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Research Article

Effect of a Single Injection of GnRH Analog Alone and at the Time of AI on Reproductive Performance of Nili Ravi Buffaloes

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ABSTRACT

Introduction: The gonadotropin-releasing hormone (GnRH) is a decapeptide hormone released from basal neurons of the hypothalamus. It stimulates the production of follicle-stimulating hormone and luteinizing hormone in the pituitary. The objective of the study was to evaluate the effect of a single injection of GnRH analog at the time of artificial insemination (AI) on buffalo estrus, conception, pregnancy, and calving rate.
Materials and methods: The anestrus nulliparous Nili Ravi buffalo heifers (n=80) were divided into 4 groups randomly. Group A (n=20) received only a single injection of GnRH analog at start of the experiment (Day 0), group B (n=20) received a single injection of GnRH analog at Day 0 and prostaglandine (PGF2α) at Day 7, group C (n=20) received progesterone sponge for 7 days, and group D (n=20) received progesterone sponge for 7 days and PGF2α at Day 9. In the 2nd study, buffaloes (n=60) expressing first (n=20), second (n=20), and third (n=20) postpartum estrus were enrolled. Moreover, heifers (n=20), expressing estrus first time were also included. Animals (n=10) from each group, received a single injection of GnRH analog at the time of AI were named as treatment group and the control (n=10) did not receive any treatment. Pregnancy tests were performed with the help of ultrasound on days 35 and 65 post-AI.
Results: The pregnancy and calving rates were lower in GnRH group compared to other treatments. Similarly, the administration of GnRH analog did not increase pregnancy rates in treatment groups.
Conclusion: The single injection of GnRH analog alone at the time of AI did not improve the reproductive performance of buffaloes.

1. Introduction

Buffalo (*Babulus bubalis*) is the second most important dairy animal across the world contributing more than 15% of milk production globally[1]. Buffalo faces different reproductive challenges like silent estrous[2], lack of homosexual behavior[3], delayed ovulation[4], and seasonal nature of breeding[5]. Buffalo is a short-day polyestrous animal and October to December is its peak breeding season[6]. The buffalo express maximum estrus activity from late night to early morning[7]. The estrus detection of buffalo is poor due to inherent problems and requires continuous and closer observation[8].

One possible option is to induce estrus by using different hormones[9]. The gonadotropin-releasing hormone (GnRH) has been widely used for ovulation synchronization[10], treatment of repeat breeders[11], and ovarian diseases in cows[12]. The GnRH is a decapeptide

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hormone released from basal neurons of the hypothalamus. It stimulates the production of follicle-stimulating hormone and luteinizing hormone in the pituitary. The exogenous administration of GnRH decreased the calving interval in buffaloes[13]. The exogenous hormones like prostaglandine (PGF2α) and GnRH in combination improved the conception rate in buffaloes[14-16]. Different implants like CIDR[17,18] and medroxy progesterone acetate sponges[19] have been tried to improve reproductive performance in buffaloes during non-breeding season. There is limited information about the comparison of GnRH analog, PGF2α, progesterone implants, and administration of GnRH analog at the time of AI. Therefore, the objective of the first study was to compare four different hormonal protocols during the breeding season in Nili Ravi buffaloes, while the objective of the second study was to compare the reproductive performance of buffaloes expressing natural estrus after injection of GnRH analog at the time of AI.

2. Materials and Methods

2.1. Ethical approval

Both studies were conducted following ethical regulations for animals and after proper approval from the farm manager Livestock Experiment Station (LES), Bhunikey, Pattoki District Kasur, Punjab, Pakistan located (31°1′30.0324″ N and 73°50′52.3608″ E) authorities.

2.2. Location and season

The studies were conducted at LES, Bhunikey, Pattoki District Kasur, Punjab, Pakistan located (31°1′30.0324″ N and 73°50′52.3608″ E) during the breeding season of buffaloes from October to December, 2022.

2.3. Selection of animal

A total of (n=80) anestrus nulliparous Nili Ravi buffalo heifers aged (30 ± 3 months) and body weight (350 ± 25 kg) were selected and placed in four groups randomly in the first study. A total of (n=80) Nili Ravi buffaloes having parity 0-4, 7 ± 3 years, and body weight 500 ± 75 kg expressing natural estrus were selected for the second study. All animals remained under the same feeding and managemental conditions throughout the studies.

2.4. Treatments

2.4.1. Study 1

The design of the study is presented in Figure 1. The group A received GnRH analog (Lecirelin, 50 mcg, Italy) at day 0 (50 days after calving). While, group B received GnRH analog (Lecirelin, 50 mcg) at day 0 and PGF2α (+ cloprostenol, 0.075 mg) at day 7. The sponge was implanted in the fornix vagina of animals in group C. The medroxy progesterone acetate sponge (Pakistan) contained (250 mg) medroxy progestin and was prepared according to the method[20]. Group D received a medroxy progesterone acetate-impregnated sponge for 7 days and PGF2α (+ cloprostenol, 0.075 mg, Italy) on day 9. The animals expressing estrus within 13 days were inseminated with frozen semen.

2.4.2. Study 2

The multiparous buffaloes (n=20) expressing first-time postpartum estrus were placed in Group A. Similarly, the buffaloes expressing 2nd (n=20) and 3rd times (n=20) post-partum estrus were placed in groups B and C. Moreover, (n=20) buffalo heifers expressing first-time estrus were placed in group D. The treatment group (n=10) received GnRH analog (Lecirelin, 50 mcg) at the time of AI, while the control group did not receive any GnRH analog at the time of AI 9 (Figure 2).

2.5. Ultrasonography

The animals were scanned at days 35 and 65, for conception and pregnancy rate through ultrasound (Honda, Japan 7400 7.5 M Hz). The animals were checked between 285-295 days for calving after AI.

2.6. Estrus detection and insemination

The estrus was detected by a trained technician and with a single pineal deviated teaser bull. The animals

Figure 1. Synchronization protocol used for estrus onset in Nili Ravi buffalo heifers

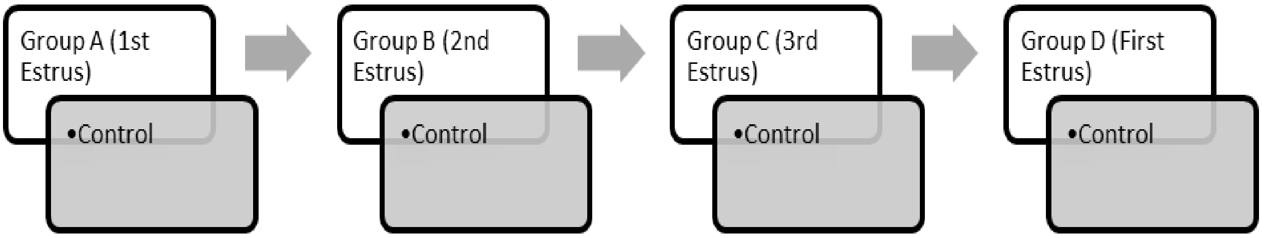

Figure 2. Experimental design for use of gondaotropine releasing hormone at the time of artificial insemination in Nili Ravi buffaloes

expressing estrus within 13 days were inseminated after 24 hours of standing heat by a single technician using the semen of 2 bulls uniformly[4].

2.7. Statistical Analysis

The data was compared through the Chi-square test by using SPSS version 13 and the P value was set significant at ($p < 0.05$).

3. Results and Discussion

The objective of the first study was to compare the efficacy of synch protocol during the breeding season. The pregnancy and calving rates were significantly lower ($p < 0.05$) in the GnRH group compared to GnRH+ PG, Sponge, and Sponge + PG groups (Table 1). The first study results are in line with Charoennam[21] and Majid[22], who reported better reproductive performance after combination of GnRH+ PG administration in buffaloes. These results are in line with Ramkrishnan[23] and Deshmukh[24], who reported 100 % estrus response after GnRH and PG administration in buffaloes. These results are supported by Singh[25], who reported GnRH and PG are effective for fertility improvement in buffaloes.

While in the second study, the administration of GnRH analog did not increase pregnancy rates (P = 0.98) in any treatment group (Table 2). The pregnancy rates were lower in the control group (40%) of heifers compared to the treatment group (60%), but non-significantly (P= 0.58). While, in the second study results are in line with Rosenberg[26], who reported GnRH administration 13-16 hours post estrus did not increase in conception rate. Similarly Stevenson[27], reported that exogenous GnRH administration did not improve the overall conception rate. These results are contrary to Kaim et al.[28], Gatius et al.[29], and Roth et al.[30] who reported higher conception rates after GnRH administration prior to AI.

Table 1. Reproductive performance of buffaloes after different hormonal protocols in Buffalo Heifers

	GnRH	GnRH + PG	Sponge	Sponge + PG	P-Value
Estrus rate	8/20 (40%)	20/20 (100%)	16/20 (80%)	20/20 (100%)	0.27
Conception rate	2/20 (10%)	6/20 (30%)	12/20 (60%)	10/20 (50%)	0.10
Pregnancy rate	0/20 ^{a} (0%)	6/20 ^{b} (30%)	10/20 ^{b} (50%)	8/20 ^{b} (40%)	0.04
Calving rate	0/20 ^{a} (0%)	6/20 ^{b} (30%)	8/20 ^{b} (40%)	8/20 ^{b} (40%)	0.01

The mean bearing different superscripts in a row differ significantly ($p < 0.05$) from each other, PG: Prostaglandine

Table 2. Effect of single injection of GnRH at the time of artificial insemination on pregnancy rates of buffaloes

	Group A	Group B	Group C	Group D	P-Value
Treatments	06/10 (60%)	07/10 (70%)	06/10 (60%)	06/10 (60%)	0.98
Control	06/10 (60%)	06/10 (60%)	06/10 (60%)	4/10 (40%)	0.58

4. Conclusion

It is concluded from both studies that a single injection of GnRH alone and at the time of AI is not sufficient to improve the reproductive performance of buffaloes during the breeding season. Further studies are required to check the effect of a single injection of GnRH on 11th days post-AI on the reproductive performance of buffaloes.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Muhammad Binyameen, Muhammad Imran, Muhammad Waseem designed and performed the research, Saba Anwar , Hina Tahir, Burhan Azam, Asim Tauseef performed the write up, and experiment design while Muhammad Saleem did the data analysis. All authors read and approved the final version of the manuscript.

Authors' relationships and activities

Authors are responsible for disclosing all relationships and activities that might bias or be seen to bias their work.

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Not Applicable.

Availability of data and materials

The data used in this study can be made available from corresponding author on reasonable request.

Ethical considerations

Ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors of the current study.

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