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RESEARCH ARTICLE

Effect of insemination timing and body condition score onpregnancy rate in crossbred dairy cows

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ABSTRACT

The objective of this study was to investigate the relationship in crossbred dairy cows between the timing of artificial insemination (AI) relative to the onset of estrus and body condition. 120 clinically healthy cows were selected from local farms for procedures of AI. Cows in the trial were categorized into two groups, A and B, each comprising 60 animals. Group A had a body condition score of 3–3.5, while Group B had a BCS of 2–2.5. Each group was subdivided into three treatment subgroups based on the AI timing following the onset of estrus: T1 (12–18 hours), T2 (18–24 hours), and T3 (24–30 hours). Statistical analyses were performed using SPSS version 16.0, with the chi-square test employed to assess differences between groups, and for calculation of frequency distributions. The results revealed that cows in group A (BCS 3–3.5) subjected to AI in the T_III interval (24–30 hours post-estrus) achieved a significantly higher pregnancy rate of 80% ($P>0.05$) compared to 45% in those inseminated during T_I (12–18 hours). Similarly, cows in group B (BCS 2–2.5) inseminated at T_III exhibited a significantly higher pregnancy rate of 70% ($P<0.05$) compared to 35% in cows inseminated during T_I. Although the BCS did not exhibit a statistically significant impact on pregnancy rates across all groups, cows with a BCS of 3–3.5 demonstrated higher pregnancy success compared to those with a BCS of 2–2.5. Additionally, while parity was not significantly correlated with pregnancy outcomes, a numerically higher pregnancy rate was observed in cows of third parity, followed by second-parity animals. In conclusion, optimizing the timing of artificial insemination to estrus onset, combined with consideration of body condition score, can enhance fertility outcomes in crossbred dairy cows.

1. Introduction

To the best of our knowledge, the present research was performed in cross-bred dairy cows on 'The effect of Artificial Insemination timing and body condition score on pregnancy rate'. Artificial insemination significantly influences the economy through its applications in genetic improvement, species conservation, selective breeding, and addressing infertility in both livestock and companion animals [1]. This reproductive technology involves challenges such as ensuring proper storage conditions, selecting high-quality semen from a fertile male, and accurately determining the optimal time for insemination, all of which are critical for its success [2]. By facilitating the union of gametes oocytes from females and spermatozoa from males AI represents a cornerstone among assisted reproductive technologies, contributing substantially to advancements in animal reproduction [3]. The approval of Artificial insemination is considered a breakthrough in the field of animal reproduction for robust multiplication of genetic resources from elite bulls [4]. Artificial insemination is one of

the most important components in assisted reproductive technologies, intra-cytoplasmic sperm injection (ICSI), in vitro fertilization (IVF), and pre-implantation genetic testing (PGT) [5]**.** Semen were collected, cryo-preserved, stored, and used for Artificial insemination from selected bulls that were shown to be superior to disease and well-deserved traits, as well as their progeny milk production capabilities [6]. AI is widely used in the cattle population and has a significant economic impact. Several studies have found that minor changes in Artificial insemination methods have a significant positive and negative impact on fertility. The conception rate in dairy crossbred cows is influenced by a variety of factors, many of which are directly associated with the animals themselves. Key determinants include milk production levels, body condition scoring, genetic attributes, and reproductive management practices. Additionally, advancements in agricultural methodologies and the strategic application of AI play a pivotal role in optimizing reproductive outcomes in crossbred dairy cows.

Several factors affect pregnancy, including parity, nutrition, body condition score, cyclicity status, expression of estrus, and hormonal treatments [7]. For assessing the condition of livestock, a body condition score is the appropriate and easy technique used at regular intervals. The valuation of body fat reserves in farm animals can be effectively achieved by visually inspecting and manually evaluating the thickness of fat coverage and the prominence of bones at key anatomical sites such as the tail head and loin region. The Body Condition Scoring system is a widely recognized method for quantifying fatness, offering a rapid, cost-effective, and straightforward approach. This technique is particularly critical as a pre-calving management tool for dry cows, facilitating a smooth transition into the lactation phase and minimizing potential complications at calving. Moreover, Body Condition Scoring is strongly associated with important reproductive and productive parameters, including the duration of postpartum an-estrus and overall milk yield [8].

Effective AI in dairy cattle requires precise estrus detection and timely insemination, adhering to the traditional AM-PM guideline. This protocol recommends inseminating cows observed in estrus during the morning in the evening and those identified in the evening the following morning. Research exhibits that the optimal pregnancy success rate, reaching 51.1%, occurs when insemination is performed between 8 to 12 hours after the onset of estrus. This finding emphasizes the importance of accurately timing insemination to maximize reproductive efficiency in dairy herds [9].

Before insemination, cross-bred cows with the following prominent signs were chosen for insemination: excitation of the animal, restlessness and nervousness, in-appetite with reduced feed, licking and mounting of other animals, frequent urination, and mucous secretion from the vulva, swelling of the vulva, raised tail, and reduced milk production [10]. AI plays a crucial role in enhancing milk quality in dairy cows, mitigating genetic disorders, addressing reproductive challenges, and improving breeding efficiency [11]. Several studies have explored critical factors influencing AI outcomes, including the hormonal effects on reproductive success, the relationship between body condition score and conception rates, as well as the significance of precise timing for insemination and its impact on subsequent fertility [7].

The relationship between BCS, fertility, and reproductive performance has been extensively documented, with evidence showing strong correlations among these parameters. A body condition score of 2.25 or lower, commonly observed in open cows and those with longer calving intervals, is consistently associated with diminished fertility and poor pregnancy outcomes in cattle. This study focuses on evaluating the interplay between Body Condition Score, timing of artificial insemination, and conception rates, emphasizing the critical role of optimal body condition in enhancing breeding efficiency and reproductive success in cows [12].

The estrous cycle in crossbred dairy cattle is often highly prolonged and irregular, presenting significant challenges in determining the optimal timing for artificial insemination [13]. In field conditions, such irregularities are observed with increasing frequency, yet Artificial Inseminators frequently lack precise guidelines for identifying the ideal insemination window to maximize conception rates. Therefore, this study aims to evaluate the influence of insemination timing, body condition scores, and parity on the pregnancy outcomes of crossbred dairy cows in the Peshawar region.

The trial was designed in Peshawar, Khyber Pakhtunkhwa Province, Pakistan, to address the issue of conception/pregnancy caused by the widespread use of artificial insemination in the field, which did not follow the required hours and schedule.

2. Materials and methods

2.1. Animals Selection

At the Agricultural University of Peshawar-Pakistan (34.0206° N, 71.4814° E), 120 crossbred fertile cows owned by local farmers underwent artificial insemination (AI). A single Artificial Inseminator assessed the cows' health before the procedure, utilizing frozen semen from selected bulls for insemination [14,15]. Pregnancy evaluations were conducted 45–60 days post-insemination on cows that did not show signs of returning to estrus. Cows were categorized based on the body condition scores (BCS) of 2–2.5 and 3–3.5, and insemination was performed at varying intervals post-estrus: 12–18 hours (T_I), 18–24 hours (T_II), and 24–30 hours (T_III).

2.2 Data Collection

In the current study, detailed records were maintained before artificial insemination (AI), including the timing of estrus commencement, insemination status, body condition score, mucus discharge (rated on a scale of 1 to 5), as well as the cow's pre- and post-calving history. Additionally, the feeding management practices for each cow were carefully documented in the Performa.

2.3 Animal Management

The cows were categorized into two groups (A and B) based on their body condition scores recorded in the Performa. Group A included 60 cows with BCS ranging from 2.25 to 2.5, while Group B consisted of 60 cows with BCS between 3.5 and 3.5. To further investigate the timing of insemination relative to estrus commencement, cows within each BCS group were subdivided into three subgroups (1, 2, and 3) according to the interval between estrus onset and insemination. Subgroup-I comprised 20 cows from each BCS group that were inseminated between 12 to 18 hours after the onset of estrus [11]. Subgroup--II included cows inseminated 18 to 24 hours following estrus onset, while subgroup -III consisted of cows inseminated between 24 to 30 hours after the start of estrus.

2.4 Body Condition Score

All of the experimental cows' BCS ratings (1–5) were recorded as stated [16]. This approach evaluated the thickness of fat across the lumber and tail head region, assigning a score ranging from 1 (extremely weak) to 5 (very fat).

2.5 The method is described as

The transverse process is pointed with a little cover, and the spine is noticeable. Although they are rounded by a thin layer of fat, transverse processes are perceptible. To feel distinct transverse spinal processes, firm pressure is necessary. The transverse processes are covered in a thick layer of fat and are not palpable [17].

2.6 Scoring method procedure

To obtain a more precise assessment of the tail head region, palpation was used to evaluate fat deposition, as this method accounts for the coat thickness and tail head positioning, offering a more reliable estimate compared to visual evaluation alone. Additionally, the same hand was employed to score the loin area of the cattle while they were in a relaxed state. The body score of a cow was calculated to the nearest half-point on a 5-point scale. Cows were handled to ensure the correct assessment of half points on a 5-point scale [18].

2.7 Procedure of Artificial Insemination

The standard procedure for insemination, as described by relevant literature, was adopted. All the procedures were performed carefully. In this study, all animals underwent artificial insemination using the recto-vaginal method, specifically for crossbred dairy cows [19].

2.8 Data evaluation and statistical analysis

The data collected was organized and entered into a Microsoft Excel spreadsheet. Subsequent analysis was performed using SPSS version 16.0, where frequencies were calculated, and the Chi-square test was applied to determine statistical differences between groups. The crosstabs function in SPSS was utilized for this analysis.

3 Results

3.1 Impact of A. I Timing on Pregnancy Rate in Crossbred Dairy Cows (BCS 3-3.5)

A significant difference was observed in the pregnancy rates of crossbred cows with a body condition score (BCS) of 3-3.5, inseminated at various intervals after the onset of estrus (Table 1). The data suggest that the timing of insemination plays a crucial role in pregnancy outcomes for these cows. Specifically, cows with a BCS of 3-3.5, inseminated at different time points—T_I (12 to 18 hours), T_II (18 to 24 hours), and T_III (24 to 30 hours) following estrus onset—exhibited conception rates of 45%, 70%, and 80%, respectively. The lowest conception rate (45%) was seen in cows inseminated during T_I (12-18 hours post-estrus), while the highest rate (80%) occurred in cows inseminated at T_III (24-30 hours). Notably, cows inseminated between 18 and 24 hours after the onset of estrus (T_II) had a significantly higher pregnancy rate (70%) than those inseminated during the earlier T_I interval. These findings emphasize the importance of timing in the AI process to optimize pregnancy rates in crossbred cows with a BCS of 3-3.5.

3.2 Effect of Estrus Timing on P/A.I in Crossbred Dairy Cows (BCS 2-2.5)

The pregnancy per artificial insemination rate of crossbred cows with a body condition score between 2 and 2.5 was significantly higher for cows inseminated at a 24–30 hour interval (T_III) post-estrus onset, reaching 75%, compared to those inseminated at earlier intervals (12–18 hours, T_I) with a P/AI of 35%, and at 18–24 hours (T_II) with a P/AI of 45%. These results suggest that shorter insemination intervals following estrus commencement led to sub-optimal P/AI in crossbred cows, whereas insemination conducted at extended intervals, specifically between 24 and 30 hours after heat detection, resulted in improved outcomes. As indicated in Table 2; 35%, 45%, and 75% of the cows were inseminated using T_I (12 to 18 hours), T_II (18 to 24 hours), and T_3 (24 to 30 hours), respectively, following first estrous signs.

3.3 BCS and time interval of A. I in crossbred cows

In the current trial, cows were observed with a high BCS of 3-3.5, and exhibited superior pregnancy rates following insemination at various time intervals, compared to those with a medium BCS of 2-2.5. Specifically, 70% and 80% of the cows with optimal BCS (3-3.5) conceived when inseminated at T-2 and T-3, respectively. Conversely, maximum conception rate was observed by insemination of cross-bred cows, at extended intervals of 24-30 hours after the commencement of estrous.

3.4 Overall P/AI among crossbred dairy cows, irrespective of body conditionscore

The overall pregnancy rate exhibited a significant difference ($P < 0.003$) as shown in Table 3. Crossbred dairy cows inseminated after a prolonged estrus period (T-3; 24–30 hours post-estrus) achieved the highest pregnancy rate of 77.5%. This was followed by cows inseminated within a moderate timeframe (T-

2; 18–24 hours), which recorded a pregnancy rate of 57.5%. In contrast, the lowest pregnancy rate of 40% was observed in cows inseminated within the shortest interval (T-1; 12–18 hours post-estrus).

Table 1. The impact of artificial insemination timing on pregnancy outcomes in crossbred Dairy cows exhibiting a body condition score (BCS) of 3 to 3.5 following the onset of estrus.

Time (after onset of estrus)	Animals inseminated	+ve	-ve	Animal's Pregnant (%)	Chi- square	P-Value
Time-I (12-18 Hours)	20	9	11	45		
Time-II (18-24 Hours)	20	14	6	70	5.71	0.05
Time-III (24-30 Hoursrs)	20	16	4	80		

Table 2. The influence of the timing of artificial insemination on conception rates in crossbred dairy cows with body condition scores (BCS) ranging from 2 to 2.5, following the onset of estrus.

Time (after onset of estrus)	Animals inseminated	+ve	-ve	Animal's Pregnant (%)	Chi- square	P-Value
Time-I (12-18 Hours)	20	7	13	35		
Time-II (18-24 Hours)	20	9	11	45	6.94	0.03
Time-III (24-30 Hoursrs)	20	15	5	75		

Table 3. Evaluation of pregnancy rate in cross- bred cow subjected to A. I at various timing intervals following the onset of estrus.

Time (after onset of estrus)	Animals inseminated	+ve	-ve	Animal's Pregnant (%)	Chi- square	P-Value
Time-I (12-18)	40	16	24	40		
Time-II (18-24)	40	23	17	57.5	11.58	.003
Time-III (24-30)	40	31	09	77.5		

3.4 Impact of Body Condition Score and Parity on the Pregnancy Rate in Cross-Bred Dairy Cows

The Pregnancy outcomes following AI were evaluated in crossbred dairy cows exhibiting optimal BCS of 3 to 3.5 across various parities as shown in Table 4. The pregnancy rates per artificial insemination (P/AIs) for cows in the first, second, and third parties were recorded as 57.14%, 70%, and 75%, respectively. The first parity exhibited the lowest conception rate at 57.14%, while the second parity had the highest at 70%. The third parity demonstrated an even higher pregnancy rate of 75%, indicating a trend of improved fertility with increasing parity.

3.5 Influence of Parity on the conception rate in cross-bred dairy cows

Insemination of crossbred dairy cows with an average body condition score of 2 to 2.5 resulted in varying pregnancy per artificial insemination (P/AI) rates across different parties **(Table 5).** The P/AI observed in first-parity cows was 37.5%,

while second-parity cows exhibited a higher P/AI of 60%. Third-parity cows showed the highest P/AI rate at 62.5%. Notably, the lowest P/AI was recorded in first-parity cows at 37.5%, while the highest was observed in third-parity cows at 62.5%. Additionally, 2nd parity cows had a superior P/AI (60%) when compared to 1st parity cows, which had a P/AI of 37.5%.

3.5 Overall Parity Wise Pregnancy Rate in Cross-Bred Dairy Cows

Table 6 revealed the overall pregnancy per artificial insemination (AI) in crossbred cows, independent of body condition score (BCS), for the trial. The P/AI values across various parities were observed to increase gradually: 48% in the 1st parity, 65% in the 2nd, and 68% in the 3rd parity. The highest P/AI (68%) was recorded in cows of the 3rd parity, while the lowermost (48%) was found in the 1st parity. This investigation indicates a linear increase in the P/AI as parity progresses, with P/AI rising from 48% in the 1st parity to 68% in the 3rd.

Table 4. Influence of Parity on the pregnancy rates of crossbred dairy cows with BCS ranging between 3-3.5

Parity	Animals inseminated	+ve	-ve	Animal's Pregnant (%)	Chi- square	P-Value
1st	28	16	12	57.14		
Parity					1.50	.47
2nd Parity	20	14	6	70		
3rd Parity	12	9	3	75		

Table 5. Influence of Parity on the pregnancy rates of crossbred dairy cows with BCS ranging between 2-2.5

Parity	Animals inseminated	+ve	-ve	Animal's Pregnant (%)	Chi- square	P-Value
1st Parity	24	9	15	37.5		
2nd Parity	20	12	8	60	3.23	0.19
3rd Parity	16	10	6	62.5		

Table 6. The overall pregnancy rate in crossbred dairy cows analyzed by parity.

Parity	Animals inseminated	+ve	-ve	Animal's Pregnant (%)	Chi-square	P-Value
1st Parity	52	25	27	48		
2nd Parity	40	26	14	65	4.02	0.13
3rd Parity	28	19	9	68		

4 Discussion

Artificial insemination (AI) is essential for the effective establishment of pregnancy in cattle, which is critical for the commencement of the subsequent lactation. It also quickens up genetic improvement and makes it easier for semen from genetically superior sires to be disseminated. The results of the present study highlighted that the timing of artificial

insemination, and post-estrus has a significant effect on the pregnancy rate (%) in crossbred dairy cows. Specifically, the pregnancy rates of cows with Body Condition Scores of 3-3.5 and 2-2.5 (as shown in Tables 3.1 and 3.2) were notably influenced ($P<0.05$) by the longer time interval after the commencement of estrus before AI. The highest conception rates were observed in crossbred cows with BCSs of 3-3.5 and 2-2.5 when AI was performed 24-30 hours after the commencement of estrus, with rates of 80% and 75%, respectively. In contrast, when AI was conducted after prolonged estrus signs, the overall pregnancy rate across all BCS groups was 77.5%, as summarized in Table 3.3. In contrast, cows with a BCS categorized as ordinary or excellent (A) exhibited the lowest pregnancy rates, at 35% and 45%, respectively. The study observed a brief window of 12 to 18 hours following the onset of estrus symptoms. The elevated pregnancy rates in crossbred dairy cows in this study may be attributed to extended estrous cycles and delayed ovulation. Dorsey et al. [20] reported that the timing of artificial insemination (AI) about the onset of estrus significantly influenced pregnancy outcomes ($P<0.01$) in crossbred cows. Heifers inseminated between 4 to 24 hours after estrus onset had higher AI pregnancy rates (63.7%) compared to those inseminated within 0–4 hours (48.1%) or beyond 24 hours (55.9%), with differences being statistically significant ($P<0.05$). The findings of this study indicate that crossbred cows exhibiting optimal physical condition scores (ranging from 3 to 3.5) had a positive effect on the pregnancy rates, as detailed in Table 3.1. These results align with the work of López-Gatius et al. [21], who highlighted the body condition score (BCS) as an effective management tool for assessing the nutritional status and reproductive health of dairy cows. Furthermore, although the observed relationship was not statistically significant, there was a noticeable trend suggesting that higher body condition scores were linked to improved conception rates [22]. Throughout the four-month supplementation period, cows receiving supplementation demonstrated higher pregnancy rates compared to control cows (42.9% vs. 25%; $P>0.05$). Additionally, 60% of supplemented cows exhibited signs of estrus, compared to 30% of the control group ($P>0.05$). In the current study, crossbred cows with a Body Condition Score (BCS) ranging from 2 to 2.5, subjected to artificial insemination (AI), exhibited a reduced pregnancy rate of 35% in cow A.I. when insemination occurred within a brief time frame (12-18 hours) following the onset of estrus (Table 3.2). These results indicate that low to average BCS in crossbred cows adversely affects pregnancy outcomes. This aligns with the findings of Mulliniks et al. [16], those who reported that crossbred cows with lower BCS tend to have lower conception rates compared to those in optimal condition. Additionally, the present study revealed that parity significantly influenced pregnancy rates, with variations observed across different physical condition scores. In the current study, pregnancy rates were observed to be 48%, 62%, and 68% for the first, second, and third parities, respectively. For cows with a body condition score ranging from 3 to 3.5, the pregnancy rates were 57.14%, 70%, and 75% for the 1st, 2nd, and 3rd parities, respectively. In comparison, crossbred cows with a Body Condition Score between 2 and 2.5 exhibited pregnancy rates of 37.5%, 60%, and 62.5% for the 1st, 2nd, and 3rd parties, respectively. The efficacy of parity on pregnancy outcomes was insignificant, with the 3rd parity showing the highest pregnancy rate, followed by the 2nd and 1st parities. These findings are in line with Khan et al. [23], who reported conception rates of 75%, 73.5%, and 61.9% for the 3rd, 2nd, and 1st parities, respectively.

5 Conclusions

The current Research experiment investigated the impact of insemination timing and body condition score on the pregnancy rate in crossbred dairy cows. Results exhibited that delaying artificial insemination to a period between 24-30 hrs following estrous commencement significantly enhanced the pregnancy rate. Cows exhibiting an optimal Body Condition Score range of 3 to 3.5, when inseminated at longer intervals post-estrus onset, revealed a notably higher conception rate per AI. Similarly, crossbred cows with a moderate BCS of 2 to 2.5 also exhibited improved pregnancy rates when inseminated at these prolonged intervals following estrus commencement.

Ethical Approval

All authors agree to submit this research article to the journal "Journal of Animal and Plant Research.

Authors' contribution

FZK, IU, FU and AK designed and conceived the study. FU, FUK, and MIK wrote the manuscript. FZK, IU, FU, FU, FUK, MIK, AN, IUK, MIK, I, MA and AK critically revised the manuscript. FU and AK supervised and funded this study. All the authors approved the manuscript for publication.

Author's declaration

The authors of the manuscript declare no conflict of interest.

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Conflicts of interest

There are no conflicts of interest.

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