/l.

2.5. Data analysis

Statistical analysis was performed using SPSS for Windows, version 11.0.1 (SPSS Inc., Chicago, IL, USA) and SAS/STAT (SAS Institute Inc., Cary, NC).

The mean rectal temperature was calculated from the average temperature value of each bitch during 8 h periods around the time of the expulsion of the puppies. Changes in mean PCV and rectal temperatures were analyzed with Repeated Measures ANOVA. Differences
in mean PCVs between the spontaneously whelping and the induced group were compared with Student's t-test with Bonferroni correction.

Litter size, age, and parity of the two groups of bitches were compared using Student’s t-test. The number of interventions during parturition, the length of the expulsion phase, i.e. the time from the expulsion of the first until that of the last pup, the mean inter-pup interval, i.e. the duration of the expulsion phase divided by the number of puppies minus 1, and the live birth weight of the puppies were also compared with Student’s t-test.

Mean growth of the puppies between days 0 and 10 and between days 0 and 31 was analyzed in a mixed model with treatment, litter size, and birth weight as fixed factors. Litter number was included as a random factor. Pup weights were log-transformed to increase linearity.

In order to compare the mean plasma progesterone concentrations between the two groups, the complete sampling period was divided into five time intervals. Period 1: from day 54 until 10:00 h on day 58 of gestation (‘late gestation’); period 2: the 30 h period before expulsion of the first pup (‘before parturition’); period 3: from the expulsion of the first pup until the expulsion of the last pup (‘during parturition’); period 4: 0–24 h after the expulsion of the last pup (‘the day after parturition’); period 5: 24–72 h after the expulsion of the last pup (‘the second and third day after parturition’). For each bitch, a mean plasma progesterone concentration was calculated for each of the periods. This mean value was entered into the statistical analysis. It should be noted that the dogs in the induced group received the first dose of aglépristone at the end of period 1.

Because blood samples were not available for each dog during parturition (period 3), an ANOVA for Repeated Measures was performed in each group for the periods 1, 2, 4, and 5 only. To apply this ANOVA to the data in the induced group, the data were made homogeneous by transformation with a natural logarithm (ln). Mean plasma hormone concentrations in the spontaneously whelping group and the induced group were compared with Student’s t-test with Bonferroni correction. Data are expressed as mean ± S.E.M. P ≤ 0.05 was considered significant.

3. Results

Local side effects from the aglépristone treatments were observed in five out of six treated bitches. In four of these a thickening of the subdermis which lasted for about 2–3 weeks after treatment could be discerned. In one bitch local necrosis of the skin overlying the injection site occurred. All side effects disappeared without any treatment.

On days 58 and 59 of pregnancy, no dead fetuses were observed during transabdominal ultrasonography of bitches in the induced group. Vaginoscopy on day 59, at 31 h after the first aglépristone treatment, revealed fetal membranes in the cranial part of the vagina indicating cervical dilatation in five of the six treated bitches. In one bitch (No. 10) no cervical dilatation was seen at that time, but when vaginoscopy was repeated 20 h later, the cervix of this bitch had also dilated.

Changes of the mean rectal temperature in the spontaneously whelping and the induced group did not differ significantly (Fig. 1). Mean rectal temperatures started to decline between 40 and 48 h before expulsion of the first pup and reached a nadir of 37.1 ± 0.1 °C
at 8–16 h before expulsion of the first pup \((P < 0.05)\). After parturition, mean rectal temperatures were significantly higher than the temperatures measured prior to whelping \((38.7 \pm 0.1 ^\circ C, P = 0.004)\).

The litter size averaged \(6.0 \pm 1.1\) in the spontaneously whelping group, and \(7.0 \pm 0.4\) in the induced group \((P = 0.44)\). Expulsion of the first pup occurred between 32 and 56 h after the first treatment with aglépristone with an average of \(41.0 \pm 3.7\) h. As a result, gestation length of the bitches in the induced group \((59.5 \pm 0.2\) days) was significantly shorter \((P = 0.001)\) than that of the bitches in the spontaneously whelping group \((62.2 \pm 0.5\) days).

The course of parturition of each bitch is depicted in Fig. 2. In the spontaneously whelping group vaginal exploration was performed 18 times. In addition, eight doses of oxytocin were administered, with six of these treatments being administered to a single bitch (No. 1). Because bitch No. 2 showed non-intense straining efforts for 1.5 h without giving birth to a pup, vaginal exploration was done at which time a pup could just be touched. Fifteen minutes later, the first pup was born. In the induced group, 36 vaginal explorations were performed and 16 treatments with oxytocin were given, with eight of these being administered to a single bitch (No. 10). The number of interventions during parturition between the groups did not differ significantly \((P = 0.26)\).

No significant difference was observed between the spontaneously whelping and the induced group regarding the length of the expulsion phase \((6.7 \pm 1.2\) and \(10.6 \pm 2.2\) h, respectively, \(P = 0.15\)) or the mean inter-pup interval \((1.9 \pm 0.8\) and \(1.7 \pm 0.3\) h, respectively, \(P = 0.80\)).

In the spontaneously whelping group, 3 out of 36 puppies were born dead versus 5 out of 42 in the induced group. Post-mortem examination revealed asphyxia as the cause of death.
The mean birth weight of the puppies in the spontaneously whelping group was 293 ± 13 g (n = 33) versus 310 ± 5 g (n = 37) in the induced group (P = 0.23). In both groups, 2 out of 6 litters needed supplementary tube feeding for 1–2 weeks after parturition.
Aglépristone treatment did not significantly influence the mean growth rates. The mean growth rate during the first 10 days was $30 \pm 0.8$ g/day ($n = 35$) in the induced group and $40 \pm 0.7$ g/day ($n = 33$) in the spontaneously whelping group ($P = 0.11$). Throughout the entire follow-up period of 31 days the mean growth rates were $48 \pm 0.5$ g/day ($n = 35$) and $52 \pm 0.4$ g/day ($n = 31$) in the induced and spontaneously whelping group, respectively ($P = 0.20$).

During the first 7 weeks, 2 puppies out of 33 in the spontaneously whelping group died and 2 out of 37 in the induced group. In the spontaneously whelping group, one pup was euthanized on day 22 after parturition because of severe growth retardation despite supplementary tube feeding. Post-mortem examination revealed no abnormalities that could explain the retarded growth. The second pup in the spontaneously whelping group died from an ileocolic intussusception. In the induced group, one pup died from an intussusception of the jejunum, the other from an infection with coccoid bacteria.

The plasma progesterone profiles of an individual bitch from the spontaneously whelping and one from the induced group are shown in Fig. 3. Between day 54 and day 58 of pregnancy, the mean plasma progesterone concentration in bitches from the spontaneously whelping group was significantly higher ($P < 0.01$) than in the 30 h period before parturition (Fig. 4). In the days after parturition, mean plasma progesterone concentrations were significantly lower ($P < 0.05$) than during the two periods before parturition. In the bitches from the induced group, the mean plasma progesterone concentration did not differ significantly between late gestation and the 30 h period before parturition. The day after parturition, the mean plasma progesterone concentration had decreased significantly ($P < 0.01$). On the second and third day after parturition the mean

![Fig. 3. Plasma progesterone concentrations around the time of expulsion of the first pup (0) of a spontaneously whelping 3-year old beagle bitch (A) and a 1.5-year old beagle bitch in which parturition was induced with aglépristone (B). The black triangles indicate the time of aglépristone treatment.](image-url)
plasma progesterone concentration was significantly lower than during the previous period ($P < 0.05$).

In late gestation, before aglépristone treatment, mean plasma progesterone concentrations of the spontaneously whelping and the induced group did not differ significantly. During the last 30 h before parturition, the mean plasma progesterone concentration in the bitches from the induced group was significantly higher ($P < 0.01$) than in the spontaneously whelping group. During the expulsion phase, the mean plasma progesterone concentration in the induced group ($22.1 \pm 4.9 \text{ nmol/l}, n = 5$) was significantly higher than the concentration in the spontaneously whelping group ($5.9 \pm 1.2 \text{ nmol/l}, n = 6, P = 0.04$). In the post-partum periods, the mean plasma progesterone concentrations did not differ significantly between the two groups.

4. Discussion

The results of this study demonstrate that the progesterone-receptor blocker aglépristone is an efficient and safe drug for the induction of parturition in the dog. Aglépristone treatment induced parturition with a high efficiency. The bitches in the induced group had a significantly shorter gestation length compared with the spontaneously whelping bitches. In addition, parturition occurred within a relatively short and predictable time frame, on average at 41 h (range 32–56 h) after the first aglépristone treatment. No side effects were observed in the bitches, except a local inflammation reaction at the injection site, which has been reported previously in dogs and cats [31–33]. No significant difference was found between the two groups with regard to
the length of the expulsion phase and the mean inter-pup intervals. In addition, the number of puppies born dead and the growth rate of living puppies were similar between the groups.

In the induced group the expulsion of puppies occurred despite the presence of high plasma concentrations of progesterone, much higher than the value considered to be necessary for maintaining pregnancy (approximately 6.4 nmol/l) [34]. This indicates an effective progesterone-receptor blocking action by aglépristone.

Previous studies were variably successful in inducing parturition with progesterone-receptor blockers. In one study, repeated treatments with mifepristone (6 mg/kg, sc) between days 57 and 59 of gestation in three beagle bitches resulted in an incomplete parturition within 26–40 h after the start of treatment. Only one bitch gave vaginal birth to a single pup, and the three animals had to undergo a caesarean section [26]. In another study, mifepristone (7.5 mg/kg bw per day) was administered orally to five beagle bitches from day 57 after mating until the birth of the first pup. Parturition occurred between 26 and 70 h after the first treatment, when plasma progesterone concentrations ranged between 8.6 and 29.6 nmol/l. One bitch needed additional treatment with oxytocin (two doses of 1 IU each) to complete whelping [3]. Fieni et al. [27] induced parturition with aglépristone (a single dose of 15 mg/kg bw, sc) on day 58 of gestation, with either 0.08 mg/kg bw of the PGF2α analogue alfaprostol, or 0.15 IU/kg bw oxytocin given 24 h later and every 2 h onwards as a standard treatment until the expulsion of the last pup. On average, parturition in these bitches occurred 32 h after the aglépristone treatment, but alfaprostol or oxytocin had also been administered at that time. In the present study, the two subcutaneous treatments with aglépristone, administered with an interval of 9 h, appeared to induce a whelping process that was highly similar to that of the spontaneously whelping animals. This suggests that aglépristone treatment induces a normal parturition.

In spontaneously whelping bitches increasing plasma prostaglandin concentrations probably induce luteolysis, which results in the onset of labor [4,18,35]. In this study, the expulsion of the first pup in bitches from the induced group occurred in the presence of high plasma progesterone concentrations, indicating that luteolysis had not been completed yet. In the post-partum periods, however, the plasma progesterone concentrations in the induced group had decreased significantly, and were similarly low to those in the spontaneously whelping group. This indicates that in both groups the corpora lutea have a decreased function at this stage. Linde-Forsberg et al. [24] induced abortion in mid-pregnancy with a different progesterone-receptor blocker, mifepristone, and found plasma concentrations of prostaglandin F2α-metabolite to increase after treatment, while progesterone concentrations were decreasing. This premature cessation of the luteal phase was also observed after treatment of bitches for mid-gestation abortion with aglépristone [23]. It is likely that plasma prostaglandin concentrations also increase after aglépristone treatment for parturition induction, possibly due to placental dehiscence. Furthermore, it may be hypothesized that, in the induced group, the occupation of the progesterone receptors by aglépristone had reduced the auto-regulatory positive feedback of progesterone on its own secretion [36].

The determination of the optimal mating time by measurement of the plasma progesterone concentration after the start of pro-estrus was 100% successful. All bitches became pregnant after a single mating. The bitches in the induced group were treated with
aglépristone at day 58 of pregnancy, at which stage the puppies would be viable for birth, but at which time in the vast majority of cases the normal parturition process would not yet have started [5,37,38]. Our findings on postnatal survival of pups demonstrate that with accurately defined gestational age day 58 is a safe time for the induction of whelping in healthy dogs.

A significant drop in rectal temperature was measured on the day before parturition in both the spontaneously whelping and the induced group. In accordance with previous reports [3,4,34], the pre-partum drop of rectal temperatures in the spontaneously whelping group was temporally related with a decrease in plasma progesterone concentrations. It has been suggested that in dogs progesterone has a thermogenic effect within the thermoregulatory system. Similar observations about the effects of progesterone on the thermoregulatory center have been made in intact and ovariectomized rats in which exogenous progesterone was found to be thermogenic [39]. The rapid decline in progesterone concentrations before parturition results in a transient drop in body temperature of dogs until other thermoregulatory factors become readjusted and restore the balance [4,18,35]. In the induced group, the drop in rectal temperature before parturition occurred in the presence of a high plasma progesterone concentration, which may be indicative of a progesterone antagonistic effect of aglépristone on the thermoregulation center.

In contrast to the protocol used by Fieni et al. [27], who administered alfaprostol or oxytocin as a standard treatment starting 24 h after administration of aglépristone, in the present study no standard treatment with uterotonic drugs was applied after aglépristone administration. This protocol enabled us to study the exclusive effects of aglépristone on the induction of parturition and avoided the possible administration of uterotonic drugs to bitches with a closed cervix. In fact, one bitch in our study did not show cervical dilatation at vaginoscopy 31 h after the first treatment with aglépristone. In all bitches, the aglépristone treatment alone was sufficient to lead to the expulsion of the first pup, within 32–56 h after the start of treatment. This is somewhat longer than the time interval observed by Fieni et al. [27]. However, a comparison between the two studies is not possible since a different protocol was used; furthermore, other factors that differ between the research colonies of beagle dogs, such as genetic make-up and body condition, may very well have an influence on the course of parturition.

A strict protocol for the management of parturition was used in both groups to minimize the risks for the bitches and puppies around parturition. This did not interfere with the aim of the study, i.e. to investigate the efficacy and safety of aglépristone for the induction of parturition. The length of the expulsion phase did not differ significantly between the spontaneously whelping and induced group. There was a large variation between bitches with respect to the number of interventions during parturition, but no significant difference was found between the groups. This suggests that aglépristone treatment does not affect the course of parturition.

Despite the significant difference in gestation length between the groups, mean birth weight of the puppies was not significantly different between the groups. This was an unexpected finding because Evans and Sack [40], Salazar and Yllera [41] and Moriyoshi et al. [42] reported a very pronounced growth rate of canine fetuses in late pregnancy in the dog.
After parturition most puppies grew steadily, but two litters in both groups received supplementary tube feedings. This suggests that aglépristone treatment had no effect on either the milk production of the bitches or the weight gain of the puppies. Also, there were no indications that aglépristone treatment affected the pre-weaning mortality rate, since survival to weaning was similar in both groups.

In conclusion, premature parturition was successfully induced in six healthy beagle bitches with the progesterone-receptor blocker aglépristone. The onset of whelping occurred within a short and predictable time frame. There was no need for additional treatment before the start of the expulsion phase, but just as in the group with the spontaneous onset of whelping it was necessary to monitor parturition closely to ensure that labor proceeded in due course. Aglépristone treatment did not affect pup survival and only minor side effects were seen in the bitches. These observations indicate that aglépristone is an effective and safe drug for the induction of parturition in dogs.

A future application of treatment with aglépristone could be the induction of parturition in bitches with a prolonged pregnancy. This would be a practical alternative to a caesarean section, which at present is the sole effective treatment for prolonged gestation. However, more research is needed to determine the etiology behind a prolonged pregnancy and the potential use of a progesterone-receptor blocker for the induction of parturition in such a case.

Acknowledgements

The authors thank Ms. D.M. Blankenstein and Ms. C.H.Y. Oei for the plasma progesterone determinations. We also express our gratitude to Ms. H.A. van Oord and the veterinary technicians for their assistance in the care for the bitches and puppies. We thank Dr. W.E. van den Brom and Mr. J.C.M. Vernooij for their help with the statistical analyses. The critical reading of the manuscript by Mr. E.J. Schaefers is highly appreciated. This study was financially supported by Virbac Nederland B.V., Barneveld, The Netherlands.

References


ABSTRACTS

6th International Symposium on Canine and Feline Reproduction

&

6th Biennial EVSSAR Congress

European Veterinary Society for Small Animal Reproduction

"Reproductive biology and medicine of domestic and exotic carnivores"

University of Veterinary Sciences
9th – 11th July 2008
Vienna, Austria

Editors: G. England, P. Concannon, S. Schäfer-Somi

Reprinted in IVIS with the permission of the Symposium Organizers
SCHEDULED CAESAREAN-SECTIONS IN THE BITCH USING AGLEPRISTONE AND TAKING INTO ACCOUNT THE DATE OF OVULATION: A NEW APPROACH

X. Levy¹, E. Fontaine¹, A. Grellet¹, V. Segalini¹, A. Fontbonne¹
¹CERCA, ENVA, 7 av. General de Gaulle, 94 700 Maisons-Alfort Paris, France.
E-mail: vetlevy@yahoo.fr

Objective of the work. 66% of bitches suffering from dystocia are treated by Caesarean sections (CS) (1). The likelihood of all foetuses remaining alive is increased if the CS is not performed in emergency (4). Recently, some authors described rationale criteria in pregnant bitches to define the events leading to an elective CS: single pup syndrome, pelvis fracture, metabolic disorders, vaginal strictures, hydrocephalus, previous C-section … (3). So far, most veterinarians don’t schedule C-section precisely, but they wait until the first signs of parturition (often at night) or until the progesterone blood level falls under 2 ng/mL. The aim of this study was to try to schedule C-section before the pre-partum decrease of progesterone and to evaluate the innocuity of this procedure for the dam and her puppies.

Materials and methods. 36 bitches were included in our study: 12 English Bulldogs, 6 French Bulldogs, 6 Beagles, and 12 other purebred bitches (5 weights over 40 kg, 4 between 20 and 40 kg and 3 less than 20 kg). All bitches, except Beagles, were brought for elective C-sections: five bitches were pregnant with one single pup, one had a history of a primary uterine inertia, one suffered from a severe stenosis at the vestibule-vaginal junction (a resection of a stage 4 vaginal fold hyperplasia was performed at mid-gestation) and the others underwent previous CS for various other reasons (in English Bulldogs, some owners asked for planned CS). Beagle bitches were included in an experimental protocol in which a C-section was needed. Bitches were monitored during their heats and the day of ovulation was determined by progesterone quantitative assays (± 6 ng/mL, Chemiluminescence assay, Progesterone II®, Elecsys 2010, Roche Diagnostics, Germany) (6). 60 days after the estimated date of ovulation, all bitches received an injection of aglepristone (Alizine®, 15 mg/kg SC) according to the protocol suggested to induce parturition (2). CS were performed 61 days after ovulation. The vitality of the foetuses was assessed by a transabdominal ultrasonography (ATL, HDI®, 7.5 MHz probes) performed before surgery. Progesterone assays were performed to confirm that the pre-partum drop had not yet occured. All the puppies were monitored during the first 48 hours.

Results. Progesterone remained above 2 ng/ml at the time of C-sections (mean=5.25, SD=1.28). No post-operative clinical complications were reported in any of the bitches. All the bitches were able to nurse and feed their puppies in the first hours following surgery. 4 out of 181 puppies died in the first 48 hours (2.2%) belonging to different bitches. No neonate showed any signs of prematurity and they were all vigorous.

Discussion and Conclusion. The determination of the ovulation date helps estimating the parturition date (63±1 day) (6, personal data). This criterion helps to avoid to perform a C-sections too early, and also to avoid getting premature puppies and high death rates. In our study, the survival rate of the neonates (2.2%) was not affected by the time of the C-sections and was in agreement with other studies (8%) (4). Even if we should keep in mind the importance to genetically select bitches able to whelp naturally, our study is the first to show that a C-section may be performed in average 2 days before the date of expected parturition, without any harmful consequence for the dam and her neonates. This has been demonstrated also in the case of induced parturition (2). However, the interest of using Alizine® to avoid prematurity cannot be confirmed by our study. This new approach may reveal to be very
useful for owners and veterinarians in some pathological conditions (pelvis fracture, single pup syndrome, metabolic disorders …) (3).

References

ABSTRACTS

6th International Symposium on Canine and Feline Reproduction

&

6th Biennial EVSSAR Congress

European Veterinary Society for Small Animal Reproduction

"Reproductive biology and medicine of domestic and exotic carnivores"

University of Veterinary Sciences
9th – 11th July 2008
Vienna, Austria

Editors: G. England, P. Concannon, S. Schäfer-Somi

Reprinted in IVIS with the permission of the Symposium Organizers
INDUCTION OF PARTURITION WITH AGLEPRISTONE IN VARIOUS SIZED BITCHES BELONGING TO DIFFERENT BREEDS

Fontbonne A. 1, Levy X 1., Fontaine E. 1, Bachellerie R. 2, Bernex F. 1, Atam-Kassigadou S. 1, Guffroy M. 1, Leblond E 1, and E.Briant 2.
1Ecole Nationale Vétérinaire, 7 avenue du Général de Gaulle, 94700 Maisons-Alfort, France
2 Virbac Inc., Carros, France. Email : afontbonne@vet-alfort.fr

Objectives - The aim of this study was to apply the protocol of induction of parturition using aglepristone and oxytocin, published by Fieni et al. (1) in Beagle bitches, to various sized bitches belonging to different breeds, in order to confirm if this medical induction of parturition remained effective in other canine breeds, to record the delay between medical induction and expulsion of the first foetus and to study the conditions of this medically induced parturition.

Material and methods - 13 pluriparous bitches, aged 18 months – 5 years, belonging to the same breeding kennel were included in this study. These bitches had no history of previous reproductive or obstetrical problems. According to their size, they were dispatched into three categories:

- Group 1: small breeds (Yorkshire Terrier: n=2, Lhassa Apso: n=2)
- Group 2: large breeds (Golden Retriever: n=3, Labrador Retriever: n=2)
- Group 3: giant breeds (Bernese: n=2, Newfoundland: n=2).

Medium sized bitches (around the same size as Beagle bitches) were voluntarily not included in this study, as this protocol aimed to study bitches different in size from Beagles. During their heats (proestrus and oestrus), daily blood samples were performed around 9 am. Blood was collected in Heparin test-tubes, centrifuged within 30 minutes and plasma was kept frozen at – 20°C until assaying. Oestrus bitches were housed with a male and matings occurred ad libitum. All bitches were vaccinated against Canine Herpes Virus (Eurican Herpes®, Merial, France) during their pregnancy.

Around 25 days after the last mating, pregnancy diagnosis by ultrasound was performed. In pregnant bitches, ovulation was determined a posteriori by assaying quantitative progesterone (Chemiluminescence – Elecsys 2010, Roche Diagnostics, Germany) in the daily blood samples previously taken during the heats. According to Marseloo et al. (2), ovulation was supposed to have occurred the day at which progesterone plasma concentrations reached 6 ng/mL and increased significantly one day later.

Around 50 days of pregnancy, bitches were housed in a nursing kennel at the Alfort Veterinary College, in large cages with special nesting boxes. A radiographic examination was performed around 55 days of pregnancy to determine the number of pups to be born. In the week preceding parturition, daily progesterone assays were performed and rectal temperature was recorded 4 times daily.

Within each breed, one bitch was randomly assigned to have its parturition medically induced, while the other remained untreated (control group) and underwent natural whelping. 2/3 Golden Retrievers were included in the induced group.

In the treated group, parturition was induced 59 days after the estimated day of ovulation (except two bitches – a Labrador retriever bitch – which was induced 60 days after ovulation and a Golden Retriever bitch which was induced 61 days after ovulation). The protocol was identical to what had been described by Fieni et al. (1): aglepristone 15 mg/kg SC (Alizine®, Virbac, France), followed 24 hours later by oxytocin injections every two hours 0.15 UI/kg SC (Ocytocine S®, Intervet, The Netherlands). The number of pups born alive, alive 48 hours later and stillborn was recorded. Their weight was recorded daily during the first 48 hours.
Results - In the control group, natural parturition occurred 61 (1/6 bitch), 62 (2/6 bitches) and 63 (3/6 days after ovulation. In the induced group, the first pup was born 25.9 ± 3.29 hours after aglepristone administration (21 to 30 hours). 2/7 bitches (one Yorkshire Terrier, one Lhassa Apso) with respectively 5/8 and 1/4 pups, were born before the first administration of oxytocin. In the induced group, 6/7 bitches required no human assistance during parturition. The Labrador Retriever bitch delivered 5/7 pups naturally but the last two pups had to be delivered by operative C-section due to a secondary obstructive dystocia. In the six remaining bitches of the induced group, the duration of parturition lasted from 3.7 hours (220 minutes - Yorkshire Terrier) to 17.5 hours (1050 mn - Bernese). Its mean duration was 9.6 hours (578.2 mn) ± 5.4 hours (321.8 mn) vs 8.0 hours (478.4 mn) in the control group (2.1 hours (126 mn) to 12.4 (744 mn)). The mean duration of induced parturition was shorter in small bitches (3.8 hours) than in large (11.2 hours) or giant (14.0 hours) bitches. Excluding the last two Labrador pups born after C-section, in the induced group, the mean interval between two successive foetal expulsions was 115.6 ± 82.8 minutes (34 to 265) vs 68.8 ± 24.5 minutes in the control group (p<0.01). 7.3 ± 2.3 pups were born in the induced group (vs 7.7 ± 2.2 in the control group). One pup was stillborn in the induced group vs two pups in the control group. After 48 hours, 6.1 ± 3.4 pups were alive in the induced group (vs 7 ± 2.4 in the control group). The mean weight at parturition did not differ significantly between the two groups (355.0 ± 174.0 g in the induced group vs 363.3 ± 176.0 g in the control group).

All pups gained weight during the first 48 hours except the four Yorkshire Terrier pups from the induced group which looked premature at the time of birth (thin hairless skin) and died between 19 to 29 hours post-delivery. Their mean weight at birth (101 g) was smaller than the Yorkshire Terrier pups in the control group (136 g). Necropsy concluded that they were premature.

Discussion - This study shows that a protocol combining aglepristone + oxytocin successfully induces parturition in bitches, whatever the size and the breed. The mean duration of induced parturition (9.6 hours ± 5.4) was longer that what had been found by Fieni et al. (1) using the same protocol in Beagle bitches (4.5 ± 1.8 hours). This was especially true in large and giant breeds. For example, it took 17.5 hours for one Bernese bitch to fully deliver. The mean interval between two successive births of pups was longer in the induced group. We may therefore think that this protocol cannot fully predict that the duration of the entire parturition process, as it seemed to be the case in Beagles. The Yorkshire Terrier bitch from the induced group who lost her 4 pups may have been induced to whelp prematurely, may be due to a wrong determination of the ovulation date.

References