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Effect of GnRh Administration at Artificial Insemination and on Day 6 Post Insemination on Pregnancy Outcomes in Holstein Friesian Cows

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ABSTRACT

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This study aimed to compare the effects of GnRH treated at artificial insemination (AI), at day 6 post-AI and at both times on pregnancy success in Holstein Friesian cows. The experiment was carried out at Aslam and Sons Dairy Farm at Dijkot, Faisalabad, Pakistan from February to June 2026. Eighty second parity cows were randomly divided into 4 groups (20 cows per group). G1 were the controls, G2 were treated with GnRH at AI, and G3 and G4 were treated with GnRH at AI and at 6 days post-AI. Each administration consisted of 5 mL of Conceptal intramuscularly, equivalent to 20 µg of buserelin. Artificial insemination was performed approximately 12 hours after the onset of natural estrus. Pregnancy diagnosis was performed using ultrasonography on days 28 and 45 post-AI to evaluate pregnancy establishment and maintenance. Overall, 45/80 cows (56.25%) were pregnant on day 28. Pregnancy rates were 45%, 60%, 55%, and 65% in G1, G2, G3, and G4, respectively; differences among groups were not statistically significant ($\chi^2 = 1.778$, $p = 0.620$). Of the 45 initially pregnant cows, nine (20%) experienced subsequent pregnancy loss by day 45. Pregnancy loss was 33.33% in G1, 25.00% in G2, 18.18% in G3, and 7.69% in G4, with no significant difference among groups ($p = 0.481$). Maintained pregnancy rates per inseminated cow were 30%, 45%, 45%, and 60%, respectively ($\chi^2 = 3.636$, $p = 0.304$). In conclusion, combined GnRH administration at AI and on day 6 post-AI produced the most favourable numerical outcomes for pregnancy establishment and maintenance. However, these differences were not statistically significant, warranting further investigation in larger studies.

Introduction

While planned breeding and genetic improvement through artificial insemination are possible, the success of insemination is dependent on establishment and maintenance of pregnancy (Pohler et al., 2012). If the service is not successful, another attempt might be necessary, more semen and technician fees would be incurred, and a longer interval between the next service and the next calving. To a herd veterinarian, an intervention at AI is appealing because the cow is already being handled and an accurate record of a reproductive event can be made (Perry et al., 2005). However, convenience is not a justification for treating. The intervention needs to address a biologically relevant limitation, yield a measurable benefit and be practical to the farm's reproductive management system.

Maintenance of pregnancy is a different component of reproductive function (Bazer et al., 2009). A later negative examination will detect a loss between tests if there is a viable pregnancy. This loss is clinically significant as the animal was apparently responding well to breeding (Al-Suhaimi et al., 2022). But an interval-based loss measure only measures pregnancy losses that survive long enough for detection. It is unable to restore any losses that have taken place before it has been observed. (Spencer and Bazer, 2004) argued that pregnancy failure should not be considered as a single uniform risk period, but rather distinct periods.

In the past, AI treatment with GnRH has been investigated to alter ovarian events associated with AI (Burnett et al., 2022). A potential benefit is significant if the animal's physiological reaction is not suitable to the desired breeding time. However, the reproductive status, estrous expression, previous AI service history, and management of cows submitted for AI vary (Giordano and Laplacette, 2026). If a preovulatory response occurs normally, the animal may have limited chance for the additional stimulus to be of benefit. There may be problems that are not corrected by hormonal treatment prior to insemination in a cow that has a reproductive dysfunction. So, the question is: does the intervention work in a clearly defined population, not does it work for all inseminated cows.

There is some research available to support this question, but it does not give a consistent answer. A historical meta-analysis showed a positive overall relationship with GnRH at insemination (Hassanein et al., 2024), and there have been trials with both positive and nonsignificant comparisons. Recent research has also examined whether the response is modified by estrous expression (Burnett et al., 2022). These observations make it worthwhile to make a careful record of insemination conditions and to make a controlled comparison. These do not rationalize building an empirical conclusion before gathering data.

The main aim of the proposed study was to assess the impact of administration of a GnRH analogue on day of AI and day 6 post AI on pregnancy at day 28. The secondary objectives were to 1) compare pregnancy loss from day 28 to day 42 in cows that initially were confirmed viable and 2) compare the proportion of cows with a viable pregnancy at day 42 that were maintained versus those that did not. The pre-specified directional hypothesis is that subsequent loss will be decreased and pregnancy per AI increased by treatment.

Methodology

Study location and period

The study was initiated during February to June 2026 at Aslam and Sons Dairy Farm, 264, near Dijkot, Faisalabad, Pakistan. The experiment evaluated the effects of gonadotropin-releasing hormone (GnRH) administration at artificial insemination (AI), on day 6 after insemination, and at both time points on pregnancy establishment and subsequent pregnancy maintenance in American Holstein Friesian cows. Consent was obtained from the farm owner before the animals were enrolled.

Experimental animals and study design

Eighty American Holstein Friesian cows in their second parity were included. Cows were randomly allocated to four groups of 20 animals each. Restriction to second-parity cows reduced variation attributable to parity. The individual cow was the experimental unit, and day 0 was defined as the day of artificial insemination. The four treatment groups represented a factorial arrangement comprising GnRH administration at AI, administration on day 6, both treatments, or neither treatment.

Table 1: Experimental groups and GnRH administration schedule

Group	Cows	At AI on day 0	Day 6 after AI
G1 Control	20	AI alone	No GnRH
G2 GnRH at AI	20	AI with GnRH	No GnRH
G3 GnRH on day 6	20	AI alone	GnRH
G4 Combined	20	AI with GnRH	GnRH

Note. Each GnRH administration consisted of 5 mL Conceptal intramuscularly, equivalent to 20 µg buserelin.

A method of detecting oestrus and artificial insemination, Cows were bred in synchrony by giving them their natural heat periods. All inseminations were made about 12 hours after the first sign of estrus.

Use of the GnRH preparation and administration

Conceptal was injected intramuscularly at each treatment with 5mL per cow. According to the manufacturer, the product contains 0.0042 mg buserelin acetate per millilitre (mL) or 0.004 mg (4 µg) of buserelin per mL (Star Laboratories, n.d.). The 5 mL injection was thus equivalent to 20 µg buserelin. At AI cows in G2 were injected once and cows in G3 were injected once on day 6. Cows in G4 were injected twice, thus delivering a total of 40 µg buserelin equivalent. Control cows were not given any GnRH.

Diagnosis and follow up of pregnancy

Ultrasound was used to diagnose pregnancy on day 28 after AI. All cows diagnosed with pregnancy initially were re-evaluated on day 45 by ultrasonography for pregnancy maintenance. Incorporating AI, pregnancy per AI was defined as the percent of cows that were inseminated and diagnosed as pregnant on day 28. This is sometimes referred to as "conception rate" but is a measure of pregnancy achieved and carried to term instead of actual conception. Pregnancy loss was considered a positive diagnosis at the 28-day exam with a negative diagnosis at re-examination. All cows that were originally pregnant were re-examined and the denominator for pregnancy loss was the number pregnant on day 28.

Calculation of reproductive outcomes

Pregnancy per AI (%) = (cows pregnant on day 28 ÷ cows inseminated) × 100. Pregnancy loss (%) = (pregnancies lost after day-28 diagnosis ÷ cows pregnant on day 28) × 100. Maintained pregnancy per AI (%) = (cows remaining pregnant at re-examination ÷ cows inseminated) × 100. Pregnancy maintenance among initially pregnant cows was calculated using initially pregnant cows as the denominator.

Statistical analysis

The aggregate counts supplied by the researcher were analyzed as categorical outcomes. Day-28 pregnancy status and maintained pregnancy status were compared across groups using Pearson chi-square tests. Expected cell frequencies were adequate for these comparisons. Pregnancy loss was compared using a two-sided Fisher–Freeman–Halton exact test for the four-group contingency table because expected numbers of losses were small. The exact p value was calculated by summing fixed-margin table probabilities no greater than that of the observed table. Statistical significance was assessed at $P < 0.05$. Analyses were performed in Minitab 20.0.

Results

Pregnancy rate at day 28

Forty-five of the 80 inseminated cows were diagnosed pregnant on day 28, giving an overall pregnancy rate of 56.25%. Pregnancy rates were 45.00% in G1, 60.00% in G2, 55.00% in G3, and 65.00% in G4. The combined-treatment group had the highest observed pregnancy rate, with 13 pregnant cows, while the control group had the lowest rate, with nine. GnRH at AI produced 12 pregnancies, and GnRH on day 6 produced 11 pregnancies.

Table 2: Pregnancy status on day 32 after AI

Group	Inseminated	Pregnant	Nonpregnant	Pregnancy %
G1 Control	20	9	11	45.00
G2 GnRH at AI	20	12	8	60.00
G3 Day 6	20	11	9	55.00
G4 Combined	20	13	7	65.00
Total	80	45	35	56.25

Note. Overall Pearson comparison: $\chi^2(3) = 1.778$, $p = .620$.

Relative to controls, pregnancy rate was higher by 15 percentage points in G2, 10 in G3, and 20 in G4. The numerical ranking was $G4 > G2 > G3 > G1$. The overall comparison did not demonstrate a statistically significant treatment effect ($p = .620$).

Pregnancy loss following initial diagnosis

Nine of the 45 pregnancies identified on day 28 were subsequently lost, giving an overall loss proportion of 20.00%. Three pregnancies were lost in G1, three in G2, two in G3, and one in G4. Using initially pregnant cows as the denominator, loss was 33.33%, 25.00%, 18.18%, and 7.69%, respectively. The lowest observed loss occurred in G4, followed by G3, while controls had the highest observed loss.

Table 3: Pregnancy loss and maintenance after day 32

Group	Initially pregnant	Lost	Loss %	Maintained
G1 Control	9	3	33.33	6
G2 GnRH at AI	12	3	25.00	9
G3 Day 6	11	2	18.18	9
G4 Combined	13	1	7.69	12
Total	45	9	20.00	36

Note. Loss percentages use pregnant cows as the denominator. Four-group exact comparison: $p = .481$. Repeat-examination day remains to be supplied.

Pregnancy maintenance among pregnant cows was 66.67% in G1, 75.00% in G2, 81.82% in G3, and 92.31% in G4. Relative to controls, losses were lower by 8.33 percentage points in G2, 15.15 in G3, and 25.64 in G4. Although G1 and G2 each lost three pregnancies, their loss proportions differed because G2 had more pregnant cows. The overall exact comparison was not statistically significant ($p = .481$).

Maintained pregnancy per inseminated cow

Thirty-six of the 80 inseminated cows remained pregnant at re-examination, giving an overall maintained pregnancy rate of 45.00%. Rates were 30.00% in G1, 45.00% in G2, 45.00% in G3, and 60.00% in G4.

Table 4: Maintained pregnancy relative to all inseminated cows

Group	Inseminated	Maintained	Maintained pregnancy %
G1 Control	20	6	30.00
G2 GnRH at AI	20	9	45.00
G3 Day 6	20	9	45.00
G4 Combined	20	12	60.00
Total	80	36	45.00

Note. Overall Pearson comparison: $\chi^2(3) = 3.636$, $p = 0.304$.

G4 maintained six more pregnancies than controls, corresponding to a 30-percentage-point difference. G2 and G3 each maintained nine pregnancies despite different initial pregnancy counts, reflecting fewer losses in G3. Nevertheless, the overall difference in maintained pregnancy status was not statistically significant ($p = 0.304$).

Discussion

Overall interpretation

The combined GnRH protocol produced the most favorable numerical outcomes: the highest day-28 pregnancy rate, lowest subsequent pregnancy-loss proportion, and highest maintained pregnancy rate. These observations suggest a potential benefit under the conditions of this farm, but none of the overall comparisons reached statistical significance. The results therefore identify a numerical pattern for further investigation rather than establish the superiority of the combined protocol.

With 20 cows per group, each additional pregnancy changed the group rate by five percentage points. The loss analysis involved only nine events across the four groups. Estimates based on these small numbers are sensitive to individual outcomes, and lack of significance should not be interpreted as proof that the protocols are equivalent.

GnRH administration at insemination

Pregnancy rate was numerically higher with GnRH at AI than in controls, at 60.00% versus 45.00%. A possible explanation is an effect on ovulatory timing relative to insemination. Such an effect could be relevant where endogenous hormonal responses vary, but ovulation was not monitored in the present study. Improved ovulatory timing cannot therefore be confirmed as the mechanism underlying the difference.

Burnett et al. (2022) indicated that GnRH was more beneficial for pregnancy per AI in cows that had weaker estrus expression, but not cows that had stronger estrous expression. This suggests that estrous features have the potential to influence reproductive response. In this instance, the number of times the estrus was observed was not quantified and its contribution is unknown. All cows were inseminated about 12-hour after onset of the natural estrus allowing a common standard, but this may have affected the insemination to ovulation interval due to the accuracy of the onset.

On day 6, GnRH was administered

The pregnancy rate was 55.00%, and subsequent loss proportion was 18.18% in G3, while the pregnancy rate and subsequent loss proportion of the controls were 45.00% and 33.33%, respectively. Early luteal GnRH may cause a responsive follicle to ovulate and form accessory corpus luteum. Physiological evidence for exploring this approach was obtained in the experimental work carried out on day 6 (Wiltbank et al., 2011). However, neither accessory luteal organs nor concentration of progesterone was reported in the present cows. Luteal support is a hypothesized treatment response and not a measured treatment response.

There is no consistent literature that supports a fertility advantage for early post-AI GnRH (Mendonça et al., 2017). There was no significant difference in pregnancy rate between the administration days 5 and 7 groups and the control group with rates of 31.6%, 33.8%, and 31.9% respectively (Kasimanickam et al., 2011). This demonstrates that a treatment appropriate for a particular herd does not have to result in a measurable improvement in that herd. The results here are variable and might be due to differences in follicular status, cow characteristics, and management that were not tested (Mahmood et al., 2026)

G4 pregnancy rate for day 32 was 65.00% (1 loss of 13 initially pregnant cows) and maintained pregnancy rate was 60.00%. The rates of the control group were 45.00% and 30.00%, respectively, with 3 losses in 9 pregnancies. The two treatment times may affect different processes: ovulatory timing at AI and/or subsequent ovarian/luteal function.

The results are not indicative of an additive or synergistic effect. An appropriate interaction analysis is needed to demonstrate synergy and the increase in the proportion in G4 alone is not enough. The same protocol also called for two injections instead of one. Cost of medications and handling should be considered when making any practical recommendation along with reproductive outcome.

The control estimate of 33.33% was based on 3 losses out of 9 pregnancies while the G4 estimate was based on one loss out of 13 pregnancies. With these small denominators comes a lot of uncertainty. In addition, the loss analysis applies only to those cows that were pregnant on day 32. Even though the treatment may influence which cows get that pregnant condition, comparison of losses will not maintain the same level of comparability as would exist if all 80 cows were randomly allocated.

The proportion of cows that have a fetus maintained after insemination is therefore a useful ancillary endpoint. It includes establishment of pregnancy, and subsequent pregnancies were maintained on the original denominator. The 4-group difference was not significant, but the highest value was for G4. Both the endpoints of pregnancy diagnosis (initially favorable and successful maintenance during follow-up) should be reported.

Wiltbank et al. (2016) identified specific crucial phases of pregnancy loss in the first trimester. This study could not measure losses at earlier stages of gestation but could diagnose pregnancies that had made it to the 32nd day. A cow that was diagnosed nonpregnant at day 28 could have failed to be bred or could have had a dying conceptus that was not diagnosed.

Suggestions for future investigation

The advantages of combined administration may be repeatable if the data could be replicated in a larger experiment on several farms. Base-line reproductive and production data should be collected and standardized examinations of pregnancy should be done at specified times. Ovulation, luteal structures and progesterone should be monitored to differentiate between the 2 treatment times. More direct assessment of independent effects and interaction would be possible by a factorial analysis of the data and by a prior calculation of sample size.

References

- AL-SUHAIMI, E. A., KHAN, F. A. & HOMEIDA, A. 2022. Regulation of male and female reproductive functions. *Emerging concepts in endocrine structure and functions*. Springer.
- BAZER, F. W., WU, G., SPENCER, T. E., JOHNSON, G. A., BURGHARDT, R. C. & BAYLESS, K. 2009. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. *MHR: Basic science of reproductive medicine*, 16, 135-152.

- BURNETT, T. A., MADUREIRA, A. M., BAUER, J. W. & CERRI, R. L. 2022. Impact of gonadotropin-releasing hormone administration at the time of artificial insemination on conception risk and its association with estrous expression. *Journal of dairy science*, 105, 1743-1753.
- GIORDANO, J. O. & LAPLACETTE, A. L. 2026. Present and future opportunities for artificial intelligence applications in dairy cattle reproduction. *Animal Frontiers*, 16, 86-98.
- HASSANEIN, E. M., SZELÉNYI, Z. & SZENCI, O. 2024. Gonadotropin-releasing hormone (GnRH) and its agonists in bovine reproduction II: Diverse applications during insemination, post-insemination, pregnancy, and postpartum periods. *Animals*, 14, 1575.
- KASIMANICKAM, R. K., MINK, M. R. & NEBEL, R. L. 2011. Effect Of GnRH Administration At Day 5 Or Day 7 After AI On Progesterone Concentrations, Corpus Luteum Volume And Pregnancy In Lactating Dairy Cows. *Clinical Theriogenology*, 3, 455-459.
- MAHMOOD, M., REHMAN, M. Z. & MOHSIN, A. 2026. Metabolic Reprogramming of Hepatic Gluconeogenesis in Transition Dairy Cows: Integrative Strategies from Nutritional Precursors to Epigenetic Modulation.
- MENDONÇA, L., MANTELO, F. & STEVENSON, J. 2017. Fertility of lactating dairy cows treated with gonadotropin-releasing hormone at AI, 5 days after AI, or both, during summer heat stress. *Theriogenology*, 91, 9-16.
- PERRY, G. A., SMITH, M. F., LUCY, M. C., GREEN, J. A., PARKS, T. E., MACNEIL, M. D., ROBERTS, A. J. & GEARY, T. W. 2005. Relationship between follicle size at insemination and pregnancy success. *Proceedings of the National Academy of Sciences*, 102, 5268-5273.
- POHLER, K. G., GEARY, T. W., ATKINS, J. A., PERRY, G. A., JINKS, E. M. & SMITH, M. F. 2012. Follicular determinants of pregnancy establishment and maintenance. *Cell and tissue research*, 349, 649-664.
- SPENCER, T. E. & BAZER, F. W. 2004. Conceptus signals for establishment and maintenance of pregnancy. *Reproductive Biology and Endocrinology*, 2, 49.
- WILTBANK, M. C., SOUZA, A. H., CARVALHO, P. D., BENDER, R. W. & NASCIMENTO, A. B. 2011. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. *Reproduction, Fertility and Development*, 24, 238-243.



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