PROGESTERONE PROFILES AND CHANGE IN RECTAL TEMPERATURE IN INDUCED WHELPING BITCHES

K.RAM CHANDRA REDDY*[1], K.SADASIVA RAO[2], K.G.S.RAJU[3] AND A.G.REDDY[4]

Dept. of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, S.P.V.N.R. Telangana State Veterinary Animal Fisheries Science University, Rajendranagar, Hyderabad -500030 (T.S.)

Received : 26.01.2014

ABSTRACT

Accepted : 01.04.2014

The present study was conducted in 29 clinical cases. Whelping was induced by using cloprostenol, dinoprostone plus cloprostenol and mifepristone plus cloprostenol. The mean duration of time interval from starting of treatment to beginning of whelping was 34.46 ± 4.70, 40.48 ± 6.47and 26.98 ± 4.51 hours in three treatment groups respectively. The mean plasma progesterone concentrations were 3.89 ± 0.54, 3.54 ± 0.31, 2.72 ± 0.41 and 3.83 ± 0.2 ng/ml before starting of treatment; 1.92 ± 0.13, 1.86 ± 0.12, 2.87 ± 0.5 and 1.55 ± 0.21 ng/ml at 2 to 12 hours before whelping and 0.56 ± 0.06, 0.53 ± 0.08, 1.18 ± 0.4 and 0.74 ± 0.13 ng/ml during 0 to 6 hours after whelping in three treatment groups respectively. The mean rectal temperature was 101.23 ± 0.20, 101.62 ± 0.17, 101.86 ± 0.30 and 102.08 ± 0.08 °F before starting of treatment; 99.98 ± 0.53, 100.85 ± 0.28, 101.43 ± 0.35 and 100.5 ± 0.22 °F at 12 hours after treatment; 99.94 ± 0.21, 100.15 ±0.3, 101.5 ± 0.38 and 99.95 ± 0.24 °F at 24 hours after treatment; 100.75 ± 0.48, 101.79 ± 0.10, 101.88 ± 0.32 and 101.3 ± 0.14 °F at 36 hours after treatment and 101.5 ± 0.75, 102.0 ± 0.5 and 101.8 ± 0.16 °F at 48 hours after treatment in cloprostenol, dinoprostone plus cloprostenol, mifepristone plus cloprostenol and control groups respectively.

Keywords: Bitches, Whelping, Induction, Progesterone, Rectal temperature.

INTRODUCTION

Progesterone is necessary for maintaining pregnancy in mammals (Heap *et al.*, 1997). In dogs, Progesterone is secreted only from the corpus luteum (Tsutsui *et al.,* 1989). In bitches whelping takes place with an average gestation length of 61.4 days, when bitches are mated once on the guidance of the preovulatory increase of the plasma progesterone concentration (Okkens *et al.,* 2001). One to two days prior to whelping, plasma progesterone concentration decreases rapidly (Vander Weyden *et al.,* 1989). As a result of decrease in progesterone and rise in $PGF_{2\alpha}$ concentration, myometrial activity gradually increases, which leads to onset of whelping. It remains unclear which signal(s) trigger(s) these hormonal changes associated with whelping in bitches. In bitches information about foetal hormone secretion is not available and circulating estrogen concentration decreased rather than increase towards parturition in bitches (Onclin *et al.,* 2002). In addition, the circulating estrogens seem to be ovarian rather than placental origin in dogs. In bitches whelping dates are difficult to predict due to lot of variation in ovulation time. The objective of the present study was to observe the changes in progesterone hormone levels and also to monitor the change in rectal temperature during parturition in bitches, in addition to compare the changes of these two observations in normal and induced whelping bitches.

*Part of Ph.D. Thesis submitted by the first author to S.V.V.U., Tirupati.[1]corresponding author and Associate professor, Mobile 91-9849235761; E-mail krcreddy_scientist@yahoo.co.in, [2]Professor &University Head, [3]Professor TVCC, Produtoor, [4]Professor &University Head, Dept. Vey, Pharmacology & Toxicology

MATERIALS AND METHODS

Clinical cases of 29 bitches of different breeds and age groups presented to the teaching veterinary hospitals of College of Veterinary Science, Rajendranagar, Hyderabad, (AP) with history of more than 65 days of gestation, but not showing any signs of whelping were utilized for medical induction of parturition(for easy whelping in multiple fetus and avoid prolonged gestation in single & twine fetus pregnancy). This study was carried out from October 2007 to May 2009. Pregnancy in these bitches was confirmed by ultrasonography using a B-mode ultrasound scanner (Esaote piemedical Benelux B.V., P.O.Box 1132, 6201 BC. Maastricht) with 5 MHz linear transducer by Trans abdominal method. Gestational age was estimated by correlating the crown rump length(possible in only few cases), abdominal diameter, by monitoring the growth of organs and cardiac diameter of foetus with the standard graph recommended by Khan (2004). During these examinations foetal heart beats were counted and cases which had > 200 foetal heart beats per minute were only used for the induction of parturition. They were also observed for foetal viability and since the fetal heart beat was less than 180 / minute cesearean section was suggested.

The bitches were randomly distributed into four groups: Group 1: Cloprostenol group (n= 6) were treated with cloprostenol 2.5µg/kg bwt, sc, as single dose (Vetmate 2 ml vial, each ml contains 250 µg cloprostenol, Vetcare, Bangalore, 560 106, India), Atropine sulphate 0.04 mg/kg b wt, sc (Tropine 1 ml ampoule contain 0.6mg Atropine sulphate) 10-15 minutes prior to cloprostenol administration. Group 2: Dinoprostone plus Cloprostenol group (n=10) were administered dinoprostone gel 0.5mg (Cerviprime gel contains 0.5mg of dinoprostone in 3.0 gm base, its supplied in a syringe with detachable plastic nozzle, Astrazeneca Pharma India Limited, 12th Mile, Bellary Road, Bangalore, India.) intra cervical and anterior portion of vagina by using the syringe and the plastic nozzle supplied with the packing. This syringe was inserted intra vaginal until feeling the resistance from the cervix to push it further forward. Following this cloprostenol and atropine sulphate was administered in the same manner as in the Group 1. Group 3: Mifepristone group (n=7) bitches were treated with mifepristone 10mg/kg body weight po (MT Pill, each tablet contains 200 mg mifepristone, Cipla Ltd., Kumrek, Rangpo Sikkim -737132, India.) as a single dose (Instead of multiple doses to have quick action and initiation of whelping shortl after treatment). If the bitch did not whelp within 24 hours, cloprostenol and atropine sulphate was administered in the same manner as in the Group 1. Group 4: Control Group (n=6) bitches which had a normal whelping.

For estimation of plasma progesterone concentration three milliliters of whole blood was collected from the cephalic or saphenous vein into heparinized vaccutainers. The blood samples were centrifuged within 30 minutes at 2000 RPM for 20 minutes. The supernatant plasma was collected into sterile cryotubes and stored at -20 °C until the hormone assay was performed. For Progesterone estimation, blood sample was collected at the beginning of treatment and subsequently 2-12 hours before whelping and between 0-6 hours of whelping. Plasma progesterone concentration was estimated by using Enzyme Linked Immuno Sorbent Assay (ELISA) using ELISA kit (United Biotech INC, California, USA). This test provides quantitative measurement of progesterone and results were expressed in ng / ml. The progesterone estimation sensitivity of this kit is 0.01 ng /ml. Changes in rectal temperature were recorded with digital thermometer and was recorded in °F, before the starting of treatment and at every 12 hours after the starting of the treatment until the beginning of whelping for each bitch. The data collected were subjected to statistical analysis by ANOVA (Snedecor and Cocheron, 1989).

RESULTS AND DISCUSSION

The mean gestation length from first mating to the time of initiation of treatment was 66.5 ± 1.2, 67.7 ± 0.58 and 65.14 ± 1.65 days in cloprostenol, dinoprostone plus cloprostenol and mifepristone plus

cloprostenol groups, respectively. The treatment requires about one or two more days from initiation of treatment to complete the whelping. So with the addition of this period, mean gestation length in treatment groups were extended by one to two days. In control group, the gestation length was 62.67 ± 0.92 days. Statistically significant difference ($p<0.05$) was observed between treatment and control groups. Iron *et al.* (1997) recorded prolonged pregnancy in bitch due to lack of luteal regression. Longer gestation lengths in treatment groups might be due to the presence of single fetuses (1/6, 2/10, 2/7 bitches in group I, II, III respectively) and two foetuses (1/6, 3/10, 1/7 bitches in group I, II, III respectively) in pregnant bitches, This was in agreement with the observation of Jackson (2004) (Some times it may also be due to presence of more number of foetuses). Whereas Moriyoshi *et al.* (1999) and Baan *et al.* (2008) experimentally induced parturition at 56th day and 59.5 ± 0.2 day of gestation length respectively.

Mean plasma progesterone concentrations of the treatment and control group are presented in Table 1. The main reason for decrease of progesterone concentration was due to luteolytic effect of cloprostenol (Baan et al 2008; Meier and Wright 2000 and Moriyoshi *et al.,* 1999). There is no statistical significance between the treatment and control groups before starting of treatment, at 2 to 12 hours before whelping. Statistical analysis revealed that mifepristone plus cloprostenol group had significantly ($p < 0.05$) higher level of plasma progesterone concentration, cloprostenol, dinoprostone plus cloprostenol and control groups had similar levels of plasma progesterone concentrations. The mean plasma progesterone concentration during 0 to 6 hours after whelping had no significant difference ($P < 0.05$) between the treatment and control groups, but slightly increased plasma progesterone concentration was observed 0 to 6 hours after completion of whelping in mifepristone plus cloprostenol group.

The mean plasma progesterone profiles recorded in the bitches treated with cloprostenol alone were slightly higher at the beginning of treatment where compared to observations of Baan *et al.,* (2008), Meier and Wright (2000) and Moriyoshi *et al.,* (1999) who recorded 12.4±3.2 ng/ml, 5.69 ± 1.19 ng/ml and 6.4 to 17.0 ng/ ml respectively. The Progesterone concentration observed 2 to 12 hours before whelping was comparable with the findings of above authors who recorded 1.2 to 2.6, 1.0 and 2ng/ml, respectively 7 to 24 hours after prostaglandin administration. After the end of treatment Meier and Wright (2000) was recorded 0.54 ± 0.1 ng/ml of progesterone, which was similar to the progesterone levels observed at 0 to 6 hours after whelping in present study. The reasons for lower progesterone concentrations before induction of whelping in this study than those observed by the above authors were attributed to the early induction of whelping at 56-59 days (from the beginning of diestrum) of gestation in their studies, where as induction started after 66.5± 1.2 days (from first mating) of gestation in the present study.

The decrease in progesterone concentration was similar to that of control group of bitches. Progesterone concentration was 0.5 ng / ml on the day of whelping (Concannon *et al.,* 1978). In this study also, whelping occurred after progesterone concentration reached around the same level. The exogenous administration of PGF2α (cloprostenol) might have caused regression of the corpus luteum and decrease in the plasma progesterone concentration in the present study. The myometrial activity might also have been gradually increased to facilitate the whelping (Chakraborty 1987).

The mean plasma progesterone concentrations in mifepristone plus cloprostenol group were in agreement with the findings of Fieni *et al.* (2001), Who recorded increased progesterone concentration (8 nmol/L) at the time of parturition in aglepristone induced parturition in bitches. The increase in progesterone concentration 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 result in increased pituitary secretions (FSH and LH). High progesterone levels were observed in induced parturition bitches at the time of whelping (Fieni and Gogny, 2008; Baan *et al.,* 2005), This was supporting the findings of the present study

Rectal temperatures of bitches were recorded and presented in Table No. 2, significant difference was observed between the treatment and control groups with respect to rectal temperatures before starting of treatment, 12, 36, 48 hours after treatment. But at 24 hours after the treatment mifepristone plus cloprostenol group had significantly ($p < 0.05$) higher rectal temperature than the cloprostenol group, dinoprostone plus cloprostenol group and control groups, where as other three groups had statistically similar rectal temperatures. Slight decrease in rectal temperature was observed 12 to 24 hours after treatment and again it reached to the normal rectal temperature around 12 hours or less before parturition in cloprostenol, dinoprostone plus cloprostenol and control groups. But such change was not observed in mifepristone plus cloprostenol group. In this group, the rectal temperature remained constant from the initiation of treatment and up to whelping. Findings of the present study were in accordance with the observations of Baan *et al.,* (2008); Meier and Wright (2000); Moriyoshi *et al.,* (1999). The decrease in plasma progesterone concentrations causes a decrease in the thermogenic effect of progesterone. Decrease of 1-2 °C rectal temperature was observed both in induced and normal whelping bitches (Baan *et al.,*2008; Concannon *et al.,* 1977). Similarly drop of 1 to 2 °F in body temperature from 24 to 48 hours before parturition was noticed by Copley (2002).

In conclusion, the results of this study indicating that the progesterone levels decrease was observed in cloprostenol group, dinoprostone plus cloprostenol and control groups at the time of parturition close to 0.5ng/ml. where as in mifepristone plus cloprostenol group higher levels of progesterone was observed at the time of parturition it indicating that in this group corpus luteum is not completely regressed at the time of whelping, but whelping had taken place due to progesterone receptor blocking action of mifepristone. Similarly decreases in rectal temperature is observed 12- 24 hours before parturition in all groups except in mifepristone plus cloprostenol group, this is in correlation with the decrease in progesterone hormone levels and its thermogenic action in pregnant bitches.

Table 1. Plasma progesterone profiles (ng/ml) in induced and natural whelping bitches.

S.No.	Period	Cloprostenol	Dinoprostone + Cloprostenol	Mifepristone + Cloprostenol	Control*
1	At the time of treatment*	3.89 ±0.54	3.54 ± 0.31	2.74 ± 0.41	3.83 ± 0.2
2	2-12 hours before whelping	1.92 ± 0.13[a]	1.86 ± 0.12 [a]	2.87 ±0.5[b]	1.55 ± 0.21[a]
3	0-6 hours after whelping	0.56 ±0.06	0.53 ±0.08	1.18 ±0.4	0.74 ±0.13

Means bearing different superscripts row wise differed significantly ($p< 0.05$).

*Plasma progesterone concentration was measured 2 days before parturition in control group. (Approximate time of whelping was predicted based on ultrasound scanning, radiography, clinical symptoms and mating history)

Table 2.Change of rectal temperature in induced and natural whelping bitches

S.NO.	Period	Therapeutic groups			
		Cloprostenol (Mean ± SE)	Dinoprostone + Cloprostenol (Mean ± SE)	Mifepristone + Cloprostenol* (Mean ± SE)	Control (Mean ± SE)
1	At the time of treatment	101.23± 0.20	101.62 ± 0.17	101.86 ± 0.3	102.08 ± 0.08
2	12 hours after treatment	99.98 ± 0.53	100.85 ± 0.28	101.43± 0.35	101.5 ± 0.22
3	24 hours after treatment	99.94± 0.21[a]	100.15± 0.30[a]	101.5± 0.38[b]	99.95±0.24[a]
4	36 hours after treatment	100.75±0.48	101.79 ± 0.10	101.88 ± 0.32	101.3 ±0.14
5	48 hours after treatment	101.5± 0.75	102± 0.50	-	101.8 ± 0.16

Means bearing different superscripts row wise differed significantly ($p< 0.05$).

* All bitches delivered before 48 hours

REFERENCES

Baan, M., Tavverne, M.A.M., deGier, J., Kooistra, H.S., Kindahl, H., Dieleman, S.J. and Okkens, A.C. (2008). Hormonal changes in spontaneous and aglepristone induced parturition in dogs. *Theriogenology,* **63:** 1958-72.

Baan, M., Tavverne, M.A.M., Kooistra, H.S., deGier, J., Dieleman, S.J. and Okkens, A.C. (2005). Induction of parturition in the bitch with the progesterone – receptor blocker aglepristone. *Theriogenology,* **63:** 1958-72.

Chakraborty, P.K. (1987). Reproductive hormone concentrations during estrus, pregnancy and pseudo pregnancy in the Labrador bitch. *Theriogenology,* **27:** 827 – 40.

Concannon, P.W., Powers, M. E., Holder, W. and Hansel, W. (1977). Pregnancy and parturition in the bitch. *Biol. Reprod.,* **19:** 517-26

Concannon, P., Butler, W.R., Hansel, W., Knight, P.J. and Hamilton, J.M. (1978). Parturition and lactation in the bitch: serum progesterone, cortisol and prolactin. *Biol. Reprod.,* **19:** 1113 -1118.

Copley, K. (2002). Comparison of traditional methods for evaluating parturition in the bitch versus using external fetal and uterine monitoring. In Proceedings of the society of Theriogenology Annual Conference, Colorado Springs, CO, 2002. *Society of Theriogenology,* P. 375 – 82.

Fieni, F. and Gogny, A. (2008). Proceedings of the 6th International Symposium on Canine and Feline Reproduction and 6th Biannual European Veterinary Society for Small Animal Reproduction Congress. Vienna, Austria, 2008. cited in International Veterinary Information Service; (www. ivis. Org)

Fieni, F., Bruyas, J. F., Batut, I. and Tainturier, D. (2001). Clinical use of Anti- progestins in the bitch. Cited in www. ivis. Org.

Heap, R.B., Gall. A.K., Horrison, F.A., Jenkin, G. and Perry, J.S. (1997). Progesterone and estrogen in pregnancy and parturition: comparative aspects

and hierarchical control. *Ciba Found Symp.* **47:** 127-57.

Iron, P.C., Nothing, J.O. and Volkmann, D.H. (1997). Failure of luteolysis leads to prolonged gestation in a bitch: A case report. *Theriogenology,* **48:**353-359.

Jackson, P.G.G. (2004). Hand book of veterinary obstetrics, 2nd edition. WB Saunders company, Philadelphia. Page No. 27 & 49

Khan, W. (2004). Veterinary Reproductive Ultrasonography. 2nd Edition Schluetersche Verlags gesellschaft mbH & Co. KG. pp.242

Meier, S. and Wright, P.J. (2000). The induction of parturition in the bitch using sodium cloprostenol. *Theriogenology,* **54 :** 457 – 465.

Moriyoshi, M., Maruyama, Y., Iseki, H., Nakada, K. and Nakao, T. (1999). Induction of parturition in bitches with minimal side effects by two injections of a low dose of fenprostalene, a prostaglandin F2 alpha analogue, and pretreatment with prifinium bromide. *J.Vet.Med.Sci.,* **61**(7): 781-786

Okkens, A.C., Teunissen, J.M., VanOsch, W., Van Den Brom WE, Dieleman SJ, Kooistra HS. (2001). Influence of litter size and breed on the duration of gestation in dogs. *J Reprod Fertil., Suppl.* **57:** 193-197.

Onclin, K., Murphy, B. Verstegen, J.P. (2002). Comparisons of estradiol, LH and FSH patterns in pregnant and non pregnant beagle bitches. *Theriogenology,* **57 :** 1957 -72.

Snedecor, G.W. and Cochron, W.G. (1989). Statistical methods 8th Edition, Iowa State University Press, Ames, Iowa.

Tsutsui, T., Kawakami, E., Orima, H., Yamauchi, M., Okubo, T. and Stabenfeldt, G.H. (1989). Effect of prostaglandin F2 alpha -analogue administration on luteal function, implantation of embryos and maintenance of pregnancy in bitches. *J.J.Vet.Sci.,* **51:** 496-504 cited in CAB abstract.

Vander Weyden, G.C., Taverne, M.A., Dieleman, S.J., Wurth, Y., Bevers, M.M. and Van Oord, H.A.(1989) Physiological aspects of pregnancy and parturition in dogs. *J Reprod Fertil., Suppl.* **39 :** 211 – 24.