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RESEARCH ARTICLE

Determination of Ovsynch Efficiency for Estrus Synchronization by Plasma LH and P4 Levels in Nili Ravi Buffalo during Peak and Low Breeding Seasons

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ABSTRACT

Oestrus synchronization may be used to overcome poor oestrus expression and seasonality of breeding in buffalo (*Bubalus bubalis*). The present work was conducted to compare the efficiency of an oestrus synchronization protocol "ovsynch" in Nili Ravi buffalo during peak (n=8) and low breeding seasons (n=11) by determining luteinizing hormone (LH) peak and progesterone (P4) rise in blood plasma using ELISA. Buffaloes were administered gonadotropin releasing hormone (GnRH) analogue (50 µg lecirelin; day 0) followed by prostaglandinF2α (PGF2α) analogue (150 µg cloprostenol; day 7) and again GnRH analogue at 36 hours after PGF2α. Blood sampling for LH was started 12 h after PGF2α injection and done at 3 h interval up to 108 h. An animal was considered to have responded to ovsynch protocol if it showed LH peak within 48 h after PGF2α injection (and within 3-6 h after second GnRH injection) and showed a P4 concentration of >2.0 ng/ml on day 18 after the 1st GnRH injection. 87.5% animals showed positive response to ovsynch protocol in term of oestrus and ovulation synchronization during the peak season as assessed by LH peak and P4 level while only 36.36 % animals responded to synchronization treatment during low breeding season ($P<0.05$). The LH peak in responsive animals was 11.48±1.98 and 10.38±1.77 ng/ml in peak and low breeding season respectively. It is concluded that ovsynch protocol in buffaloes resulted in a significantly better response during peak breeding season as compared to that in low breeding season.

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INTRODUCTION

Silent estrus and seasonality of breeding are two important factors limiting reproductive efficiency of buffalo. Estrus synchronization may be used to overcome these problems. Progestogens, gonadotropin releasing hormone (GnRH), prostaglandin F2α (PGF2α) and/or their different synthetic analogues have been used for oestrus control in buffalo with variable results (Brito *et al*., 2002). Environmental factors cause seasonal anoestrus in buffalo by affecting ovarian and hypophysial hormonal secretion and lead to lower peaks of luteinizing hormone (LH) and variable progesterone (P4) levels (De Rensis *et al*., 2005). Successful synchronization protocol requires a clear knowledge of ovarian secretary function in relation to control of follicle development, luteal phase of the cycle and ovulation (De Rensis and López-Gatiuss, 2007; Perera, 2010; Mwaanga *et al*., 2012). Furthermore, in order to overcome seasonality of breeding, a synchronization protocol should initiate follicular development by activating hypothalamo-ovarian axis. The ovsynch protocol is a sequence of GnRH - PGF2α - GnRH treatments that became popular for oestrus synchronization in cattle over the last decade, resulting in an acceptable fertility to timed AI (TAI) (Pursley *et al*., 1997). Numerous variations of the protocol have also been tested and developed to meet demands of different physiological situations (Macmillan, 2010; Stevenson *et al*., 2012). The GnRH injection, at any phase of estrous cycle, results in release of high amount of LH that ovulates an existing dominant follicle (Bodensteiner *et al*., 1996) or causes lutenization of non viable follicles, and starts emergence of

a new follicular wave 2 or 3 days later (Twagiramungu *et al*., 1995). With the ovulation or lutenization of the dominant follicles, P4 levels will remain high; therefore PGF2α is given on day 7 to induce luteolysis and promote the ovulation of the follicle of the new wave of follicular growth. The second GnRH injection is recommended after 48 h of PGF2α injection for better synchronization of ovulation and to allow the fixed timed AI (De Rensis and Peters, 1999). Ovsynch treatment has been successfully used in buffaloes with acceptable fertility rates (Chaikhun *et al*., 2010). It reduced the incidence of anestrous from 45% before treatment to 18% after treatment (Roy and Prakash, 2009). Information regarding use of ovsynch protocol in Nili-Ravi buffalo in various seasons of the year is meager. Therefore, the present work was conducted to compare the efficiency of "ovsynch" protocol in Nili Ravi buffalo during peak and low breeding seasons. Parameters for this purpose were LH peak and P4 rise in blood plasma of buffaloes post ovsynch treatment.

MATERIALS AND METHODS

Animals and their feeding: The study was conducted at National Agricultural Research Center, Islamabad, Pakistan (33° 42' N, 73° 10' E). Eleven Nili-Ravi buffaloes that had calved 45 days ago were used in the trial. Animals had normal calving history and were in their 2nd to 4th lactation. All the animals were in good body condition. Animals were offered seasonal green fodder (40 kg/buffalo/day). A concentrate comprising of cotton seed cake (2kg) and wheat bran (1kg) mixed with 2kg wheat straw was offered per animal/day. Animals were sent for grazing for two h (8 am to 10 am) every day. Ad lib clean water was available for drinking. Animals were also given a bath twice daily. Animals were milked manually twice a day at 12 h interval (2 am and 2 pm). Calves were suckled for 5 minutes before milking.

Ovsynch treatment and blood sampling: Buffaloes were treated with ovsynch protocol in November and December of 2007 (i.e. the peak breeding season of buffalo). The buffaloes used for heat synchronization during peak breeding season were kept non pregnant and same were used for studying the effect of ovsynch during low breeding season (May, 2008). Heat detection was continued in these animals to determine their cyclic status from peak into low breeding season. Each animal was treated with ovsynch protocol (Neglia *et al*., 2003) as described below.

1. DAY0 (10:00 am): Lecirelin 50 µg (GnRH analogue; 2cc i.m. Dalmarelin; FATRO, Italy).
2. DAY7 (9:00 pm): Cloprostenol 150 µg (PGF2α analogue, 2cc i.m. Dalmazin, FATRO).
3. DAY9 (9:00 am): Dalmarelin 2cc.

For P4, blood was collected on day 0, 4, 7, 11 and then once weekly for 8 weeks. For LH sampling was started 12 h after PGF2α injection at 3 h interval up to 108 h. At each occasion 10 ml blood was collected in a heparinized vial. For the frequent sampling for LH an intravenous catheter (14g x 5.25 inch "Clear Pebax®", Jorgensen Laboratories, Inc. Colorado, USA) was placed in jugular vein. Blood samples were immediately centrifuged at 3000 rpm for 15 min. Plasma was stored at -20°C until analyzed.

LH assay: LH was determined by using an ELISA specific for buffaloes (LH DETECT®, INRA, France). LH DETECT® is an ELISA sandwich type assay using two polyclonal antibodies produced from the same antigen, buffalo LH. Essays for each sample were conducted in duplicate. The sensitivity of the assay was 0.1 ng/ml, with inter- and intra-assay coefficients of variation being less than 10%.

P4 assay: Plasma P4 was estimated by using an ELISA kit (MicroLISA®; Amgenic, USA). Essays were conducted in duplicate. The sensitivity of the assay was 0.3 ng/ml, with inter- and intra-assay coefficients of variation being less than 15%.

Criteria to determine responsive animals: An animal was considered to have responded to ovsynch protocol if it showed LH peak within 48 h after PGF2α injection and showed a P4 concentration of >2.0 ng/ml on day 18 after the 1st GnRH (considered an indicator of a functional corpus luteum (CL) assumed to have been formed as a result of ovulation after PGF2α administration). A four-fold increase in LH concentration over its basal level was considered "LH peak" (Paul and Prakash, 2005).

Statistical analysis: A Chi-square test was used to compare animals responding to ovsynch protocol during peak and low breeding seasons (MINITAB, 1998). $P<0.05$ was considered indicative of a significant difference.

RESULTS

LH peak was noted at 39 h after PGF2α in all the eight animals in which blood sampling could be accomplished during peak breeding season however, seven (87.5%) animals had P4 concentration >2 ng/ml and were considered as responsive. Fig. 1 shows mean plasma LH profile and weekly P4 levels in seven buffaloes with positive response. Fig. 2 shows blood LH profile and weekly P4 levels in the buffalo with no ovulation. The range of LH peak was 4.43-19.37 ng/ml with a mean of 10.71±1.88 ng/ml during peak breeding season.

Seven out of 11 (63.6%) buffaloes became acyclic over low breeding season. Based on LH peak and P4 concentration, 4 out of 11 (36.4%) buffaloes responded to ovsynch protocol during low breeding season. Three out of these 4 buffaloes had been showing heat over low breeding season, and the last heat in these animals was observed during first half of May. Only one out of seven (14.3%) buffaloes with ceased heat activity responded to ovsynch protocol during low breeding season. Fig. 3 shows plasma LH profile and weekly P4 levels in four buffalos that responded positively. Fig. 4 shows plasma LH profile and weekly P4 levels in seven buffalos that did not respond to ovsynch. The range of LH peak during low breeding season was 4.42-13.60 ng/ml with a mean of 7.38±1.13 ng/ml. A significantly higher number of buffaloes responded to ovsynch protocol during peak breeding (87.5%) season as compared to animals in low breeding season (36.4%) ($P<0.05$).

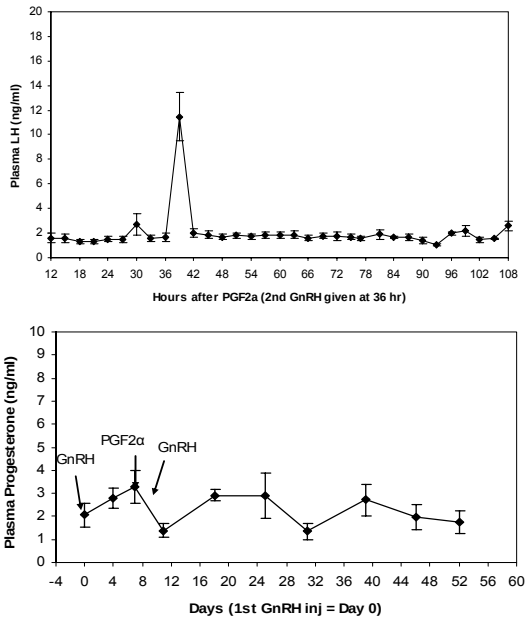

Fig. 1: Plasma LH (a) and progesterone (b) concentration (mean±SE) in seven buffalos that responded to ovsynch protocol during peak breeding season. LH peak is evident at 3 h after 2nd GnRH inj. A P4 rise of >2 ng / ml on day 18 after the 1st GnRH injection is visible.

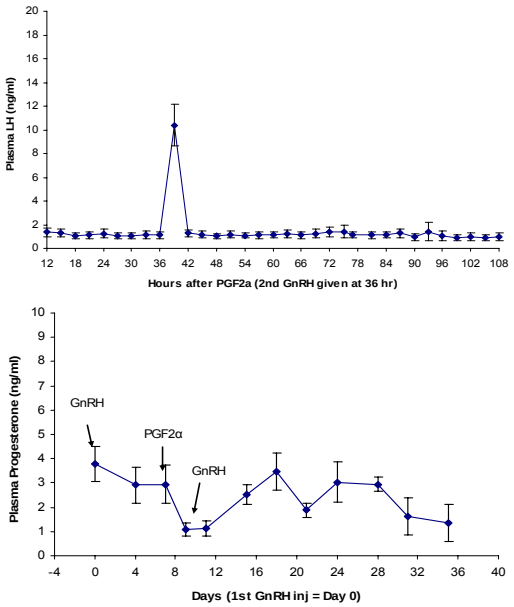

Fig. 3: Plasma LH (a) and progesterone (b) concentration (mean±SE) in four buffalos that responded to ovsynch protocol during low breeding season. LH peak is evident at 3 h after 2nd GnRH inj. A P4 rise of >2 ng / ml on day 18 after the 1st GnRH injection is visible.

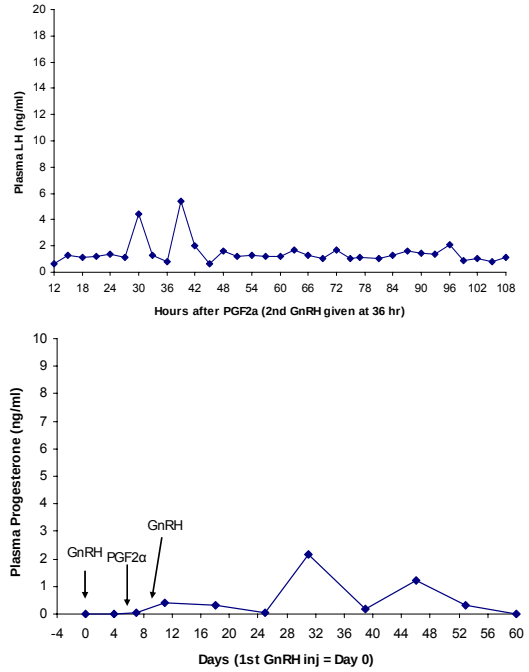

Fig. 2: Plasma LH (a) and progesterone (b) concentration in the buffalo that did not respond to ovsynch protocol during peak breeding with no progesterone rise following the LH peak.

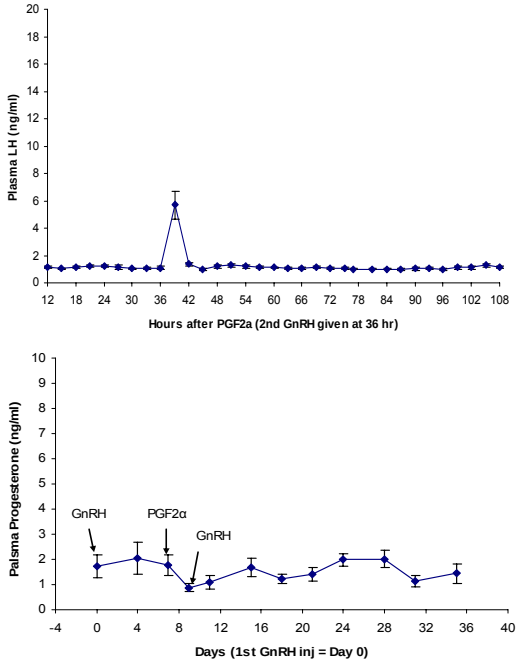

Fig. 4: Plasma LH (a) and progesterone (b) concentration (mean±SE) in buffalos (n=7) that did not respond to ovsynch protocol during low breeding season with no progesterone rise following the LH peak.

DISCUSSION

A well distinguishable pre-ovulatory LH peak was observed after 3 h of 2nd GnRH injection of ovsynch protocol in the present study. The means of LH peak were lower in the present study as compared to that reported by Paul and Prakash (2005) who observed peak LH concentrations of 13.5±3.5 ng/ml at 2.1±0.1 h (range 1.2-3.0 h) after the second-GnRH treatment. Probable reason might be a higher frequency of blood sampling in the later study. Roy and Prakash (2009) conducted sampling at 2 h interval and observed LH peak within 2 h after 2nd GnRH administration in 10 out of 11 buffalo heifers. If we look at the results of present study in combination with observations of Paul and Prakash (2005) and Roy and Prakash (2009), it seems that LH peak during ovsynch protocol in buffalo occurs between 1 and 3 h after second GnRH injection.

During low breeding season, 36.36% buffaloes responded to ovsynch protocol in terms of LH peak and P4 concentration ≥2ng/ml indicating synchronization of ovulation and heat. Das and Khan (2010) observed that in anoestrus buffaloes, the progesterone concentration generally remained below 1 ng⁄ml. Accordingly, we considered a buffalo responsive to ovsynch protocol if the concentration of progesterone was more than 2 ng/ml on day 18th after the 1st GnRH injection. Seven 87.5% animals showed positive response to ovsynch during peak breeding season. The number of buffaloes responding to ovsynch protocol in the present study, in terms of LH peak and plasma P4 concentration, was significantly higher in animals treated during peak breeding season as compared to animals treated during low breeding season. The use of ovsynch protocol for fixed time artificial insemination during favorable season has been shown to give satisfactory results in buffalo (Baruselli *et al*. 2003), but it gave very low conception rate in non-cyclic buffaloes (De Rensis *et al*., 2005; Karen and Darwish, 2010). However a recent report indicated that a doublesynch protocol effectively synchronized ovulation in Murrah buffaloes with enhanced pregnancy rate in cycling and anestrus animals during low breeding season (Mirmahmoudi and Prakash, 2012).

It is well documented that the breeding frequency in buffaloes is highest during winter and lowest in summer in Pakistan (Shah, 1988). Majority of the buffaloes (63.63%) stopped showing heat from January to March in the present study. This may be called transition period between peak and low breeding season. However 4 out of 11 (36.36%) buffaloes continued showing heat over low breeding season indicating that oestrus activity continues in a portion of buffaloes over low breeding season. Major portion of responding buffaloes (3 out of 4) in low breeding season came from the lot that was cyclic even during May. On the other hand the response of acyclic buffaloes to ovsynch seemed poor. It may be attributed to a true deep acyclic condition that is characterized by an absent or strongly reduced follicle turnover. Therefore, attainment of an adequate size of a dominant follicle required for responsiveness to GnRH may not have been reached. Another explanation is that animals may be unresponsive to prostaglandin administration due to insufficient or absent luteal tissue (Ali and Fahmy, 2007). De Rensis and López-Gatiuss (2007) also observed that ovsynch protocol is not effective during the low breeding season in buffaloes.

Conclusion: It is evident from LH peak and plasma P4 rise that Ovsynch protocol resulted in a better synchrony of heat in Nili-Ravi buffaloes in peak breeding season as compared to that in low breeding season. There is a need to explore an effective oestrus induction protocol for low breeding season in buffalo. Furthermore results of the study should be confirmed by fertility trials in Nili Ravi buffalo during peak and low breeding seasons.

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