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## Comparative efficacy of GnRH analogue and HCG during the luteal phase on conception rate in non- infectious repeat breeder buffaloes

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### Abstract

Repeat breeding is a major reproductive disorder in dairy buffaloes that causes economic losses due to reduced fertility and prolonged calving intervals. This study evaluated the effect of Gonadotropin-Releasing Hormone (GnRH) analogue and human chorionic gonadotropin (hCG) administered during the luteal phase in non-infectious repeat breeder buffaloes. Sixty buffaloes from dairy farms in Parbhani district were screened through gynaeco-clinical examination, cervical mucus pH, White Side Test, and PMNL count, and were divided into GnRH-treated, hCG-treated, and control groups. The hCG-treated group showed the highest pregnancy rate (70%), followed by the GnRH group (60%), while the control group recorded 40%. The study concludes that hormonal treatment during the early luteal phase improves luteal function, progesterone secretion, and fertility, with hCG being the most effective treatment.

**Keywords:** Repeat breeding, buffalo, GnRH, hCG, progesterone, conception rate and luteal phase

### Introduction

Repeat breeding is a common infertility problem in dairy animals and is defined as the failure to conceive after three or more successive services with fertile semen despite normal estrous cycles and absence of detectable reproductive abnormalities. The incidence of repeat breeding in bovines ranges from 10 to 30% and is often higher in buffaloes due to inherent reproductive limitations (Bartlett *et al.*, 1986) <sup>[1]</sup>.

The corpus luteum plays a vital role in maintaining pregnancy through the secretion of progesterone, which is essential for uterine preparation, embryo development, and maintenance of uterine quiescence. Luteal dysfunction and inadequate progesterone secretion during the early luteal phase are major causes of early embryonic mortality in repeat breeder animals (Mann *et al.*, 1995; Kimura *et al.*, 1987) <sup>[5, 4]</sup>.

Hormonal treatments such as Gonadotropin-Releasing Hormone (GnRH) and human chorionic gonadotropin (hCG) have been used to improve fertility by inducing ovulation, promoting luteal function, and increasing progesterone secretion. Administration of these hormones during the early luteal phase can stimulate accessory corpus luteum formation and enhance conception rate in repeat breeder animals (Pandey *et al.*, 2016) <sup>[6]</sup>.

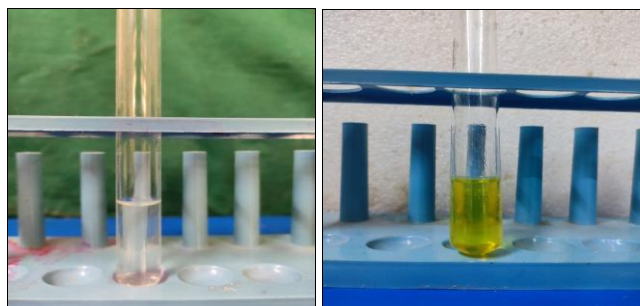
Therefore, the present study was undertaken to evaluate the effect of GnRH analogue and human chorionic gonadotropin administered during the luteal phase on conception rate in non-infectious repeat breeder buffaloes.

### Materials and Methods

The present study entitled “Studies on GnRH analogue and hCG during the luteal phase on conception rate in non-infectious repeat breeder buffaloes” was conducted from September 2025 to February 2026 in and around Parbhani district, Maharashtra. A total of 30 pluriparous repeat breeder buffaloes with regular estrous cycles and a history of failure to conceive after three or more services were selected from cooperative dairy societies and private dairy farms. The animals were examined through per-rectal palpation to confirm the normalcy of the

reproductive tract. Non-infectious status was confirmed by cervical mucus pH estimation, White Side Test, and polymorphonuclear leukocyte (PMNL) cell count, and animals with reproductive tract infections or anatomical abnormalities were excluded from the study.

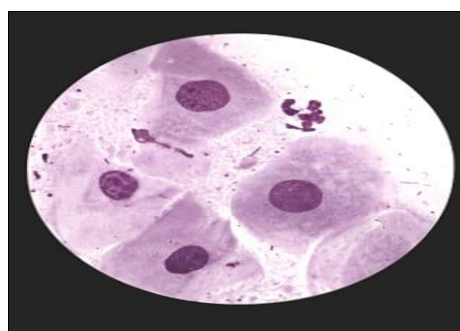
The selected buffaloes were randomly divided into three groups ( $n = 10$  each): Group I animals were treated with GnRH analogue (buserelin acetate) @ 10 µg intramuscularly on the 5th day post-artificial insemination (AI), Group II animals received hCG @ 1500 IU intramuscularly on the 5th day post-AI, while Group III animals served as untreated controls.



White Side Test



Measurement of pH



Observation and counting of PMN cells.

## Results and Discussion

Accurate diagnosis of non-infectious repeat breeding is essential prior to initiating therapeutic interventions. In the present study, the classification of repeat breeder buffaloes as non-infectious was confirmed through evaluation of cervical mucus pH, White Side Test (WST), and polymorphonuclear leukocyte (PMNL) cell count. The findings obtained from these diagnostic parameters are summarized in Table 1.

The cervical mucus pH ranged from 7.0 to 7.5 in 65% of buffaloes, which falls within the normal physiological range observed during estrus, while 35% of animals exhibited slightly alkaline values (7.5-8.0). Such variation in pH during estrus is generally associated with increased estrogen

concentration, which enhances cervical mucus secretion and electrolyte content, thereby creating a favorable environment for sperm survival and transport. Similar observations have been reported by Verma *et al.* (2014) [8], who noted that cervical mucus tends to become slightly alkaline during estrus due to elevated estrogen levels.

The White Side Test was negative in all animals (100%), indicating the absence of uterine infection in the selected buffaloes. The absence of colour change following the addition of sodium hydroxide suggests that no inflammatory exudates or bacterial contamination were present in the cervical mucus. Similar findings were reported by Bhat *et al.* (2014) [2], who documented negative White Side Test results in a substantial proportion of non-infectious repeat breeder animals.

Furthermore, the PMNL cell count was less than four cells per 100 endometrial cells in all animals, with an average value of  $2.47 \pm 0.01$ , confirming the absence of endometrial inflammation. Elevated PMNL cell counts are commonly associated with uterine infections or subclinical endometritis. Kasimanickam *et al.* (2004) [3] suggested that PMNL percentages exceeding 10% are indicative of subclinical endometritis, whereas the values recorded in the present study were well within the normal range. Therefore, the combined results of cervical mucus pH, White Side Test and PMNL cell count clearly confirm that the infertility cases included in this investigation were of non-infectious origin, thereby justifying the use of hormonal therapy aimed at correcting functional luteal insufficiency.

**Table 1:** Details of investigative parameters recorded for diagnosis of non-infectious repeat breeder Buffaloes

| Investigative parameter |               | Observation  |                 |
|-------------------------|---------------|--------------|-----------------|
| pH value                | Category      | Range        | Buffaloes       |
|                         | Normal        | 7.0-7.5      | 39 (65%)        |
|                         | Alkaline      | 7.5-8        | 21 (35%)        |
| WST result              | Negative      | No infection | 60(100%)        |
| PMNL cells              | Presence      | Less than 4  | 60(100%)        |
|                         | Average value | 0-04         | $2.47 \pm 0.01$ |

The effect of hormonal treatments on conception and pregnancy rates in non-infectious repeat breeder buffaloes is presented in Table 2. Buffaloes treated with GnRH on the fifth day post-artificial insemination exhibited a conception rate of 70% and pregnancy rate of 60%, compared with 50% conception and 40% pregnancy rate in the control group. The improvement in reproductive performance following GnRH administration may be attributed to the stimulation of luteinizing hormone (LH) release from the anterior pituitary, which promotes luteinization of follicles and enhances corpus luteum development. Increased luteal activity subsequently elevates progesterone secretion during the early luteal phase, thereby providing an optimal uterine environment for embryo development and implantation.

These findings are consistent with the reports Pandey *et al.* (2016) [6] reported improved fertility outcomes in repeat breeder animals following GnRH treatment during the luteal phase.

A comparatively higher reproductive response was observed in buffaloes treated with hCG, which showed a conception rate of 80% and pregnancy rate of 70%, whereas the control group recorded only 50% conception and 40% pregnancy rate. The superior efficacy of hCG treatment may be attributed to its luteinizing hormone-like biological activity, which directly stimulates luteal tissue by binding to LH

receptors present on granulosa and luteal cells. This action induces ovulation of dominant follicles during the luteal phase and promotes the formation of accessory corpus luteum, thereby enhancing progesterone secretion during the critical period of early embryonic development.

The formation of additional luteal tissue plays a crucial role in sustaining elevated progesterone concentrations, which are essential for maintenance of pregnancy and prevention of early embryonic mortality. Similar improvements in conception rates following hCG administration have been reported by Schmitt *et al.* (1996) [7]. Furthermore, Pandey *et al.* (2016) [6] also reported significantly higher pregnancy rates in repeat breeder cows treated with hCG during the early luteal phase.

The comparatively lower conception and pregnancy rates observed in the control group (50% and 40%, respectively) may be attributed to inadequate luteal function and suboptimal progesterone secretion during the early stages of embryonic development. Insufficient progesterone levels during this critical period can impair uterine receptivity and compromise embryo survival, leading to repeat breeding.

Overall, the findings of the present investigation clearly demonstrate that hormonal intervention during the early luteal phase significantly improves reproductive performance in non-infectious repeat breeder buffaloes. Among the treatment protocols evaluated, hCG administration on the fifth day post-insemination produced comparatively better conception and pregnancy rates than GnRH, indicating its greater efficacy in enhancing luteal function, increasing progesterone secretion and improving fertility in repeat breeder buffaloes.

**Table 2:** Comparison of conception rate and pregnancy rate in different group

| Group               | Conception Rate% | Pregnancy Rate% |
|---------------------|------------------|-----------------|
| GnRH (GROUP-I)      | 70%              | 60%             |
| hCG (GROUP-II)      | 80%              | 70%             |
| Control (GROUP-III) | 50%              | 40%             |

## Conclusion

The findings of the present study clearly demonstrate that hormonal intervention during the early luteal phase significantly improves reproductive performance in non-infectious repeat breeder buffaloes. Accurate diagnosis using cervical mucus pH, White Side Test, and PMNL cell count effectively confirmed the non-infectious nature of repeat breeding cases included in the study.

Administration of GnRH and hCG on the fifth day post-artificial insemination resulted in improved conception and pregnancy rates compared to untreated controls. Among the two treatment protocols, hCG produced comparatively better reproductive outcomes, with higher conception and pregnancy rates, indicating its superior efficacy in enhancing luteal function and progesterone secretion. The improved fertility observed following hormonal treatment may be attributed to the formation of accessory corpus luteum and increased progesterone levels, which support embryo survival during the critical period of early pregnancy.

Therefore, administration of hCG during the early luteal phase can be considered an effective therapeutic strategy for improving fertility in non-infectious repeat breeder buffaloes under field conditions.

## Conflict of Interest

Not available.

## Financial Support

Not available.

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## How to Cite This Article

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