

Dystocia in the Bitch

Epidemiology, aetiology and treatment

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Abstract

Dystocia means difficult birth or inability to expel foetuses through the birth canal. The aetiology of dystocia may be maternal or foetal. Primary uterine inertia is the most common reason for dystocia in the bitch approaching 75% of the cases.

The objective of this thesis was to investigate the incidence of dystocia and to find causes of primary uterine inertia. Also the treatment regimes for bitches with primary uterine inertia were evaluated.

An epidemiologic investigation was performed. A large animal insurance data-base was used which covered healthy as well as diseased animals over time. Incidence of dystocia and the frequency of caesarean section in affected bitches were calculated. The overall incidence of dystocia was 5.7 cases per 1 000 dog years at risk. The frequency of caesarean section in bitches with dystocia was 64%.

The other three studies included healthy bitches and bitches with abnormal parturition and the hormonal concentrations were analysed. An increase in prostaglandin $F_{2\alpha}$ -metabolite concentration was observed from the last week of pregnancy. At labour stage II the plasma concentrations of prostaglandin $F_{2\alpha}$ -metabolite, oxytocin, vasopressin and cortisol all increased in normal labour. Bitches with primary uterine inertia had plasma concentration of prostaglandin $F_{2\alpha}$ -metabolite which was significantly lower compared to the normal bitches.

The serum electrolytes was analysed in bitches with normal and abnormal parturition. No evidence was found indicating that abnormal serum concentration of electrolytes was a cause of primary uterine inertia in the bitch.

Evaluation of two different treatment regimes revealed no significant difference in labour outcome if the bitch was treated with calcium solution in combination with oxytocin or with oxytocin only.

In summary, this thesis provides information about the incidence of dystocia which was earlier unknown. The results suggest that abnormal release or production of prostaglandin $F_{2\alpha}$ may be a cause of primary uterine inertia in bitches.

Keywords: bitch, cortisol, dystocia, electrolytes, epidemiology, estradiol, oxytocin, progesterone, prostaglandin $F_{2\alpha}$, vasopressin.

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Dedication

To my Family

Det är skönare lyss till en sträng, som brast, än att aldrig spänna en båge.

Verner von Heidenstam

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Bergström, A., Nødtvedt, A., Lagerstedt, AS., Egenvall, A. (2006). Incidence and breed predilection of dystocia and risk factors for caesarean section in a Swedish population of insured dogs. *Veterinary Surgery* 35, 786-791.
- II Olsson, K., Bergström, A., Kindahl, H., Lagerstedt, AS. (2003). Increased plasma concentrations of, vasopressin, oxytocin, cortisol and the prostaglandin F2alpha metabolite during labour in the dog. *Acta Physiologica Scandinavica* 179, 281-287.
- III Bergström, A., Fransson, B., Lagerstedt, AS., Olsson, K. (2006). Primary uterine inertia in 27 bitches: aetiology and treatment. *Journal of Small Animal Practice* 47, 456-460.
- IV Bergström, A., Fransson, B., Lagerstedt, AS., Kindahl, H., Olsson K. Hormonal and electrolyte concentrations in bitches with primary uterine inertia (manuscript).

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Abbreviations

ATP	Adenosine triphosphate
CS	Caesarean section
DYAR	Dog years at risk
IV	Intravenous
LH	Luteinizing hormone
NSAID	Non steroid anti-inflammatory drug
OR	Odds ratio
PGF _{2α}	Prostaglandin F _{2α}
PGFM	PGF _{2α} -metabolite
SR	Sarcoplasmatic reticulum

1 Background

Dystocia means difficult birth or inability to expel foetuses through the birth canal. The term dystocia stems from the Greek where *dys* means difficult and *tokos* means birth. The incidence of dystocia is not known in bitches, but according to some authors the frequency may be approximately 5% in the pregnant bitches. The Brachycephalic Breeds are reported to have a high frequency of dystocia (Linde-Forsberg, 2005).

Dystocia is caused by abnormalities originating from the foetus or from the mother (Linde-Forsberg, 2005; Feldman & Nelson, 2003b; Johnson, 1986; Gaudet, 1985). The treatment may be medical or surgical. Primary uterine inertia is the most common reason for dystocia in the bitch approaching 75% of the cases (Darvelid & Linde-Forsberg, 1994).

1.1 Normal labour

1.1.1 Gestational length

When handling a pregnant bitch it is important to evaluate gestational length and the expected day of parturition. The gestational length varies from 57 to 70 days when calculated from the date of first mating to parturition. Gestational length from ovulation to parturition is nearly constant, 64 days (Tsutsui *et al.*, 2006; Concannon *et al.*, 1983). Like other mammals the ovulation in bitches is preceded by the luteinizing hormone (LH) peak. Canine sperm can survive several days in the female tract before fertilization (Concannon *et al.*, 1983). The day of mating may be many days from day of fertilization, explaining the variation in gestational length. In bitches unlike other mammals, the progesterone starts to increase before the ovulation and at ovulation it reaches 12–32 nmol/L (Feldman & Nelson, 2003a). Approximately two days after ovulation the oocytes have matured and may

become fertilized (Feldman & Nelson, 2003a). In bitches, the unique preovulatory rise in serum progesterone is associated with luteinization of the follicles. Repeated measurements of plasma progesterone concentration are commonly used to determine the optimal day for mating. In one study 100% accuracy of predicting parturition dates were within 65 ± 3 days after the first progesterone increase (at the time of the LH peak) (Kutzler *et al.*, 2003). Post ovulation the increase in progesterone is rapid and the estimated day of ovulation and predicted day of labour will not be as accurate (Concannon *et al.*, 1975).

1.1.2 Stages of parturition

Progesterone is expected to decrease before initiation of labour. Therefore progesterone may be measured in the prepartum period. A temperature decrease coincidences with progesterone withdrawal. If a decrease in rectal temperature is followed by a return to normal temperature labour is expected to start.

The labour process is by convenience split into three stages (Linde-Forsberg, 2005; Johnston *et al.*, 2001).

Stage I is the time when the body prepares for parturition. It lasts normally for 6-12 hours and includes the start of cervical dilatation. The myometrial contractions are not visible externally. The bitch shows change in behaviour such as decreased appetite, panting, restlessness, shivering and occasional vomiting.

Stage II is the time with abdominal contractions, when cervix is fully dilated and puppies are expelled through the birth canal. Rectal temperature is normal and the stage is usually accomplished within 12 hours. Stretch sensitive cells in cervix and vagina transmit nerve impulses to the neurons in the hypothalamus resulting in release of oxytocin from the pituitary gland leading to increased myometrial contractions (Ferguson, 1941).

Sixty percent of puppies are born in cranial presentation and 40% in caudal presentation and both positions are considered to be normal. Normal interval between the puppies may range from a few minutes to approximately two hours (Linde-Forsberg, 2005; van der Weijden *et al.*, 1989; Gaudet, 1985). The green discharge that follows expulsion of puppies represents breakdown products of biliverdin in the blood to uteroverdin from the placental margins.

Stage III refers to the period when placentas are expelled. They normally pass within 5 to 15 minutes after each puppy.

1.1.3 Physiological aspects

Induction of parturition requires interaction of many hormones. In rodents which are dependent of an active corpus luteum during pregnancy for progesterone synthesis the onset of labour is triggered by the luteolysis which is mediated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Sugimoto *et al.*, 1998; Sugimoto *et al.*, 1997). The bitch is dependent of the ovaries for progesterone production throughout pregnancy (Concannon & Hansel, 1977) and the induction of labour may therefore be similar to the small rodents.

A high plasma *progesterone* concentration is necessary to maintain pregnancy in the bitch (Verstegen-Onclin & Verstegen, 2008). The hormone start to rise preovulatory in the bitch and the increase continues to high levels which are maintained during pregnancy. The labour starts when this hormone has decreased to less than 1 ng/mL (3.18 nmol/L) (Verstegen-Onclin & Verstegen, 2008; Concannon & Hansel, 1977). Progesterone is produced exclusively by the ovaries in the bitch and the decrease in plasma progesterone concentration is probably achieved by the luteolytic effect of $PGF_{2\alpha}$ (Veronesi *et al.*, 2002; Williams *et al.*, 1999; Concannon *et al.*, 1988; Concannon & Hansel, 1977)

The change in estrogen:progesterone ratio has been considered to be an important factor for the induction of parturition. In dogs increased ratio may mainly be caused by a decrease in progesterone, which stimulates the synthesis of prostaglandin. The estrogen concentration appears to fluctuate during late gestation and prepartum period in the bitch without an evident decrease (Baan *et al.*, 2008; Luz *et al.*, 2006; Hadley, 1975).

Beside its luteolytic effect, $PGF_{2\alpha}$ stimulates uterine contractility together with oxytocin. Several studies have shown increased plasma concentration of $PGF_{2\alpha}$ -metabolite (PGFM) in the normal prepartum and parturition periods in bitches (Luz *et al.*, 2006; Veronesi *et al.*, 2002; Concannon *et al.*, 1988; Concannon & Hansel, 1977). Studies in mice and rats strengthen the importance of $PGF_{2\alpha}$ in labour (Sugimoto *et al.*, 1998; Sugimoto *et al.*, 1997).

Oxytocin is produced in the hypothalamus and stored in the pituitary gland. In some mammals oxytocin also is produced in the uterus and foetal membranes (Chibbar *et al.*, 1993), but this has not been shown in the bitch. It is known that oxytocin causes myometrial contractions during labour in mammals while its importance for induction of labour is questioned. As long as progesterone concentration in plasma is high the oxytocin receptors in the myometrium stays inactive (Lopez Bernal, 2003). When estrogen and not progesterone dominates the uterus the oxytocin receptors starts to increase

in number and sensitivity. Oxytocin is routinely used to induce labour in women (Bakker *et al.*, 2009; Waxman, 1962), and it was therefore believed to be important in the normal induction of labour, but this is still controversial.

The uterine response to oxytocin increases at the time close to labour in many species for example the bovine and human (Fuchs *et al.*, 1992; Fuchs *et al.*, 1984), but no similar studies have been performed in dogs. Both in vitro (Fuchs *et al.*, 1981) and in vivo (Fuchs *et al.*, 1983) studies have reported an increase of PGFM during oxytocin infusions in the human uterus at term. It is possible that there is a positive feedback system between oxytocin receptors and PGF_{2α} causing the increased myometrial activity (Mitchell *et al.*, 1998). However, oxytocin may not be essential for parturition as oxytocin knock-out mice can deliver their offspring (Nishimori *et al.*, 1996).

Vasopressin, also called antidiuretic hormone is well known for regulating the body's retention of water. It releases when the body is dehydrated and cause the kidneys to reabsorb water. In high concentrations it also increases the blood pressure by vasoconstriction. In the uterus vasopressin causes myometrial contractions by acting on both oxytocin- and vasopressin V1a receptors (Zingg, 1996). Each of these hormones is able to bind to both receptor types (Åkerlund, 2002; Åkerlund *et al.*, 1999). In both cattle and goats the plasma vasopressin concentration has been shown to increase during labour (Hydbring *et al.*, 1999). Vasopressin has also been seen to increase during abdominal surgery and traction of viscera (Goldmann *et al.*, 2008; Hauptman *et al.*, 2000; Melville *et al.*, 1985; Haas & Glick, 1978).

Cortisol has been used as an index for physiological stress and is known to increase during the trauma of surgery in bitches (Fox *et al.*, 1998; Fox *et al.*, 1994). Plasma cortisol concentration increases before and during parturition in bitches (Baan *et al.*, 2008; Veronesi *et al.*, 2002; Concannon *et al.*, 1978). This increase may be secondary to pain and stress during parturition. An alternative explanation is that the cortisol originates from the foetus. The foetal adrenals secrete huge amounts of cortisol near term which is believed to initiate parturition in some mammals (Liggins *et al.*, 1977) and this had been suggested also in dogs (Concannon *et al.*, 1978).

Calcium is the most important electrolyte regulating myometrial contractions. The myometrial resting membrane potential is generally -35 to -60 mV, being low during most of pregnancy and increases near term (Sanborn, 2000). The extracellular calcium ion concentration is approximately 1.5 mmol/L and the estimated myometrial intracellular

concentration is 0.13 $\mu\text{mol/L}$ (Sanborn, 2000). Adenosine triphosphate (ATP) supplies energy for contraction (Guyton & Hall, 2000). The myometrium contains a significant intracellular pool of bound calcium ions stored in the sarcoplasmic reticulum (SR), and the myometrial cells has a high concentration of SR (Carsten, 1969). Calcium can be mobilized from the SR as well as from the extracellular fluid.

Formation of gap junction occurs near term which simplifies coordination between the smooth muscle cells (Guyton & Hall, 2000).

The SR actively absorbs free calcium in the myometrial cells. As long as the uterus is under progesterone dominance the uptake of calcium ions to the SR inhibit contractions. Both $\text{PGF}_{2\alpha}$ and oxytocin block the calcium uptake to the SR thereby stimulating uterine contractions (Carsten, 1974). In addition oxytocin mobilize the extracellular free calcium (Thornton *et al.*, 1992). Vasopressin increases myometrial calcium ion concentration and contractility by acting primarily on oxytocin receptors in pigs (Yu *et al.*, 1995). The oxytocin and the vasopressin receptor in the uterus operate through different types of G proteins (Zingg, 1996). Activated receptors cause release of calcium from the SR and stimulate influx of calcium through myometrial calcium-channels (Sanborn, 2000; Carsten, 1974). If there is a critical concentration of extracellular calcium ions required for myometrial contractions is not known. A correlation between blood calcium concentration and intensity of uterine contractility has not been found in bitches with dystocia (Kraus & Schwab, 1990).

Magnesium infusion is used to prevent preterm labour in humans. Magnesium causes both smooth muscle relaxation and inhibition of myometrial contraction by both intracellular and extracellular mechanisms (Fomin *et al.*, 2006; Popper *et al.*, 1989). Therefore, magnesium may be of importance when evaluating causes of dystocia.

The myometrial intracellular *phosphorous* and *potassium* concentrations have been compared in myometrial strips obtained from women in normal labour and in labour that was oxytocin resistant. The concentration of phosphorous was significantly higher in the normal group (Rezapour *et al.*, 1996). The authors suggest that a dysfunction in the sodium-potassium pump can result in dystocia.

1.2 Dystocia

The aetiology of dystocia may be maternal or foetal. Primary uterine inertia is the most common reason for dystocia in the bitch approaching 75% of the cases (Darvelid & Linde-Forsberg, 1994). Primary uterine inertia is of

maternal origin and may be caused by anatomical abnormalities or disturbances of the physiological interaction between hormones and electrolytes. Secondary uterine inertia is due to an obstruction in the birth canal. The labour may begin normally but myometrial contractions ceases with time due to exhaustion (Linde-Forsberg, 2005). Primary uterine inertia is divided into complete primary uterine inertia which occurs in 50% of dystocia cases and partial uterine inertia which is seen in 23% of dystocia cases (Darvelid & Linde-Forsberg, 1994).

Foetal causes of dystocia may be foetal death, malpresentations, malformations and foetal oversize.

Clinical signs of dystocia include

- Any condition causing depressed general health at the time of expected labour
- Prolonged gestational length
- Vaginal discharge (green or bloody), i.e. lochia, in the end of gestation without delivery of the first foetus. Foetal fluids that have passed but are not followed by delivery of a foetus
- Progression of labour is not accurate: if more than 2 hours has elapsed since the last born puppy, foetal survival may be at risk (Gaudet, 1985). Weak contractions without delivery of a foetus. Strong uterine contractions without delivery of foetuses may be caused by obstruction of the birth canal

1.2.1 Clinical examination of the dystocia bitch

Bitches with dystocia should be examined regarding obstruction of the birth canal and signs of foetal malposition as this require immediate attention. Vaginal digital palpation and radiographs may be used and blood samples to evaluate electrolytes, glucose and acid-base disturbances are recommended. Doppler or ultrasonography is used to count foetal heart rate which is important to ascertain foetal survival and wellbeing. A decrease in foetal heart rate is an indication for caesarean section.

To evaluate the uterine contractions a commercial external monitoring devices which record the uterine activity are available (WhelpWise; Veterinary Perinatal Specialties, Wheat Ridge, CO, USA). The device may aid in evaluation of phase I and II of labour by determining if prolonged or abnormal contractions occur.

1.2.2 Treatment of the dystocia bitch

Medical

Bitches with primary uterine inertia are subjected to a variety of treatments. The veterinarian often initially treats the bitches medically. Commonly used protocols for medical treatment include oxytocin administration, which can be repeated after 30 minutes if successful. If the oxytocin injection does not result in the birth of a pup within 30 minutes, intravenous (IV) calcium gluconate may be administered, especially in hypocalcemic bitches. The calcium infusion may also be followed by another oxytocin injection. (Johnston *et al.*, 2001). In contrast to many other countries, veterinarians in Sweden commonly give IV infusions of calcium solutions initially, which is supplemented with oxytocin injections as needed. However, evaluation of different treatment regimes is lacking.

If the dog is hypoglycaemic, glucose solutions is given intravenously. Crystalloid solutions are administered IV to correct fluid, electrolyte and acid-base imbalances (Linde-Forsberg, 2005).

If the response to medical treatment is incomplete or in cases of secondary uterine inertia and birth canal obstruction CS or manual delivery is indicated.

Surgical treatment

Anaesthesia and analgesia

Before induction of anaesthesia the bitch should be preoxygenated as apnoea is commonly seen after induction which may cause maternal and foetal hypoxemia and distress. It is important to know that all drugs that pass the blood brain barrier also pass the placental barrier and affect the foetus (Pascoe & Moon, 2001). Pain relief is given to the bitch, and opioids may be used, as they can be reversed with naloxone after delivery (Mathews, 2005; Mathews & Dyson, 2005). Benzodiazepines may also be considered for sedation of the bitch and use of anticholinergics may improve neonatal outcome (Ryan & Wagner, 2006).

The most commonly used anaesthetic protocols are isoflurane for induction and maintenance of anaesthesia or propofol for induction followed by isoflurane for maintenance of anaesthesia (Moon *et al.*, 1998). Epidural anaesthesia is another accepted method, but canine patients frequently require additional sedation. Bitches treated with epidurals may develop hypotension, urine retention and hindlimb paresis (Ryan & Wagner, 2006), although the puppy survival may be optimal (Funkquist, 2002).

Caesarean Section

Celiotomy from the umbilicus to the pubis is most commonly performed, but a flank incision is described as an alternative (Gilson, 2003). The approach to the uterine lumen is preferentially an incision in the uterine body or the uterine horn that reduces the amount of vessels being severed. After delivery of all puppies and inspection of the uterus, the uterus is closed using two layer continuous patterns; first layer may be appositional with care taken to avoid penetration of the mucosa, followed by a layer of inverting Cushing pattern. Placentas are removed if possible. The abdominal wall and cutis is closed in a standard way (Gilson, 2003). Oxytocin is commonly administered IV after closure of the uterus to reduce haemorrhage and to allow for detachment of remaining foetal membranes. If spillage of uterine contents, the abdomen is lavaged with crystalloid fluids.

The mortality of the bitch associated with CS is reported to be approximately 1% and the neonatal mortality is 14% within 24 hours after surgery (Moon *et al.*, 1998).

2 Aims

The aims of this thesis were:

- To evaluate the incidence of dystocia in Swedish bitches and the frequency of caesarean section
- To evaluate estradiol, $\text{PGF}_{2\alpha}$, progesterone, oxytocin, vasopressin and cortisol changes in bitches during normal labour
- To evaluate estradiol, $\text{PGF}_{2\alpha}$, progesterone, oxytocin, vasopressin, cortisol and calcium, magnesium potassium, phosphorous and glucose changes during primary uterine inertia in bitches
- To evaluate two different medical treatment regimes in bitches with primary uterine inertia

3 Methods

3.1 Animals

3.1.1 Paper I

This study was based on a database obtained from a Swedish animal insurance company (Agria) for evaluation of the epidemiology of dystocia and the frequency of caesarean section. Breeds with 300 or more insured females, under the age of the ten years during the observation period were included.

Approximately 70% of the Swedish canine population has an insurance plan for veterinary care and 30% of the entire population is covered by the insurance company Agria (Egenvall *et al.*, 1999). The database was validated and it was concluded that it was acceptable for research purposes (Egenvall *et al.*, 1998).

3.1.2 Paper II

Five healthy research Beagle bitches were studied. They were part of a colony of research dogs, born and housed at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden. Series of blood samples were obtained from these dogs through different reproductive phases.

3.1.3 Paper III

Twenty-seven privately owned bitches that were presented to the University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala, Sweden were included. The bitches were diagnosed with primary

uterine inertia and were randomly allocated into two treatment groups. Group I was treated with IV calcium solution followed by oxytocin injection IV, and group II was treated with oxytocin only.

3.1.4 Paper IV

Seventeen privately owned bitches presented to the University Animal Hospital, Swedish University of Agricultural Sciences, Uppsala, Sweden were included. The bitches were diagnosed with primary uterine inertia and underwent CS. A control group consisting of six research Beagle bitches without signs of dystocia were included. A CS was performed in those bitches at the end of labour phase I or at the beginning of labour phase II. Blood samples were collected at predetermined occasions before and during CS.

In all studies using research animals or privately owned animals the local Ethical Committee in Uppsala and the Swedish Board of Agriculture approved the experimental designs. The owners to the privately owned bitches all provided written consent to participation in the study.

3.2 Sampling

Blood samples were obtained in paper II, III and IV. The blood was collected from a catheter in the cephalic vein or the jugular vein. A contralateral catheter was inserted if infusions were administered.

In paper II, blood samples were obtained from the bitches repeatedly in anoestrous, pro-oestrous, oestrous, pregnancy, parturition and lactation. Plasma PGFM, oxytocin, vasopressin and cortisol were analysed.

In paper III, oxytocin was analysed from samples prior to medical treatment. Total calcium and glucose was analysed before and after the medical treatment.

In paper IV, blood samples were taken before and repeatedly during CS. Plasma PGFM, progesterone, oxytocin, vasopressin, cortisol and serum estradiol and electrolytes were analysed.

All blood samples for hormone analyses except estradiol were collected into ice-chilled tubes containing K₃-ethylenediaminetetra-acetic acid (EDTA). They were centrifuged within 60 min after collection at 4°C for 10 minutes at 1000 x g (Universal 16R) and stored at -70°C until assayed. Blood samples for estradiol and electrolytes, except ionized calcium, were collected in serum tubes and centrifuged within 60 min at 4°C for 10 minutes at 1000

x g and stored at -70°C until assayed. Ionized calcium was collected in airtight heparinised tubes.

3.3 Laboratory Analysis

Oxytocin, vasopressin, cortisol and progesterone were analysed at the department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences.

PGFM was analysed at the Department of Clinical Sciences, Swedish University of Agricultural Sciences.

Electrolytes and estradiol was analysed at the Laboratory of Clinical Pathology, University Animal Hospital, Swedish University of Agricultural Sciences, using routine laboratory methods.

Ionized calcium was analyzed on whole blood by an I-stat (Abbott, Illinois, USA). For details, see each manuscript.

3.4 Statistical Methods

For study I the statistical software package Stata version 8.12 (Stata Corporation, Collage Station, Texas, USA) was used for data handling and analysis. The incidence rate (IR) was calculated by dividing the number of cases with dog years at risk (DYAR) which was possible as the time at risk was known for all insured dogs included. The IR was calculated both overall and for habitat, region and for breed. Habitat referred to if the bitch lived in one of the three major cities in Sweden whereas region defined as latitude, included “North”, “Central” and “Southern” Sweden. The frequency of CS was also calculated based on the insurance data information. A logistic regression model was used to evaluate factors that could be associated with the risk for CS. For more detailed information of the statistical methods, see paper I.

For paper II, III and IV the data was presented as mean \pm SD, and a one way ANOVA was performed to compare groups, using JMP (SAS Institute, Cary, NC, USA) for samples only analysed once.

For series of hormonal measures, repeated measures ANOVA (procedure mixed) was applied to the hormone data (SAS Institute, 2005). For pairwise comparisons Bonferroni adjustment was used to limit the risk for false mass significance. For details, see each manuscript.

4 Results and Discussion

4.1 Paper I, epidemiology

The population studied included 195 931 insured bitches younger than ten years of age with 103 breeds represented. Of those, 1 408 and 2 486 bitches had an insurance claim for dystocia or CS, respectively. The two categories were combined into the group “dystocia cases” (n=3 894). Accordingly, 2% of all bitches were affected, including pregnant as well as non-pregnant bitches. The mean age at diagnosis was 4.6 years. The overall incidence rate of dystocia was 5.7 cases per 1 000 DYAR. The incidence rate did not vary by habitat, but to some extent by region (North 6.0, Central 5.8 and Southern Sweden 5.4 cases per 1 000 DYAR, respectively). The 20 breeds with the highest and the 20 breeds with the lowest incidence are presented in tables within paper I.

According to some authors, approximately 5% of canine pregnancies led to dystocia (Linde-Forsberg, 2005; Jackson, 1995). In this study, 2% of almost 200 000 female dogs had an insurance claim for dystocia. The study included all female dogs and not only pregnant bitches.

During the study period, according to the Swedish Kennel Club, 81 306 litters were born and registered. It is known that 30% of the Swedish dog population was insured in the Agria insurance company and an estimate can be made that that approximately 24 000 litters were born from bitches included in Agria insurance ($81\,306 \times 0.3$). From these 24 000 litters, 3 894 had claims for dystocia which represents approximately 16% of all pregnancies. This figure is of course a rough estimate, but it shows a higher frequency of dystocia compared to earlier reports. To the author's knowledge previous estimates of dystocia are not based on accurate incidence data, but on the frequency of cases presented to different hospitals

without any knowledge of the background population. Therefore, it seems reasonable that our higher estimate is a more accurate representation of the dystocia frequency in Swedish dogs. Importantly, our study only included bitches eligible for insurance claims. Bitches of three breeds with a known high frequency of dystocia: the English and French Bulldogs and the Boston terrier were not covered for a CS by the insurance company and were therefore excluded. However, they are uncommon, representing 0.49% of all born litters in Sweden during the study period and would therefore not influence the total incidence.

Overall, 64% of the bitches with dystocia in this material underwent a CS.

Bitches in the north of Sweden were more frequently treated with CS compared to bitches in central Sweden (odds ratio (OR) 1.93). Bitches older than 7 years were more frequently treated with a CS compared to younger bitches (OR 1.74) and high risk breeds had an increased risk to have CS compared to other breeds (OR 1.34). These effects were statistically significant, but may still not be biologically important.

The frequency of CS in this study was similar to earlier reports (Darvelid & Linde-Forsberg, 1994; Gaudet, 1985). An increased age has earlier been discussed as a cause for dystocia which is in agreement with this study, as the older bitches (>7 years) had a higher risk for being subjected to a CS.

4.2 Paper II, III, and IV

4.2.1 Hormones

Blood plasma concentrations of oxytocin, vasopressin, and cortisol did not differ between samples taken during anoestrous, pro-oestrous, oestrous, pregnancy and lactation. The plasma levels of all analysed hormones increased during normal parturition (paper II).

Estradiol did not differ between normal bitches and bitches with primary uterine inertia ($p=0.3603$) (paper IV). The change in estradiol:progesterone ratio has been discussed as an important factor for the induction of parturition in some mammals (Liggins *et al.*, 1977). The plasma estradiol concentration fluctuates during late pregnancy in the bitch (Luz *et al.*, 2006) and immediately before parturition the concentration decreases to levels comparable with our results (Baan *et al.*, 2008). It appears that in bitches both progesterone and estradiol declines at parturition. The role of estradiol in bitches remains unknown.

Progesterone was analysed in paper IV. The results from the sample taken before induction of anaesthesia was 4.5 ± 3.4 nmol/L in the bitches with primary uterine inertia and 3.3 ± 3.1 nmol/L in the normal bitches. This difference was not significant. The overall progesterone concentration differed significantly ($p = 0.031$) between groups, being higher in the bitches with uterine inertia. At the time of normal parturition the progesterone concentration in several studies was 3.2 nmol/L or less (Luz *et al.*, 2006; Veronesi *et al.*, 2002; Concannon *et al.*, 1988) which show that the progesterone level in the Beagle bitches had fallen below the critical value for parturition to start. The bitches with primary uterine inertia ought to have equally low concentrations considering the longer gestational length, but this was not the case. Instead the progesterone concentration increased throughout the CS.

PGFM was analysed in paper II and IV. The PGFM increased during the last week of pregnancy from 0.2 ± 0.0 nmol/L to 4.2 ± 0.2 nmol/L (paper II). During labour the PGFM concentration increased, and at the time when the first puppy was born the concentration was 66 ± 17 nmol/L and when one of the last puppies were born the concentration was 92 ± 18 nmol/L. In paper IV the PGFM concentration was significantly higher in normal Beagle bitches compared to bitches with primary uterine inertia ($p = 0.0014$). In the first sample the PGFM concentration was 28 ± 14 nmol/L in bitches with primary uterine inertia and 76 ± 23 nmol/L ($p = 0.04$) in bitches with normal parturition. The latter value corresponds to another study of bitches in normal parturition (Veronesi *et al.*, 2002). The healthy bitches had high PGFM and low progesterone levels which show that the CS was performed in late labour phase I or early labour phase II. The bitches with primary uterine inertia would have been expected to have higher values as they were later in gestation but instead the PGFM concentration was low. This could have been one of the reasons for the uterine inertia. The combination of a lower PGFM and a higher progesterone concentration in the bitches with primary uterine inertia suggests that the luteolytic effect of the prostaglandin was not complete, causing the inertia.

Prostaglandin is used locally in humans for induction of labour (Rozenberg *et al.*, 2001). This has not been investigated in bitches, however, $\text{PGF}_{2\alpha}$ injections are not recommended due to side effects like cramp, diarrhea and vomiting (Williams *et al.*, 1999).

The *oxytocin* concentration was analysed in paper II, III and IV. In the healthy bitches in paper II the hormone concentration increased from labour

phase II and not before. Oxytocin differs in respect to prostaglandin which starts to increase before parturition.

In paper III the oxytocin concentration was analysed once, before the medical treatment of primary uterine inertia was initiated. The mean concentration was approximately 30 pmol/L in the both groups. Some of the bitches were in stage II of labour and had delivered one or more puppies before treatment, others suffered from total primary uterine inertia and had not initiated labour. At the time of sampling none of the bitches had visible abdominal contractions; therefore high oxytocin concentrations were not expected.

In paper IV, the concentration of oxytocin did not differ between Beagle bitches with normal parturition and bitches with primary uterine inertia. The mean plasma concentration in the bitches with primary uterine inertia was higher than in paper III, but lower than the concentrations observed at birth of the first puppy in healthy bitches (paper II). The Beagle bitches were not allowed to enter labor stage II, which explains that the oxytocin concentration was low in the first sample in paper IV. In both groups the plasma oxytocin concentration started to increase when the first puppy was taken out and in the Beagle bitches it had increased to levels comparable to those seen in paper II when the last puppy was delivered. This suggests that manipulation of the uterus had started sensory nerves signals and elicited increased oxytocin release from the pituitary (Ferguson, 1941).

The low plasma concentration of PGFM and the progesterone concentration at the upper normal level indicate that the normal interaction between the hormones was disturbed in the bitches with primary uterine inertia. Impaired signals and activation of oxytocin receptors are possible effects of this disturbance. Therefore measuring the oxytocin receptor concentration in the normal and dystocia bitch would be of great interest.

There is evidence that oxytocin from the pituitary is not needed for induction of parturition but play an important role in the progress of labour. Oxytocin was earlier believed to be produced only by the hypothalamus and released from the pituitary, but other organs also have oxytocin production, one being the human pregnant uterus (Chibbar *et al.*, 1993). It is possible that local release of oxytocin in the uterus is sufficient to cause myometrial contractions, but that the production does not influence the plasma concentration.

Oxytocin is often administered to stimulate uterine contractions in bitches with primary uterine inertia (Cain, 1998; England, 1998; Bennett, 1974). It is also commonly given during CS, after delivery of all puppies. This decreases the bleeding from the uterus and allows for contraction which commonly is seen momentarily after IV injections.

Oxytocin does not seem to be the single cause for uterine inertia in bitches.

Vasopressin was analysed in paper II and IV. In paper II the concentration increased significantly when the first puppy was born. The vasopressin concentration increased until the time when the second puppy was born, thereafter it decreased again. At the birth of the last puppies the vasopressin was no longer elevated compared to prepartum. In paper IV the concentration did not differ between normal Beagle bitches and bitches with primary uterine inertia. There was an obvious increase of vasopressin at the last sample in the normal bitches similar to the oxytocin peak. In one report evaluating vasopressin during anaesthesia and surgery in dogs the vasopressin increased during surgery. At one hour of surgery the mean value was comparable with the elevations seen in the present study (Hauptman *et al.*, 2000). This may indicate a difference between the bitches in surgery with open abdomen and manipulation of abdominal viscera and bitches experiencing natural parturition, and it also shows the ability in the dystocia bitches to release the hormone. None of the bitches in paper IV had substantial blood losses. In contrast to humans, term pregnant dogs in supine position do not develop hypotension and pregnancy has not shown significant effect on systemic blood pressure (Probst *et al.*, 1987; Probst & Webb, 1983). Therefore vasopressin concentrations were unlikely to be elevated secondary to changes in blood pressure in this study. Pain has been shown to increase the plasma vasopressin concentration in humans (Kendler *et al.*, 1978). It may be that pain during labour is one important cause to the vasopressin increase. Bitches in anaesthesia and abdominal surgery increased even more prominent and longstanding (paper IV). However, there is another possibility; in humans, vasopressin is involved in the myometrial contractions. Vasopressin receptors in the human uterus increases myometrial calcium ion concentration and contractility by binding to both uterine vasopressin and oxytocin receptors, this occurs at the time of parturition (Åkerlund *et al.*, 1999). It may be that vasopressin is important for normal labour contractions also in bitches, but this has not been shown.

Cortisol was measured in paper II and IV. In paper II the cortisol concentration started to increase in labour phase I and the first sample in paper IV was in the same range. In normal labour the cortisol concentration peaked at birth of the second puppy (paper II). The cortisol concentration increased throughout the CS in paper IV which may be due to anaesthesia and surgical trauma (Fox *et al.*, 1998).

The cortisol concentration in paper II correlated well with that of vasopressin and this may be a response to stress and pain during labour. Cortisol has been shown to be important for initiation of labour in some mammals (Liggins *et al.*, 1977), and a plasma cortisol increase has been reported in bitches both before and during labour (Baan *et al.*, 2008; Veronesi *et al.*, 2002; Concannon *et al.*, 1978). In paper II the increase of cortisol was not seen until the bitches had entered labour phase II. The rise in foetal cortisol concentrations is unlikely to be able to raise the maternal concentrations to these levels. Still the foetal cortisol release may be of importance for induction of parturition (Concannon *et al.*, 1978; Liggins *et al.*, 1977).

4.2.2 Electrolytes

The serum concentration of calcium was analysed in paper III and IV. In paper IV the calcium concentration was within the reference range in both bitches with normal parturition and bitches with primary uterine inertia. In paper III some bitches with primary uterine inertia had hypocalcemia. No difference in treatment effect was seen between the treatment groups (with and without calcium infusion, respectively). Therefore calcium infusion is not always effective in the hypocalcemic bitch. One reason for this may be that it is the intracellular calcium which is involved in myometrial contractions and the calcium ions in serum have to pass into the cell via channels. Both the transport of extracellular calcium into the myometrial cell as well as the release of calcium from the SR is regulated by hormones (Sanborn, 2000; Carsten, 1974).

The myometrial intracellular concentration has not been investigated in bitches and it may be that a defect in calcium-channels or low free intracellular calcium concentration occurs. This can explain why normocalcemic bitches sometimes respond to calcium administration, although it is desirable to avoid such infusions as it may cause cardiac arrhythmias.

In paper IV also magnesium, potassium and phosphorous were analysed. They were within normal reference range with no signs of abnormality in bitches with primary uterine inertia. It is unlikely that the serum concentration of the electrolytes is a cause to uterine inertia in the bitch. To evaluate this further, intracellular studies in the bitch may be required.

4.2.3 Glucose

Blood glucose was measured in paper III. The average concentration was at or above the upper reference value for the two groups respectively, but the

difference was not significant. After the medical treatments the glucose concentration increased further in both groups. The high glucose concentration may be secondary to the high cortisol release that is seen during stress in connection with labour. It seems like the labour initiates catabolic processes in the body and this may be useful for the energy needed at expulsion of foetuses. Hypoglycaemia may still occur, and glucose concentration should be evaluated in every case, especially in toy breeds as they may be more prone to develop hypoglycaemia (Linde-Forsberg, 2005).

4.2.4 Medical treatment

In study III the bitches with primary uterine inertia were treated with calcium solution followed by oxytocin or with oxytocin only. Both groups were also treated with an IV balanced electrolyte solution. The number of born puppies did not differ between treatments. Although the number of animals was limited, the results give an indication that calcium is not needed to initiate the labour. In many cases oxytocin may be sufficient, but if not, an IV infusion of calcium gluconate can be given. Avoiding IV calcium solutions administration is desirable due to its negative effect on the heart.

5 Conclusions

- Two percent of all insured bitches below the age of ten years had claims for dystocia. The frequency of dystocia among pregnant bitches is considerably higher and may be approximately 15%
- The overall incidence rate of dystocia was 5.7 cases per 1000 dog years at risk. The incidence varies greatly depending on breed
- Caesarean section was performed in 64% of the bitches diagnosed with dystocia
- Plasma concentration of PGFM started to increase the week before labour and is believed to be important for induction of labour in the bitch. The plasma concentrations of oxytocin, vasopressin and cortisol did not increase until labour stage II
- Bitches with primary uterine inertia showed significantly lower concentrations of PGFM, compared to healthy bitches. These results suggest that inadequate prostaglandin release or aberrant metabolism of this hormone may play an important role in the development of primary uterine inertia in bitches
- Findings from paper IV did not support that serum concentrations of electrolytes, including calcium, are abnormal in bitches with primary uterine inertia
- Hypoglycaemia was uncommon in bitches with primary uterine inertia

- Hyperglycaemia was a frequent finding
- Medical treatment with a combination of calcium solution and oxytocin was not more successful than oxytocin alone in bitches with primary uterine inertia

6 Clinical implication

According to the results from studies performed here and from earlier reports the following treatment policy is suggested:

First of all maternal and foetal wellbeing should be examined and any signs of obstructed birth canal should be treated. This may require a CS or manual delivery of the foetuses immediately. If no obstruction is found a blood sample is recommended to exclude any electrolyte, acid-base or glucose abnormalities. In the normocalcemic bitch without hypoglycemia, oxytocin is the first treatment of choice. If abdominal contractions and expulsion of a foetus is seen the treatment may be repeated. The risk with overuse of oxytocin should be carefully considered during treatment, since this may result in hyperactive uterine contractions in bitches (Johnston *et al.*, 2001) as well as women (Jonsson *et al.*, 2008). Calcium gluconate may be given IV infusion once after the first oxytocin treatment if no response has been seen to oxytocin. After the calcium infusion oxytocin may be administered once more. If no results are seen a CS is recommended (Johnston *et al.*, 2001). The recommendation to initiate treatment with oxytocin is based on findings that have shown that most bitches with primary uterine inertia are normocalcemic. The relation between intracellular and extracellular calcium ions in the myometrial cell is not known. It may be a dysfunction of the membrane transport of calcium ions causing the inertia. This could be one explanation for the observation that normocalcemic bitches sometimes respond to calcium infusion. Calcium solutions can therefore be attempted as a complement to oxytocin, but careful heart auscultation is recommended during treatment. In any case of primary uterine inertia that is reluctant to medical therapy or where the bitch or foetuses show signs of distress a CS should be performed.

7 Future perspectives

The results from the epidemiologic study gave information on the incidence of dystocia in all bitches but the database could not separate pregnant from non pregnant bitches. It would be of great interest to continue the initial study with investigations of the number of bitches used for breeding purposes and the labour outcome in these bitches. This may be possible in cooperation with the Swedish Kennel Club and different Breed clubs.

In study II, III and IV plasma and serum hormone concentrations were analysed. In the future, to find the cause of primary uterine inertia hormonal receptor studies are required in the bitch. The interaction between the hormonal effects and free intracellular calcium concentration is important for control of myometrial activity. To answer these questions it is necessary to study the interaction at the cell level throughout normal labour as well as primary uterine inertia in bitches.

According to Liggins (Liggins *et al.*, 1977) parturition in the sheep is initiated by the foetal cortisol released from the adrenal glands. If this occurs in the dog is unknown, but could be investigated by analysis of plasma cortisol concentration in newborn puppies. Also in cases with dead puppies the adrenals could be investigated and weight.

The number of bitches included in the clinical studies was limited for practical and ethical reasons. If increasing the number of bitches the conclusion will be more reliable.

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