Repeat Breeding Syndrome and its Management Protocols in a

Dairy Farm

***Original Research Article***

# ABSTRACT

**Aims:** The study involved sixty dairy cows aimed at identifying causes of repeat breeding syndrome, assessing the responses to three hormonal protocols, and evaluating conception rates following natural and artificial insemination.

**Place and Duration of Study:** The study was conducted at Dairy Castle Farm in Birol, Dinajpur (25°31'–25°46' N; 88°26'–88°38' E), from June 2018 to May 2019. The area has a humid subtropical climate (20–35°C, 70% humidity).

**Methodology:** Three protocols—Select synch, Ovsynch, and PGF2α plus PGF2α—were administered based on rectal palpation findings. Estrus was detected via visual signs and vaginal electrical resistance thrice daily. Cows in estrus were inseminated by Artificial Insemination and natural service. Pregnancy was confirmed by non-return to estrus within 21 days and rectal palpation at 90 days post-insemination.

**Results:** Approximately 54.72% of cows were diagnosed with cystic ovarian degeneration. Estrous response rates were high across all protocols: 100% for Select synch and Ovsynch, and 89.47% for PGF2α plus PGF2α. The mean onset of estrus was similar among groups: 49 ± 3.41 hours for Select synch, 48.6 ± 2.98 hours for Ovsynch, and 44.26 ± 15.8 hours for PGF2α plus PGF2α, with no significant differences observed. Duration of estrus also showed no significant differences: 40.1

± 3.85 hours for Select synch, 47.1 ± 5.61 hours for Ovsynch, and 37.5 ± 13.44 hours for PGF2α plus PGF2α. The conception rates following induction of estrus by Select synch (GnRH+PGF2α) protocol, Ovsynch (GnRH+PGF2α+GnRH) and PGF2α plus PGF2α protocol in dairy cows served naturally used in this experiment were 100%. It can be concluded that hormonal treatments may effectively use for improving conception rates in repeat breeder dairy cows.

**Conclusion:** Hormonal protocols effectively synchronized estrus in Repeat Breeding Syndrome (RBS) cows. To improve RBS management: Ensure strict semen quality control during storage/transport, train AI technicians in proper insemination techniques and timing and prioritize natural service where AI infrastructure is inadequate.

*Keywords: Artificial insemination; conception rate; dairy cows; estrous; hormones; repeat breeding syndrome.*

# INTRODUCTION

Livestock is a significant part of agro-based countries, playing socio-economic roles in rural livelihoods. These socio-economic factors are expanding in significance as the division develops due to an increasing human population, rising salaries, and urbanization rates. Bangladesh has a tropical monsoon climate which creates a huge scope for livestock development. There is a livestock population of 3,931.37 lakh in Bangladesh (DLS 2017-2018). Approximately 1.53% of the national GDP (Bangladesh Economic Review, 2019) is accounted for by the livestock sector in Bangladesh. Despite the cattle population in Bangladesh being considerably high (240.86 lakh, DLS 2017-2018), milk and calf production are not satisfactory. It is necessary to improve the reproductive efficiency of cows to achieve the maximum return from a dairy farm (Kapse et al., 2017). An unhealthy reproductive system can interrupt animal production (Maruf et al., 2012). The low conception rate, lack of appropriate breeding policies, inadequate nutrition, and reproductive diseases compromise productivity (Gröhn et al., 2000). The repeat breeding syndrome is the major cause of low conception rate in zebu cows. A repeat breeder is generally defined as any cow that has a normal estrus cycle, is free from any palpable abnormalities, shows no abnormal vaginal discharges and has not conceived after three or more successive services (Asaduzzaman et al., 2016). It is still a

considerable issue in cattle breeding, leading to financial misfortune and lower benefit for dairy farmers, because it requires more inseminations, increases calving intervals, culling rates and producer cost (Temesgen et al., 2022). The culling rate of repeat breeder dairy cattle can be reduced by improving their conception rate through careful handling of genitalia during insemination, diagnosis and management of uterine infections, and the use of hormones to enhance fertilization rates and lower embryonic mortality (Singh, 2017). Some researchers agree that the use of hormonal protocols is one of the best treatments for repeat-breeding cows (López-Gatius et al., 2020). Repeat-breeding cows with delayed ovulation can be recovered by GnRH treatment (Asaduzzaman et al., 2016). AI using double doses of semen or single AI with GnRH administration showed better responses to increase the pregnancy rate in repeat breeder cows (Asaduzzaman et al., 2016). The combined use of GnRH and PGF2α improved the pregnancy rate of repeat breeding crossbred cows (Jayaganthan et al., 2016; Sahoo et al., 2014). In Bangladesh, several dairy farms are facing the problem of repeat breeding and conception failure, resulting in economic losses. It is necessary to increase the conception rate in dairy farms by early detection of causes to reduce the intercalving interval in repeat-breeding cows (Fogwell et al., 1986). Therefore, the research was conducted to detect the causes of repeat breeding syndrome of dairy cows in a farm, to

observe the responses of three hormonal protocols for management of repeat breeding syndrome and to estimate the conception rate in hormonally treated cows after insemination naturally and artificially.

# MATERIALS AND METHODS

## Study Area and Period

The study was conducted at Dairy Castle Farm in Birol, Dinajpur (25°31'–25°46' N; 88°26'–88°38'

E), from June 2018 to May 2019. The area has a humid subtropical climate (20–35°C, 70% humidity).

## Experimental Animals

Sixty lactating Holstein Friesian crossbred cows (age: 4–5 years; weight: 350-400 kg) with RBS history (≥3 unsuccessful AI/NS) were selected. Cows underwent clinical examination and rectal palpation to exclude anatomical abnormalities, infections, or systemic diseases.

## Hormonal Protocols

Cows were assigned to three groups:

Select synch (n=20): GnRH (Ovurelin®; 100 μg IM) on Day 0; PGF₂α (Ovuprost®; 500 μg IM) on Day 7; AI/NS 72 h post-PGF₂α.

Ovsynch (n=20): GnRH (Day 0); PGF₂α (Day 7); second GnRH 48 h post-PGF₂α; timed AI 16 h later.

PGF₂α+PGF₂α (n=20): Two PGF₂α injections 11 days apart; AI/NS 72 h after the second injection.

## Estrus Detection and Insemination

Estrus was detected via visual signs (restlessness, vulval swelling, and clear mucus discharge) and vaginal electrical resistance (VER: 170–210 Ω) three times daily using a Draminski estrous detector (Poland). All estrous cows were artificially inseminated by using commercial frozen semen by a technician or naturally mated with proven fertile bull of farm.

## Pregnancy Diagnosis

Pregnancy was confirmed by non-return to estrus within 21 days and rectal palpation at 90 days post-insemination.

## Statistical Analysis

Data were analyzed using SPSS® 15.0. Estrus response, onset/duration, and CR were

compared using ANOVA and Chi-square tests. Significance was set at *P* < 0.05.

# RESULTS AND DISCUSSION

## The Basal Findings of Rectal Palpation Found Before the Start of the Experimental Protocols

The findings of rectal palpation, conducted prior to the initiation of the experimental protocols, are presented in Table 1. Among all the experimental cows, 54.72 of % cows had cystic ovarian degeneration diagnosed by rectal palpation. The cows of this farm were also diagnosed with 9.43% hypertrophied ovaries, 7.55% inactive (small rudimentary), 5.66% anovulatory heat, 1.89% fibrosis of the ovary, and 1.89% corrugated ovaries, which are considered as the female factors of repeat breeding syndrome. Correct deposition of semen, timing of deposition, preservation of semen, thawing of semen, and anatomical knowledge of the female reproductive system were identified as management factors (18.87%) contributing to the repeat breeding problem in this study (Table 1).

## Time of Onset of Estrus and Estrus Duration

The effects of Select synch, Ovsynch and PGF2α plus PGF2α protocols on estrus response, time of onset of estrus and duration of estrus in repeat breeder cows are summarized in Table 2. All cows of Select synch (n=20) and Ovsynch (n=20) showed estrus following treatment. Out of 20 dairy cows, 89.47% cows showed estrus by using PGF2α plus PGF2α protocol. The onset of estrus in cows of three protocols ranged from 42-55 hr. of PGF2α injection. The mean time of onset of estrus was 49 ± 3.41 hr. in Select synch, 48.6 ± 2.98 hr. in Ovsynch and 44.26 ± 15.8 hr. in PGF2α plus PGF2α protocol, respectively. Though there was no significant difference (P>0.05) among the groups of dairy cows on the time of onset of estrus, therefore, the time was comparatively higher Select synch (49 ± 3.41 hr.) and lowest (44.26 ± 15.8) in PGF2α plus PGF2α protocol.

The mean time of duration of estrus was 40.1 ±

3.85 hr. Select synch, 47.1 ± 5.61 hr. in Ovsynch and 37.5 ± 13.44 hr. in PGF2α plus PGF2α protocol, respectively. There was no significant difference (*P*>0.05) on the duration of estrus among the three groups of cows.

### Table 1. The basal findings of rectal palpation found before the starting of the experimental protocols

|  |  |
| --- | --- |
| **Findings** | **Percentage** |
| **Cystic ovaries** | 54.72a |
| **No reproductive pathology** | 18.87b |
| **Hypertrophy ovaries** | 9.43c |
| **Inactive ovaries (Small & rudimentary)** | 7.55 c |
| **Anovulatory heat** | 5.66 c |
| **Fibrosed ovaries** | 1.89d |
| **Ovary corrugated** | 1.89d |

*Values within the same column followed by different superscript letters differ significantly (P < 0.05) by Chi-square test*

### Table 2. The effects of Select synch, Ovsynch and PGF2α plusPGF2α protocols on estrus response, time of onset of onset of estrus and duration of estrus in repeat breeding cows

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Groups** | **No. of cows** | **Estrus response rate** | **Mean ± s.e.m. time of onset of estrus (hr.)** | **Mean ± s.e.m duration of estrus (hr.)** |
| Select synch (GnRH+ PGF2α) protocol | 20 | 100 % a | 49 ± 3.41 a | 40.1 ± 3.85 a |
| Ovsynch (GnRH+ PGF2α +GnRH) protocol | 20 | 100 % a | 48.6 ± 2.98a | 47.1 ± 5.61 a |
| PGF2α plus PGF2α protocol | 20 | 89.47 % b | 44.26 ± 15.8 a | 37.5 ± 13.44 a |

*Mean within the same column followed by same superscript letter does not differ significantly (P > 0.05) by ANOVA test*

## Conception Rate

The effects of select synch, ovsynch and PGF2α Plus PGF2α protocol on conception rate in dairy cows served either by artificially or naturally are presented in Table 3. The conception rates following induction of estrus served naturally in different protocol used in this experiment were 100%, whereas the conception rates following induction of estrus by Select synch (GnRH+PGF2α) protocol, Ovsynch (GnRH+PGF2α+GnRH), and PGF2α plus PGF2α protocol in dairy cows inseminated artificially with commercial frozen semen by a technician were 0%, 10% and 11.11%, respectively.

Detecting the cause of repeat breeding syndrome and its management is necessary to increase the conception rate in a dairy farm. Management factors can be addressed by a skilled Artificial Insemination technician, a nutritional balanced diet, and owner awareness. Hormonal treatment can easily solve the anatomical and functional defects of repeat breeder cows. For this reason, the present work was designed to investigate the

Factors responsible for repeat breeding syndrome in dairy cows and its management with hormonal treatment.

The female factors of repeat breeding syndrome investigated by rectal palpation in this experiment was 54.72% cystic ovarian degeneration, 9.43% hypertrophied ovaries, 7.55% inactive (small rudimentary), 5.66% anovulatory heat, 1.89% fibrosed ovary and 1.89% corrugated ovaries. The incidence of cystic ovarian degeneration was

8.8 – 27.4% in cattle (Nasution et al., 2021) and

0.9 – 2.0% in buffaloes (Mahrous et al., 2022) found in the studies of other researchers which are lower than the observation of this experiment. This indiscrimination may be due to stress from higher milk production, as well as management and nutritional factors in dairy cows (Sumi et al., 2022). Repeat breeding syndrome may also be associated with ovulatory disturbances and reproductive tract infection. Along with cystic ovarian degeneration, other ovarian dysfunction and hormonal aberration may also observe as cause of repeat breeding syndrome (Long et al., 2021).

### Table 3. Conception rate following induction of estrus by Select synch, Ovsynch and PGF2α plus PGF2α injection protocols in dairy cows inseminated either naturally or artificially

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Protocols** | **Methods of service** | **Number of cows served** | **Number of cows conceived** | **Conception rate,****%** |
| Select synch (GnRH+ PGF2α) protocol | AI | 6 | 0 | 0 |
| NS | 8 | 8 | 100 |
| Ovsynch (GnRH+ PGF2α +GnRH) protocol | AI | 10 | 1 | 10 |
| NS | 10 | 10 | 100 |
| PGF2α Plus PGF2α protocol | AI | 9 | 1 | 11.11 |
|  | NS | 8 | 8 | 100 |

*AI= Artificial Insemination, NS=Natural Service*

The dairy cows treated with Select synch and Ovsynch protocol showed 100% estrus response in this experiment. The results of this experiment were similar to those of other researchers, who found that Ovsynch resulted in 87 to 100% estrus response (Abuelhamd, 2023), and Select synch resulted in 91% estrus response (Yusuf, 2024). Another study showed lower estrus response of 17.70 (Ovsynch) and 63% (Select synch) (Kasimanickam, 2005). The estrus response to the PGF2α plus PGF2α protocol showed 89.47%, which is similar to the results of others (Wondim et al., 2022). The response rate was higher (Heidari et al., 2017) and lower (Qu et al., 2022) than other studies. This difference may be due to individual body physiology responses to hormones.

The average time of onset of estrus were 49 ±

3.41 hr., 48.6 ± 2.98 hr. and 44.26 ± 15.8 hr.

observed in cows treated with Select synch, Ovsynch and PGF2α plus PGF2α protocol, respectively. The onset of estrus after the last PGF2α injection in the Select Synch group fell within the range reported by other researchers (Yusuf, 2024). Ovsynch Protocol showed similarity with others (White et al., 2002). However, the average time of onset of estrus in cows treated with the PGF2α plus PGF2α protocol was lower than that reported by another researcher (Okawa et al., 2017).

The mean time of duration of estrus was 40.1 ±

3.85 hr. in Select synch, 47.1 ± 5.61 hr. in Ovsynch and 37.5 ± 13.44 hr. in PGF2α plus PGF2α protocols. This result showed a shorter duration of estrus in the Ovsynch protocol than the findings reported by López-Gatius (2020), and a longer duration in the results. This result showed similarity in the PGF2α plus PGF2α protocol with the findings of some researchers who experimented with ewes and was lower than other researchers (Heidari et al., 2017). This dissimilarity may be due to the seasonal effects (Mohan, 2023; Almadaly et al., 2023).

The conception rates following induction of estrus, as served naturally in the different protocols used in this experiment, were 100%. The conception rate achieved through natural service was within the range of results reported by others (Smith et al., 2004; Dahlen, 2014). However, many researchers have found a lower pregnancy rate in synchronized cows after they were served naturally (Stevenson et al., 2000; Baruselli et al., 2002; Bó et al., 2003). The higher conception rate found in the present study is due to the bulls used for natural service in this research, which were reared on the farm and had proven fertility. The conception rate of cows treated by

different synchronization in our study with timed AI was lower than natural services. This finding was inconsistent with other researchers (Buhecha et al., 2016; Jayaganthan et al., 2016). The causes of this lower conception rate may be the use of poor-quality semen by AI technicians for artificial insemination (Hamid et al., 2021). The sperm motility of remaining semen straws was lower preserved in liquid nitrogen container. The technician did not check the quality of semen before artificial insemination. A recent study demonstrated that impaired oocyte competence at the earlier phase of folliculogenesis reduces the probability of normal fertilization, embryo development, and embryo viability, which may also be responsible for repeat breeding syndrome in cows (Sood et al., 2017). The existence of an inherent inferior quality of the ovulatory follicle also found in repeat breeding Holstein heifers (Kafi et al. 2017; Sudano et al., 2011).

# CONCLUSION

Hormonal protocols (Select synch: GnRH+PGF2α, Ovsynch: GnRH+PGF2α+GnRH, PGF2α plus PGF2α) effectively synchronized estrus in Repeat breeding syndrome cows. However, conception rates via artificial insemination remained critically low (8%) while natural service achieved 100% conception. To improve repeat breeding syndrome management, ensure strict semen quality control during storage/transport, train AI technicians, and prioritize natural service where AI infrastructure is inadequate.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that no generative AI technologies such as large language models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

# ETHICS APPROVAL

Ethics committee approval for this study was received from Institutional Ethical Committee (IEC), Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh {Date: 26.11.2023, Number:4356 (1)}.

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