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RESEARCH ARTICLE

Serum Progesterone and Estradiol-17β Profiles in Nili Ravi Buffaloes (*Bubalus bubalis*) with and without Dystocia

Muhammad Amjad Ali, Laeeq Akbar Lodhi and Faiz-ul-Hassan[1]*

Department of Theriogenology; [1]Department of Animal Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: faizabg@gmail.com

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ABSTRACT

The aim of this study was to compare serum hormone profiles (progesterone and estradiol-17β) in buffaloes with dystocia and unassisted calving in three agro-ecological zones of Punjab, Pakistan. One hundred and seventy three buffaloes (n=173) with assisted (dystocia) and unassisted calving (normal birth) were sampled for study. The results showed that the buffaloes suffering with dystocia had significantly higher (P<0.05) mean serum progesterone level compared with those having normal calving. The comparison amongst the agro-ecological zones revealed that serum progesterone level of dystocia cases in buffaloes of northern irrigated zone was significantly lower (P<0.05) compared with those in the southern irrigated zone and the arid zone, whereas the latter two did not differ between each other. No difference was observed in serum progesterone levels in normal buffaloes when compared amongst three agro-ecological zones. The serum estradiol-17β profile showed a significant (P<0.05) lower level in buffaloes with dystocia as compared to those with normal calving. Mean serum estradiol-17β level in the buffaloes affected with dystocia in the northern irrigated zone was significantly lower (P<0.05) compared to those in the southern irrigated zone and the arid zone wherein the latter two did not differ between each other.

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INTRODUCTION

Pakistan is the fourth largest milk producing country in the world with annual milk production of 46.66 million tones. Major share of this production is contributed by the buffalo, which is predominant dairy specie in Pakistan. The total milk produced in the country is contributed by 28.69 million heads of buffalo (GOP, 2011). The serious issue behind the poor individual production per animal is low production potential, poor reproductive efficiency and lack of proper breeding management. There are many factors which hamper better reproductive efficiency of the buffalo, which include silent heat, poor feeding management and incidence of reproductive disorders such as dystocia (Prakash *et al*., 2005; Khan and Das, 2011).

The incidence of calving related disorders is high in the buffalo compared to cattle (Laven and Peters, 1996; Rabbani *et al.*, 2010). Samad *et al.* (1987) reported highest incidence of genital prolapse (42.9%), followed by retention of fetal membranes (23.2%) and dystocia (20.7%) among the reproductive disorders in the riverine buffalo.

Dystocia is characterized as abnormal birth that requires assistance and is one of the most serious complications of buffalo and cattle at parturition. Its incidence rate is about 3-25% of all pregnancies in cattle and results in reduced productive performance and economic loss (Oakes *et al.*, 2001; Linden *et al.*, 2009). Numerous factors are considered to increase the incidence of dystocia such as size of pelvic cavity of the dam, sire breed, birth weight of calf, dam's age, twin pregnancies, hormonal disturbances, nutritional status of dam during pregnancy etc. and other unknown factors (Cítek *et al.*, 2009; Nahkur *et al.*, 2011).

Dystocia is one of the most important reproductive disorders resulting in an increased incidence of other calving related disorders and a loss of milk production ultimately affecting economics of the farmer and the herd health (Lorenz *et al.*, 2011). However, mechanisms whereby dystocia results in reduced fertility and productivity are yet to be clarified.

The secretions of endocrine glands regulate the complex birth process in animals. Both estradiol-17β and

progesterone have fundamental role in maintenance of gestation and calving. They also mitigate synthesis of prostaglandins which play functional role in regression of corpora lutea and birth process. Prostaglandin E2 (PGE2) production is stimulated by progesterone (Wooding *et al*., 1996), and estrogens function to increase the prostaglandin F2α (PGF2α) levels in the uterus (Rasmussen *et al*., 1996). In normal calving, the reduced levels of progesterone before parturition and the enhanced levels of estradiol-17β and prostaglandins on the day of calving appear to be important. Decreased level of estrogen and delay in lysis of the corpora lutea resulting in increased level of progesterone, are suggested to be associated with calving related disorders like dystocia and retention of fetal membranes in dairy cows (Zhang *et al*., 1999; Ali *et al*., 2009).

Information on the postpartum endocrinology in buffaloes is limited (Prakash *et al*., 2005), especially in buffaloes with calving disorders like dystocia (Mishra and Parkash, 2005). Therefore, the present study was planned to determine serum progesterone and estradiol-17β concentrations in buffaloes affected with dystocia in three agro-ecological zones of Punjab, Pakistan viz. Southern irrigated, Northern irrigated and arid zones.

MATERIALS AND METHODS

Animals and sample collection: A total of 173 buffaloes of different parities with and without dystocia were selected from three different agro-ecological zones of Punjab province, Pakistan. The number of buffaloes with dystocia located at Southern irrigated zone, Northern irrigated zone and Arid zone were 3, 5 and 15, respectively. Fifty buffaloes having normal calf birth from each zone were taken as control. Blood samples were collected aseptically from jugular vein between 12 to 24 h after calving for hormonal assay (progesterone and estradiol-17-β), serum was separated and stored at −20°C.

Hormones concentration: Progesterone concentrations in blood serum were determined through solid phase competitive ELISA by using commercially available kit (Bio Check, Inc. USA, Lot. RN-28387). The sensitivity of the progesterone ELISA assay was 0.3 ng/ml, intra-assay precision was less than 7.1% and inter-assay precision was less than 12.6%. Estradiol-17β concentrations in blood serum were determined through ELISA by using commercially available kit (Bio Check, Inc. USA, Lot. RN-27637). The sensitivity of the estradiol ELISA assay was 10 pg/ml, intra-assay precision was less than 24.1% and inter-assay precision was less than 26.7%.

Statistical analysis: The data collected for serum progesterone and estradiol-17β concentrations were analyzed statistically through analysis of variance (ANOVA), using completely randomized design. Significant means were compared using Duncan's Multiple Range (DMR) test (Steel and Torrie, 1990).

RESULTS

Progesterone: The results showed that the buffaloes with normal calving had significantly lower (P<0.05) mean serum progesterone concentration compared with the buffaloes suffering from dystocia. The comparison amongst the agro-ecological zones revealed that in cases of dystocia the level in buffaloes of northern irrigated zone was significantly lower (P<0.05) compared with those in the southern irrigated zone and the arid zone, whereas the latter two did not differ between each other. No difference was recorded among the mean serum progesterone levels of buffaloes with unassisted calving when compared amongst the three agro-ecological zones (Table 1).

Estradiol-17β: The hormone profile showed a significant (P<0.05) higher levels of serum estradiol-17β in buffaloes with normal calving as compared to those with dystocia. Mean serum estradiol-17β level in the buffaloes affected with dystocia in the northern irrigated zone was significantly lower (P<0.05) compared to those in the southern irrigated zones and the arid zone wherein the latter two did not differ between each other (Table 2). In healthy control buffaloes, significantly higher (P<0.05) mean serum estradiol-17β level was recorded in the arid zone compared with those in the southern irrigated zone and the northern irrigated zone, wherein the latter two did not differ significantly between each other (Table 2).

Table 1: Serum progesterone (mean±SE) levels (ng/ml) in buffaloes with and without dystocia maintained in three agro-ecological zones of Punjab, Pakistan

Calving Disorder	Southern Irrigated Zone	Northern Irrigated Zone	Arid Zone
Difficult Birth (Dystocia)	0.69 ± 0.05^{aA} (n=3)	0.50 ± 0.03^{bA} (n=5)	0.64 ± 0.02^{aA} (n=15)
Unassisted Birth	0.22 ± 0.01^{B} (n=50)	0.23 ± 0.01^{B} (n=50)	0.19 ± 0.02^{B} (n=50)

Values with different superscripts in the same row (small letters) and in the same column (capital letters) differ significantly (P<0.05).

Table 2: Serum estradiol-17β (mean±SE) levels (pg/ml) in buffaloes with and without dystocia maintained in three agro-ecological zones of Punjab, Pakistan

Calving Disorder	Southern Irrigated Zone	Northern Irrigated Zone	Arid Zone
Difficult Birth (Dystocia)	28.77 ± 0.52^{aB} (n=3)	24.45 ± 0.96^{bB} (n=5)	29.53 ± 0.70^{aB} (n=15)
Unassisted Birth	35.88 ± 0.71^{bA} (n=50)	34.59 ± 0.33^{bA} (n=50)	41.87 ± 0.67^{aA} (n=50)

Values with different superscripts in the same row (small letters) and in the same column (capital letters) differ significantly (P<0.05).

DISCUSSION

The significantly higher (P<0.05) mean serum progesterone concentration were found in the buffaloes suffering from dystocia, compared with the buffaloes with unassisted calving. The reason behind this increased level of progesterone concentration is reduced level of Estradiol-17β and delayed regression of corpora lutea (Zhang *et al*., 1999). Other workers also reported higher serum progesterone concentrations in dystocia (Zhang *et al*., 1999). The mean serum progesterone concentrations recorded in healthy controls in this study are similar to those in spontaneous parturition (El-Wishy, 2007). Nevertheless, the higher serum progesterone in dystocial animals might be related to the incidence of calving difficulty and stillborn calf in animals (Kornmatitsuk, 2002). Similar findings were reported by Zhang *et al*.

(1999), who reported that the levels of progesterone were still higher and longer than normal in cows with dystocia.

The comparison amongst the agro-ecological zones revealed that in cases of dystocia the serum progesterone level in buffaloes of northern irrigated zone was significantly lower ($P<0.05$) compared with those in the southern irrigated zone and the arid zone, whereas the latter two did not differ between each other. No difference was recorded among the mean serum progesterone levels of control buffaloes amongst the three agro-ecological zones.

In this study, serum estradiol-17β concentrations were found to be lower in buffaloes affected with dystocia compared with healthy controls. This lower level of estrogen is also reported earlier (Erb *et al*., 1981) and these concentrations caused reduced synthesis of PGF2α resulting in calving difficulty (Wischral *et al*., 2001). The serum estradiol-17β concentration observed in this study in buffaloes with unassisted calving is in agreement with earlier reports (Zhang *et al*., 1999; El-Wishy, 2007).

In healthy control buffaloes significantly higher ($P<0.05$) mean serum estradiol-17β level was recorded in the arid zone compared with those in the southern irrigated zone and the northern irrigated zone, wherein the latter two did not differ significantly, between each other. The higher concentration of serum estradiol-17β in arid zone as compared to irrigated zones may be due to variations of feeding, management and agro-climatic conditions among zones.

Hormonal synchronization plays an important role at calving. The reciprocal changes in estradiol and progesterone synchronize the parturition process. Asynchrony of this hormonal mechanism can lead to calving problems (Olujohungbe *et al.*, 1998). In our study higher progesterone and lower estradiol concentrations were observed in buffaloes with calving difficulty which contradicts normal hormonal synchronization at calving. Contrarily, higher estradiol and lower progesterone concentration in dystocial cattle as compared to controls were reported by Yokus *et al*., 2010. However, these hormonal changes were not significant. Similarly, DeGraaf *et al*. (1982) observed non-significant differences in plasma progesterone or estradiol concentrations between heifers with and without dystocia. Zhang *et al*. (1999) also reported no significant difference for progesterone or estradiol concentrations between eutocial and dystocial cattle until the last two weeks before parturition.

Conclusion: The difference in hormone concentration and patterns of changes between the normal and dystocial buffaloes suggest that the sequence of hormones change (Estradiole-17β and progesterone) is late and not as pronounced in dystocial animals before parturition. Therefore, animals having dystocia are not as well-prepared hormonally for parturition.

This study indicates that concentrations of progesterone and estradiole-17β vary between buffaloes with and without dystocia which is indicative of possible role of these hormones in incidence of this disorder. However, detailed studies including pre-partum hormone profiles in buffaloes are needed for further elucidation of underlying mechanism. Moreover, variation in serum concentrations of hormones in different agro-ecological zones may be due to different factors such as agro-climatic conditions, feeding and management differences. These factors vary among different agro-ecological zones and might be responsible for the variation in hormonal profile and, thus, affecting the incidence of the problem.

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