Influence of hypophysectomy on the lifespan of the corpus luteum in the cyclic dog

A. C. Okkens, S. J. Dieleman, M. M. Bevers, A. A. M. E. Lubberink* and A. H. Willemsen

Clinic for Veterinary Obstetrics, A.I. & Reproduction, State University of Utrecht, Yalelaan 7, De Uithof, 3508 TD Utrecht, and* Small Animal Clinic, State University of Utrecht, Yalelaan 8, De Uithof, 3584 CM Utrecht, The Netherlands

Summary. Five dogs were hypophysectomized on Day 4 and 9 on Day 18. Prolactin and LH stimulation tests showed that hypophysectomy was complete in 6 dogs only. In these dogs, the progesterone concentration was measured in the peripheral blood; it decreased sharply immediately after surgery. It regained normal values in 3 of the 4 dogs hypophysectomized on Day 4, and remained low in the 2 dogs hypophysectomized on Day 18. This indicates that, in the dog, luteal function is autonomous during a certain period. The luteal period of the 3 dogs hypophysectomized on Day 4 was shorter than that of control animals, although the time of onset of luteal regression appeared to be similar. This indicates that pituitary luteotropic support is required during the second part of the oestrous cycle of the dog.

Introduction

In general, the lifespan of the corpus luteum of the cycle is controlled by pituitary luteotropic and uterine luteolytic factors; in the cow and the sheep, the corpus luteum persists when hysterectomy is performed before luteolysis (Hansel, Concannon & Lukaszewska, 1973). However, hysterectomy of bitches during the luteal phase did not change the lifespan of the corpus luteum (Olson, Bowen, Behrendt, Olson & Nett, 1984; Okkens, Dieleman, Bevers & Willemsen, 1985b).

The role of luteotropic factors in controlling the corpus luteum function varies among mammalian species (Rothchild, 1981). In the cow, only luteinizing hormone (LH) is required for the maintenance of the corpus luteum after ovulation (Hoffman et al., 1974); in the sheep, prolactin is also necessary (Kann & Denamur, 1974). In rhesus monkeys, however, hypophysectomy immediately after ovulation did not shorten the luteal phase of the ovarian cycle (Asch, Abou-Samra, Braunstein & Pauerstein, 1982) and administration of an LH-releasing hormone (LHRH) antagonist (Balmaceda, Borghi, Coy, Schally & Asch, 1983) during the post-ovulatory period showed that in this species luteal function during the cycle is independent of continued pituitary gonadotrophin secretion. In the dog, limited data are available indicating that LH (Concannon, 1980) and prolactin (Okkens, Bevers, Dieleman & Willemsen, 1985a) are important for the maintenance of the corpus luteum.

We have therefore investigated whether hypophysectomy after ovulation does shorten the luteal period of the oestrous cycle in the dog.

Materials and Methods

Animals. Nulliparous beagle bitches 1–7 years of age were used for hypophysectomy on Day 4 (N = 5) or Day 18 (N = 9); Day 1 is defined as the day on which the progesterone concentration in the peripheral blood reaches 5 ng/ml for the first time after onset of pro-oestrus. General animal maintenance procedures and methods for detection of pro-oestrus were as previously described.
(Okkens et al., 1985b). Blood samples were collected by jugular venepuncture once every day from the onset of pro-oestrus until Day 36 and, thereafter, every other day until Day 65. About the time of hypophysectomy blood samples were collected 4 times a day. The progesterone patterns of 3 normally cyclic dogs from a previous experiment (Okkens et al., 1985b) served as control values.

Hypophysectomy. Hypophysectomy was carried out by the technique described by Markowitz, Archibald & Downie (1964). Briefly, after incision of the soft palate an elongated burr hole was drilled in the exposed sphenoid bone in which the pituitary is located in an excavation; thereafter the pituitary was removed from the hypophysial fossa (Lubberink, 1977). Sham-operations have not been carried out, since the mere making of the hole in the sphenoid bone will cause damage to the pituitary. Premedication of the fasting animals consisted of methadone HC1 (s.c., 1-2 mg/kg bodyweight; Symoron: Gist-Brocades, Delft, The Netherlands), droperidol (1 mg/kg: Dehydrobenzperidol: Janssen Pharmaceutica, Beerse, Belgium) and atropine (0-1 mg/kg). Anaesthesia was induced with thiopentone sodium (10-15 mg/kg i.v.; Pentothal: Abbott, Amsterdam, The Netherlands) after which the animals were intubated. Anaesthesia was sustained by inhalation of oxygen, nitrous oxide and halothane (Fluothane: I.C.I., Rotterdam, The Netherlands), and artificial ventilation by intermittent positive pressure respiration (Monaghan M300; The Monaghan Company, Denver, CO, U.S.A.); respiration was monitored by the concentration of carbon dioxide in the end expiratory flow (capnography). The fluid balance was controlled by infusion with lactated Ringer’s solution using an over-the-needle catheter in a saphenous vein; this was also used to correct immediately for eventual blood losses.

About 3 h after surgery pitressin tannate (s.c., 3-5 i.u. in oil; Parke-Davis Company, Pontypool, U.K.) was administered to prevent post-surgical diabetes insipidus, and cortisol acetate (2-3 mg s.c.; Hydro-adreson: Organon, Oss, The Netherlands) and ampicillin (20 mg/kg s.c.; Albipen: Gist-Brocades) were administered every 6 h during the first day after surgery. On the 2nd to 7th day after surgery the animals were treated as follows: cortisone acetate (0-5 mg/kg, twice a day orally; O.P.G., Utrecht, The Netherlands), ampicillin (20 mg/kg, 3 times a day orally) and, if necessary, desmopressin (one drip twice a day intranasally; Min Rin, Ferring, West Germany) at 48 h after operation. When appetite returned thyroid hormone substitution was started (20 mg/kg, once a day orally; Thyranon: Organon). Further maintenance therapy consisted of cortisone acetate (5 mg twice a day orally).

Stimulation tests. To examine whether hypophysectomy was complete, a prolactin stimulation test with thyrotrophin-releasing hormone (TRH) was carried out in all animals on 1-4 occasions at 2 weeks to 6 months after surgery. Richards et al. (1980) demonstrated that, in the dog, TRH causes a significant increase of prolactin in peripheral blood. The stimulation test was repeated in several animals to examine eventual reactivation of pituitary remnants. Blood samples were collected at -15, 0, 5, 10, 15 and 30 min after injection of TRH (200 µg i.v.; Hoffman La Roche, Mijdrecht, The Netherlands). Animals that did not respond to the prolactin stimulation test also underwent an LH stimulation test with an LHRII agonist. Blood samples were collected at -15, 0, 5, 10, 15 and 30 min after injection of buserelin acetate (1 ml i.v. containing 4 µg buserelin; Receptal: Hoechst Veterinär GmbH, Frankfurt, West Germany). In control bitches this dose caused a 5- to 20-fold increase of LH in peripheral blood.

Radioimmunoassays. Concentrations of progesterone in the peripheral blood were estimated by a previously validated radioimmunoassay (Dieleman & Schoenmakers, 1979); the intra- and interassay coefficients of variation were <11 and <14% (n = >12), respectively, and the sensitivity was 20 pg/RIA tube.

Prolactin concentrations were determined by a previously validated heterologous radioimmunoassay (Okkens et al., 1985b). LH concentrations were determined by a heterologous radioimmunoassay as described by Nett et al. (1975). The sheep LH antibody, GDN No. 15, radioiodinated NIAMDD-bLH-4 and a canine pituitary standard LER1685-1 were used in this assay.
## Results

During the first 4–5 days after surgery the animals commonly showed upper respiratory dyspnoea. After the first week, however, no adverse effects were observed on health.

Injection of TRH produced a 1·8–21-fold increase of the prolactin concentration (before TRH: 7·1 ± 2·3 (s.e.m.) µg/l; mean maximum after TRH: 34·1 ± 9·4 µg/l) in the peripheral blood of 8 animals of which 7 were hypophysectomized on Day 18 and one on Day 4; these animals were therefore excluded from the experiments. The remaining 6 animals did not respond to TRH treatment and had mean prolactin concentrations of 4·5 ± 0·7 and 2·2 ± 0·2 µg/l (i.e. at the detection limit of the assay) during the periods before and after surgery throughout the experiment; in control animals the average prolactin concentration is 7·0 µg/l during the luteal period of the oestrous cycle (Okkens et al., 1985b). These 6 animals also did not respond to treatment with the LHRH agonist, since the LH concentration remained at or below the detection limit of the assay; 4 of these dogs were hypophysectomized on Day 4 and 2 on Day 18.

![Fig. 1. Mean progesterone concentrations (± s.e.m.) of 3 dogs hypophysectomized on Day 4; time of surgery is indicated by the arrow.](image)

The progesterone concentration in the peripheral blood sharply decreased immediately after surgery; it regained a normal level by 6–10 days after operation in 3 animals which were hypophysectomized on Day 4 (Fig. 1). One bitch hypophysectomized on Day 4 showed a similar pattern for the progesterone concentration until Day 13; thereafter, however, the progesterone concentration decreased to reach basal values from Day 16 onwards. In the 2 animals hypophysectomized on Day 18 the progesterone concentration also did not recover (see Fig. 2). The lifespan of the corpus luteum is expressed as the length of the luteal period and is considered to be terminated when the progesterone concentration initially falls below 1 ng/ml. Corpus luteum lifespan was significantly \( (P < 0·01; \text{two-tailed Student's } t\text{-test}) \) shorter in the 3 animals in which the progesterone concentration did recover than in the control animals \( (N = 3) \), i.e. 49·3 ± 1·2 and 74·7 ± 3·5 days respectively. Regression of the corpus luteum, however, was estimated to start at about the same time in hypophysectomized and control animals, i.e. at Day 18. This onset of regression was assessed by calculation of the day on which the relative progesterone concentration is 100% from a model developed by linear regression analysis (Fig. 3). Least square fit was performed on progesterone concentrations after transformation into percentages relative to the mean of the 5 highest progesterone concentrations; percentages between 90 and 20 were used.
Fig. 2. Progesterone concentrations of 2 dogs hypophysectomized on Day 18; time of surgery is indicated by the arrow.

Fig. 3. Regression lines for the progesterone concentrations after and relative to the 5 highest progesterone values in (a) 3 control animals (●), \( r = 0.94 \), and (b) 3 animals hypophysectomized on Day 4 (○), \( r = 0.93 \).

Discussion

Hypophysectomy was complete in 6 dogs only. The increase of the prolactin concentration after injection of TRH showed that it was incomplete in 8 dogs that were hypophysectomized on Day 4 (\( N = 1 \)) and Day 18 (\( N = 7 \)); these animals were excluded from the experiments. Whether this indicates that hypophysectomy during metoestrus is significantly more often incomplete than shortly after ovulation, is doubtful. However, it does show that examination of the completeness of hypophysectomy is essential, preferentially by stimulation tests. Upon post-mortem examination

In all animals a sharp decrease of the progesterone concentration occurred immediately after surgery. However, this does not appear to be due to loss of pituitary luteotrophic support, since the progesterone concentration regained normal values after 6–10 days in 3 of the 4 animals hypophysectomized on Day 4. The acute decrease is probably a consequence of the surgical stress. This effect of surgery may have been more severe in the one Day 4 animal in which the progesterone concentration did not recover. It has been reported (Olson et al., 1984; Okkens et al., 1985b) that surgical trauma is probably the cause of the marked, sometimes 80% drop of the progesterone concentration lasting 5–10 days in bitches that were hysterectomized during the luteal period.

The finding that the corpus luteum of the bitch was able to produce progesterone in the absence of the pituitary strongly suggests that luteal function in this species is autonomous during a certain time after initial stimulation by the preovulatory LH surge, as in rhesus monkeys (Asch et al., 1982; Balmaceda et al., 1983). This autonomous period appears to end at about Day 24–28, since the progesterone concentration did not recover after 6–10 days in animals hypophysectomized on Day 18, although Concannon (1980) has reported that luteal function in the dog is supposed to be chronically dependent on pituitary luteotrophic support. However, 4 of the 5 animals described had been hypophysectomized after the autonomous period assumed here.

No difference was observed between animals hypophysectomized on Day 4 and controls with respect to the onset of luteal regression. The progesterone concentration in the hypophysectomized animals, however, reached basal values at an earlier date, as is reflected by the significantly shorter luteal period. This indicates that the corpus luteum of the bitch is dependent on pituitary luteotrophic factors during the second part of the luteal period. Administration of bovine LH during this part of the canine oestrous cycle indicated that LH may be such a factor (Concannon, 1980), and the shortening of the luteal period due to chronic suppression of prolactin (Okkens et al., 1985a) showed that prolactin also constitutes part of the luteotrophic complex in the dog.

The results of this study show that the corpus luteum of the bitch functions autonomously after ovulation, followed by a period in which pituitary luteotrophic support is required. Hypophysectomy appears to be unsuitable for investigation of when this transition takes place because of the severe consequences of surgery.

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