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C Das

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

PM Barua

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

D Borpujari

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

A Das

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

PJ Nath

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

M Dutta

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

M Dasgupta

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

M Hazarika

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

S Tamuly

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

Corresponding Author:**PM Barua**

College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati, Assam
India

Role of progesterone, relaxin, C - reactive protein during gestation and whelping in crossbred bitch

C Das, PM Barua, D Borpujari, A Das, PJ Nath, M Dutta, M Dasgupta, M Hazarika and S Tamuly

Abstract

Background: Detection of pregnancy and prediction of whelping time are important for reproductive management in canine. The present study was aimed to study the role of certain blood biochemical profile during gestation *viz.* progesterone, relaxin and C - reactive protein in detection of pregnancy and whelping time in bitch.

Methods: A total of twenty (20) pregnant crossbred bitches presented to the Veterinary Clinical Complex, Khanapara, Guwahati were selected for the study. The gestational length was divided into first, second and third phase consisting 0(day of mating)-20 day, 21-40 day and 41 days to term (day of whelping). Blood samples were collected to estimate serum progesterone, relaxin, and C - reactive protein using ELISA on 0 (day of mating), 10th, 20th, 30th, 40th, 50th day of gestation and on the day of whelping.

Result: The mean serum progesterone level increased from 10th day (29.97±0.54ng/ml) to 30th day (40.42±0.57ng/ml), while decreasing from 40th day of gestation (35.60±0.33) and recorded lowest (1.66±0.25 ng/ml) on day of whelping. The mean serum concentration of relaxin gradually increased from 10th day (0.39 ±0.03ng/ml) till 50th day of gestation (5.54 ±0.39ng/ml). On day of whelping it dropped to 3.71 ±0.86 ng/ml. The mean serum level of C - reactive protein increased from the 0 day (5.15±0.20 mg/L) to 40th day (86.27±1.55 mg/L) of gestation which decreased then from 50th day gestation (57.95±2.37) and on the day of whelping recorded as 11.62±2.32 mg/L. The mean serum concentration of progesterone, relaxin and C - reactive protein significantly ($p<0.01$) differed between days of gestation in crossbred bitches.

Keywords: bitch, C-reactive protein, progesterone, relaxin, whelping

Introduction

Accurate prediction of the whelping date is necessary to plan an elective C-section and protect the life of the bitch as well as her pups and for better management. In recent years there is a quest for reliable biochemical tests for easy and early detection of pregnancy in canine. Progesterone, relaxin and C-Reactive Protein estimation are found to be helpful to confirm pregnancy in canine. Progesterone hormone is one of the most important female hormones and is essential for establishment and maintenance of pregnancy. In canine, relaxin hormone is detectable only during gestation and hence serves as a useful endocrine diagnostic marker of pregnancy in this species (Fusi and Veronesi., 2022) [2]. Measurement of relaxin hormone concentration has been used for pregnancy diagnosis between 19 and 28 days after LH surge in dogs (Buff *et al.*, 2001) [1]. Implantation of the developing embryo in the endometrium and development of placenta may be the likely cause of an acute phase response leading to an increase in the concentration of C-reactive protein during mid-gestation (Kuribayashi and Shimada, 2003) [14]. The present study was therefore undertaken to evaluate the serum profile of Progesterone, relaxin and C-reactive protein in pregnant bitches to verify how these profiles could be applied for diagnosis of gestational stage and whelping time.

Materials and Methods

The present study was conducted in the Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam persevering a humid sub-tropical climate where wet season is mostly cloudy, hot and dry season is warm and clear. A total of twenty (20) pregnant crossbred bitches free from any history of reproductive diseases were selected. The gestational length of the bitch was divided into first phase, second phase and third phase consisting 0-20 days, 21-40 days and 41 days to term, respectively.

Blood samples were collected on 0 (day of mating), 10th, 20th, 30th, 40th, 50th day of gestation and on the day of whelping. Serum Progesterone, Relaxin, and C-Reactive Protein were assayed for quantitative estimation by ELISA. Statistical analysis was performed as per Snedecor and Cochran (1989) [23] and MS Excel 2019 software was used for analysis of data.

Results and Discussion

A) Serum Progesterone Concentration

The present study revealed that, the mean serum progesterone concentration increased from 10th day of gestation which recorded highest on 30th day of gestation (40.42±0.57 ng/ml). On 40th day of gestation progesterone level decreased to 35.60±0.33 ng/ml and recorded lowest 1.66±0.25 ng/ml on the day of whelping (Table 1, Fig.1). Progesterone, in bitch, is solely produced from CL which supports development of endometrium and placenta and by inhibiting uterine contractions, which 24 h prior to parturition falls below 2 ng/ml due to rapid luteolysis (Concannon, 2002) [5]. Concannon and Digregorio (1986) [4] observed that during the last third of pregnancy, progesterone levels slowly decreased to 4-16 ng/ml which was maintained for 1-2 weeks and then dropped abruptly to a value below 2 ng/ml 12-24 hours prior to whelping. Though, fetoplacental endocrine changes initiated by maturation of the fetal hypothalamic-pituitary-adrenal axis in dog, cortisol might act directly to stimulate prostaglandin release in the placenta or uterus to rapid luteolysis. Therefore, pre-partum luteolysis occurs around 60 days after ovulation, following PGF_{2α} release from the utero/placental compartment (Kowalewski, 2014) [13].

Margaret and Root (2005) [17] reported that in all normal dogs, serum progesterone concentrations remain high in diestrus. Because, absence of release of an effective luteolysin from the uterus helps maintenance of corpora lutea after pregnancy recognition. This prolonged luteal phase in dogs precludes ability to use measurement of serum progesterone concentrations for pregnancy diagnosis. Serum progesterone concentrations can also be used to assess the time of the

impending parturition of bitches (Rota *et al.*, 2019; De Cramer and Nöthling, 2018) [21, 6]. However, the progesterone concentrations are not different during the last 3 days of pregnancy. In the present study, variation of mean serum progesterone level was found significantly ($p<0.05$) differed between days of gestation.

B) Serum Relaxin Concentration

In the present study, serum relaxin level 0.61 ±0.13 ng/ml was found at 0-day (day of mating) which gradually increased upto 5.54 ±0.39 ng/ml at 50th day of gestation. On the day of whelping mean serum concentration of relaxin reduced to 3.71 ±0.86 ng/ml (Table 1, Fig. 1). Margaret and Root (2005) [17] reported that serum relaxin concentrations in pregnant dogs rise significantly at beginning 20-30 days of gestation, and peak at mid-gestation. Sebastian (2020) [22] stated that concentrations of relaxin begin to increase from day 25 and peak at days from 40 to 50, followed by a slight decline before parturition. Relaxin is produced primarily by the canine placenta and therefore, can be considered as a pregnancy-specific hormone in the dog. In the present study, the similar trend of serum relaxin level which observed with that of progesterone on different days of gestation can be stated that relaxin has luteotropic effect to maintain progesterone throughout gestation. Thus, relaxin can be taken as a useful marker of early pregnancy diagnosis (Einspanier *et al.*, 2002; Purohit and Gaur, 2004; Kustritz 2005; Özyurtlu *et al.*, 2006; Onclin and Verstegen, 2008; Umamageswari *et al.*, 2016) [8, 20, 15, 19, 18, 25]. Relaxin causes softening of the connective tissue in the cervix and promotes elasticity of the pelvic ligaments (Sebastian, 2020) [22]. In the present study, the level was found increased at 30th day gestation. Pregnancy may be diagnosed as early as 21-day post breeding by detecting relaxin in canine maternal blood (Margaret and Root, 2005, Kowalewski *et al.*, 2020) [17, 12].

In the present study, the serum relaxin concentration differed significantly ($p<0.05$) amongst 40th, 50th days of gestation and on the day of whelping.

Table 1: Serum progesterone, relaxin and c-reactive protein (Crp) level (Mean±se) during different phases of gestation in crossbred bitch

Hormone	Phases of gestation						
	First phase (0-20 days)			Second phase (21-40 days)		Third phase (41-term)	
	0 (Day Of Mating)	10 TH	20 TH	30 TH	40 TH	50 TH	Term (Day Of Whelping)
Progesterone (ng/ml)	5.01 ^a ±0.39	29.97 ^b ±0.54	32.62 ^c ±0.16	40.42 ^d ±0.57	35.60 ^e ±0.33	23.80 ^f ±1.40	1.66 ^g ±0.25
Relaxin (ng/ml)	0.61 ^a ±0.13	0.39 ^a ±0.03	0.59 ^a ±0.01	1.13 ^a ±0.69	3.78 ^b ±0.48	5.54 ^c ±0.39	3.71 ^d ±0.86
CRP (mg/L)	5.15 ^a ±0.20	5.92 ^a ±0.83	31.03 ^b ±2.59	79.93 ^c ±46	86.27 ^d ±1.55	57.95 ^e ±2.37	11.62 ^f ±2.32

Means bearing different superscripts within a row differ significantly ($p<0.05$)

C) Serum C-reactive protein (CRP) Concentration

In the present investigation, the mean serum concentration of CRP was found 86.27±1.55 mg/L at 40th day of gestation and decreased to 57.95±2.37mg/L at 50th day of gestation. On the day of whelping Serum C-reactive protein level was recorded 11.62±2.32mg/L (Table 1, Fig. 1). The lowest level of CRP was recorded 5.15±0.20mg/L at 0-day of mating which triggered to 31.03±2.59mg/L by 20th day of gestation. The C-reactive protein (CRP) is an acute-phase protein (APP) that produced upon stimulation of pro-inflammatory cytokines by any tissue injury and develops following either infectious, immunologic, neoplastic, traumatic events or from other causes (Ceron *et al.*, 2005) [2]. Eckersall *et al.*, (1993) [7] suggested that implantation of developing embryo in the endometrium and placental development are the results of an

acute phase response, which ascribed as 'the influence of endocrine hormones during pregnancy' (Kuribayashi and Shimada, 2003) [14]. In the present study, the increased level of CRP 31.03±2.59mg/L at 20th day of gestation, can be ascribed as acute phase response occurred due to embryo and placental development. Kuribayashi and Shimada (2003) [14] reported that the peak reached between 70 and 90 mg/L within 1-1.5 months after ovulation. The reaction of the uterus to the foreign antigen appeared to produce an antigenic reaction which results in the uterine swellings around the blastocyst and eventually an acute phase of elevated protein production from liver. Eckersall *et al.* (1993) [7] reported similar findings of acute phase response which had been identified during mid-gestation in bitches by determination C-reactive protein (CRP) concentration in blood serum.

Harvey (1991)^[10] postulated that the high concentrations of CRP could occur in female dogs during pregnancy might be in response to processing of blastocyst hatching which does not occur until almost 3 week after ovulation. Moreover, the increasing concentrations of CRP in dogs can be evaluated in clinical exams as a marker of both acute and chronic inflammatory disorders (Ceron *et al.*, 2008)^[3]. Ceron *et al.*, (2005)^[2] claimed that the increasing concentrations of CRP in dogs can be detected after 4 h from the time of the insult with a peak concentration at 24–48 h. From the findings of various

study conducted on female pregnant dogs it can be stated that CRP concentration increase in the first or second trimester of pregnancy in the bitch, followed by a decrease before parturition (Eckersall *et al.*, 1993; Kuribayashi and Shimada, 2003)^[7, 14]. The present study indicates that parturition induces CRP concentrations, likely due to physiological uterine inflammatory conditions (Thomson *et al.*, 1999; Leong *et al.*, 2008)^[24, 16]. It has ‘moderate’ accuracy as a marker of impending parturition in dog by C - reactive protein (Kjelgaard-Hansen *et al.*, 2003)^[11].

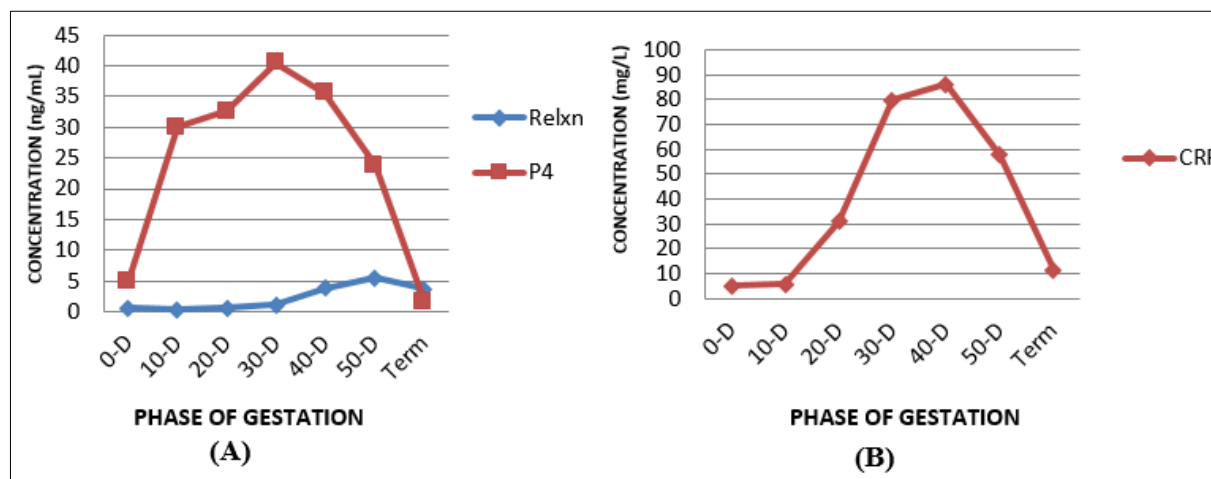


Fig 1: Mean serum concentration of Progesterone, Relaxin (A) and C-reactive protein (B) during different phases of gestation in crossbred bitch

Conclusion

From the present study, it could be concluded that serum progesterone supports pregnancy in dog which occurs from day of mating with highest peak by 30th day of gestation. The serum relaxin maintains a similar trend of level to that of serum progesterone and supportive in maintenance of progesterone level during gestation. Detection of C - reactive protein in blood serum confirms embryo developed in gravid uterus.

On the day of whelping, serum progesterone and relaxin level significantly ($p < 0.05$) reduced to 1.66 ± 0.25 ng/ml and 3.71 ± 0.86 ng/ml, respectively. C-reactive protein level recorded 11.62 ± 2.32 mg/L on the day of whelping might be associated with uterine inflammatory conditions to induce parturition following serum progesterone and relaxin level suddenly decreased.

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