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Efficacy of the Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes (*Bubalus bubalis*)

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Abstract

Two experiments were conducted to assess the timing and synchrony of ovulation, plasma LH concentrations, and pregnancy rate in Murrah buffaloes (*Bubalus bubalis*) treated with the Ovsynch (GnRH-$PGF_{2\alpha}$-GnRH) protocol. In Experiment 1, 10 non-lactating cycling buffaloes received 10 μg of a GnRH analogue i.m. (buserelin acetate) without regard to the stage of the estrous cycle (day of treatment, day 0), followed by 25 mg of $PGF_{2\alpha}$ i.m. (dinoprost thromethamine) 7 days later. A second-treatment of the same GnRH analogue (10 μg, i.m.) was given 48 h after $PGF_{2\alpha}$. Ovulation was confirmed by transrectal palpation (at 2-h intervals) from the second-GnRH treatment to detection of ovulation or up to 96 h after the second-GnRH treatment. Plasma LH concentrations were determined in blood samples collected at 15-min intervals for 6 h, starting at the second-GnRH treatment, and thereafter at 2-h intervals until 2 h after detection of ovulation. Ovulation occurred in 9/10 buffalo (90%) 23.3 ± 1.3 h (mean ± S.E.M.; range 20–32 h) after the second-GnRH treatment. Peak LH concentrations 13.5 ± 3.5 ng/mL (range 3.9–40.0 ng/mL) occurred 2.1 ± 0.1 h (range 1.2–3.0 h) after the second-GnRH treatment. In Experiment 2, 15 lactating, cycling buffaloes were subjected to the Ovsynch protocol, with fixed-time AI 12 and 24 h after the second-GnRH treatment and 75 lactating buffaloes were inseminated, approximately 12 h after detection of spontaneous estrus. Pregnancy rates were 33.3% for TAI and were 30.7% for buffaloes inseminated following spontaneous estrus ($P = 0.84$). In conclusion, the Ovsynch protocol effectively synchronized

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ovulation in Murrah buffaloes and resulted in conception rates (to two fixed-time inseminations) that were comparable to those achieved with a single AI after detection of spontaneous estrus.

Keywords: Murrah buffalo; Ovsynch; Estrus synchronization; Ovulation

1. Introduction

Buffaloes play a prominent role in livestock production and the economy in India; they contribute >50% of total milk production [1]. However, their productivity is limited by poor reproductive efficiency. Silent estrus is the single factor most responsible for poor reproductive efficiency in buffaloes [2–7]. Various estrus synchronization protocols, i.e. use of progesterone and progestagens [8–12] and prostaglandin $PGF_{2\alpha}$ and its synthetic analogues [13–21] have been utilized to enhance estrus detection, thereby facilitating the use of AI. However, the efficacy of these estrus synchronization protocols is limited because dairy producers must rely on visual estrus detection, which is inefficient on most farms, to determine when to inseminate. The Ovsynch protocol, a sequence of GnRH, $PGF_{2\alpha}$, and GnRH treatments [22], has successfully synchronized ovulation in lactating dairy cows, resulting in fertility to timed AI (TAI) that was similar to that of cows inseminated after detection of estrus [23–24]. However, there are only three recent reports using the Ovsynch protocol in buffalo: half-bred (Murrah × Mediterranean) buffaloes [25], Mediterranean buffaloes [26], and Egyptian buffaloes [27]. It is noteworthy that the use of the Ovsynch protocol in Murrah buffalo in its native tropical environment has not been reported. The objective of the present study was to determine the efficacy of the Ovsynch protocol for synchronization of ovulation in Murrah buffaloes and to determine pregnancy rates to TAI compared to those achieved with AI after detection of estrus. Secondary objectives were to determine the timing of the preovulatory LH surge and ovulation following the second-GnRH treatment.

2. Materials and methods

2.1. Animals and management

Two experiments were conducted (from November to March) using non-lactating ($n = 10$) and lactating ($n = 15$) second- and third-parity Murrah buffaloes from the herd maintained at the National Dairy Research Institute, Karnal, India. The buffaloes used were free from any apparent anatomical, physiological or reproductive disorders. Prior to these experiments, each buffalo had experienced at least one estrous cycle. Based on plasma progesterone concentrations different stages of estrous cycle were estrus (<0.4 ng/mL), early luteal (from 0.4 to 2.0 ng/mL), mid luteal (from 2.0 to 5.0 ng/mL), and late luteal (from >2.0 to <0.4 ng/mL). The buffaloes were kept under loose housing conditions in clean, hygienic paddocks with brick flooring, asbestos roofing, and sufficient space for the free movement of the animals. All buffaloes were fed a ration consisting of concentrates

(maize grain, groundnut cake, mustard cake, wheat bran), roughages (either berseem and maize or oat fodder), a mineral mixture, and salt. Fresh tap water was available ad libitum. All experimental protocols and animal care met institutional regulations regarding the care and use of experimental animals.

2.2. Experiment 1: endocrine changes and timing of ovulation

This experiment was carried out to determine the timing of ovulation and plasma concentrations of progesterone and LH in non-lactating Murrah buffaloes ($n = 10$) treated with an Ovsynch protocol.

2.2.1. Treatments

Estrus was synchronized by administering 10 μg of a GnRH analogue (Buserelin Acetate, Receptãl®VET, Intervet India Private Ltd., Pune, Maharashtra, India) without regarding to the stage of the estrous cycle (day of GnRH treatment, day 0), followed by 25 mg of $PGF_{2\alpha}$ (dinoprost thromethamine; Lutalyse™, Novartis India Limited, Maharashtra, India) on day 7, and a second-GnRH treatment (10 μg) 48 h after $PGF_{2\alpha}$ (day 9). All treatments were given as i.m. injections in the neck.

2.2.2. Collection of blood samples

On alternate days (from days −10 to 22), blood samples (5 mL for determination of progesterone concentrations) were collected by jugular venipuncture into heparinized (20 IU heparin/mL blood) polystyrene tubes. These samples were maintained at 4 °C and transported to the laboratory within 1 h of collection. In addition, to determine changes in plasma LH concentrations, blood samples were collected (via an indwelling jugular catheter) every 15 min for 6 h after the second-GnRH treatment, and thereafter at 2-h intervals until 2 h after confirmation of ovulation (or until 96 h after the second-GnRH treatment if ovulation was not detected). Before catheterization, local anaesthesia (Lidocaine hydrochloride, Xylocaine® Astra Zeneca Pharma India Ltd., Bangalore, India) was given, and after the removal of catheter, the animals were given antibiotic treatment (Terramycin, Oxytetracyclin®, Pfizer Ltd., Aanna salai, Chennai 600015, India) for the next 3 days. For all blood samples, plasma was separated by centrifugation (1200 × g for 20 min at 4 °C) and stored (at −20 °C) in 2 mL vials until assayed.

2.2.3. Detection of ovulation

To detect ovulation, transrectal palpation of the ovaries was conducted every 2 h from the second-GnRH treatment to detection of ovulation (or to 96 h after the second-GnRH treatment if ovulation was not detected). Ovulation was confirmed by the change of ovarian surface from turgid to flaccid [28].

2.3. Experiment 2: pregnancy rates to TAI (after Ovsynch protocol) and after detection of estrus

This experiment was conducted to compare pregnancy rates to TAI in buffaloes synchronized with the Ovsynch protocol, compared to those bred after detection of

spontaneous estrus. Fifteen lactating, cycling Murrah buffaloes were treated with the Ovsynch protocol (as in Experiment 1) and TAI was performed at 12 and 24 h after the second-GnRH treatment. The timing of insemination was based on the time of ovulation in Experiment 1. Concurrently, 75 lactating buffaloes (part of an institute herd of 100) were inseminated approximately 12 h after detection of spontaneous estrus. Estrus was detected twice a day (06:00 and 17:30) by a vasectomized bull (teaser) parading. Pregnancy diagnosis post-insemination was determined by transrectal palpation 60 days post-AI in animals, which had not returned to estrus.

2.3.1. Collection of samples to determine milk progesterone concentration

Milk samples were collected from all Ovsynch treated buffaloes twice weekly from days −10 to 56 to monitor the ovarian status (CL) and to detect buffaloes returning to estrus. Milk samples were also collected on alternate days from days 0 to 6, and thereafter to day 14 to monitor the Ovsynch response. All milk samples were collected between 18:30 and 19:30 and placed into 20 mL plastic storage vials. After addition of 100 μL of a preservative (2.64 g potassium dichromate and 0.61 g mercuric chloride per 100 mL distilled water) for every 10 mL of milk, the sample was placed in a refrigerator (approximately 4 °C). Assays for milk progesterone were conducted within 2 weeks.

2.4. Hormone analyses

Plasma progesterone concentrations were determined by radioimmunoassay, as described by Kamboj and Prakash [29]. The anti-progesterone serum used in the present study (bspNR#2) was highly specific for progesterone [30]. The sensitivity of the assay was 8 pg/tube (corresponded to plasma concentrations of 400 pg/mL) and the 50% binding sensitivity was 70 pg/tube. The intra- and inter-assay coefficients of variations were 7.1 and 12.5%, respectively. Whole milk progesterone was determined by a direct radioimmunoassay, as described by Gupta and Prakash [31]. The sensitivity of the assay was 12.5 pg/tube (corresponded to milk progesterone concentrations of 1.25 ng/mL) and the 50% binding sensitivity was 102 pg/tube. The intra- and inter-assay coefficients of variation were 8.1 and 12.5%, respectively. Buffalo plasma LH concentrations were determined with a highly sensitive, direct enzyme-immunoassay using the second-antibody coating technique developed in the laboratory [32]. The sensitivity of the assay for LH was 6.25 pg/well/20 μL plasma (corresponded to plasma concentrations of 0.31 ng/mL). The 50% relative binding sensitivity was 50 pg/well/20 μL (corresponded to 2.5 ng/mL of plasma). The intra- and inter-assay coefficients of variation (from the measurement of pooled plasma containing 0.62 and 5.0 ng/mL) were 7.7 and 3.2% and 16.0 and 10.9%, respectively. The bovine LH antiserum (USDA-309-684P) was highly specific for LH (USDAbLH-B-6). The cross-reactivity of the bLH antiserum is provided by Prakash et al. [32].

2.5. Statistical analyses

Mean and standard errors were calculated with Graph Pad Prism-3 software package (GraphPad Software Inc., San Diego, CA, USA). An LH surge was defined as having occurred when the value of the first sample forming the peak exceeded the preceding sample

Table 1
Plasma LH peak characteristics and timing of ovulation in buffaloes ($n = 10$) treated with the Ovsynch protocol

Parameters	No.	Mean ± S.E.M.	Range
LH peak concentration (ng/mL)	10	13.5 ± 3.5	3.9–40.0
Duration of LH surge (h)	10	6.4 ± 0.4	4.0–8.0
Time of onset of LH peak after second-GnRH injection (h)	10	2.1 ± 0.1	1.2–3.0
Time of ovulation after second-GnRH injection (h)	9[a]	23.3 ± 1.3	20.0–32.0
Time of ovulation after end of LH surge (h)	9[a]	17.3 ± 1.2	14.0–26.0

[a] Nine of 10 buffaloes ovulated after the second-GnRH treatment.

(onset of LH peak) by at least a four-fold increase in LH concentrations over its basal level. There had to be at least one more value either on the ascending slope or the descending slope before basal concentrations were reached again (end of LH peak). The time elapsed between the onset and end of LH peak was considered as the duration of the LH peak. Changes in plasma progesterone concentrations during treatment and plasma LH concentrations after the second-GnRH treatment were analyzed using repeated measure ANOVA. A Chi-square test was used to compare pregnancy rates to TAI versus spontaneous estrus.

3. Results

3.1. Experiment 1

3.1.1. Synchronization and timing of ovulation

Based on progesterone concentrations (≤0.4 ng/mL after 48 h of $PGF_{2\alpha}$ administration) following $PGF_{2\alpha}$ treatment and transrectal palpation of ovaries (change of ovarian surface

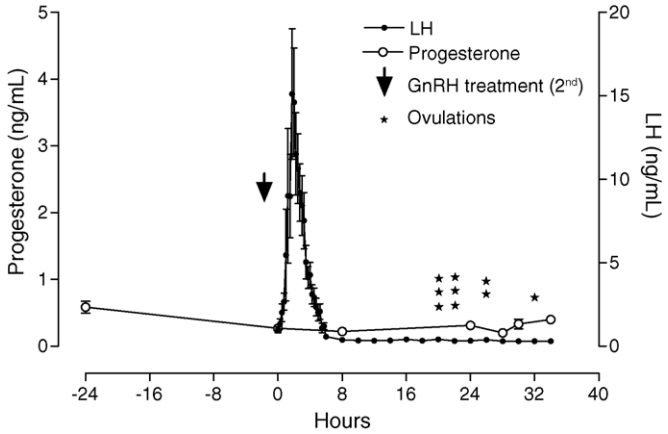

Fig. 1. Mean (±S.E.M.) plasma concentrations of LH and progesterone in Murrah buffaloes ($n = 9$) treated with the Ovsynch protocol. A GnRH analogue (10 μg) was given i.m. and blood samples were collected at 15-min intervals, from the second-GnRH treatment (0 = time of GnRH treatment) to 6 h post-treatment, and thereafter at 2-h intervals until 2 h after ovulation.

from turgid to flaccid) following the second-GnRH treatment, estrus and ovulation were synchronized in 9 of 10 (90%) buffaloes. Characteristics of the LH surge and ovulation are shown in Table 1.

3.1.2. Endocrine changes

Mean plasma LH and progesterone concentrations after the second-GnRH treatment are presented in Fig. 1. Plasma progesterone concentrations prior to, during and post-Ovsynch treatment and plasma LH concentrations after the second-GnRH treatment for the buffaloes treated with the Ovsynch protocol are presented in Figs. 2 and 3. Since the buffaloes were treated with the Ovsynch protocol irrespective of stage (early luteal phase, mid luteal phase, late luteal phase, and estrus phase) of estrous cycle at the time of the first

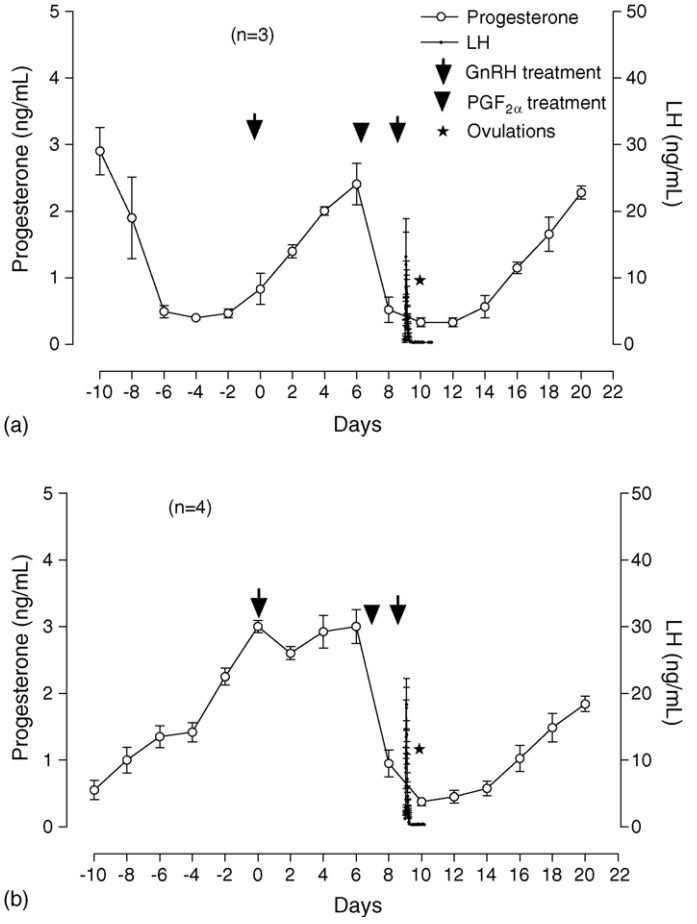

Fig. 2. Plasma progesterone concentrations prior to, during and after an Ovsynch protocol and plasma LH concentrations after the second-GnRH treatment in buffaloes ($n = 9$), which responded to treatment. In these buffaloes, the Ovsynch protocol (i.e. first GnRH treatment) was started at: (a) early-luteal phase; (b) mid-luteal phase; (c) late-luteal phase; and (d) estrus (day of first GnRH treatment, day 0).

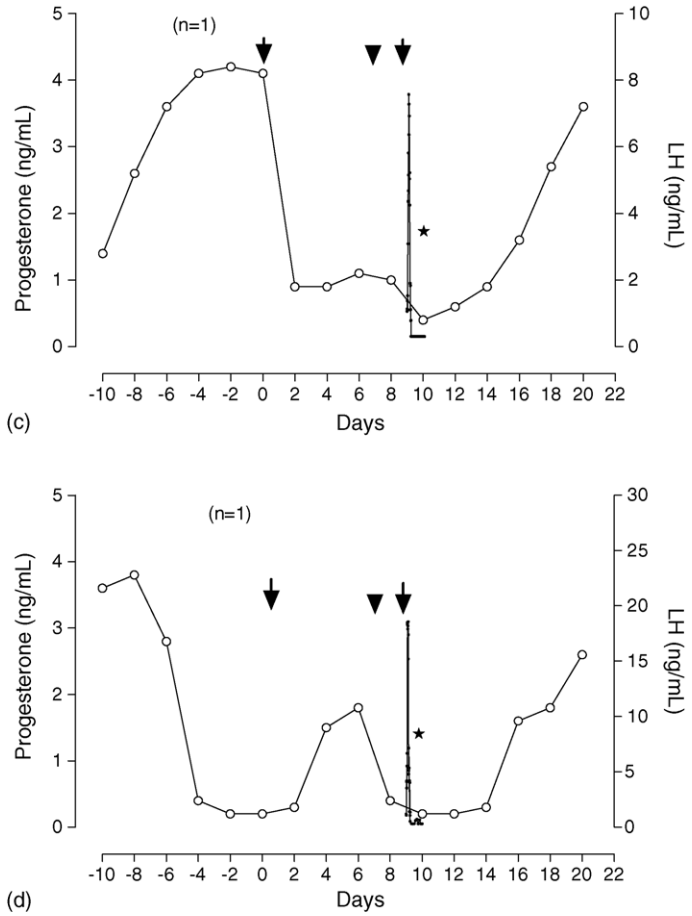

Fig. 2. (*Continued*).

GnRH treatment, plasma progesterone profiles differed among animals (Fig. 2). The first GnRH treatment apparently did not influence the progesterone profiles in individual buffaloes (Fig. 2). However, plasma progesterone concentrations were basal (≤0.4 ng/mL) at the time of the second-GnRH treatment in responder buffaloes due to the luteolytic action of $PGF_{2\alpha}$. In the case of the single non-responder buffalo, plasma progesterone concentrations did not decline after $PGF_{2\alpha}$ (Fig. 3).

3.2. Experiment 2

On the basis of milk progesterone analysis and transrectal palpation (60 days post-TAI), the conception rate was 33.3% (5/15) for buffaloes treated with the Ovsynch protocol. Milk progesterone concentrations declined sharply after $PGF_{2\alpha}$ administration to basal concentrations (≤1.25 ng/mL) within 48 h of treatment and continued to stay low until

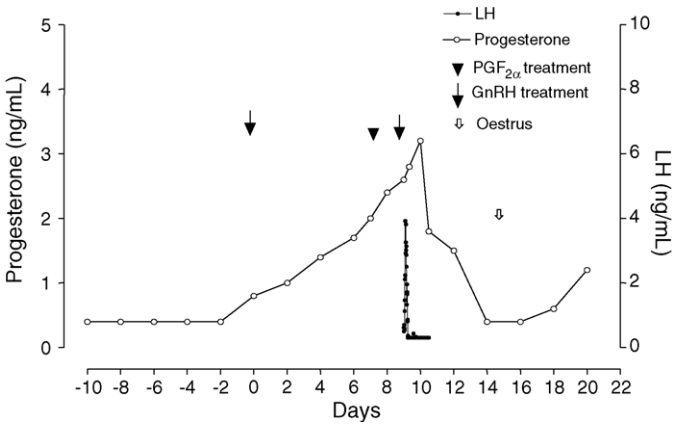

Fig. 3. Plasma progesterone concentrations prior to, during and after an Ovsynch protocol and plasma LH concentrations after the second-GnRH treatment in one buffalo that did not respond to the protocol (day of first GnRH treatment, day 0).

TAI in the 14 of 15 buffaloes that responded to treatment. However, one buffalo did not respond to $PGF_{2\alpha}$ administration (Fig. 4b). Milk progesterone concentrations in pregnant buffaloes ($n = 5$; Fig. 4a) after increasing from basal values of $\leq$1.25 to up to 50 ng/mL by 20 days after TAI continued to stay high (>10 ng/mL); however, in the remaining 10 non-pregnant buffaloes, progesterone concentrations dropped sharply to baseline ($\leq$1.25 ng/mL) 16 days ($n = 1$; Fig. 4c), 21 days ($n = 2$; Fig. 4e), 26 days ($n = 4$; Fig. 4d), and 33 days ($n = 2$; Fig. 4f) post-TAI, respectively.

In the control group of buffaloes inseminated after spontaneous estrus, 23 of 75 (30.7%) were confirmed pregnant at 60 days. The conception rates obtained on TAI (33.3%) were similar ($P = 0.84$) to those achieved with a single AI (30.7%) after detection of spontaneous estrus.

4. Discussion

This study was designed to evaluate the efficacy of the Ovsynch protocol for ovulation synchronization and conception rate in Murrah buffaloes. In the present study, conducted on Murrah buffaloes in their native tropical environment, the Ovsynch protocol precisely synchronized ovulation within a 12-h range (20–32 h) after the second-GnRH treatment in 9 of 10 buffaloes. These findings were consistent with previous reports. In crossbred (Murrah × Mediterranean) buffaloes, ovulations occurred, on average, 26.5 and 24.4 h after the second-GnRH or LH treatments, respectively [25]. In cattle, ovulation occurred 24–32 h after the second-GnRH treatment [22].

In the present investigation, peak LH concentrations occurred within 2 h after the second-GnRH treatment in 10 buffaloes treated with the Ovsynch protocol. The study of Aboul-Ela et al. [33] in Swamp buffaloes, where peak LH concentrations were recorded within 50–130 min after administration of GnRH analogue further support the present

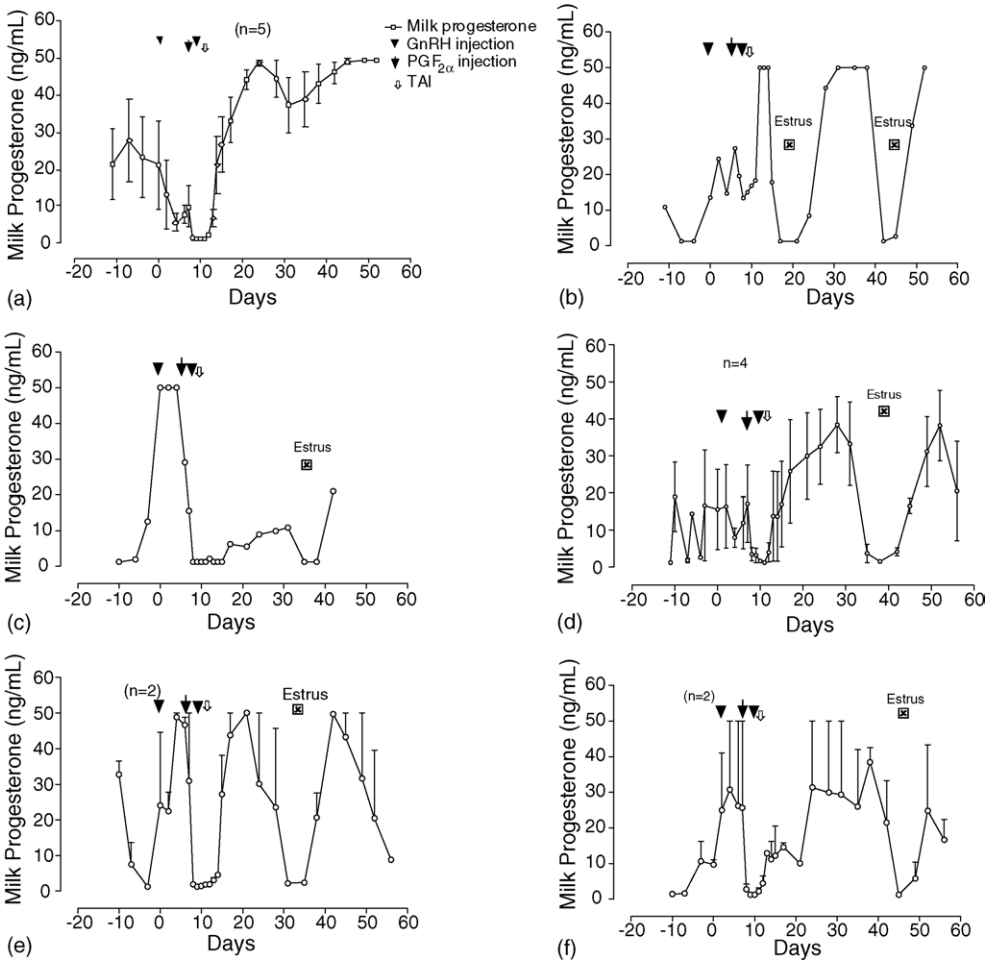

Fig. 4. Plasma progesterone concentrations prior to, during and after an Ovsynch protocol in pregnant (a, $n = 5$) and non-pregnant (b, $n = 1$; c, $n = 1$; d, $n = 4$; e, $n = 2$; f, $n = 2$) lactating buffaloes.

findings. Similarly, administration of an agonist of GnRH induced peaks of LH within 2–3 h in mature, cycling cows [34,35]. The study of Peters et al. [36] for induction of ovulation in cows, where peak LH concentrations were recorded within 2 h after GnRH agonist administration, is in agreement with the present findings in buffaloes. The decline in plasma progesterone concentrations to basal levels ($\leq$0.4 ng/mL) in response to $PGF_{2\alpha}$ treatment confirmed luteolysis at the time of the second-GnRH treatment in the nine buffaloes that responded to treatment. In a previous study protocol, in cross-bred (Murrah $\times$ Mediterranean) buffaloes [25], plasma progesterone concentrations declined from 3.2 ng/mL on day 7 (just before $PGF_{2\alpha}$ treatment) to 0.3 ng/mL 2 days later. Similarly, plasma progesterone concentrations declined to basal concentrations ($\leq$0.1 ng/mL) within 24 h after $PGF_{2\alpha}$ in cows treated with the Ovsynch protocol [36]. In the single

non-responder buffalo in the present study, plasma progesterone concentrations did not decline after $PGF_{2\alpha}$ treatment. Therefore, this buffalo did not have a synchronous ovulation.

In Experiment 2, the Ovsych treatment resulted in five pregnancies, i.e., 33.3% conception rate, which compared ($P = 0.84$) well with a conception rate of 30.7% in control buffaloes inseminated at detected estrus. In the study of Berber et al. [25] in cross-bred (Murrah × Mediterranean buffaloes) and in Brazilian buffaloes [27], conception rates of 55.6–64.2 were reported after TAI, which seemed higher than that of present investigation. Possible reasons for this marked difference in response to the same treatment in different studies may be attributed to differences in breed, managemental, and environmental conditions. In that regard, substantial variations in conception rates have also been reported in cattle treated with this protocol. In beef cows, conception rates exceeding 60% were obtained [37], whereas in dairy cows, conception rates ranged from 40 to 55% [22–24,38]. Burke et al. [39] compared the effectiveness of TAI and AI at detected estrus, in lactating dairy cows; conception rates of 30.5 and 29.0% were observed for TAI and AI at observed estrus, respectively. In post-partum (43–57 days), lactating dairy cows, Momcilovic et al. [40] reported conception rates of 33% with the Ovsynch protocol, compared to conception rates of only 6% in those inseminated after spontaneous estrus.

In conclusion, in the present study, the Ovsynch protocol effectively synchronized ovulation in Murrah buffaloes and resulted in conception rates (to two fixed-time inseminations) that were comparable to those achieved with a single AI after detection of spontaneous estrus. Therefore, this study clearly indicates the opportunity for practical application of the Ovsynch protocol for TAI in Murrah buffaloes.

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