A CLINICAL STUDY ON INDUCTION OF PARTURITION IN FEMALE DOGS

ABHIGNYA KRISHNA

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS
VETERINARY COLLEGE, HEBBAL, BANGALORE
KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR- 585 401

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ABHIGNYA KRISHNA

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS
VETERINARY COLLEGE, HEBBAL, BANGALORE
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CERTIFICATE

This is to certify that the thesis entitled "***A CLINICAL STUDY ON INDUCTION OF PARTURITION IN FEMALE DOGS***" submitted by **Ms. ABHIGNYA KRISHNA,** ID No. **MVHK-1227** in partial fulfillment of the requirements for the award of ***MASTERS OF VETERINARY SCIENCE*** in ***VETERINARY GYNAECOLOGY AND OBSTETRICS*** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision. And the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
June, 2014

Dr. A. KRISHNASWAMY
Major Advisor
Professor and Head,
Department of Veterinary Gynaecology and Obstetrics

Approved by :

Chairman: ________________________
(A. KRISHNASWAMY)

Members : 1. ________________________
(T.G. HONNAPPA)

2. ________________________
(G.SUDHA)

3. ________________________
(SUGUNA RAO)

Affectionately Dedicated to,

MY BELOVED

PARENTS, BROTHER AND SISTER.

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"Gratitude is when memory is stored in the heart and not in the mind".

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June, 2014 ***(Abhignya Krishna)***

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LIST OF ABBREVIATIONS

LH	Lutenizing Hormone
GnRH	Gonadotropin Releasing Hormone
FSH	Follicle Stimulating Hormone
PGF2α	Prostaglandin F2 α
CRH	Corticotropin Releasing Hormone
P4	Progesterone
PRL	Prolactin
PGFM	13,14 dihydro 15-keto-Prostaglandin F2α
PGF	Prostaglandin
CL	Corpus Luteum
COX-2	Cyclo Oxygenase-2
ng/ml	Nanogram per mili litre
nmol/L	Nano mol per litre
µg/kg	Microgram per kilogram
mg/kg	Milligram per kilogram
mEq/ mm	Miliequivalent per millimetre
Lb	Pound
Kg	Kilogram
kg^{-1}	Per kilogram
Ml	Mili litre
IU	International Units
b.wt	Body Weight
$<$	Less than
%	Per cent
-	To
/	Or
±	Plus or minus
H	Hour
WBC	White blood corpuscles
RBC	Red blood corpuscles
PCV	Packed cell volume

Introduction

I. INTRODUCTION

The reproductive pattern of female dog is considered to be peculiar in many ways. It is monoestrus, exhibiting estrus only once or twice a year. It exhibits proestrus period ranging from 0 – 17 days and has an extended period of estrus ranging from 4 to 24 days (Bell and Cristie, 1971). The ova is ovulated as a primary oocyte which is ready for fertilization only after 48 – 72 hours after ovulation and the delivery can occur over a wide period of 58-72 days after the multiple matings (Johnston *et al.,* 2001). Howerever, most breeders believe that the process of parturition has to begin spontaneously anywhere between day 62 to day 65 after the first of the multiple matings. He would become extremely anxious in cases where the delivery is not initiated by day 65 after the first of multiple matings. Such animals are invariably presented to the veterinarians with a request for either medical induction of parturition or for elective caesarean as the owner believes that waiting anymore may jeopardise the viability status of the newborns.

The duration of pregnancy in dogs is 63 – 66 days determined as interval from LH surge (Concannon, 2000). The first of the multiple matings may however occur before or after the LH surge and therefore, may apparently contribute to the animal delivering before 63 days or after 65 days. Most of them mate their female dogs on the basis of physical signs, predetermined date or on the basis of exfoliative cytology, all of which have a poor correlation with the preovulatory LH surge. Hence determination of the age of pregnancy becomes extremely difficult for a veterinarian. Although, ultrasonographic techniques are now available for determination of foetal age, the technique is still considered not very accurate due to the varied size of different breeds (Luvoni and

Grioni, 2000). Therefore, a practicing veterinarian is at crossroads when presented with a complaint of failure of initiation of delivery even after completing 65 days after the first of multiple matings. He would have to decide whether to wait for few more days, going for an elective caesarean or attempt medical induction of parturition.

Perhaps, the most difficult step is to convince the owner to wait for few more days to allow the animal for spontaneous initiation of labor in cases where matings has not been carried out on the basis of preovulatory LH or progesterone rise which is the case in most cases. Many a times the breeder may not be prepared to wait and may insist for either elective caesarean section or medical induction of labor. Caesarean section invariably involves anaesthetic and surgical risks, is expensive and may lead to unsatisfactory pup survival rate, often due to the use of improper anaesthetic protocols. These complications can be overcome by medical induction of whelping (Reddy *et al.*, 2012). However, a safe, fast and reliable protocol for successful medical induction of parturition in the bitch remains to be established. Ideally, the drug should induce whelping with a high efficacy and within a predictable short time frame after treatment. In addition, treatment should be safe for the bitch and her puppies (Azawi *et al*., 2012).

Progesterone receptor blockers such as aglepristone and mifepristone are competitive antagonists for the progesterone receptors and because of the antiprogestin effect, these drugs have been investigated for their use as agents for induction of whelping in dogs (Baan *et al*., 2005). Studies with progesterone receptor blockers have shown variable results as some could only induce an incomplete parturition (Nohr *et al*., 1993) while others reported a normal course of parturition (Baan *et al*., 2005). Attempts have also been made to improve the response by adding prostaglandins F2α (PGF2α) or oxytocin to mifepristone (Fieni *et al*., 2001). However, studies on medical induction of parturition in the bitch are few and therefore, the present study was planned with the objective of evaluating the efficacy of the progesterone receptor blocker alone for induction of parturition in the bitch. An attempt was also made to study the efficacy of mifepristone in combination with administration of prostaglandins.

Review of Literature

II. REVIEW OF LITERATURE

2.1. Endocrinology of pregnancy and parturition in dogs

The essential requirement for any experiment aimed at induction of parturition in bitch is to have a clear understanding of major events and endocrine changes associated with pregnancy and parturition in bitch. There are several research reviews on luteal function and maintenance of pregnancy in bitch (Parkes *et al.*, 1972; Tsutsui *et al.*, 1982; Shille *et al*., 1984; Lein *et al*., 1986).

In general, the major endocrine feature of pregnancy in bitch is the maintenance of elevated levels of progesterone throughout gestation (Concannon and Hansel, 1977). The ovaries are the major, if not the only source of progesterone for maintaining canine pregnancy (Sokolowski, 1971; Concannon and Hansel, 1977; Concannon *et al.*, 1988). Ovariectomy at any time during pregnancy resulted in resorption of foetuses or abortion (Sokolowski, 1971; Tsutsui, 1983; Steinetz *et al*., 1989). Further progesterone appears to be the only sex steroid required for maintenance of pregnancy in bitch. The synthetic progestin, medroxy progesterone acetate maintained pregnancy in ovariectomised bitches (Concannon *et al*., 1977). The canine placenta did not appear to naturally synthesise large amounts of progesterone (Kisko and Yamuchi, 1984).

Secretion of progesterone from corpora lutea appears to be regulated by both luteotrohpic and luteolytic factors (Okkens, 1986; Concannon *et al.,* 1987; Onclin *et al.*, 1993). The major source of luteotrophic hormone is the pituitary gland (Johnston *et al*., 2001).The luteal secretion of progesterone depends on both pituitary LH and prolactin (Concannon *et al*., 2001). The canine luteal phase is negatively impacted; progesterone secretion is suppressed and pregnancy is terminated by GnRH-antagonist treatment as early as day 10 and any time thereafter (Vickery *et al.*, 1989; Valiente *et al*., 2009a,b) indicating LH and ⁄ or FSH to be required for luteotrophic support throughout the luteal phase. LH acutely stimulates progesterone synthesis by dispersed luteal cells in vitro (Concannon, 1993).

During the second half of canine pregnancy, prolactin is the main luteotrophic factor. Prolactin likewise has a major luteotrophic role beginning as early as day 13, at which time prolactin-lowering doses of dopamine agonist severely reduce progesterone (Concannon, 1993). Prolactin quickly becomes a required luteotrophin by day 25, after which time administration of dopamine agonist cannot only reduce but can also permanently or transiently suppress luteal function fully, cause abortifacient lowering of progesterone concentrations and shorten the luteal phase (Concannon, 2009).

Factors that maintain uterine quiescence during pregnancy include progesterone and relaxin (Johnson, 2008). Relaxin is produced by the placenta. It reaches detectable concentrations in serum or plasma as early as 20 days after the LH surge, and peaks 30–35 days after the LH surge. Serum concentrations of prolactin and relaxin increase during the second half of pregnancy in bitches. Relaxin remains high throughout pregnancy, until parturition or abortion, when it declines precipitously. In some species relaxin stimulates prolactin secretion, but its possible role in regulation of prolactin secretion in bitches remains to be elucidated (Concannon *et al*., 2001).

Mechanisms of parturition in the bitch are still not completely understood, but the available data indicate that they are not unlike the mechanism proposed for several other species. Factors that stimulate uterine activity include prostaglandin and oxytocin (Johnson, 2008). In several species, the serum estradiol concentration increases before parturition and stimulates prostaglandin secretion, but in the bitch, PGF2α increases without an increase in estradiol (Luz *et al*., 2006). It is suggested that the trigger for parturition may involve the maturation of foetal hypothalamo pituitary-adrenal axis as suggested for several other species (Concannon *et al*., 1989). Pregnancy specific increase in maternal plasma cortisol concentration have been recorded in late pregnancy in bitch (Concannon *et al*., 1977) and it is suggested that increased release of fetal corticotrophin (CRH) near parturition stimulates release of fetal adrenocorticotrophic hormone and subsequent release of fetal cortisol and rise in maternal plasma cortisol concentration which are considered important in intiation of parturition (Concannon *et al*., 1977). Maternal cortisol concentration fluctuated within the normal range during the last week of gestation (15-25 ng/ml) and in most bitches was elevated on the day before parturition (40-80 ng/ml) and reduced (10-25 ng/ml) during parturition (Concannon *et al*., 1977). Increase concentration of cortisol, however was erratic and failed to parallel the decline in progesterone and subsequent onset of parturition (Johnston *et al*., 2001). Concannon (1989) also suggested that increase in maternal concentration of cortisol probably represent a far greater increase in the fetal cortisol at the feto placental level are probably more intimately involved in the mechanism of luteolysis than the corresponding plasma concentration. It is not clear, however, whether cortisol would act directly or indirectly via altered placental steroid production to promote the release of luteolytic amounts of prostaglandin F2 α from the placenta. It is therefore, possible that cortisol at the feto placental level may directly promote the release of PGF2α fom the uterus. It has also been suggested that corticosteroid may have an up regulating effect on placental or uterine prostaglandin synthesis (Wanke and Concannon, 2001).

A prepartum rise in plasma 13-14dihydro 15-keto-PGF2α (PGFM, the major metabolite of PGF2α) has been demonstrated to occur in bitch (Concannon *et al*., 1988). The PGFM increase may reflect increased synthesis and release of prostaglandins by the fetoplacental unit in response to increased fetal cortisol secretion (Johnston *et al*., 2001). Concannon *et al*. (1988) observed that the increase in PGFM concentration suggesting that the PGF2α initiate prepartum luteolysis (Concannon and Hansel, 1977; Concannon and Verstegen, 2000; Eilts, 2002). Placental and or uterine release of luteolytic amounts of PGF2α has been suggested to play a role in initiation or completion of prepartum luteolysis and the reduction in plasma progesterone concentration.

Progesterone withdrawal following luteolysis and the resultant increase in estrogen to progesterone ratio has been considered as the major cause of placental separation, dilation of cervix, and increased uterine contractility (Johnson, 1986). Decreased plasma progesterone concentration following luteolysis is expected to increase the myometrial sensitivity to oxytocin and play an increasingly important role in increasing uterine contractions. However, measurements of oxytocin or relaxin levels around the time of parturition have not been reported in bitch.

Among other mechanisms, oxytocin is released in response to pressure against the cervix. The decrease in progesterone and increase in prostaglandin cause the placenta to separate. Relaxin, which is produced by the placenta, abruptly declines at parturition but may remain detectable for up to 9-week postpartum, due to invasion of the trophoblast cells into the endometrium and delayed sloughing of these cells with physiologic involution of the canine uterus (Klonisch *et al*., 1999).

2.2. Progesterone profile during pregnancy and parturition

During pregnancy the progesterone concentration in the plasma fluctuates in the manner similar to that during oestrous cycle, until it declines to a plateau of 16-48nmols/L. It is maintained at this level for one to two weeks, then falls rapidly to 3-6nmols/L just before parturition. The decrease in progesterone is essential for parturition and is negatively correlated with the progressive qualitative change in the uterine activity (Rijnberk and Kooistra, 2010).

Progesterone, produced by corpora lutea (CLs), is essential throughout pregnancy in the bitch. The serum progesterone concentration can be used to assess corpora luteal function. After ovulation, it should be greater than 5–8 ng/mL (approximately 16–25 nmol/L) and should continue to increase for the next 15–25 days. Serum progesterone concentrations remain at peak levels for 7–14 days and then gradually decline throughout the remainder of pregnancy. In pregnant bitches, a rapid, prepartum decrease to <2 ng/mL (approximately 6.4 nmol/L) is consistently found within 48 h of whelping. This abrupt decline in progesterone is the result of an acute rise in prostaglandin F2a concentrations, which does not occur during the non-pregnant cycle. Peak concentrations of progesterone occur during early to mid diestrus, then gradually decline to basal values 51 to 82 days after LH peak in nonpregnant bitches or 24 to 48 hours prior to parturition. The luteal phase, or diestrus, ends when concentrations of progesterone decrease to and remain at, a level inadequate to support pregnancy (i.e., < 1-2 ng/ml) (Johnson, 2008).

Peak concentrations of progesterone during diestrus are variable among bitches, ranging from 10 to 70 ng/ml. Total progesterone secreted by multiple corpora lutea in pregnant bitches with large litters has not been compared to total progesterone secreted by pregnant bitches with small litters (Johnston *et al.*, 2001). Ovarian P4 production is independent of luteotropic support from the pituitary during the first half of gestation in this species (Okkens *et al.*, 1986). Progesterone levels in pregnant and non-pregnant dogs are similar within the first 60 days after ovulation (Concannon *et al.*, 1978) with the course of P4-concentrations in peripheral blood being virtually identical shortly until parturition, when it declines around day 60 of pregnancy to basal levels preceding the onset of fetal expulsions (Concannon *et al.*, 1978).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 (Onclin *et al.*, 2000).

According to *Kowalewski et al. (2009),* mean progesterone concentrations were: 35.71±7.9 ng/ml in the pre-implantation period, 29.73±13.23 ng/ml in the post-implantation period, 13.32±8.66 ng/ml at mid-gestation and 2.07±0.99 ng/ml during the prepartal progesterone decline; the effect of time was highly significant. It has been stated that serum progesterone concentrations must be sustained at less than 2.0 ng/mL for at least 2 days to result in termination of pregnancy (Johnston *et al.*, 2001). Serum progesterone is at peak concentration between Days 15 and 30, and may reach peaks as high as 80 ng/ml (240 nmol/L) or as low as 15 ng/ml (45 nmol/L). In late gestation, Day 50 - 60, progesterone can be as high as 15 ng/ml (45 nmol/l) or as low as 3 ng/ml (9 nmol/L). Progesterone typically declines from 4 - 5 ng /ml (12 - 15 nmol/L) to near or below 2 ng/ml (6 - 7 nmol/L) during the 24 h before the onset of labor (Concannon *et al* ., 2000).

2.3. Duration of pregnancy

Duration of gestation in the bitches has been reported using the the following criteria.

A.) The interval from the day mating to parturition.

B.) Interval from luteinizing hormone (LH) surge to parturition

C.) Interval from estrus to parturition and

D.) Interval from preovulatory rise serum progesterone concentration to parturition

2.3.1. The interval from the day mating to parturition.

Using the day of mating as a reference point, as observed in cases of just a single mating or insemination, parturition can occur as early as 56 days later and as late as 68 days later. Similarly, a large variation in apparent gestation length can be encountered when counting from the first of multiple matings or the last of multiple matings, the extremes encountered differing by 2 weeks. For instance, if a bitch is held for an aggressive stud dog and forced to mate starting 3 - 5 days before the LH surge, the interval from first mating to whelping may be as long as 69 - 70 days. And, in rare instances where a bitch is still fertile 9 or 10 days after the LH surge and is bred then, the interval from mating to whelping can be as short as 55 or 56 days (Concannon *et al*., 2000). However full-term gestation, calculated from insemination, is reported to range from 57 to 72 days (Concannon *et al*., 1983). The difference between these measurements was attributed to the potential 6 day viability of sperm in the female reproductive tract and the long period of receptivity in the bitch of 3 to 21 days (Holst and Phemister, 1974; Concannon *et al*., 1983).

2.3.2. Interval from luteinizing hormone (LH) surge to parturition

In the vast majority of bitches parturition occurs 64, 65 or 66 days after the ovulatory surge in LH (Concannon and Lein, 1989). A 64 - 66 day gestation length measured from the LH surge to parturition is the same as a 62, 63 or 64 day interval between ovulation and parturition, since ovulation has been estimated to occur 2 days after the surge in LH (Concannon and Verstegen, 1998). The actual gestation length in the bitch is 65±1 days when timed from the preovulatory LH surge in peripheral blood and the period between the preovulatory LH surge and parturition was almost constant (Concannon *et al*., 1983) and the key to timing the duration of canine gestation was neither insemination date nor estrus onset, but rather the preovulatory LH surge and concomitant increase in serum progesterone concentrations (Meyers-Wallen, 1995).

2.3.3. Interval from estrus to parturition

In bitches, identifying the first day of diestrus on the basis of vaginal cytologic findings can be used to predict when labor should occur, because most bitches whelp 57 ± 3 days after Day 1 of cytologic diestrus (Johnson 2008). Gestation length as measured from the first day of diestrus based on vaginal cytology (Holst and Phemister, 1974), has a large range (51–60 days), although 80% of bitches in that study gave birth on the 57th day of diestrus.

2.3.4. Interval from preovulatory rise serum progesterone concentration to parturition

Prediction of the duration of pregnancy on the basis of LH surge is difficult for a praciting clinicaian as identification of LH surge require frequent sampling of the blood, sophisticated laboratory facilities, besides being cost effective. Therefore, recommendations have been made for identifications of time of LH surge on the basis of changes in the serum concentration of progesterone (P4) during late proestrus and estrus (Concannon *et al*., 1977a; 1977b; Nett *et al*., 1975). Goodman (1998), opined that if accurate serial quantitative serum P4 concentrations are obtained, the LH surge might be estimated as the day when a distinct rise in levels of serum P4 is seen and that fertile matings should result in whelping 64 to 66 days later. Concannon *et al*. (1983) demonstrated that the changes in the serum P4 concentrations during the later part of proestrus and early estrus characterised by a gradual increase with a sudden sharp rise which coincided with the LH surge. Basal serum P4 levels have been reported to typically range between 0 to 1 ng/ml during an estrus and proestrus (Concannon *et al*., 1977a; 1977b). At the time of LH surge, serum P4 concentration rose rapidly (0.8 to 3ng/ml), continued to rise at ovulation (1.02 to 8.0 ng/ml), and was even higher (4.02 to 20 ng/ml) towards the end of fertile period (Concannon, 1986; Jeffcoate and Lindsey, 1989).

Bouchard *et al*. (1991) stated that ovulation was estimated to occur when serum P4 concentration was 4.9±1.0 ng/ml (3.4 to 6.6 ng/ml). Wright (1990), reported that LH surge was located to the period when serum P4 concentrations were between 2 to 4 ng/ml and that ovulation occurred when the serum P4 concentrations was 5.4 ng/ml (range 3 to

8 ng/ml). Renton *et al*. (1991) reported that in bitches, the concentrations of serum P4 reached 3ng/ml two days before ovulation, and recommended the determination of serum P4 concentration for identifying the time of ovulations. In another study carried out to compare the endocrine changes and ultrasound as means of idenitifying the ovulation in the bitch. Renton *et al*. (1992), reported that serum P4 concentrations were around 3 ng/ml on the day of LH surge in 12 out of 20 bitches and within 1 day in 7 out of 8 bitches. Ultrasound was less accurate in that, only 4 of the 16 estimates agreed. It was concluded that the measurements of serum P4 concentration was a better indicator for ovulation than ultrasonograhy.

Zoldog *et al*. (1994), reported that ovulation in bitches occurred when serum P4 concentrations reached 5 ng/ml. Barr (1995) reported that in all 14 beagle bitches studied, the serum P4 concentration was high, ranging from1.9 to 11.7 ng/ml around the time of ovulation. Johston and Root (1995) in a retrospective study of 49 bitches reported that serum P4 concentrations began to rise concurrently with LH surge, reaching 1.0 to 1.9 ng/ml; on the day of LH surge. The day after LH surge, the serum P4 concentration was 2 to 9 ng/ml. On the day of ovulation, the serum P4 concentration measured 4.0 to 10 ng/ml.

2.4. Medical Induction of parturition

The criteria for use of drugs to induce parturition is that 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. Several drugs such as nitroglycerine (Azawi *et al.*, 2012), antiprogestins (Baan *et al.*, 2005), prostaglandins (Meier and Wright, 2000) either alone or in combination (Reddy *et al*., 2012) have been evaluated for their efficacy in induction of parturition in bitch.

2.4.1. Induction of parturition using nitroglycerine

There is a solitary report of use of nitric oxide for induction of parturition in bitch (Azawi *et al*., 2012). Chwalisz *et al*. (1997) suggested that nitric oxide is a factor in cervical ripening and this ripening can be mediated via the application of a nitric oxide donor. Nitric oxide has been showed to stimulate prostaglandin production via induction of COX-2 and cytokine release (Sennström *et al*., 2000). Cervial ripening is associated with changes in local cytokines and prostaglandins as well as in other bioregulators. During parturition, the cervix undergoes changes in two phases: ripening, which involves collagen realignment, and dilation (Azawi *et al*., 2012). Azawi *et al*. (2012), reported successful induction of parturition in a bitch employing 50mg of nitroglycerine mixed in 30 ml of normal saline administered intravenously over a period of 30 minutes after sedation with xylazine. The animal was over 58 days pregnant as calculated from the day of mating. Endoscopic examination of the cervix after nitroglycerine administration revealed complete cervical dilation. The animal also received 10 IU of oxytocin intramuscularly in three doses 10 minutes apart after cervical dilatation was observed. The animal started straining two hours after third oxytocin injection and delivery of first pup was completed after three hours of third oxytocin injection. The remaining puppies delivered in less than one hour.

2.5. Antiprogestins

2.5.1. Mifepristone

Antiprogestins are compounds which have been developed to block the actions of endogenous hormone progesterone. A number of antiprogestins have been developed by the pharmaceutical industry. However so far, only mifepristone (RU38486) has been autherised as drug for human use and aglepristone (RU46534) for use in dogs (Hoffmann *et al*., 2011).

Mifepristone was discovered in the early 1980s at the French pharmaceutical company RU (Roussel-Uclaf) as part of a special research project to develop antiglucocorticoid compounds (Ulmann *et al.*, 1990).

Mifepristone is a derivative of norethindrone, a synthetic 19-nor-steroid, and is also known as RU486. Mifepristone strongly binds to progesterone as well as glucocorticoid receptors and thus, acts as an antagonist to progestational and glucocorticoid functions. Chemically it is 17b -hydroxy-11b-(4 dimethyl aminophenyl)-17a-(1-propynyl)-estra-4,9-dien-3-one (Spitz *et al.*, 1993).

It was found that the combination of mifepristone with a prostaglandin resulted in complete abortion in almost 100% of cases. These findings were so impressive that RU 486 became primarily known as the ‘abortion pill’, although a multitude of potential clinical applications had been predicted at its discovery (Kettel, 1995).

Mifepristone is an orally active compound with an approximately 70% absorption rate from the gut. However, it undergoes first-pass effect in the liver and gets partially metabolized and eventually its bioavailability is reduced to 40% in human beings and rats and 15% in monkeys (Sartos and Figg, 1996).

Experimental assessment of enterohepatic cycling of this compound after an oral dose in normal subjects has suggested that mifepristone may be partly pooled in the enterohepatic cycle (Heikinheimo *et al.*, 1989).

Three metabolites of mifepristone have been identified. This compound undergoes demethylation to produce mono- demethylated (RU42633) and di-demethylated (RU42848) derivatives as well as hydroxylation of the propynyl group to yield hydroxylated metabolite (RU42698). The study showed that metabolism of RU486 to RU42633 and RU24698 was rapid but removal of the second methyl group leading to formation of RU42848 occurred much more slowly and to much lesser extent than removal of the first (Yong-en *et al*., 1993).

Like mifepristone, these metabolites are immunologically and biologically active and retain anti-progestational and anti-glucocorticoid properties (Yong-en *et al*., 1993).

Elimination of mifepristone and its metabolites from the body is mainly through feces (83%) and urine (8.8%) within 6–7 days after administration of a single oral dose (Sartos and Figg, 1996).

Progesterone (Baulieu, 1989) exerts its biological effect by entering into the cell and binding to specific proteins, progesterone receptors, which are situated in the nucleus of the cell. When this binding occurs, the receptor gets activated. These receptors also have domain for DNA binding.

Progesterone (Baulieu, 1989) and other progestins cause a dramatic change in the conformation of the progesterone receptor, which transforms the receptor from non-DNA binding into one that will bind to DNA. This transformation is associated with a loss of heat shock proteins and dimerization of the receptor molecules. The activated receptor dimer then increases the gene transcription rates producing progesterone agonistic effects at cellular and tissue level which are essential for continuation of pregnancy.

When mifepristone (Spitz and Agranat, 1995) binds to the inactive receptor, it induces equally dramatic change in the receptor conformation, loss of heat shock protein and dimerization. However, in this case, the DNA-bound receptors are transcriptionally inactive, suppressing progestin effects, thus terminating the pregnancy.

In the presence of progestins, mifepristone acts as a competitive receptor antagonist for both A and B forms of progesterone receptors. When administered in early stages of pregnancy, mifepristone causes decidual breakdown by blockade of uterine progesterone receptors (Hardman, 1996). This leads to detachment of blastocyst and subsequent decrease in hCG production. This in turn causes a decrease in progesterone secretion from corpus luteum which further accentuated decidual breakdown. Decreased endogenous progesterone coupled with blockade of progesterone receptors in the uterus, increases the uterine PGF2α levels and sensitizes the myometrium to the contractile action of prostaglandins (Tripati, 1993). The property of mifepristone for cervical ripening may help to manage clinical situations in humans such as, preparation for second trimester for abortion, preparation for labor induction at term and preparation of the cervix when labor must be induced because of intrauterine foetal demise (Donaldson *et al*., 1993).

Antiprogestins also have antiglucocorticoid activity. For this reason, mifepristone (Spitz and Agranat, 1995) could be used to treat the patients with over reactivity of the adrenal glands known as Cushing's syndrome which may arise from inoperable tumors (Spitz, and Bardin, 1993). Other applications may include eye drops containing mifepristone to lower pressure of the eyes of the patients of glaucoma and oral administration of the drug to prevent viral diseases. It is also a potent antiglucocorticoid and shows promising activity in treating estrogen-dependent gynaecological disorders and hormone-deficient tumors.

Like most of the synthetic drugs, mifepristone also shows some unwanted side effects. A few women who received single doses of mifepristone to interrupt pregnancy experienced side effects, which include heavy bleeding, nausea, vomiting, abdominal pain and fatigue. Other side effects are slight weight loss and skin rashes (Braja and Vandana, 2001).

2.5.2. Aglepristone

Agle´pristone, a progesterone receptor antagonist, has proved to be safe and effective in early and midgestation pregnancy termination in the bitch (Fieni *et al*., 2001). In addition to pregnancy termination, endocrine effects such as temporary elevation of plasma prolactin concentration, premature cessation of the luteal phase, and shortening of the interestrous interval have been observed after administration of progesterone receptor antagonists (Fieni *et al*., 2001).

Progesterone receptor antagonists terminate pregnancy by binding with high affinity to the uterine progesterone receptor, thereby preventing progesterone from exerting its biological effect (Hoffman and Schuler, 2000).

After 2 injections of 10 mg/kg/day at 24 hours interval, the peak plasma concentration (Cmax) is about 280 ng/ml. This peak is reached within 2.7 days (Tmax). The Mean Residence Time (MRT) is about 6 days, which includes the mean absorption time from the injection site. The results showed that aglepristone follows a linear kinetic pattern because the concentration peak (Cmax observed) is proportional to the dose and appears in a time period (Tmax observed) independent of the dose after one administration. After administration at a dose rate of 10 mg/kg, excretion is very slow. Only 60% of the administered dose is excreted during the first 10 days and about 80% over 24 days. Excretion is essentially via the faeces (about 90%) with the urine route remaining minor (7%). This corresponds to what we know about excretion of steroid hormones. The slowness of excretion is explained by the marked lipophilia and the almost certain existence of an entero -hepatic cycle (Scientific Update 2010).

2.5.3. Mifepristone / Aglepristone for induction of parturition

Weyden *et al.* (1989) in his study, administered mifepristone (7.5 mg/kg b.wt per day) 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.

In another study conducted by Nohr *et al.* (1993), 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.

Fieni and Gogny, (2009) demonstrated that at day 58 of pregnancy, all of the bitches were injected subcutaneously with 15 mg kg-1 aglepristone (Alizine, Virbac-Carros, France) and 24 h later and subsequently at every hour intervals until delivery of the last pup with 0.15 IU kg-1 oxytocin, Parturition was obtained in all bitches at an average of 29.7±5.6 hours after aglepristone administration; the shortest interval being 15 hours and the longest 40.5 hours. Average expulsion time at parturition was 5.9±1.9 hours, which was equivalent to an average of 1.1±0.4 hours per pup; 121 pups were born. Onset of parturition had occurred in 95% of bitches between 27.4 and 32.0 hours after aglepristone administration. The use of oxytocin as an uterotonic agent therefore proved to be very effective. Duration of parturition was 1.1±0.4 hours per pup on average. And the author clearly demonstrates that the association of aglepristone and oxytocin can be effectively used to induce parturition in bitches and provides safe conditions for the bitch and the litter.

Baan *et al.* (2005) induced parturition by administering Aglepristone twice with a 9 h interval on day 58 of pregnancy. The litter size averaged 6.0±1.1 in the spontaneously whelping group, and 7.0±0.4 in the induced group. Expulsion of the first pup occurred between 32 and 56 h after the first treatment with aglepristone with an average of 41.0 ± 3.7 h. As a result, gestation length of the bitches in the induced group (59.5±0.2 days) was significantly shorter than that of the bitches in the spontaneously whelping group (62.2 ± 0.5 days). Aglepristone treatment did not significantly influence the mean growth rates. The mean growth rate during the first 10 days was 30 ± 0.8 g/day (n = 35) in the induced group and 40 ± 0.7 g/day (n = 33) in the spontaneously whelping group. Aglepristone is an efficient and safe drug for the induction of parturition in the dog. Aglepristone 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 aglepristone 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 (Galac *et al.*, 2004). 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) (Concannon, 1977). And in all bitches, the aglepristone treatment alone was sufficient to lead to the expulsion of the first pup, within 32–56 h after the start of treatment.

2.5.3.1. Progesterone concentration after induction of parturition with antiprogestins

After Alizine® application and as presented by Kowalewski *et al.* (2009), the progesterone concentration before the first treatment was 15.11 ± 6.7 ng/ml; at the second aglepristone treatment 13.61 ± 8.2 ng/ml; 5.1 ± 2.7 ng/ml 24 h later and 2.33 ± 1.44 ng/ ml, respectively 1.2 ± 0.6 ng/ml 48 and 72 hour later whereas according to Baan *et al.* (2008), the general linear model for repeated measures showed that the P4 concentrations did not change significantly after aglepristone administration in any of the bitches. 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. Mean P4 concentration in the spontaneously whelping group decreased significantly in the 30-hour period before parturition, whereas the mean PGFM concentration increased significantly during that same period and the P4 concentrations in the samples taken during the expulsion phase were significantly higher in the induced group than in the spontaneously whelping group (Baan *et al.*, 2008).

At the time of aglepristone administration, the average peripheral plasma progesterone concentration was 8.5 ± 2.2 nmol l-1, and all of bitches had a progesterone concentration of more than 6.2 nmol (Fieni and Gogny, 2008). While in a study conducted by Baan *et al. (*2005) between day 54 and day 58 of pregnancy, the mean plasma progesterone concentration in bitches from the spontaneously whelping group was significantly higher than in the 30 hour period before parturition. In the days after parturition, mean plasma progesterone concentrations were significantly lower 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. On the second and third day after parturition the mean plasma progesterone concentration was significantly lower than during the previous period. In late gestation, before aglepristone treatment, mean plasma progesterone concentrations of the spontaneously whelping and the induced group did not differ significantly. During the last 30 hour before parturition, the mean plasma progesterone concentration in the bitches from the induced group was significantly higher than in the spontaneously whelping group. During the expulsion phase, the mean plasma progesterone concentration in the induced group was significantly higher than the concentration in the spontaneously whelping group. In the post-partum periods, the mean plasma progesterone concentrations did not differ significantly between the two groups. 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 concentration in 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. Furthermore, because maximum plasma aglepristone levels are only reached after approximately 2.5 days, 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 PGF2α secretion. In addition, P4 might have a stimulating paracrine/autocrine effect on its own production within the luteal cells (Hoffmann *et al*., 2004).

Fieni *et al*. (2001) reported that progesterone peripheral plasma concentrations increase after injection of aglepristone and bitches finish parturition with a progesterone concentration higher than 8 nmol/L. This increase could be due to the binding of progesterone receptors by aglepristone in place of natural hormone. The increase in plasma progesterone concentrations might also be due to a hypothalamic effect of aglepristone on GnRH neurons that results in increased pituitary secretions (FSH and LH) which might stimulate corpora lutea cells.

2.5.4. Prostaglandins

The prostaglandins, leukotrienes and related hydroxyl fatty acids are members of a group of compounds collectively known as the 'eicosanoids' (Smith, 1989).

Prostaglandins are 20-carbon chain fatty acids that function as local hormones and are produced by all cells of the body. Biologically active eicosanoids (PGs, leukotrienes, lipoxins, and other 20-carbon fatty acids) are formed from the polyunsaturated fatty acid, arachidonic acid (5,8,11,14-cis eicosatetraenoic acid). Arachidonic acid is a common constituent of phospholipids in all membranes within a cell, cholesteryl esters and triglycerides. The liberation of arachidonic acid from phospholipids is the initial step in the synthesis of PGs. This is accomplished directly by the catalytic action of members of the phospholipase A2 (PLA2) family of enzymes, or indirectly by the action of phospholipase C (PLC) (Olson, 2003).

Parturition is composed of five separate but integrated physiological events: fetal membrane rupture, cervical dilatation, myometrial contractility, placental separation and uterine involution. Prostaglandins (PGs) have central roles in each of these, but the most studied is myometrial contraction. Elevated uterine PGs or the enhanced sensitivity of the myometrium to PGs leads to contractions and labour. The regulator of PG synthesis is the mRNA expression of PGHS-2 (Olson, 2003).

Exogenous PGF is luteolytic in dogs but the early- and mid-luteal phases are relatively resistant to PGF in that, compared with the single doses effective in large artiodactyls, a comparable dose on a body weight basis must be administered repeatedly two or more times a day for several days.

Canine gestation is terminated by a foeto-placental mechanism that releases large amounts of uterine and ⁄ or placental PGF2α reflected in a large increase in PGFM beginning 24–36 h prior to parturition and results in a rapid decline in progesterone to below 1–2 ng ⁄ ml by 8–12 h before parturition (Concannon, 2009).

Prostaglandin F-receptors are constitutively expressed in canine CL, increasing between days 5 and 25 (Kowalewski *et al*., 2007). Sensitivity to PGF increases as progesterone secretion wanes with lower doses required, especially after days 35–40, a phenomenon important in late pregnancy when placental PGF must effect a functional pre-partum luteolysis. The luteal effects of PGF in dogs as in other species likely involves in addition to the observed vascular disruptions, luteal cell decreases in LHR and StAR, decreased progesterone synthesis, increases in PG synthesase and endogenous PGF and promotion of apoptosis. Exogenous PGF caused 60–80% increase in the apoptotic DNA fragmentation index of days 15–50 canine CL but only minimal increases in Bax-gene expression index (Aiudi *et al.,* 2006).

The simultaneous rise in cortisol, presumably of foetal adrenal origin, suggests a direct role for cortisol as proposed for sheep and humans, including the upregulation of placental PGH2 and PGH-synthestase-2 and suppression of PG catabolism, perhaps acting as progesterone antagonist (Challis *et al.,* 2002). The process is presumably accelerated in a cascade-like manner, as in other species, with the simultaneous declines in progesterone reducing its inhibitory effects on PGF release, as suggested for other species. Progesterone antagonist administration in pregnant bitches induces a rise in PGFM (Baan *et al*., 2008) in late pregnancy but not when administered in mid-gestation (Fieni *et al*., 2001) suggesting PGF is more available for release closer to term. The capacity for PGF production is higher in the endometrium than in the placenta on a per tissue weight basis late in canine pregnancy, but on an organ weight basis the majority of the PGF likely comes from placental sources (Luz *et al*., 2006). The pre-partum rise in prolactin could play a role in ongoing PGF release and the progression of parturition. In rats, the administration of a prolactin receptor antagonist can disrupt and ⁄ or delay parturition independent of its ability to reduce oxytocin secretion (Nephew *et al*., 2007) and prolactin seems to be important in luteal responsiveness to PGF.

However, day 65 luteal tissue in vitro produced small but detectable amounts of PGF suggesting a possible autocrine or paracrine role for PGF in the slow regression of the canine CL at least in the latter stages. Luteal PGF production was not stimulated by phorbol ester (Luz *et al*., 2006) suggesting that it may be constitutive and not under exogenous control. Although the canine endometrium has low but detectable capacity to produce PGF in vitro (Luz *et al*., 2006), any luteal effect is negligible as hysterectomy has no protective effect (Olson *et al*., 1984).

The early developing CL is very resistant to the effects of exogenous PGF2α or PRL-lowering doses of dopamine agonist (Concannon, 1998). After 30 days, PGF2α treatments or suppression of LH or PRL activity can render progesterone concentrations nondetectable (or nearly or so) (Concannon, 1995).

In dogs, as in several other mammalian species, exogenous PGF2α is considered to be a primary luteolytic agent that causes an abortifacient decline in luteal progesterone production, similar to what occurs naturally prior to parturition, although the exact mechanism by which PGF2α elicits its response in the CL remains unclear (Wanke *et al*., 2002). The action of PGF2α is likely mediated via specific PGF2α receptors located on the plasma membrane of luteal cells, as observed in the CL of the ovarian cycle in domestic ruminants) (Cunningham, 2002). In the bitch, the efficacy of exogenous PGF2α in inducing abortion is not only dose and frequency dependent, but is also related to the stage of pregnancy.

Higher doses are needed to induce a complete luteolysis in early than in late pregnancy, despite the occurrence of more severe side effects (Romagnoli *et al*., 1993). The clinical side effects after PGF2α administration could be related to the action of acetylcholine (Ach), through an increase in the concentration of the cholinergic neurotransmitter, probably via an inhibition of Ach-esterase activity, or through direct action on a cyclase receptor (Sciorsci *et al*., 1992).

2.5.5. Prostaglandins for induction of parturition

Natural PGF and the more potent PGF-analogs are effective in the termination of pregnancy because (a) PGF is luteolytic in dogs as in most species (Concannon and Hansel, 1977) and (b) corpora lutea are the only source of progesterone in the pregnant bitch (Concannon, 1995). A PGF induced luteolysis causes a decline in progesterone, withdrawal of progesterone action and, as a result, termination of pregnancy. Prostaglandin is also utero-tonic and the uterine contractions caused by PGF facilitate its abortifacient action. While the use of PGF to terminate pregnancy is an extra-label and experimental use of the drug, it is being used increasingly for this purpose in many veterinary practices. PGF administration is by injection, at intervals typically more frequent than once a day, and thus can become labor intensive and expensive due to the costs of hospitalization and professional time (Wanke *et al*., 2002).

The abortifacient efficacy of PGF involves induction of luteolysis, stimulation of uterine contraction and cervical dilation. Of these, the luteolytic effect is the most important. In dogs, the progesterone supporting pregnancy comes entirely from the corpora lutea throughout gestation. Prostaglandin-F2alpha will induce luteolysis and depress progesterone concentrations to nearly non-detectable levels more readily after Day 25 or 30, than earlier in pregnancy. Prostaglandin-F2alpha is rarely capable of inducing luteolysis in very early pregnancy (Day 1 to 15) if treatment is not continued well beyond Day 15 or 20. The later in the cycle PGF is administered, the easier and more rapidly the induction of luteolysis. Use of PGF requires intramuscular or subcutaneous administration 2 or 3 times a day, for 4 to 6 days or longer. Most reviewers consider hospitalization as part of the protocol, to allow for the monitoring of adverse side effects and confirmation of efficacy (Lein *et al*., 1989). Some clinicians allow bitches to be returned home after side effects have been carefully checked by the clinician after the first PGF administration, and the treatment continued at home by the owner where local regulations and liability issues permit. The PGF product most frequently used in dogs in North America is PGF-2alpha thamsalt, commonly referred to as dinoprost (Lutalyse), and is marketed for use in cattle. Use of this product in small animals, as for all prostaglandin products, is an experimental, extra-label use and a release form or statement of understanding should be used to document consent of the pet owner. No PGF products are marketed in North America with an indication for use in dogs or cats. Other veterinary PGF, i.e., dinoprost, produced in other countries include: SincroBovis and Dinolytic (Wanke *et al*., 2002).

The attendant side effects include emesis, salivation, defecation, urination, and respiratory distress. Side effects are typically acute and short-lived, dose dependent and self-limiting, and decreasing in intensity with repeated dosing. Since side effects are often self limiting, and since dogs vary in the extent of side effects, it may be best to initiate treatment with low doses of 50 ug/kg or less, and to then increase the dose over time in relation to the response of the patient. The half-life of PGF is only seconds, and it is only in the circulation for a few minutes following an IM injection, or perhaps a little longer when it is administered by subcutaneous injection. Therefore, administration multiple times per day is an absolute requirement for efficacy whether using low, moderate or high doses. Treatment must be continued until verification of efficacy by ultrasound or palpation. Partial abortion of litters can occur if treatment is discontinued prematurely. With any dose, 9 or more days may be required to terminate some pregnancies, although 5 to 7 days is usually sufficient (Wanke *et al*., 2002).

Highly potent, synthetic PGF-analogs, such as cloprostenol (Estrumate, Veteglan), have not been extensively promoted for pregnancy termination in dogs in

North America, because there are no dose-response studies to demonstrate the minimal effective doses. Furthermore, an error dosing by mistakenly using doses commonly suggested for natural PGF could be fatal. However, cloprostenol has been routinely and effectively used in Europe for pregnancy termination in dogs, being used at a dose of 2.5 ug/kg, administered three times, at 48 hour intervals (Fieni *et al*., 1989). Side effects have been reduced by administration of various drugs, including anti-cholinergic drugs like atropine. A study of 67 pregnant bitches demonstrated a 100% efficacy in termination of pregnancy using cloprostenol at the dose of 2.5 ug/kg, subcutaneously, administered three times, at 48 h intervals, starting at Day 30 of pregnancy (Fieni *et al*., 1997). Pre-medication given at 15 minutes before prostaglandin-analog injection included atropine sulfate, prifinium bromide, and metopimazine, and it eliminated side effects in 58% of the bitches, and presumably reduced them in others. Cloprostenol at even lower doses been used in combination with dopamine agonist treatment to terminate pregnancy in dogs shortly after implantation, as reviewed below. The PGF agonist alphaprostol (Gabbrostim) has also been used to terminate pregnancy in bitches, at a doses to 20 ug/kg, BID. Another PGF agonist is luprostiol (Prosolvin) (Wanke *et al*., 2002).

Reddy *et al*. (2012), induced bitches with subcutaneous injection of cloprostenol at the rate of 2.5μg/kg body weight. Atropine sulphate at the rate of 0.04 mg/kg was subcutaneously administered 10-15 minutes prior to cloprostenol administration. The mean duration of time interval from starting of treatment to beginning of whelping (hours), duration of whelping (minutes) and inter pup intervals (minutes) were 34.46 ± 4.70, 490 ± 96.94 and 192.24±62.58 respectively in cloprostenol group. Whelping occurred in cloprostenol and *control* groups when the mean plasma progesterone concentrations reached around 1.0ng/ml whereas, Meier and Wright, (2000), has demonstrated that parturition can be induced in late pregnant bitch, without adverse effects, by the administration of sodium cloprostenol as a continuous low dose for 24h (1µ/kg/24h).The duration of parturition, viability of pups and maternal behaviour were similar to those observed in normal parturition of bitches in their facility. The timing of the birth of the first pup occurred within 40 to 46 h after the start of the treatment in all but 2 out of 6 bitches. The first puppy was born 37.7±2.9 h after the start of treatment (range 28-46 h). The duration of whelping was approximately 15.7±2.2 h (range 10 to 24 h). The litter size was 9.2±0.8 pups (range 6 to 12 pups), and the pup survival rate was 6.0±0.8 per litter (range 4 to 9 pups).

Moriyoshi *et al.* (1999), conducted an experiment using 16 Beagle bitches (aged 11 months to 6 years and 2 months) in their 56th to 58th day of pregnancy to investigate the effects of two injections of a low dose of fenprostalene, a long-acting prostaglandin F2alpha analogue, and pretreatment with prifinium bromide, a parasympathetic nerve blocking agent, on the induction of parturition and severity of side effects. The results showed that pretreatment with prifinium bromide and two injections of 2.5 microg/kg of fenprostalene can alleviate side effects following fenprostalene administration and have no adverse effect on the survival of newborn puppies, indicating that this method is a reliable and safe way of inducing parturition in bitches.

2.5.6. Combined use of Mifepristone and Prostaglandin F2 alpha :

Blendinger *et al*. (1994), initiated parturition in bitches by injecting the antiprogestin aglepristone Alizine® in the dose of 10 mg/kg, a second injection was given 24 hours later. The treatment with PGF2α (Dinolytic® 160µg/kg) commenced 12 hours after the first antiprogestin treatment and was repeated 12 hours later. Four hours after the second PGF2α injection first puppy was born. Combined treatment of this type antiprogestin with PGF2α is a useful approach to terminate prolonged pregnancies in the dog.

Reddy *et al*. (2012), induced parturition in bitches with mifepristone at the rate of 10mg/kg body weight orally as a single dose. If the bitch did not whelp within 24 hours they were administered cloprostenol at the rate of 2.5µg/kg body weight.and atropine sulphate at the rate of 0.04 mg/kg was subcutaneously administered 10-15 minutes prior to cloprostenol administration. Thus he concluded the use of mifepristone plus cloprostenol group and cloprostenol group as the alternative choices in the induction of whelping in bitches.

2.6. Haematology and Serum biochemistry during pregnancy and parturition

2.6.1. Haematology during pregnancy

There are published reports of haemodilution and alterations in plasma volume as well as gastro-intestinal absorption efficiency during pregnancy (Hytten and Leitch, 1984). These observed changes during pregnancy further resulted in alterations in haematological and plasma biochemical profiles (Allard *et al.*, 1989).

Krishnamurthy *et al.* (2012), indicated that there will be significant raise in total leucuocyte and neutrophil count by 42nd day of pregnancy, changes in the estrogen concentration during pregnancy might be a reason for this change in hematological parameters and the leucocytosis observed during 42nd day can be used to differentiate pseudopregnant and pregnant bitches. Further mild inflammation or tissue injury occurs around 20 days of gestation due to implantation which could also be a reason for mild leucocytosis as observed by (Eckersall *et al.,* 1993).

Mshelia *et al.* (2005) found the significant difference in the WBC counts of bitches with transition from oestrus to dioestrus non-pregnant bitches. The value in non-pregnant dioestrus bitches was almost double of that recorded in pregnant bitches, this shows that WBC counts may be useful in pregnancy diagnosis in the Mongrel bitch. Further the author showed in a study that RBC counts elevated during anoestrus and dropped gradually during proestrus and oestrus to the lowest value recorded during pregnancy, this low value recorded during pregnancy might be due to the anaemia associated with pregnancy (Concannon and Lein, 1989) and might also reflect the poor nutritional status. In the non-pregnant dioestrus bitches, RBC value was non significantly higher than the oestrous animals.

Mshelia *et al.* (2005) reported that PCV was lowest during pregnancy, although this did not vary significantly with anoestrus values. Contrary to the findings above Concannon and Lein, (1989) reported that during pregnancy, maternal haematocrit declined after implantation, with PCV normally reaching 40% by day 35 and fell below 35% at term. This might be due to the haemodilution effects of increased plasma volume, because total blood volume increases along with body weight increase of 20 to 55% over the course of gestation. And the haemoglobin concentration was also found to be low during pregnancy (Concannon, 1986).

2.6.2. Serum Calcium

For proper function and response, neuromuscular tissues are dependent upon a normal balance of electrolytes within the body. In particular, uterine contractions are dependent upon adequate levels of calcium (Pamela, 2001). In cases where calcium metabolism has been comprised (i.e. by inadequate diet, by dietary supplementation of a nutritionally balanced diet exogenous calcium during pregnancy, or by extended periods of uterine contractions as seen in long deliveries), mildly depleted levels of serum calcium within a whelping bitch might inhibit the normal progression of delivery by interfering with uterine contractions (Pamela, 2001). The myometrium needs calcium ions to contract. There is evidence that serum calcium concentration in bitches with primary inertia is similar to that in those with normal myometrial contractions (Kraus and Schwab, 1990) but it is not known if a low intracellular calcium content is the cause of the weak myometrial contractions.

Bergstrom *et al.* (2006), showed in an experiment that the calcium concentrations were below normal range for non-pregnant animals in seven bitches in group I and five in group II. Three bitches in group I started to deliver when the calcium solution was given and two more when oxytocin was also administered. Further the administration of calcium gluconate to treat dystocia can be directed and tailored based on the results of monitoring. And the author opined that the administration of calcium increases the strength of myometrial activity, when in effective weak uterine contractions are detected calcium gluconate as 10 per cent solution (0.465 $mEqCa^{++}$/mm) can be given subcutaneously at the rate of 1 mL per 4.5 kg/ 10 lb body weight (Davidson, 2003). Oxytocin mobilises intracellular stores of calcium and causes influx of extracellular calcium into human myometrial cells in vitro (Rezapour *et al.,* 1996). This shows the important interaction between oxytocin and calcium during parturition and could explain why more puppies were delivered when oxytocin was given after the calcium solution.

Darvelid and Forsberg, (1994) reported that calcium and oxytocin treatment was successful in relieving dystocia due to uterine inertia in 44 of 181 cases (24.3 %). Several other studies have also documented the beneficial effect of calcium administration in bitch with dystocia due to uterine inertia (Jones and Joshua, 1988). In hypocalcaemic bitch, slow intravenous injection of calcium borogluconate is usually recommended (Johnston, 1986).

Gaudet and Kitchell, (1985) Opined that, although dystocia due to hypocalcaemia is rarely confirmed by laboratory analysis it should be assumed to be present in cases of uterine inertia that fail to respond to oxytocin administration. Their study documented at least 16 cases in which propulsive uterine contractions recorded following calcium therapy which failed to respond to oxytocin administration.

Johnston *et al*. (2001) compared blood calcium levels between eutocia and dystocia bitches and concluded that there was no indication that blood calcium deficiency is the cause of uterine inertia in 17 of 26 animals' diagnosed dystocia because of uterine inertia.

Bergstrom *et al.* (2006), showed that two bitches did not respond and were subjected to caesarean section. In the seven bitches with normal serum calcium concentration, three dams started to deliver during treatment, while the other four were subjected to caesarean section. So the importance of the serum calcium levels therefore remains unsettled.

2.6.3. Blood glucose

Hypoglycemia has been reported to cause uterine inertia during parturition (Buckner, 1979). It has been reported that hypoglycemia often mimics a clinical picture similar to hypocalcaemia in bitch and deferential diagnosis between hypoglycemia and other causes of uterine inertia requires blood sugar tests **(**Buckner, 1979). Although hypocalcaemia is usually a problem in mid lactation, the condition can arise as a parturient complication**.** Freak, (1975) observed that, some subclinical hypocalcaemia bitches may not show the typical tremors and in coordination but, may exhibit restlessness coupled with uterine inertia.

Furthermore, it has been proposed that hypoglycemia is a cause of primary inertia, especially in small breeds of dogs (Linde-Forsberg and Eneroth, 2000). Contrary to the above observation Johnston *et al*. (2001) have stated that hypoglycaemia is uncommon in canine dystocia.

Lucio *et al*. (2008) reported that bitches exhibited normal glycaemia independent of the obstetric management. In their study on peripartum hemodynamic status of bitches under distinct obstetric conditions and also considered Labour is a stressful condition for any female, signed by reflex release of cortisol and relative hyperglycaemia. However, an endocrine control through an acute release of insulin maintains glucose level at the normal range.

Bergstorm *et al*. (2006) evaluated two treatment methods in bitches with primary uterine inertia in relation to blood concentrations of oxytocin, calcium and glucose and reported that before treatment, blood glucose values were 95.0 +/- 0.5mmol/l in group treated with combination of intravenous calcium solutions and oxytocin and 7.3 +/- 1.4mmol/l in group treated with oxytocin only. One of the treatment regimens recommended for primary uterine inertia is administrating glucose intravenously (Linde-Forsberg and Eneroth, 2000). Successful correction of uterine inertia due to hypoglycaemia by intravenous glucose administration has also been reported in bitch (Jones and Joshua, 1988).

2.6.4. Rectal Temperature

The prepartum rectal temperature drop is a particularly useful diagnostic tool in predicting onset of whelping, because it is a clinical indicator of rapid decline in serum progesterone, the thermogenic hormone that maintains pregnancy, at the end of gestation (Johnston *et al.*, 2001).

Tsutsui and Murata, (1982) found that the interval between the time when body temperature decreased to 37.5 C^0 in the late stage of pregnancy and the onset time of parturition was characterized by small individual differences among the bitches and concluded that this interval should be of great particular value as a criterion for the prediction of the onset time of parturition.

In the induced group, the drop in rectal temperature before parturition occurred in the presence of a high plasma progesterone concentration, which might be indicative of a progesterone antagonistic effect of aglepristone on the thermoregulation center (Baan *et* *al.*, 2005). 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 (Meier and Wright, 2000). The hypothermia parallels the decline in blood progesterone concentration with a delay of about 12 h, so pre-partum luteolysis can be monitored by the pre-partum decrease in rectal temperature (Concannon *et al.*, 1989). It has been suggested that in dogs progesterone has a thermogenic effect within the thermoregulatory system.

Rectal temperature did not vary until time ´0´ at the onset of whelping, the mean temperature was 37.6±0.7 C^0. After 12 hour, a significant increase as compared with time 0 (38.3±0.9 C^0) was noted body temperature also had higher levels 24 and 36 h after the onset of whelping (38.7±0.3 and 38.5±0.2 C^0). These changes were significant with respect to pre-partum values from –48 to 0 hour (Veronesi *et al.*, 2002).

Williams *et al.* (1999) reported the interesting finding that, in the bitch, post-partum mean rectal temperatures were higher than pre-partum rectal temperatures. Rectal temperature declines abruptly, atleast 1 full degree and often less than 99 °F, about 14 hours after prepartum luteolysis with drop in serum progesterone to less than 1 ng/ml. Temperature thereafter begins to rise as the bitch enters stage 1 labor, and whelping starts 8-24 hours after temperature drop (Concannon *et al.*, 1977). Further the author reported that prepartum hypothermia was observed in 98% of the bitches examined.

Veronesi *et al.* (2002) In a study found that no statistically significant difference in body temperature was recorded between time 48 hour and the beginning of whelping (time 0), suggesting that monitoring the body temperature variation could not be considered valuable in predicting the onset of parturition in the dog. They reported the lack of correlation between the decrease in plasma progesterone concentration and body temperature variation points to the uselessness of body temperature measurement to predict the impending parturition in this species, and revealed that in the thermoregulatory process in late pregnancy and at parturition in the dog, progesterone could play only a partial role in a very complex mechanism. And the author also stated that from a clinical point of view, any significant increase in body temperature recorded at the end of pregnancy without the beginning of expulsion of foetuses, is a tool to indicate problems at parturition.

Williams *et al.* (1999) found that, despite bitches showing circadian rhythm in rectal temperature profiles, the temperature changes caused by parturition were more marked than the circadian effect.

2.6.5. Ultrasonography

Real time ultrasonography has proven to be a valuable tool for diagnosing canine pregnancy and assessing foetal viability. Ultrasonography does not always determine the number of foetuses accurately, because only one sector of the abdomen is imaged a time. Therefore pups may be inadvertently imaged a second time (giving too high a number) or missed when scanning (giving too low a number) (Johston *et al*., 2001). Ultrasonography as early as 20 day might reliably detect pregnancy, but it is preferably done at 30 day after breeding so as to account for later conception dates (Matoon *et al*., 1995). Ultrasonography is used widely as a method for diagnosing early pregnancy (Ferguson, 1990) and fetal viability in the bitch (Johnston and others 1983, Barr 1988), although its application for the estimation of fetal age is limited in this species compared with humans. This is because of the great variability in dog breed sizes, which makes the measurements of fetal structures different, depending on the female's weight. Early and accurate determination of canine gestational age using trans abdominal ultrasonography is useful for predicting parturition date, management of parturition, and planning Caesarean section. Transabdominal ultrasonographic examination can be used to confirm pregnancy as early as 17 days after the luteinizing hormone (LH) surge (England and Yeager, 1993).

Ultrasonography is an excellent method of pregnancy detection. It has the advantage of also assessing fetal growth rate and viability (England and Russo, 2006) The gestational sac appears as a spherical, anechoic structure, surrounded by a hyper-echoic wall comprised of the uterine wall and placenta. Hyper-echoic fetal structures are present within the gestational sac. Although it occasionally is possible to identify the gestational sac as early as 10 d after breeding, pregnancy is more reliably detected 24–28 day after breeding in bitches. At that time, fetal structures and cardiac activity are detected within the gestational sacs. Fetal heart rates range from 200 to 250 beats/min. Fetal movement characterized by dorsiflexion of the head and extension of the limbs is common after Days 33–39. By Days 40–50, fetal anatomy is obvious (Johnson, 2008).

Gestational age cannot be determined precisely but it can be estimated based on ultrasonographic findings, and fetal maturity and impending fetal death can be assessed by the development, or lack of fetal organs (Beccaglia and Luvoni, 2006) Nonviable fetuses show no cardiac motion and within 1 day of death, lose identifiable morphology. After death, fetal size decreases and it assumes the appearance of an ovoid mass of heterogeneous echogenicity.

Materials and Methods

III. MATERIALS AND METHODS

The present study was conducted in the small animal section of the department of Veterinary Gynaecology and Obstetrics, Veterinary college Hebbal, Bangalore, on female dogs (n=18) with the history of 65 days of pregnancy and above calculated from the first day of multiple matings with no impending signs of parturition. Female dogs selected belonged to different breeds and body weight. In every case, the following history was obtained from the owner.

1.) Dates of mating

2.) Age of the animal

3.) Parity

4.) Previous gestational and parturient abnormalities if any

5.) Litter size during the previous parturition

6.) Feeding standard

Each animal was subjected to the following physical examination.

1.) Rectal temperature (ºF)

2.) Mammary gland development and the nature of mammary secretions

3.) Presence or absence of vaginal discharges and the nature of vaginal discharge

4.) Presence or absence of vulval edema

5.) Presence or absence of vaginal canal relaxation as judged by digital examination of the vagina

6.) Abdominal palpation to approximately identify the number of foetuses

7.) Transabdominal ultrasonography to determine the age of the foetus based on the foetal head diameter, the number of foetuses and the viability status of the foetus.

In each animal, blood samples were obtained for analysis of blood glucose, serum calcium and serum progesterone concentration. Subsequently, the animals were allotted into the following three groups;

Group A: Induction of parturition with mifepristone

This group consisted of six female dogs and they belonged to five different breeds and their body weight ranged between 10 to 45 kg.

The mean gestation period calculated as the interval from the first mating to the day of presentation was 66.50±0.72 days and ranged between 65 to 69 days. The mean gestation period calculated on the basis of ultrsonograghic measurement of the fetal head diameter was 63.40±0.81 days and ranged from 61 to 64 days.

Each animal allotted to this group were administered orally with 5mg/kg BW of mifepristone (MT pill®, Cipla Limited[1]) twice daily on two consecutive days. The process of parturition was described to the owners and were adviced carefully to look for the symptoms of onset of parturition. They were also adviced to record the time of expulsion of the first pup after initial oral administration of mifepristone and the time taken for the delivery of the entire litter as well as the viability status of the pups, the owners were also adviced to present the animal if the process of parturition was initiated

[1] MT pill 200 mg manufactured by Cipla Limited

but did not proceed normally. The owners were advised to present the animal again if the process of parturition had not been initiated upto 72 hours after the first dose of mifepristone and such animals were immediately subjected for caesarean section and medical induction of parturition was considered as a failure.

Group B: Induction of parturition using mifepristone and prostaglandin.

The body weight of six animals allotted to this group ranged between 25 to 47 kg and they belonged to 5 different breeds.

The mean gestation period calculated as the interval from the first mating to the day of presentation was 69.50±1.57days and ranged between 65 to 73 days. Further the mean gestation period calculated on the basis of ultrsonograghic measurement of the head diameter was 64.67±0.33 days and ranged from 64 to 65 days.

The treatment protocol for initiation of parturition in six animals allotted to this group consisted of oral administration of mifepristone at the dose rate of 5mg/kg BW twice daily for two consecutive days and sub cutaneous administration of Dinoprost tromethamine (Lutalyse®, Pharmacia and Upjohn Company[2]) at a dose of 0.1mg/kg BW twice daily for two consecutive days. As far as possible, the interval between two prostaglandin injections was kept as 8 hours. The owners were explained of the side effects of prostaglandin injections and were strictly instructed not to miss any of the scheduled injections of prostaglandins. As in the previous group, the owners were instructed to record the time of expulsion of first pup, the time taken for the completion of parturition process and viability status of the pups. If the process of parturition was not

[2] Lutalyse 5mg/ml, manufactured by Pharmacia and Upjohn Company

initiated upto 72 hours after the commencement of the treatment they were subjected for further needful treatment.

Group C: Control group

Six animals allotted to this group did not receive any treatment for induction of parturition and were kept as control. Their body weight ranged between 11 to 37 kg and belonged to 4 different breeds. The owners of these animals were adviced to wait for another 72 hours for initiation of labor and were subjected to an elective caesarean section if they did not deliver spontaneously within 72 hours after the voluntary waiting period. As in the previous two groups, the time of expulsion of first pup, the number of pups delivered, their viability status, as well as the time taken for the completion of labor process was recorded in every animal.

Blood Collection:

Blood samples were obtained by the venipuncture of the cephalic or the lateral sephenous vein using scalp vein set prior to induction of parturition. About 5ml of venous blood was drawn into a disposable 10 ml syringe. About 2ml of blood was dispensed into EDTA tube and the remaining blood sample was transferred into a serum separation tube.

The determination of blood glucose concentration was done using a glucometer (One touch) and the concentration was expressed as mg/dl.

The blood sample transferred into the serum separation tube was allowed to stand at room temperature for 1h for separation of serum. Subsequently, it was centrifuged at 1500 rpm for 20 minutes the separated serum was aliquoted for the serum calcium concentration which was carried out on the same day of collection. The serum calcium concentration was determined using (BIOSYSTEMS A15) and concentration was expressed as mg/dl.

The remaining portion of the serum sample was stored at -20 ºC for estimation of serum progesterone concentration at a later date. The progesterone concentration was determined by RIA (Immulite®, Losangeles, CA, USA) and was expressed as ng/ml.

Statistical analysis:

The data generated from the present study was subjected to one way analysis of variance to compare the efficacy of the different protocols tried for medical induction of parturition in dogs with that of control group.

Results

IV. RESULTS

The present study was conducted to evaluate the efficacy of two different protocols for medical induction of parturition in dogs. The study was conducted on three groups of six animals each which were presented with the history of having completed atleast 65 days of pregnancy calculated as the interval between the first of multiple matings and the time of presentation.

4.1. Duration of pregnancy in animals selected for the study

The mean duration of pregnancy calculated as the interval from the first day of mating to the time of presentation in 18 animals were determined as 67.56 ± 0.67 days and in individual animals, ranged between 65 to 76 days (Table 1.). However, ultrasonographic measurements of the foetal head diameter revealed the mean duration of pregnancy in these animals to be 63.75 ± 0.41 days and ranged between 61 to 65 days (Table 2.).

4.1.1. Breed of dogs which were presented with a complaint of prolonged gestation

Eighteen animals presented with a complaint of exceeding 65 days on the basis of mating dates belonged to 11 different breeds. The most commonly presented breed was Labrador retriever (7 animals) and was followed by pug and Irish setter (2 animals each). The other breeds presented were Beagle, Basset hound, St. Bernard, Siberian husky, Golden retriever, Rotwhiler and Boxer (1 animal each).

4.1.2 Age, Parity and previous history of parturition in animals presented for the study

Table No. 3. Shows the age and parity of the animals presented with a complaint of failure of initiation of parturition by day 65 of pregnancy calculated on the basis of mating dates. Among 18 animals, 4 (22.22%) were aged < 2 years, 11 animals (61.11%) were aged between 2-5 years and 3 animals (16.66%) were aged over 5 years. Further, 13 animals (72.22%) had delivered atleast once and the rest 5 (27.7%) were primiparous. In 13 animals which had delivered atleast once, the process of parturition was described as uneventful in 12 animals (92.30%) and one animal (7.70%) had a history of having undergone caesarean section during the previous delivery (Table 4.).

4.2. The physical and genital tract examination findings in the animals presented

The physical and genital tract examination findings in the animals presented with the complaint of prolonged gestation is presented in Table 5. The rectal temperature in the individual animals varied between 100°F to 103.5°F and the mean rectal tempreture was recorded as 101.9±0.2°F. Further, every animal presented exhibited mammary engorgement and secretion of milk. Scanty mucoid vaginal discharge was noticed in only 8 animals (44.44%). Moderate degree of vulval edema was observed in 12 animals (66.66%) while in the rest, the vulva only showed mild degree of edema. Digital examination of the vagina revealed that in 12 animals (66.66%), the vagina did not evince any relaxation and a tight vestibulovulval constriction was observed. The remaining 6 animals showed a moderate degree of vaginal relaxation (33.33%). Ultrasonographic evaluation of 18 animals revealed the litter size as single in 5 (27.77%) (Table 6.) of animals and multiple in the rest (72.22%). Ultrasonography also revealed that the foetus/foetuses were viable in every case as judged by heart beat (Table 6.).

4.3. The blood and serum biochemical parameters

The blood glucose concentration in all the animals included for the present study were within the physiological range. The blood glucose concentration in individual animals ranged between 75 to 120 mg/dl and the overall mean concentration was calculated as 94.55±3.25 mg/dl (Table 7). The serum calcium concentration was also within the normal physiological limits and the mean calcium concentration was recorded as 9.14 ± 0.22 mg/dl and the individual animals, its concentration ranged from 7 to 10.1 mg/dl (Table 7.) The serum progesterone concentration in individual animals even by 65 days of pregnancy ranged between 4 to 20 ng/ml and the mean serum progesterone concentration was calculated as 8.08±1.11 ng/ml (Table 7).

4.4. The efficacy of mifepristone in initiation of parturition (Group A)

The efficacy of mifepristone in initiation of parturition in the animal presented with a complaint of prolonged gestation is presented in Table 8. The mean duration of pregnancy calculated as the interval from first mating to the day of treatment was 66.50 ± 0.72 days. The mean serum Progesterone concentration before the initiation of treatment was recorded as 9.3±2.4 ng/ml. Following the initiation of treatment with mifepristone successful initiation as well as completion of process of parturition was observed in 5 out 6 treated animals (83.33%) within 72 h after the oral administration of the first dose of mifepristone. The remaining animal was presented to the clinic again with the complaint that the animal had still not shown any signs of parturition even after 72 h after the first dose of mifepristone and the treatment with mifepristone with this animal was considered a failure and the animal was subjected to caesarean section. In 5 animals in which the process of parturition was initiated following the oral administration of first dose of mifepristone, the mean time taken for the expulsion of the first pup from the time of initiation of treatment was recorded as 22.01±5.30 h and the first pup was delivered as early as 14h in one animal. In the remaining animals, the expulsion of the first pup was observed latest by 36h. The time taken for the delivery of the entire litter following first dose of mifepristone treatment averaged 23.17±5.32 h and in individual animals ranged between 14 to 36 h. The litter size was single in 60% of the delivered animals and in the rest (40%) the litter size ranged between 2 to 4 (Table 8). It was also observed that 25% of the puppies delivered were either born dead or died soon after delivery and percentage of puppies which remained viable at 24 h post delivery was 75% (Table 8).

4.5. The efficacy of a combined treatment of mifepristone and prostaglandin F2α for initiation of parturition (Group B).

The efficacy of a combined treatment of mifepristone and prostaglandin F2α for initiation of parturition in animals presented with a complaint of prolonged gestation is presented in Table 9. The mean duration of pregnancy of 6 animals allotted to this group was 69.50±1.57 days. Successful induction and completion of the prolonged parturition was recorded in 5 out of 6 animals (83.33%) treated with a combined therapy involving mifepristone and PGF2α. In the remaining one animals, there was no evidence of the signs of parturition even by 72h after the initiation of the treatment and was subjected to Caesarean section.

In 5 animals which responded to the combined therapy of mifepristone and prostaglandins, the mean time taken for the expulsion of the first pup from the time of initiation of treatment was recorded as 25.69±3.7 h and in individual animals it ranged between 15 to 44 h (Table 9). Further, the process of parturition was completed in mean time of 27.80±3.6 h from the initiation of treatment (Table 9). The litter size was recorded as single in 20% of the animals which delivered following treatment with mifepristone and PGFα and in the rest (80%), the litter size ranged between 3 to 9 (Table 9). Further, 92% of the puppies born were found to be alive at 24h after delivery.

4.6. Course of parturition in spontaneously whelping bitches (Control)

Table 10 presents the course of parturition in female dogs in which the process of parturition had not been initiated even by 65 days following mating. These animals were left untreated and was watched for spontaneous delivery over the next 72 h. The mean duration of pregnancy calculated on the basis of mating dates in 6 animals allotted to this group averaged 66.67±0.66 days. Serum progesterone concentration at the time of presentation in these animals was recorded as 6.0±0.42 ng/ml. All the 6 animals allotted to group spontaneously delivered within 72 h and the mean time taken for the expulsion of the first pup was determined as 65.33±1.8 days and in individual animals the delivery occurred between 61 to 71 h. Further the mean time taken for delivery of the entire litter was determined as 70 ± 2.07 h and ranged between 17 to 36 h (Table 10.). The litter size was multiple in every animal which delivered spontaneously. However, 25% of the puppies delivered were either born dead or died soon after and 75% of the neonates remained alive by 24h after the voluntary waiting period (Table 10).

4.7. Comparative study between the two treatment groups with that of control

Table 11. compares the efficacy of two different protocols used for induction of parturition in dogs and also compares the same with group of animals which did not receive any treatment for induction of parturition (Control). The mean serum progesterone concentration did not differ significantly between all the three groups ($P > 0.05$). The success rate of induction of parturition using mifepristone alone was 83.33% and the addition of PGF2α treatment to mifepristone did not improve the same. In comparison all the animals in the control groups delivered within 72 h after the initiation of the study. Further, the time taken for the expulsion of the first pup after the initiation of the treatment protocols did not differ significantly between the two treatment groups. However, it was significantly higher ($P<0.05$) in the control group. The time taken for the completion of the process of delivery also did not differ between two treatment protocols and was significantly shorter ($P<0.05$) as compared to the control. The highest number of viable foetus (92%) were observed in deliveries induced with a combined treatment involving mifepristone and PGF2α. The viable status of the puppies in groups of animals, where in the parturition was initiated with mifepristone alone was similar to that observed in the control group.

Table 1. Mean duration of pregnancy calculated as the interval from the day of first mating to the time of presentation in animals presented with the complaint of prolonged gestation

No. Of animals	Mean duration of pregnancy (in days)	Range (in days)
18	67.56 ± 0.67	65 – 76

Table 2. Mean duration of pregnancy calculated on the basis of ultrasonographic measurements of foetal head diameter in animals presented with the complaint of prolonged gestation

No. of animals	Mean duration of pregnancy (in days)	Range (in days)
18	63.75 ± 0.41	61 – 65

Table 3. Age and parity of animals presented with a complaint of prolonged gestation.(n=18)

Age	No. of animals	Parity	No. of animals
<2 years	4 (22.22%)	Pimipara	5(27.77%)
2-5 years	11(61.11%)	Pleuripara	13(72.22%)
>5 years	3(16.66%)		

Table 4. Previous parturition history of animals presented with a complaint of prolonged gestation.(n=13)

Type of delivery	No. of animals
Normal	12 (92.30%))
Caesarean section	1(7.70%)

Note: Out of 18 animals 5 were in their first parity, hence not included in the above table.

Table 5: Physical and genital tract examination findings in animals presented with a complaint of prolonged gestation (n=18)

Parameters	
Rectal Temperature °F (Mean ± Std error)	101.9 ± 0.2
No. Of animals exhibiting mammaray gland development (%)	18(100%)
No. Of animals exhibiting milk secretion	18 (100%)
No. Of animals exhibiting mucoid vaginal discharges	8 (44.44%)
No. Of animals exhibiting vulval edema	12(66.66%)
No. Of animals with vaginal relaxation	6 (33.33%)

Table 6: Ultrasonographic evaluation of the litter size and viability status of the foetus in animals presented with the complaint of prolonged gestation (n=18)

Litter size	No. of animals	Viability status	No. of animals
Single	5 (27.77%)	Viable	18 (100%)
Multiple	13(72.22%)	Non Viable	0

Table 7: Blood glucose, serum calcium and serum progesterone concentration in animals presented with a complaint of prolonged gestation (n=18)

Parameter	
Blood glucose concentration (mg/dl) (Mean ± std.error)	94.55 ± 3.25
Serum calcium concentration (mg/dl)	9.14 ± 0.22
Serum progesterone concentration (ng/ml)	8.08 ± 1.1

Table 8: Efficacy of Mifepristone in initiation of parturition in animals presented with the complaint of prolonged gestation (n=6)

Parameters	
Mean duration of pregnancy (interval from Ist mating to day of treatment) (in days)	66.50 ± 0.72
Mean serum progesterone concentration prior to initiation of treatment (ng/ml)	9.3 ± 2.4
No. of animals exhibiting initiation of parturition and completing the process of parturition within 72 h	5(83.33%)
Time of expulsion of first pup from initiation of treatment	Mean =22.01 ± 5.30 h (Range 14to 36h)
Time taken for the delivery of the entire litter	Mean = 23.17 ± 5.32 h (Range 14to 36h)
Litter size	
No. of animals delivering Single puppy	3(60%)
No. of animals delivering Multiple puppies	2(40%)
No. of puppies delivered live	9 out of 12 (75%)

Table 9: Efficacy of combined treatment with Mifepristone and PGF2α for initiation of parturition presented with the complaint of prolonged gestation (n=6)

Parameters	
Mean duration of pregnancy (interval from Ist mating to day of treatment) (in days)	69.50 ± 1.57
Mean serum progesterone concentration prior to initiation of treatment (ng/ml)	9.0 ± 2.3
No. of animals exhibiting initiation of parturition and completing the process of parturition within 72 h	5(83.33%)
Time of expulsion of first pup from initiation of treatment	Mean= 25.69 ± 3.7 h (Range 15 to 44)h
Time taken for the delivery of the entire litter	Mean=27.80 ± 3.6 h (Range 23 to 47)h
Litter size – No. Of animals delivering single pup	1 (20%)
No. of animals delivering Multiple pups	4 (80%)
No. of puppies delivered live	23 out of 25 (92%)

Table 10: Course of parturition in female dogs presented with the complaint of prolonged gestation and not receiving any treatment for induction of parturition (n=6)

Parameters	
Mean duration of pregnancy (interval from 1st mating to day of presentation) (in days)	66.67 ± 0.66
Mean serum progesterone concentration (ng/ml)	6.38 ± 0.26
No. of animals exhibiting initiation of parturition and completing the process of parturition within 72 h after initial presentation	6(100%)
Time of expulsion of first pup after the initial presentation	Mean =65.33 ± 1.80 (Range 61 to 71h)
Time taken for the delivery of the entire litter	Mean =70.0 ± 2.07 (Range 65 to 76h)
Litter size – No. of animals delivering single pup	Nil
No. of animals delivering Multiple pups	6 (100%)
No. of puppies delivered live	16 out 20 (75%)

Table 11: Comparison of the efficacy of two different protocols for induction of parturition in dogs presented with the complaint of prolonged parturition (n=18)

Parameters	Group I (Mifepristone treated animals) (n=6)	Group II (mifepristone + Pgf2α treatment group) (n=6)	Group III (Control) (n=6)
Mean serum progesterone concentration	9.3 ± 2.4^{a}	9.0 ± 2.3^{a}	6.00 ± 0.42^{a}
Percentage of animals delivering within 72 h after the initiation of the study (%)	83.33%	83.33%	100%
Time of expulsion of first pup from initiation of treatment	Mean $=22.01 \pm 5.30^{a}$ h	Mean$=25.69 \pm 3.7$ h^{a}	Mean $=65.33 \pm 1.8^{b}$
Time taken for the delivery of the entire litter	Mean $=23.17 \pm 5.32^{a}$ h	Mean$=27.80 \pm 3.6^{a}$	Mean $=70.0 \pm 2.07^{b}$
Litter size – single pup	60%	20%	Nil
Multiple pups	40%	80%	100%
Viability status of pups	75%	92%	75%

- **Values with common superscripts in each row are not significantly different (P>0.05)**

Discussion

V. DISCUSSION

Traditionally, breeders have assumed the parturition date in female dogs as 62 to 64 days from the day of first mating and pregnancy duration exceeds 65 days is considered by many breeders as cases of prolonged gestation. In nature, however, the interval from mating to parturition vary widely (59-59 days) (Doak *et al*., 1967; Phemister, 1973; Wildt *et al*., 1978; Concannon *et al*., 1983; Concannon *et al*., 1986; Moiser, 1986) .

Some studies have attempted to determine the factors affecting the variability in the gestational length. The influence of the breed on the length of gestation has been controversial. Okkens *et al*. (1993) reported that breed influenced the duration of gestation in bitches. The more recent study by Okkens *et al*. (2001), had at least 12 bitches representing each breed. The reported that West Highland white terrier had longer gestation duration than German Sheperd, Labrador Retrievers and Doberman. The same group analysing atleast 5 bitches from each breed had previously reported that German Sheperd dogs had shorter gestation duration when compared to Boxer, Burmese Mountain dogs, Old English sheep dog and Bouvier_Desflanders (Okkens *et al*., 1993). Eltis *et al*. (2005) reported that compared to Labrador Retrivers, German Shaperds, Golden Retrievers and Hounds were more likely to have longer gestations length. However, other investigators have found no breed effect on gestation length (Kutzler *et al*., 2003).

Litter size is another factor which has been studied for its influence on the length of gestation in bitches. Litter of a single Beagle pup had gestation in one study (Holst and

Phemister, 1974) and litter size was negatively correlated with gestation length in other studies (Okkens *et al*., 1993). However, in two other reports (Kutzler *et al*., 2003) the litter size did not affect the duration of gestation.

The effect of age or parity on gestation length has not been studied extensively in the bitches. A single study found primiparus bitches to have similar gestation duration as multiparus bitch (Okkens *et al*., 1993). Within breed, age had no effect on litter size (Okkens *et al*., 2003). Eilts *et al*. (2005) reported that age or parity had no effect on gestation length.

A more likely explanation for the apparent variability in the gestation length of bitches appears to be due to the tendency of the female to accept the male from 5 to 6 days before to 2 to 3 days after ovulation. Additional variability may be caused by the prolonged survival of ova (upto 3 days; Holst and Phemister, 1971) and of spermatozoa (upto 6 days; Doak *et al*., 1967; Concannon *et al*., 1983).

The duration of pregnancy timed as the interval from LH surge or as the interval from preovulatory rise in serum progesterone concentration to parturition has been reported to be less variable and ranged from 64 to 66 days and averaged 65.1 ± 0.1 days (Concannon *et al*., 1983; Kutzler *et al*., 2003).

However, in clinical practice methods of estimation of LH surge are not easily available, besides being expensive and time consuming. Similarly, facilities for determination of serum progesterone concentration either by RIA or by ELISA are available only in metros. Therefore, in practice, dog breeders generally mate their animals on the basis of Physical signs, predetermined dates or on the recommendations of a veterinarian who gives them the dates of mating after studying the vaginal exfoliative cytology. Unfortunately, these techniques have very little correlation with the preovulatory LH surge. And the duration of pregnancy in such cases may extend upto 72 days timed from the date of mating. In such a situation, it becomes extremely difficult for a practicing veterinarian to decide whether the animal that is presented with a complaint of having not delivered after the completion of 65 days from date of initial mating is a case of prolonged gestation or a case where the animal has only apparently completed 65 days of pregnancy but still has time to deliver. In case, he decides to wait, it may jeopardise the viability of the foetus, if its a true case of prolonged gestation. On the other hand, if he decides to intervene, he may be delivering immature pups if the animal had not completed 65 days of pregnancy. Sometimes, it also becomes necessary to heed to the request of the owners, to either perform an elective caesarean section or medically initiate parturition for the sake of his convenience.

In the present study, the animals included for the medical initiation of parturition had completed atleast 65 days of pregnancy from the date of first mating. In one animal, the interval was as long as 73 days. The mean duration of pregnancy at the time of presentation was calculated as 67.56 ± 0.67 days (Table 1). However, when the animals were subjected to transabdominal ultrasonography, the mean duration of pregnancy determined on the basis of foetal head diameter was found to be 63.75 ± 0.41 days and in individual animals, the duration of pregnancy was observed to range from 61-65 days (Table 2).

The results of the present study clearly showed a disparity between the duration of pregnancy calculated on the basis of mating dates and those recorded on the basis of ultrasonography. Although much has been learnt regarding the application of B-mode ultrasonography in small animal reproduction, concerns have been raised regarding its utility for accurate estimation of foetal age in view of the great differences in their size, conformation and litter size. The accuracy of prediction of foetal age with ultrasonography is reported to range from 50% to 90.9%. (Luvoni and Grioni, 2000 ; Kutzler *et al*., 2003; Bhagirathi, 2008). These observations further emphasise the difficulties faced by a practicing veterinarian in decision making, when presented with a case of female dog which has not delivered by day 65 after mating. Nevertheless, a decision has to be taken and it is best decided based on the physical signs of advanced pregnancy, presence of colostrums or milk in the mammary gland and ultrasonographic evaluation of the foetal growth and viability.

In the present study, medical initiation of parturition was attempted in only those animals which exhibited considerable mammary gland development along with the secretion of colostrums or milk, as this parameter is important for future viabilities of the puppies. The other factors that was taken into consideration were, the general health of the animal and the absence of any evidence that the animals are likely to deliver in the next 12 – 24 h. Evidence of the presence of the thick mucoid vaginal discharges, absence of vaginal relaxation and mild to moderate edema of the vulva were also considered to suggest that th animals were in advanced pregnancy and that the animals were not due for their delivery in the next 12 – 224 h (Table 5).

Among the animals selected for the present study 5 were primiparus and the rest were pleuriparus (Table 4) and this observation may suggest that the duration of pregnancy is apparently longer in pleuriparus. However, study by Okkens *et al*. (1993), reported that multiparus bitch have similar gestation duration as primiparus bitches (Table 4).

With regard to the breed of the animal presented with a complaint of prolonged gestation, no definite inference could be drawn as eighteen animals presented belonged to 11 different breeds. The influence of breed on the length of gestation has been highly controvertial. While, Okkens *et al*. (1993) reported that the breed influence on duration of bitches Kuzler *et al*. (2003) found no such effect.

In the present study, significant number of (61.11%) animals were aged between 2-5 years at the time of presentation (Table 3). However, Okkens *et al*. (2003) and Eltis *et al*. (2005) reported that age had no effect on the gestation length.

Ultrasonographic evaluation of the animals presented with a complaint of prolonged gestation revealed the litter size to be single in 27.77% of the animals and as multiple in 72.22% of the animals (Table 6). There are some reports that the time taken for the initiation of labor in animals carrying a single pup may be delayed as compared to those carrying multiple foetus (Roberts, 1986).

The plasma glucose and serum calcium levels of all the 18 animals included for the present study were within the normal physiological range (Table 7) suggesting that the animals were neither hyperglycaemic nor hypoglycaemic. However, hypocalcemia or hypoglycaemia has been indentified as an important cause of maternal dystocia and may play a significant role after the initiation of labor and are important for the progress of delivery rather than the initiation of delivery (Freak, 1975; Darvelid and Forsberg 1994; Freak 1962; Buckner, 1979).

The serum progesterone concentration of all 18 animals included for the present investigation were > 2ng/ml and the mean concentration was determined as 7.21 ± 1.23 ng/ml (Table 7.). The high progesterone recorded in the present study suggested the presence of an active functional corpus luteum. It has been reported that the maintenance of pregnancy require a serum progesterone concentration of atleast 2 ng/ml and the serum progesterone concentration must decrease to < 2 ng/ml for 2 days to result in termination of pregnancy (Selim, 1996). The high level of serum progesterone observed in the present study therefore suggested that the process of parturition had not been initiated in any of the animals.

In the present study, mifepristone which is a progesterone receptor blocker was evaluated for its efficacy in initiation of parturition in 6 animals which had completed 65 days of pregnancy calculated on the the basis of mating date. The serum progesterone concentrations were also above the basal levels (9.3 ± 2.4) suggesting the presence of an active functional CL. Mifepristone was used at a dose of 5mg/kg BW and was administerd orally twice daily for two days. Other studies have used at a slightly higher doses than the one used in the present investigation for induction of parturition. Weyden *et al*. (1999) administered mifepristone at a dose of 7.5 mg/kg BW per day orally until the time of birth of first pup. On the other hand, Nohr *et al*. (1993) employed mifepristone at a dose of 6 mg/kg BW subcutaneously. Mifepristone have also been used at a variable doses for induction of abortion (Concannon *et al*., 1989; Concannon and al., 1990), while Padmini, (2005), employed mifepristone at a dose of 20 mg/kg BW orally to terminate midpregnancy. Concannon *et al*. (1998), attempted termination of pregnancy with administration of 2.5 mg/kg mifepristone twice a day for 4.5 days starting at day 32 of gestation.

Aglepristone, also a progesterone receptor blocker has been evaluated more extensively for its efficacy for induction of either abortion or parturition at varying dose levels and duration (Galac *et al*., 2000; Abhilash *et al*., 2012). However, aglepristone is not available in India for clinical use.

None of these animals which were administered with mifepristone orally exhibited any side effects. On the other hand, local side effects such as thickening of the subdermis and local necrosis of the skin overlying the injection site has been reported in animals receiving aglepristone for induction of parturition (Baan *et al*., 2005).

Mifepristone has not been extensively evaluated for induction of parturition in female dogs. Weyden *et al*. (1989), reported that parturition was initiated between 26 and 78 h after oral administration of 7.5 mg/kg BW per day of mifepristone. Nohr *et al*. (1993), reported the use of mifepristone at 6 mg/kg BW administered repeatedly between day 57 and 59 of gestation in beagle bitches and reported that the initiation of parturition occurred within 26 – 40 h after the start of treatment.

Aglepristone has also been used more extensively for induction of parturition in female dogs. Fieni *et al*. (2009), the onset of parturition after administration of aglepristone in 95% of the bitches between 27.7 ± 5.6 after the treatment initiation. On the other hand, Baan *et al*. (2005), reported a slightly longer duration of 41.0 ± 3.7 h as the time taken for the initiation of parturition following the use of aglepristone. However, Fieni *et al*. (2009), uased oxytocin in association with aglepristone whereas Baan *et al*., used aglepristone alone making comparisons difficult.

The course of parturition was uneventful in all the five animals in which the parturition was initiated within 72 h after mifepristone administration . None of these animals needed vaginal digital manipulation or oxytocic therapy to assist the expulsion of pup. In contrast to these observations Baan *et al*. (2005) reported that interventions such as vaginal manipulation or injection with oxytocin were frequently necessary while inducing induction of parturition with aglepristone. Nohr *et al*. (1993), reported that aglepristone could only induce an incomplete parturition, which did not proceed beyond the stage of dilatation of the cervix.

The success rate in induction of parturition in the present study was 83.33%. The parturition was not initiated in the 6th animal and was subjected for caesarean section. The success rate observed rate in the present study similar to those reported by Weyden *et al*. (1989), who could induce parturition in 4 out of 5 mifepristone treated animals. In contrast, Nohr *et al*. (1993), could induce parturition in only one out of animal with the use of mifepristone.

Baan *et al*. (2005), demonstrated that aglepristone was an effective and a safe drug for induction of parturition in dogs. They reported that premature parturition was successfully induced in all the treated animals and that onset of whelping occurred within a short and predictable time frame.

In the present study a total of 12 puppies were delivered from 5 animals in which the induction of parturition was successfully induced with mifepristone. The low litter size obtained from 5 animals was primarily because of the fact that 3 animals delivered a single puppy. Further 25% (3 out of 12) puppies are delivered dead or died soon after delivery. On the other hand, Hoffmann *et al*. (1999), reported that administration of mifepristone alone resulted in still born pups. Fieni *et al*. (2009), reported the mean survival of puppies born after induction of parturition as 86% at birth and 68% at 48 h after birth. Baan *et al*. (2005), reported that only 5 out of 42 puppies were born dead in parturition induced with aglepristone.

The mechanism by which mifepristone induce parturition is not completely understood. In women mifepristone has been described to act as a competitive receptor antagonists for A and B forms of receptors. When administered orally, mifepristone causes decidual breakdown by blockade of uterine progesterone receptors, which increases uterine PGF2α levels and sensitizes the myometrium to the contractile actions of prostaglandins (Tripati, 1993). A separate effect of the compound is to cause cervical softening which may facilitate parturition. Results from other investigators suggests that antiprogestins may also reduce the progesterone secretion from the CL due to its inhibitory effects on hypothalamic and pituitary function (Schiason *et al*., 1985; Garzo *et al*., 1998).

In the second part of the study, trials were conducted to determine if the induction rate and puppy viability obtained following administration of mifepristone could be improved by simultaneous treatment with a potent uterotonic drug such as PGF2α. Mifepristone was used in the same dose and frequency as in the previous experiment and a natural PFG2α was administered subcutaneously twice daily or the beginning of the labor or upto 48h in cases where the labor had not begun

The owners reported that every animal which received PFG2α exhibited mild to moderate side effects such as hypersalivation, omitting and panting side effects of PFG2α administration observed in the present study has been previously reported (Renukaradhya *et al*., 2008; Abhilash *et al*., 2012).

The serum progesterone concentration of all 6 animals allotted to this group was also suggestive that the animals had not entered into the 1st stage of parturition. In 5 out of 6 treated animals the first pup was delivered at a mean time of 25.69 ± 3.7h following the first oral dose of mifepristone and first subcutaneous dose of PFG2α and in 5out of 6 animals the entire process of delivery was completed by 27.80 ± 3.6h after the initiation of treatment. The mean time taken for the onset of parturition as well as the time taken for the delivery of the entire litter was similar to the group of animals which received mifepristone alone for induction of parturition. The success rate of combined treatment with mifepristone and PFG2α for induction of parturition was also similar to the mifepristone treated group. The result of the present study therefore indicate that additional treatments with PFG2α neither enhances the success rate nor reduce the time taken for induction or completion of delivery process as compared to treatment with mifepristone alone. The time taken for the initiation of whelping was reported to be slightly longer 34.46 ± 4.70 h in the re;ports of Reddy *et al*. (2012) who administered 10 mg of mifepristone per kg BW orally followed by a single dose of clprostenol at 2.5 µg/kg BW for induction of parturition in bitch. Fieni *et al*. (2009), concluded that aglepristone at 15mg/kg on the day 58 of gestation and 24h later and subsequently at 2 h interval with 0.08mg/kg alfaprostol resulted in parturition in all the five bitches. The mean time of onset of parturition was determined as 32.6 ± 3.7 h. However, the mean expulsion time for bitches which received 0.5 IU of oxytocin every 2 h after two doses of aglepristone was significantly shorter (31. 6 ± 3.6 h). Blendinger *et al*. (1994), successfully initiated parturition by injecting aglepristone in the dose of 10 mg/kg followed by a second injection 24 h later. The animals also received PGF2α at 160 µg/kg BW commensing at 12 h after the first antiprogestin treatment and repeated 12 h later. The results of the study suggested that the combined treatment of antiprogestins with PGF2α is a useful approach to initiate parturition in a bitch.

A total of 25 pups were delivered from 5 bitches which received mifepristone and PGF2α for induction of parturition. The litter size was single in one animal and multiple in the rest. Further 92% of the puppies born were alive following induced delivery. This was a significant improvement over the mortality rate observed in puppies delivered with mifepristone alone. Fieni *et al*. (2009), reported the mean survival rate of the puppies born in bitches in which the parturition was induced with aglepristone and alfaprostol as 86%.

The improved viability of the puppies observed with the use of mifepristone and PGF2α may probably be due to rapid expulsion of puppies when uterotonics are employed. Unfortunately the owners of the animals had not recorded the time interval between the delivery of the puppies and it is probable this was shorter when PGF2α was used. In support of this statement , it was observed that a total of 25 puppies were delivered in mifepristone PGF2α treated animals over a period of 40 h where as it took almost the same time to deliver 12 puppies in the mifepristone treated group, suggesting that the puppies in the mifepristone PGF2α treated animals were delivered at more frequent intervals. Conversely, in the mifepristone treated group, the interval between the delivery of the puppies may have been much longer resulting in foetal death due to placental separation.

The administration of PGF2α along with mifepristone was expected to initiate parturition much earlier than when only mifepristone was administered because of its luteolytic properties. This was not observed in the present study and it is known that PGF2α need to be administered more frequently and over a longer period of time for complete luteolysis of the canine CL.

The efficacy of the two treatments employed for the induction of parturition was compared with another group of animals which were left untreated. The animals which did not receive treatment also had serum concentration above 2 ng/ml suggesting that spontaneous initiation of parturition had not begun in these animals. These animals had also completed atleast 65 days of pregnancy calculated from the 1st day of mating. The spontaneous expulsion of 1st pup was observed in all the six animals at an average of

65.33 ± 1.86 h and ranged between 61 to 72 h. Further, the mean time taken for the delivery of the entire litter from the time of presentation was determined as 70 ± 2.07 h. The time taken for the expulsion of the 1st pup as well as the time taken for the delivery of the entire litter was significantly longer in the untreated animals. Further, in 2 animals parturition was initiated but needed manual assistance in one and oxytocin therapy in another to complete the process of parturition.

About 25% of the puppies born during the spontaneous parturition were delivered dead, and these puppies were delivered from the animals which required manual assistance or oxytocin therapy.

The failure of initiation of parturition in one animal receiving only mifepristone and in another animal receiving mifepristone along with PGF2α was not very clear. It is possible that some animals may require either a higher dose of mifepristone or a more frequent administration than used in the present study to block all the receptors of progesterone at the uterine level.

The present study clearly established that mifepristone is capable of successfully initiating the labor in over 80% of the treated animals. The parturition was initiated rapidly and without any side effects. However, the delivery of the 25% of the puppies as dead cannot be accepted. The high mortality rate of the puppies could overcome if PGF2α was administered at frequent intervals following mifepristone administration. Although, PGF2α administration is associated with side effects, the induction was rapid, the delivery was smooth and the puppy survival rate was satisfactory.

Summary

VI. SUMMARY

A clinical study was carried out to determine the efficacy of mifepristone alone or in combination with PGF2α for induction of parturition in bitches presented with a history of having completed atleast 65 days of pregnancy calculated from the day of first mating. The study was carried out on a total of 18 animals and pregnancy period determined on the basis of mating dates averaged 67.56 ± 0.67 days. They belonged to 11 different breeds and 5 animals were in their 1st parity and the remaining had delivered 1 – 4 times. The selection of the animals was carried out on the basis of the presence of milk or colostrums in the udder, general health of the animal, signs of advanced pregnancy and finally on the basis of mating dates.

The serum progesterone concentration of all the 18 animals were determined to be >5 ng/ml indicating that spontaneous delivery had not yet been initiated. None of the animals were found to be hypoglycaemic or hypocalcemic.

The animals were randomly allotted to three groups of six animals each. Animals in Group A received oral administration of mifepristone at 5 mg/kg BW twice dailyuntil the beginning of parturition or for a maximum of four doses. Animals in Group B were also treated with mifepristone at the same dose level and frequency. In addition they were also administered a natural PGF2α at 0.1 mg/kg BW subcutaneously twice daily until the beginning of the labor and upto a maximum of 4 doses. The side effects, the success rate in initiation of labor, the interval from treatment to expulsion of the 1st pup, the course of labor, the time taken for the completion of labor from initiation of treatment and the puppy survival rate in the two treatment groups was compared to 6 other animals which

were left untreated. Administration of mifepristone was successful in initiation of parturition in 83.33% of the treated animals. The mean time taken for the expulsion of the 1^{st} pup and the delivery of the entire litter from the initial time of treatment averaged 22.01 ± 5.30 h and 23.17 ± 5.32 h respectively. However, 25% of the puppies delivered were born dead.

The success rate in the initiation of parturition with the use of combination of mifepristone and PGF2α was similar to those observed with mifepristone alone. Although, mild to moderate side effects were observed after PGF2α administration, the mean time taken for the expulsion of 1^{st} pup and the time taken for the delivery of the entire litter was comparable (25.69 ± 3.7 h and 27.80 ± 3.6 h respectively), in those observed in the animals treated with mifepristone alone. However, the puppy survival rate was as high as 92%.

In the animals which were left untreated, spontaneous delivery did eventfully occur but at a later time than in treated animals. The 1st pup was expelled at a mean time of 65.33 ± 1.80 h and the process of delivery was completed by 70.0 ± 2.07 h. Further, in some animals, interventions were necessary to complete the process of parturition.

The results of the present study clearly established that mifepristone was highly capable of initiation of parturition in bitch. It was >80% effective and the process was initiated rapidly and the delivery process was completed in a short span of time. However, puppy survival rate was unsatisfactory and it was observed that this could be over come if induction of parturition was carried out using a combination of mifepristone and PGF2α.

However, the failure of mifepristone or mifepristone and PGF2α to effectively initiate parturition in some of the animals warrants the need of further standardization of the dose and the frequency of their administration.

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Abstract

VIII. ABSTRACT

A clinical study was designed to evaluate the efficacy of mifepristone alone or its combination with PGF2α for initiation of labor in bitches which had completed at least 65 days of pregnancy on the basis of mating dates. Mifepristone was administered at a dose of 5 mg/kg BW orally twice daily until the initiation of labor or for a maximum of four doses in six bitches. Six other animals received natural PGF2α at 0.1 mg/kg BW twice daily subcutaneously in addition to mifepristone. The mean time taken for the expulsion of the first pup, the mean time taken for the expulsion of the entire litter and the puppy survival rate in the two treated groups was compared with a group of 6 other animals which did not receive any treatment. Treatment with mifepristone alone or in combination with PGF2α effectively initiated parturition in over 80% of the treated animals. The time taken for the expulsion of the first pup and the time taken for the expulsion of the entire litter in the treated animals was significantly shorter than the control group. The puppy survival rate was highest in animals receiving a combined therapy of mifepristone and PGF2α. It is concluded that initiation of parturition is best carried out using a combination of mifepristone and PGF2α.