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The endocrine changes, the timing of ovulation and the efficacy of the Doublesynch protocol in the Murrah buffalo (*Bubalus bubalis*)

R. Mirmahmoudi *, B.S. Prakash

Reproductive Physiology Laboratory, Division of Dairy Cattle Physiology, National Dairy Research Institute, Karnal, Haryana 132 001, India

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ABSTRACT

Experiments were conducted to investigate (a) the timing of ovulation and the associated endocrine changes (progesterone, estrogen and LH) during estrous cycle and (b) the efficacy, with respect to the pregnancy rate, in cycling and anestrus in Murrah buffaloes subjected to the Doublesynch protocol during the low breeding season. In experiment 1, 10 cycling buffaloes were administered $PGF_{2\alpha}$ on day 0 (without regard to the estrous cycle stage), GnRH on day 2, a second $PGF_{2\alpha}$ injection on day 9, and a second GnRH injection on day 11. Transrectal palpation was performed at 2-h intervals after the first and second GnRH treatments until ovulation was detected or for upto 96 h. The plasma progesterone and total estrogen concentrations were determined in blood samples collected at daily intervals starting 2 days before the onset of the protocol and continued until the day of the second detected ovulation. The plasma LH and total estrogen concentrations were measured in blood samples collected at 30-min intervals for 8 h following the first and second GnRH injections and thereafter at 2-h intervals until 2 h after the detection of ovulation. Ovulation occurred in 9/10 buffaloes (90%) at 22.2 ± 1.2 h (mean ± S.E.M.; range 18.0–26.0 h) and 10/10 buffaloes (100%) at 23.2 ± 1.0 h (mean ± S.E.M.; range 20.0–28.0 h) after the first and second GnRH treatments, respectively. The peak LH concentrations of 99.8 ± 28.5 ng/ml (range 37.8–320.0 ng/ml) and 62.3 ± 11.9 ng/ml (range 20.9–143.9 ng/ml) occurred 2.1 ± 0.3 h (range 1.0–3.5 h) and 2.3 ± 0.3 h (range 0.5–3.0 h) after the first and second GnRH treatments, respectively. The total estrogen concentration gradually increased from the day of both the first and second $PGF_{2\alpha}$ administrations until the LH peak (with great variability) and then gradually declined to the basal level, which was reached at the time ovulation was detected. In experiment 2, 10 cycling and 11 non-lactating anestrus buffaloes were subjected to the Doublesynch protocol with timed artificial insemination (TAI) 16 and 24 h after the second GnRH treatment, and 55 cycling buffaloes were inseminated after spontaneous estrus was detected (control group). The pregnancy rates were 60% using TAI on cycling buffaloes (experiments 1 and 2), 55% for anestrus buffaloes (experiment 2), and 27.3% for cycling buffaloes inseminated following spontaneous estrus. The overall pregnancy success rates after the Doublesynch protocol in both cycling and anestrus buffaloes increased by 30.8% compared to spontaneous estrus (58.1% vs. 27.3%).

In conclusion, the Doublesynch protocol effectively synchronized ovulation twice (after the first and second GnRH treatments) irrespective of the stage of estrous cycle in Murrah buffaloes. The study also demonstrated that the Doublesynch protocol followed by TAI significantly ($P < 0.005$) enhanced the pregnancy rate in cycling and anestrus buffaloes in comparison to untreated controls during the low breeding season.

1. Introduction

Buffaloes are a major livestock species for milk and meat production, and they contribute significantly to the economy of many countries in South and Southeast Asia, particularly in India. Delayed maturity, poor estrus expressivity and long postpartum calving intervals contribute to the low reproductive efficiency of buffaloes, especially under tropical conditions. Such limitations substantially reduce a farmer's financial returns due to a reduced number of pregnancies. In the last two decades, considerable attention has been focused on understanding the causes of the inherent reproductive limitations in buffaloes by studying their reproductive endocrinology and on developing techniques to augment their reproductive efficiency [11].

In 1995, the Ovsynch protocol, a sequence of GnRH and $PGF_{2\alpha}$ treatments, was developed to allow timed artificial insemination (TAI) without the need for detecting estrus in lactating dairy cows [26]. Paul and Prakash [22] and Mohan et al. [14] successfully performed the Ovsynch and Heatsynch protocols in Murrah buffaloes

* Corresponding author. Fax: +91 184 2250042.
E-mail address: mirmahmoodi@gmail.com (R. Mirmahmoudi).

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to synchronize ovulation and perform TAI resulting in a range of pregnancy success rates from 30% to 40%. Later, in two field trials conducted on a total of 191 (60 + 131) anestrus/repeat breeding buffaloes on farms in different villages, pregnancy rates of 40% and 51% were recorded using TAI and subsequent AI (both included) and the Ovsynch protocol [23]. Studies on dairy cows have demonstrated that the success rate of the Ovsynch protocol is dependent on the estrous cycle stage at the onset of the protocol. For example, the initiation of the Ovsynch protocol between days 13 and 17 or early in the estrous cycle (days 2–4) led to a reduced pregnancy rate [18,32]. Other studies have established that GnRH-induced follicular turnover or induction of a new follicular wave is the most efficient if ovulation is induced in response to the first GnRH treatment [30] and that resetting the follicular development can produce a new dominant follicle containing an oocyte with greater potential fertility [13].

Several strategies employing hormonal treatment before initiating the Ovsynch protocol have been utilized to minimize the proportion of cows in the above-mentioned problematic stages and to maximize the proportion of cows in favorable stages of the estrous cycle. However, these strategies have several disadvantages: either pregnancy rates are limited, or the protocol requires a long period of time to complete [3,4,7,17,19,20]. Recently, Cirit et al. [3] developed a new synchronization method that includes the administration of an additional $PGF_{2\alpha}$ injection 48 h before beginning the Ovsynch protocol. They named this new protocol the Doublesynch (the abbreviation of double synchronization) protocol, as it resulted in synchronized ovulation after both the first and second GnRH treatments. Öztürk et al. [21] confirmed the pregnancy rate success (increased by 43% compared with the Ovsynch protocol) of the Doublesynch protocol in both cyclic and anestrus cows.

This study investigates the effect of the Doublesynch protocol on (a) the timing of ovulation (following the first and second GnRH injections) and the associated endocrine changes (progesterone, total estrogen and LH) in cycling and (b) the efficacy, in terms of pregnancy rates, in both cycling and anestrus buffaloes during the low breeding season.

2. Materials and methods

2.1. Animals and management

This study was conducted with 20 cycling and 11 anestrus (ranging from 6 to 8 months postpartum) Murrah buffaloes (3–4 parity) during the period extending from the first week of April to the end of May (low breeding season) when the humidity was 75–85% and the ambient temperature was 30–39 °C. The cyclicity of the buffaloes was confirmed by progesterone analysis from blood plasma samples that were collected twice weekly over a 21-day period [8] prior to the treatment. The buffaloes were classified as anestrus when the concentration of plasma progesterone was $\leqslant$0.4 ng/ml in all six of the samples collected during the 21-day period. The cycling buffaloes exhibited a characteristic profile with at least two samples having $\geqslant$1.0 ng/ml of plasma progesterone. To classify the estrus cycle stages, the day of estrus was considered as day 0. For this purpose, the basal progesterone concentration (<0.4 ng/ml) and heat detection using a vasectomized bull (teaser) paraded twice daily for 3 weeks prior to the onset of the protocol were used to predict the day of estrus with an error of ±1 day. In addition, to classify the different stages of the estrous cycle with high accuracy, we used the plasma progesterone concentrations from 2 days before the onset of the protocol until the day of the first $PGF_{2\alpha}$ injection: <0.4 ng/ml plasma progesterone in all three samples was indicative of estrus and very early of estrous cycle, the early luteal phase was indicated by plasma progesterone levels of 0.4–1.0 ng/ml, and the luteal phase was indicated by plasma progesterone concentrations of >1.0 ng/ml.

All of the animals were selected from the herd maintained at the National Dairy Research Institute, Karnal, India. The animals were fed a diet consisting of a concentrated mixture of maize grain, groundnut cake, mustard cake, wheat bran, mineral mixture, salt and roughage (either berseem, maize or oat fodder based on availability) following the standard feeding practices employed at the NDRI farm. Ad libitum fresh drinking water was available throughout the day and night to all of the animals. The Animal Ethics Committee of the Institute approved these experiments.

2.2. Experiment 1: Endocrine changes and the timing of ovulation

This experiment was conducted to investigate the timing of ovulation after both the first and second GnRH treatments and to assess the plasma concentrations of progesterone, total estrogen, and LH in 10 cycling Murrah buffaloes subjected to the Doublesynch protocol.

2.2.1. Treatments

Estrus was synchronized by administering 25 mg of $PGF_{2\alpha}$ (dinoprost tromethamine; Lutalyse™, Novartis India Limited, Maharashtra, India) without regard to the estrous cycle stage (day of first $PGF_{2\alpha}$ treatment = day 0), followed by 10 μg of a GnRH analog (Buserelin Acetate, Receptal®VET, Intervet India Private Ltd., Pune, Maharashtra, India) on day 2, a second $PGF_{2\alpha}$ dose (25 mg) on day 9, and a second GnRH dose (10 μg) 48 h after the second $PGF_{2\alpha}$ dose (day 11). All injections were given i.m.

2.2.2. Collection of blood samples

All of the blood samples were collected by jugular venipuncture into heparinized (20 IU heparin/ml blood) polystyrene tubes. The samples were maintained at 4 °C and transported to the laboratory within 1 h of collection. The samples were then centrifuged, and the obtained plasma was stored at -20 °C until hormone analysis. Before catheterization, local anesthesia (Lidocaine Hydrochloride, Xylocaine® Astra Zeneca Pharma India Ltd., Bangalore, India) was administered, and after removal of the catheter, the animals were given an antibiotic treatment (Terramycin, Oxytetracycline®, Pfizer Ltd., Anna Salai, Chennai, India) for the next 3 days. To determine the changes in plasma LH, progesterone and total estrogen concentrations after the GnRH treatments, the blood samples were collected via an indwelling jugular catheter every 30 min for 8 h and then at 2-h intervals until 2 h after ovulation was confirmed.

2.2.3. Detection of ovulation

Transrectal palpation of the ovaries was conducted every 2 h after both the first and second GnRH treatments until ovulation were detected (or up to 96 h after the GnRH treatment if ovulation was not detected). Ovulation was confirmed by the change of the ovarian surface from turgid to flaccid [15]. All of the buffaloes were subjected to TAI at 16 and 24 h after the second GnRH injection.

2.3. Experiment 2: conception rates after TAI following the Doublesynch protocol in cycling and anestrus buffaloes and after AI of the detected spontaneous estrus

Following experiment 1, another experiment was conducted to determine the efficacy of the Doublesynch protocol after TAI in both cycling and anestrus buffaloes. 10 cycling and 11 anestrus Murrah buffaloes were treated with the Doublesynch protocol (as in experiment 1), and TAI was performed at 16 and 24 h after the second GnRH treatment. To compare the success of the Doublesynch treated with untreated (control group) buffaloes, 55 cycling buffaloes were inseminated twice, approximately 12 and 18 h after