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Responsiveness to exogenous gonadotrophin on post-partum fertility during breeding and non-breeding seasons in buffaloes

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The present study was designed to investigate the responsiveness to exogenous gonadotrophin on post-partum fertility during breeding and non-breeding seasons in buffaloes. During the breeding season, twenty-two post-partum buffaloes that calved between September and February were included and further divided into two groups: treatment (n = 12) and control (n = 10). Buffaloes in the treatment group received an exogenous GnRH analogue (Receptal; 10 µg buserelin acetate, i.v.) on day 21 post-partum, whereas the control group received no treatment. During the non-breeding season, post-partum buffaloes (n = 20) that calved between April and August were divided into two groups, treatment (n = 10) and control (n = 10), and the treatment group received exogenous GnRH analogue (Receptal; 10 µg buserelin acetate, i.v.) on day 21 post-partum. During breeding season, average pre-treatment LH concentration was similar in both treatment (1.7 ± 0.3 ng/ml, n=8) and control (1.2 ± 0.2 ng/ml, n=8) groups. During non-breeding season, pre-treatment LH concentration was comparable in both treatment (0.8 ± 0.1 ng/ml, n=8) and control (0.9 ± 0.2 ng/ml, n=8) groups. The basal LH level was lower ($P < 0.05$) during the non-breeding season compared with that during the breeding season (0.88 ± 0.1 ng/ml, n=16 vs 1.44 ± 0.2 ng/ml, n=16). GnRH administration on day 21 post-calving has been found to improve the early resumption of cyclicity during breeding calved (83.3%) and non-breeding calved buffaloes (80%) by day 28 post-partum. Administration of GnRH helped resume early cyclicity in all animals during both seasons. Following GnRH administration, cyclicity resumed in 82% of the treatment group and 45% of the control group on day 28 post-calving. By day 60 post-partum, 95.5% and 75% of buffaloes in the treatment and control groups, respectively, had resumed cyclicity. GnRH administration on day 21 post-calving did not influence conception rate, as overall conception rates within three inseminations were similar in the treatment and control groups during the breeding season (75% vs. 70%). In conclusion, administration of GnRH post-partum aids early resumption of ovarian cyclicity and reduces days open in buffaloes, irrespective of season.

KEYWORDS

buffalo, GnRH, breeding, season, fertility

1 Introduction

The post-partum period in buffalo starts with parturition and ends with complete uterine involution, normal estrus expression, and resumption of ovarian cyclicity. Hormonal changes during the peri-parturient period, besides regulating lactogenesis and parturition, have an impact on post-partum reproductive activity (El-Wishy, 2007a). Knowledge of these changes is essential to understand the factors responsible for the initiation of cyclic ovarian activity following parturition. At the time of parturition, progesterone and estradiol concentrations reduce to basal levels, which influence post-partum reproductive activity.

Buffaloes are considered difficult breeders because of their inherent susceptibility to environmental stress, which causes anestrus, delayed age at first calving, and a higher incidence of silent ovulations (El-Wishy, 2007b; Bhat and Dhaliwal, 2023). Post-partum anestrus is usually longer in buffalo than in cattle under similar management conditions (Jainudeen and Hafez, 1993). The delay in the resumption of ovarian cyclicity after parturition is mainly attributed to inadequate nutrition, seasonal effects, genital pathologies, and managemental factors. Environmental factors in buffaloes directly and adversely affect the neuroendocrine axis (Rao and Pandey, 1983). The luteinizing hormone (LH), which plays a significant role in reproduction, is directly under brain control and regulated by ovarian steroids (Phogat et al., 1997a). The peak plasma LH concentration also varies with seasons, with levels higher during the cooler months compared with the non-breeding season (Rao and Pandey, 1983).

Gonadotrophin-releasing hormone (GnRH) occupies a central role in reproductive function in mammals. It is a neuropeptide released in a synchronized, pulsatile manner from neurons terminating at the medial basal hypothalamus–median eminence, an area bathed by the hypothalamo-hypophyseal portal vessels (Hassanein et al., 2024). GnRH moves into the portal vessels and is delivered to gonadotroph cells in the anterior pituitary gland. At the gonadotroph cells, GnRH binds to specific cell-surface receptors, triggering a sequence of events for the synthesis of LH and FSH (Peters et al., 1985).

Several researchers have used GnRH agonists in cattle and buffaloes to improve post-partum fertility. Variable effects of GnRH agonists have been reported on pituitary responsiveness, post-partum ovarian cyclicity, uterine involution, and conception rate (Sharma et al., 2017). Administration of exogenous GnRH analogues causes a steep LH peak concentration within 2 h after treatment, with the duration of the surge lasting 3–5 h (McDougall et al., 1995), resulting in ovulation of the dominant follicle (DF) of 10 mm or more (Crowe et al., 1993). The pattern of LH release is similar in suckled and non-suckled buffaloes, although the quantity of LH is affected by suckling (Singh et al., 2006). GnRH administration around the second week of calving helped induce ovulation (100%) in buffaloes within 45 days post-partum, with a significantly higher conception rate (Abdel-Halim and Helmy, 2014). Resumption of ovarian cyclicity can be enhanced by using different types of hormones in the early post-partum period, with

variable results (Kandiel et al., 2013). Faster uterine involution and a higher conception rate in Indian Murrah buffaloes were observed after administration of GnRH or PGF2 α two weeks after parturition (Ingawale et al., 2014).

Considering these findings, the present study was designed to investigate the responsiveness to exogenous gonadotrophin on post-partum fertility during breeding and non-breeding seasons in buffaloes in an organized herd.

2 Materials and methods

For this study, 42 post-partum pluriparous healthy Murrah buffaloes were selected that calved normally at term with no post-partum complications. All buffaloes were suckled for milk letdown, and all calves were weaned as per standard managemental practices. According to the season of calving, animals were randomly divided into two groups, comprising 22 animals in the breeding season (September to February; Group 1) and 20 animals in the non-breeding season (April to August; Group 2). All experimental animals were fed routinely as per ICAR (2013) standards with free access to clean drinking water. All procedures were conducted with the approval of the Institutional Animal Ethics Committee of ICAR-CIRB.

2.1 Group 1

Twenty-two post-partum pluriparous Murrah buffaloes (average body weight 445 ± 5 kg; BCS 3–3.5) that calved between September and February were included in this group. During the breeding season, relative humidity ranged between 70% and 80%, and ambient temperature ranged from 11 °C to 39 °C. The animals were further divided into two groups, i.e., treatment ($n = 12$) and control ($n = 10$). Buffaloes in the treatment group received an exogenous GnRH analogue (Receptal; 10 μ g buserelin acetate, i.v.) (Sharma et al., 2017) on day 21 post-partum, whereas animals in the control group ($n = 10$) received no treatment.

2.2 Group 2

Post-partum pluriparous Murrah buffaloes ($n = 20$; average body weight 450 ± 5 kg; BCS 3–3.5) that calved between April and August were included in this group. During the non-breeding season, relative humidity ranged between 25% and 60%, and ambient temperature ranged from 35°C to 47°C. Buffaloes in this group were further divided into two groups, i.e., treatment ($n = 10$) and control ($n = 10$). Similar to Group 1, buffaloes in the treatment group received an exogenous GnRH analogue (Receptal; 10 μ g buserelin acetate, i.v.) on day 21 post-partum, whereas the remaining buffaloes ($n = 10$) served as controls and received no treatment. All animals were monitored regularly using per rectal examination, ultrasonographic scanning, and blood sampling as described below.

2.3 Blood sampling

To measure luteinizing hormone (LH) concentration, blood sampling was carried out on day 21 post-partum, when GnRH injection was administered as per the study design. Blood was collected from a minimum of eight buffaloes in each treatment and control group during both the breeding and non-breeding seasons. Samples were collected at 0, 30, 60, 90, 120, and 150 min after GnRH injection. Blood was centrifuged at 1,500 rpm for 15 min to separate plasma. The separated plasma was stored frozen at -80°C in 500 μL aliquots until LH estimation.

2.3.1 Estimation of LH

Plasma LH concentration was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory), and values were expressed as ng/mL. The procedure described by the manufacturer was followed, and a standard curve was prepared using known standards. The coefficients of intra-assay and inter-assay variation were $<10\%$ and $<12\%$, respectively.

An ELISA plate pre-coated with bovine NEFA antibody was used for the estimation of LH concentration. NEFA present in the sample was added and bound to the antibodies coated on the wells, after which biotinylated bovine NEFA antibody was added and bound to NEFA in the sample. Subsequently, streptavidin-HRP was added, which bound to the biotinylated NEFA antibody. The detection range of the assay was 0.08–33.34 ng/mL.

2.4 Transrectal ultrasonography

All ultrasound examinations were performed by a single operator using a Hitachi Aloka Color Doppler Prosound F31 ultrasound scanner equipped with an intraoperative electronic convex sector probe (IPX7). Ultrasound examinations were conducted on days 0, 7, 14, 21, 28, 35, 42, 60, and 90 post-partum to evaluate the resumption of ovarian cyclicity and uterine involution. The day of calving was considered day 0 in this study. Pregnancy diagnosis was performed after day 30 post-insemination in inseminated buffaloes.

2.4.1 Uterine involution

This refers to the process by which the uterus returns to its normal size and functional state post-calving. Rectal palpation was started from day 21 post-partum at weekly intervals until complete involution of the uterus. Uterine involution was considered complete when there was little difference between the previously gravid and non-gravid horns, and the entire uterus was palpable within the pelvic cavity (El-Wishy, 2007a).

2.4.2 Onset of ovarian cyclicity

To determine the onset of ovarian cyclicity, ultrasonography was carried out from day 0 onwards until a corpus luteum was detected in any ovary or until day 90 post-partum, whichever occurred earlier.

2.4.3 Days open

Days open were calculated as the time interval between calving and successful conception.

2.4.4 Conception rate

Conception rate was calculated at different artificial inseminations (AI) and post-partum days. All buffaloes under study exhibiting signs of estrus were inseminated as per standard procedures.

2.5 Statistical analysis

Different parameters, *viz.* LH concentration and days open, were analyzed and compared between the control and treatment groups as well as between the two seasons using two-way ANOVA. The significance between time intervals within each group was assessed using repeated measures ANOVA. Other parameters, *viz.* resumption of ovarian cyclicity, uterine involution, and conception rates between groups at different time intervals, were analyzed using Fisher's exact test. Differences with $P < 0.05$ were considered statistically significant. All statistical analyses were performed using SPSS version 16.0 for Windows (IBM Corp., Armonk, NY, USA).

3 Results

3.1 LH hormone status

During the breeding season, the average pre-treatment LH concentration was similar in both the treatment (1.7 ± 0.3 ng/mL) and control (1.2 ± 0.2 ng/mL) groups. A sharp rise in LH concentration was observed within 30 min of GnRH injection, peaking (18.3 ± 0.9 ng/mL) at 120 min post-injection in the treatment group (Table 1). However, in the control group, LH values remained static across different time intervals.

During the non-breeding season, pre-treatment LH concentration was comparable in both the treatment (0.8 ± 0.1 ng/mL) and control (0.9 ± 0.2 ng/mL) groups. Similar to the breeding season, a sharp rise in LH values was observed within 30 min of GnRH injection, peaking (15.6 ± 0.4 ng/mL) at 120 min post-injection in the treatment group, whereas LH concentrations in the control group remained static across all time intervals.

The pattern of LH release in response to buserelin acetate treatment was significantly higher ($P < 0.05$) during both the breeding (18.3 ± 0.9 ng/mL) and non-breeding (15.6 ± 0.4 ng/mL) seasons, with a peak at 120 min post-treatment. LH concentrations in the treated group during the breeding season at 0 h (1.7 ± 0.3 ng/mL), 30 min (4.8 ± 0.7 ng/mL), 120 min (18.3 ± 0.9 ng/mL), and 150 min (14.3 ± 0.8 ng/mL) were higher ($P < 0.05$) than those in the non-breeding counterparts. However, the magnitude of LH release in the treatment group was significantly lower ($P < 0.05$) during the non-breeding season at different time intervals compared with the

TABLE 1 Plasma LH concentrations (ng/ml; mean $\pm$ SE) at different time intervals following administration of GnRH analogue in post-partum buffaloes during breeding and non-breeding seasons.

Season	Group	0 hr	0 hr	30 min	60 min	90 min	120 min	150 min
Breeding	Treatment (n=8)	1.44 $\pm$ 0.2 ^a	1.7 $\pm$ 0.3 ^{aFx}	4.8 $\pm$ 0.7 ^{aEx}	8.4 $\pm$ 0.6 ^{aD}	11.6 $\pm$ 0.8 ^{aC}	18.3 $\pm$ 0.9 ^{aAx}	14.3 $\pm$ 0.8 ^{aBx}
	Control (n=8)		1.2 $\pm$ 0.2 ^a	1.2 $\pm$ 0.2 ^b	1.2 $\pm$ 0.1 ^b	1.1 $\pm$ 0.2 ^b	1.2 $\pm$ 0.2 ^b	1.2 $\pm$ 0.2 ^b
Non-Breeding	Treatment (n=8)	0.88 $\pm$ 0.1 ^b	0.8 $\pm$ 0.1 ^{aFy}	3.0 $\pm$ 0.3 ^{aEy}	7.1 $\pm$ 0.4 ^{aD}	10.3 $\pm$ 0.4 ^{aC}	15.6 $\pm$ 0.4 ^{aAy}	11.8 $\pm$ 0.3 ^{aBy}
	Control (n=8)		0.9 $\pm$ 0.2 ^a	0.9 $\pm$ 0.1 ^b	0.9 $\pm$ 0.2 ^b	0.9 $\pm$ 0.2 ^b	1.0 $\pm$ 0.1 ^b	1.0 $\pm$ 0.1 ^b

Different superscripts (a, b) differ significantly ($P < 0.05$) between groups within a particular season (column).

Different superscripts (A, B, C, D, E, F) differ significantly ($P < 0.05$) between time intervals within a group during a particular season.

Different superscripts (x,y) differ significantly ($P < 0.05$) between two seasons within a group (column).

breeding season. The basal LH level was comparable between the breeding and non-breeding seasons.

3.2 Resumption of ovarian cyclicity

Resumption of ovarian cyclicity was assessed by the appearance of the first corpus luteum following calving during regular ultrasound examinations of post-partum buffaloes. It was observed that 10% of buffaloes during the non-breeding season and 20%–25% of buffaloes during the breeding season resumed cyclicity by day 21 post-calving (Table 2). By day 28, 30% of buffaloes that calved during the non-breeding season and 60% that calved during the breeding season resumed cyclicity (Table 3).

It is evident that by day 28, the majority of buffaloes exhibited their first estrus, as indicated by the appearance of the first corpus luteum. By day 60 post-partum, 90% of buffaloes in the control group during the breeding season and 60% during the non-breeding season had developed a corpus luteum. GnRH administration on day 21 post-calving was found to improve early resumption of cyclicity in buffaloes calving during both the breeding (83.3%) and non-breeding (80%) seasons by day 28 post-partum. Moreover, 10% of buffaloes that calved during the breeding season and 40% that calved during the non-breeding season did not resume cyclicity by day 90 post-partum.

Administration of GnRH on day 21 post-partum facilitated early cyclicity resumption in all animals during both seasons. Irrespective of season, overall 18.3% of buffaloes in the treatment group and 15% in the control group had already resumed cyclicity before administration of GnRH on day 21 post-calving (Table 3). Following GnRH administration, cyclicity resumed in 82% of the treatment group and 45% of the control group by day 28 post-calving. By day 60 post-partum, 95.5% and 75% of buffaloes in the treatment and control groups, respectively, had resumed cyclicity. Beyond day 90 post-partum, 4.5% of buffaloes in the treatment group and 25% in the control group remained anovular.

3.3 Uterine involution

None of the buffaloes that calved during the breeding season had completed uterine involution before day 21 post-partum in either the treatment or control groups. However, by day 28, 58% of buffaloes in the treatment group and 40% in the control group had completed uterine involution. By day 35, uterine involution was complete in all buffaloes in the treatment group and in 70% of buffaloes in the control group. In the control group, all buffaloes completed uterine involution by day 42 post-calving (Table 4). During the non-breeding season, 20% of buffaloes in both the treatment and control groups had already completed uterine

TABLE 2 Resumption of ovarian cyclicity (%) with respect to post-partum days in treatment and control group during breeding and non-breeding seasons.

Season	Group	n	Resumption of ovarian cyclicity (%) based on appearance of First Corpus luteum at different time interval						
			Before 21 d	21–28 d	28–35 d	35–42 d	42–60 d	60–90 d	> Day 90
Breeding	Treatment	12	25% (3/12)	58.3% (7/12)	0%	8.3% (1/12)	8.3% (1/12)	0%	0%
	Control	10	20% (2/10)	40% (4/10)	10% (1/10)	20% (2/10)	0%	0%	10% (1/10)
Non-breeding	Treatment	10	10% (1/10)	70% (7/10)	10% (1/10)	0%	0%	0%	10% (1/10)
	Control	10	10% (1/10)	20% (2/10)	10% (1/10)	10% (1/10)	10% (1/10)	0%	40% (4/10)
Overall	Treatment	22	18.3% (4/22)	63.7% ^a (14/22)	4.5% (1/22)	4.5% (1/22)	4.5% (1/22)	0%	4.5% (1/22)
	Control	20	15% (3/20)	30% ^b (6/20)	10% (2/20)	15% (3/20)	5% (1/20)	0%	25% (5/20)

Different superscripts (a, b) differ significantly in a column ($P < 0.05$).

TABLE 3 Cumulative percentage of buffaloes resuming ovarian cyclicity with respect to post-partum days in treatment and control groups during breeding and non-breeding seasons.

Post-partum days	Cumulative percentage of buffaloes resuming ovarian cyclicity on different days post-partum					
	Breeding season		Non- breeding season		Overall	
	Control	Treatment	Control	Treatment	Control	Treatment
Day 21	20% (2/10)	25% (3/12)	10% (1/10)	10% (1/10)	15% (3/20)	18.3% (4/22)
Day 28	60% (6/10)	83.3% (10/12)	30% (3/10)	80% (8/10)	45% ^a (9/20)	82% ^b (18/22)
Day 35	70% (7/10)	83.3% (10/12)	40% (4/10)	90% (9/10)	55% ^a (11/20)	86.5% ^b (19/22)
Day 42	90% (9/10)	91.6% (11/12)	50% (5/10)	90% (9/10)	70% (14/20)	91% (20/22)
Day 60	90% (9/10)	100% (12/12)	60% (6/10)	90% (9/10)	75% (15/20)	95.5% (21/22)
Day 90	90% (9/10)	100% (12/12)	60% (6/10)	90% (9/10)	75% (15/20)	95.5% (21/22)

Different superscripts (a, b) differ significantly in a column ($P < 0.05$).

involution by day 21 post-partum. On days 28, 35, and 42 post-calving, the cumulative percentages of complete uterine involution were 40%, 70%, and 90%, respectively, in the control group during both the breeding and non-breeding seasons, indicating that uterine involution was not influenced by the season of calving. Similarly, there appeared to be no significant effect of GnRH administration on uterine involution, as by day 42 post-calving, uterine involution was complete in 95% of buffaloes in both the treatment and control groups (Tables 4, 5).

3.4 Days open

Buffaloes in both treatment and control groups were inseminated according to farm management practices using frozen-thawed semen. No significant difference ($P > 0.05$) was observed in days open between the treatment (121.9 ± 24.9 days) and control (162.5 ± 47.4 days) groups during the breeding season (Table 6). Similarly, days open did not differ significantly between control groups during the breeding (162.5 ± 47.4 days) and non-breeding (154.1 ± 22.7 days) seasons. However, GnRH administration on day 21 post-partum significantly reduced days

open when given during the non-breeding season (90.5 ± 17.4 vs 154.1 ± 22.7 days).

3.5 Conception rate

Buffaloes that conceived at different time intervals post-calving (<90 days, $90-120$ days, and >120 days) were further evaluated. During the breeding season, the majority of buffaloes conceived within 90 days of calving in both the treatment and control groups (Table 7). However, during the non-breeding season, GnRH administration was beneficial, as most buffaloes (60%) conceived within 90 days post-calving, whereas in the control group, the majority (70%) conceived beyond 120 days post-calving (Table 7).

GnRH administration on day 21 post-calving did not influence conception rate, as the overall conception rate within three inseminations remained comparable between the treatment and control groups during the breeding season (75% vs. 70%). However, during the non-breeding season, a higher proportion of buffaloes in the treatment group (80%) conceived within three artificial inseminations (AI) compared with only 50% in the control group (Table 8). When overall results were compared irrespective of

TABLE 4 Buffaloes completing uterine involution (%) at different post-partum days in treatment and control group during breeding and non-breeding season.

Season	Group	n	Post-partum days				
			Before 21 d	21-28 d	28-35 d	35-42 d	42-60 d
Breeding	Treatment	12	0%	58% (7/12)	42% (5/12)	0%	0%
	Control	10	0%	40% (4/10)	30% (3/10)	30% (3/10)	0%
Non-Breeding	Treatment	10	20% (2/10)	40% (4/10)	20% (2/10)	10% (1/10)	10% (1/10)
	Control	10	20% (2/10)	20% (2/10)	30% (3/10)	20% (2/10)	10% (1/10)
Overall	Treatment	22	9.2% (2/22)	50% (11/22)	31.8% (7/22)	4.5% (1/22)	4.5% (1/22)
	Control	20	10% (2/20)	30% (6/20)	30% (6/20)	25% (5/20)	5% (1/20)

TABLE 5 Cumulative uterine involution (%) at different days post-partum during breeding and non-breeding season in treatment and control groups.

Post-partum Days	Breeding		Non-Breeding		Overall	
	Treatment	Control	Treatment	Control	Treatment	Control
Before day 21	0%	0%	20% (2/10)	20% (2/10)	9.2% (2/22)	10% (2/20)
Day 28	58% (7/12)	40% (4/10)	60% (6/10)	40% (4/10)	59.2% (13/22)	40% (8/20)
Day 35	100% (12/12)	70% (7/10)	80% (8/10)	70% (7/10)	90% (20/22)	70% (14/20)
Day 42	0%	100% (10/10)	90% (9/10)	90% (9/10)	95.5% (21/22)	95% (19/20)
Day 60	0%	0%	100% (10/10)	100% (10/10)	100% (22/22)	100% (20/20)

TABLE 6 Days open (Mean ± SE) during breeding and non-breeding seasons in treatment and control group. .

Groups	Days open (± SE)		
	Breeding season	Non-breeding season	Overall
Treatment	121.9 ± 24.9 ^a (12)	90.5 ± 17.4 ^b (10)	107.6 ± 15.8 ^b (22)
Control	162.5 ± 47.4 ^a (10)	154.1 ± 22.7 ^a (10)	158.3 ± 25.6 ^a (20)

Different superscripts (a, b) differ significantly (P<0.05) between groups at particular seasons in the column.

season, 77.3% of buffaloes in the treatment group and 60% in the control group conceived within the first three inseminations.

4 Discussion

4.1 Hormone status

In the present experiment, GnRH analogue (buserelin acetate) administration on day 21 post-partum resulted in an increase in LH concentration that peaked at 120 min post-injection during both breeding and non-breeding seasons, which was in accordance with Fields et al. (2009). In our experiment, average basal LH

concentrations and the time interval for attaining peak LH levels following GnRH administration were similar to earlier reports by Singh et al. (2006) and Mehmood et al. (2024). The baseline LH concentrations ranged between 0.72 and 3.0 ng/mL during most of the estrus cycle, and peak values of 20–40 ng/mL have been observed on the day of estrus in cattle and buffaloes (Avenell et al., 1985).

The pituitary gland remains suppressed for GnRH sensitivity during the immediate post-partum period and gradually becomes sensitive to releasing LH in response to GnRH (De Rensis et al., 1993). This sensitivity is recovered by day 14 in cows and by day 20 in buffaloes after calving (Alam and Dobson, 1986). However, the time of appearance of pituitary sensitivity varies according to species and breed (Peters et al., 1981). In addition, suckling induces differences in the time of appearance and intensity of pituitary responsiveness and in the magnitude of LH release. The inhibitory effect of suckling on the resumption of cyclic activity after calving acts more on the LH and GnRH release mechanisms than on their synthesis (Singh et al., 2006).

4.2 Resumption of ovarian cyclicity

Early resumption of post-partum ovarian cyclicity is important for ensuring high reproductive efficiency in dairy animals. During the late gestation period, high levels of placental and ovarian

TABLE 7 Buffaloes conceiving (%) with respect to different post-calving intervals in treatment and control groups during breeding and non-breeding seasons.

Season	Group	n	Buffaloes conceived (%) at different time interval post-calving		
			<90 d	90–120 d	>120d
Breeding	Treatment	12	50% (6/12)	16.7% (2/12)	33.3% (4/12)
	Control	10	50% (5/10)	10% (1/10)	40% (4/10)
Non-breeding	Treatment	10	60% (6/10)	20% (2/10)	20% (2/10)
	Control	10	20% (2/10)	10% (1/10)	70% (7/10)
Overall	Treatment	22	54.5% (12/22)	18.2% (4/22)	27.3% (6/22)
	Control	20	35% (7/20)	10% (2/20)	55% (11/20)

TABLE 8 Conception rate (%) trends across different AI attempts in treatment and control groups during breeding and non-breeding seasons, AI (artificial insemination).

Season	Group	Conception rate on different AI attempts			Overall conception rate	
		First AI	Second AI	Third AI	Within 3 AI	>3AI
Breeding	Treatment (n=12)	58.4% (7/12)	8.3% (1/12)	8.3% (1/12)	75% (9/12)	25% (3/12)
	Control (n=10)	50% (5/10)	10% (1/10)	10% (1/10)	70% (7/10)	30% (3/10)
Non-Breeding	Treatment (n=10)	60% (6/10)	20% (2/10)	–	80% (8/10)	20% (2/10)
	Control (n=10)	30% (3/10)	10% (1/10)	10% (1/10)	50% (5/10)	50% (5/10)
Overall	Treatment (n=22)	59.1% (13/22)	13.6% (3/22)	4.6% (1/22)	77.3% (17/22)	22.7% (5/22)
	Control (n=20)	40% (8/20)	10% (2/20)	10% (2/20)	60% (12/20)	40% (8/20)

steroids (estrogen and progesterone) exert a negative feedback effect on the hypothalamic–pituitary axis (El-Wishy, 2007b). Resumption of ovarian cyclicity can be assessed by the appearance of the first corpus luteum using regular ultrasound examination of post-partum animals. El-Wishy (2007b) reported that 34%–49% of animals resume cyclicity during the first 90 days post-partum, while 31%–40% remain acyclic for more than 150 days. Barman et al. (2011) reported that within 30 days, 40%–45% of buffaloes resume cyclicity, and by day 60 post-partum, 80%–100% of Murrah buffaloes become regularly cyclic. In Nili-Ravi buffaloes, 25%, 54%, 15%, and 6% of buffaloes resume post-partum cyclicity in <30, 31–60, 61–90, and >90 days post-partum, respectively (Mustafa, 2019).

Our findings that by day 28, 30% of buffaloes that calved during the non-breeding season and 60% that calved during the breeding season resumed cyclicity clearly indicate that season has a great influence on the resumption of ovarian cyclicity. GnRH administration on day 21 post-partum improved the early resumption of cyclicity in buffaloes calving during both the breeding (83.3%) and non-breeding (80%) seasons by day 28 post-partum. Furthermore, 10% of buffaloes that calved during the breeding season and 40% that calved during the non-breeding season did not resume cyclicity by day 90 post-partum. Administration of GnRH on day 21 post-partum facilitated early cyclicity resumption in all animals during both seasons.

The mechanism by which GnRH produces its effect is by inducing a pulsatile release of LH, which results in ovulation of the dominant follicle present at the time of treatment (Rajamahendran et al., 1998; Mehmood et al., 2024). The ovulation induction rate in response to a single injection, multiple injections, or continuous infusion of exogenous GnRH varies from 10% to 100% in post-partum cows depending upon the size of the dominant follicle at the time of injection (Crowe et al., 1993). Dominant follicles of 10 mm or more in size usually ovulate between 24 and 48 h, with a steep LH peak concentration within 2 h after treatment and a surge duration of 3–5 h (Stevenson and Call, 1988; McDougall et al., 1995).

Days open were lower and overall conception rates were significantly higher in the treatment group than in the control group during the non-breeding season but were non-significant during the breeding season, similar to the findings of Abdel-Halim and Helmy (2014). Maintenance of an appropriate calving interval requires rapid uterine involution accompanied by the return of normal cyclic activity, which is a prerequisite for reducing days open and improving conception rates (Nanda et al., 2003). Early resumption of cyclicity and uterine involution can be enhanced by administration of PGF2 α , GnRH analogues, or other uterine ecbolics in cattle and buffaloes (Nanda et al., 2003; Abdel-Halim and Helmy, 2014; Garcia-Ispuerto et al., 2019). Our findings were similar to the above observations and confirmed that the use of GnRH analogue helps in achieving early ovarian cyclicity, thereby promoting early breeding, reducing days open, and improving overall conception rates in the treatment group compared with the control group.

4.3 Uterine involution

Maintenance of an appropriate calving interval requires rapid involution of the uterus accompanied by the return of normal cyclic activity (El-Wishy, 2007a). Uterine involution has been reported to be completed in buffaloes within 27–74 days of calving (Lohan et al., 2000). Post-partum administration of PGF2 α or other uterine ecbolics enhances uterine contractility and hastens uterine involution in buffaloes (Nanda et al., 2003). Sheshappa et al. (2002) found that cows receiving GnRH only on day 14 post-partum had a shorter uterine involution period than controls, but those receiving sequential injections of GnRH on day 14 and PGF2 α on days 22–26 post-partum had a shorter involution period than those receiving GnRH analogue only.

In our experiment, 20% of buffaloes during the non-breeding season and none during the breeding season had completed uterine involution before day 21 post-partum. The majority of buffaloes

(>70%) completed uterine involution by day 35 post-partum in the control group. Administration of GnRH on day 21 post-partum helped in early uterine involution, as 91% of buffaloes completed uterine involution by day 35 post-partum. Contrary to our findings, Abdel-Halim and Helmy (2014) did not find a significant difference in uterine involution days between the treatment group (100 µg GnRH; Receptal administered on days 12–14 post-partum) and the control group.

GnRH improved reproductive efficiency by reducing the interval from calving to conception and decreasing the number of services per conception. During the non-breeding season, however, 20% of buffaloes in both the treatment and control groups completed uterine involution by day 21 post-partum. On days 28, 35, and 42 post-calving, the cumulative percentages of complete uterine involution were 40%, 70%, and 90%, respectively, in the control group during both breeding and non-breeding seasons, indicating that uterine involution was not influenced by season of calving. Similarly, there appeared to be no significant effect of GnRH administration on uterine involution, as by day 42 post-calving, uterine involution was complete in 95% of buffaloes in both the treatment and control groups. In contrast, Kumar et al. (2010) found that administration of GnRH-PGF2α in the early post-partum period improved uterine involution in crossbred cows. More research on these lines needs to be carried out in the future.

4.4 Days open and conception rate

GnRH administration on day 21 post-partum was found to significantly reduce days open when given during the non-breeding season, while no significant difference was observed in days open between treatment and control groups during the breeding season. During the non-breeding season, GnRH administration was beneficial, as the majority of buffaloes (60%) conceived within 90 days post-calving. Administration of GnRH during the non-breeding season could assist heat-stressed follicles by providing greater potential resistance to adverse environmental conditions and enhancing the fertilizing capacity of oocytes (Gallab et al., 2022).

Abdel-Halim and Helmy (2014) reported a higher conception rate in the buserelin acetate-treated group compared with the control group (55.8 ± 2.6) during the early post-partum period of buffalo cows. GnRH treatment aids in the early post-partum period by stimulating ovulation in buffaloes, thereby enhancing post-partum behavioral estrus, shortening the number of days open, decreasing the number of services per conception, and improving conception rate (Lamb et al., 2010; Adam et al., 2021; Gallab et al., 2022). More investigations are warranted on these lines.

5 Conclusion

From the study, it is evident that the use of GnRH in the early post-partum period elicits a predictable increase in fertility that can be reliably applied to buffalo herds. We found its beneficial effects

on reproductive parameters such as the resumption of cyclicity and reduction in days open during both the breeding and non-breeding seasons. Nonetheless, more research on these lines is warranted to devise suitable reproductive management strategies for improving post-partum fertility in buffaloes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Institutional Animal Ethics Committee of ICAR-CIRB. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RK: Methodology, Validation, Formal Analysis, Data curation, Visualization, Writing – review & editing, Writing – original draft, Investigation. JP: Visualization, Data curation, Validation, Formal Analysis, Conceptualization, Writing – review & editing, Supervision, Writing – original draft, Investigation. RS: Conceptualization, Resources, Project administration, Funding acquisition, Writing – review & editing, Data curation, Methodology, Writing – original draft, Supervision, Visualization, Investigation. SP: Methodology, Investigation, Visualization, Data curation, Writing – review & editing, Writing – original draft, Formal Analysis, Validation. YB: Data curation, Writing – original draft, Methodology, Visualization, Validation, Writing – review & editing, Formal Analysis. JA: Data curation, Writing – original draft, Conceptualization, Methodology, Visualization, Validation, Writing – review & editing, Formal Analysis.

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