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Follicular, luteal dynamics and effect of synchronization methods on reproductive responses in Cholistani cows

Muhammad Awais Ajmal[1], Mubbashar Hassan[1,2], Muhammad Shahzad[1], Muhammad Ilyas Naveed[1,2], Aijaz Ali Channa[1], Nasim Ahmad[1]*

[1] Department of Theriogenology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan; [2] Department of Clinical Sciences, College of Veterinary and Animal Sciences (Sub-Campus, University of Veterinary and Animal Sciences, Lahore), Jhang, Pakistan.

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Abstract

The Cholistani cow is considered one of the milch breeds of Zebu cattle. Despite being heat and tick-resistant, it has not yet gained much popularity among Pakistan dairy farmers due to its long calving interval. This study aimed to understand the basic reproductive physiology of Cholistani cows using sonography and reproductive biotechnologies such as estrus synchronization and timed artificial insemination to improve reproductive efficiency. In experiment 1, six Cholistani cows with mixed parity 3.20 ± 1.30 and weighing 400 kg were selected to monitor ovarian dynamics on alternate days by the same sonographer from the onset of heat through ovulation until the next ovulation. Experiment 2 measured the effect of estrus synchronization methods, controlled internal drug release-gonadotropin-releasing hormone (CIDR-GnRH, n = 31) and Ovsynch (OVS, n = 32) on various reproductive parameters. The mean estrous cycle length was 19.81 ± 0.56 days with two follicular waves. The mean inter-ovulatory interval was 20.80 ± 0.52 days, with a preovulatory follicular size of 13.83 ± 2.37 mm. Estrus response was higher ($p > 0.05$) in controlled internal drug release (CIDR)-GnRH (93.54%) than in OVS (84.37%) cows. Similarly, ovulation and conception rates were higher in CIDR-GnRH (91.66% *vs.* 68.42%) than in OVS cows (41.37% *vs.* 33.33%), respectively. In conclusion, CIDR-GnRH results in a better estrus response, higher ovulation rate, and subsequently greater conception rate than OVS in *Bos indicus* dairy cows.

Introduction

In the tropics, *Bos indicus* cows have significantly supported the nutritional requirements of the human population.[1] They have played a vital role in the world's dairy industry, particularly in Pakistan, which has different breeds that vary from region to region.[2] These *Bos indicus* breeds are primarily found in tropical and subtropical countries with high tick resistance and adaptability to greater environmental temperatures. Although *Bos indicus* cows produce less milk, their reproductive performance is better than that of *Bos taurus* cows.[3] Despite these merits and demerits, less research has been performed to exploit the reproductive potential of Cholistani cows. Therefore, there is a dire need to identify the reproductive potential to enhance the productivity and profitability of *Bos indicus* dairy cows.

The Cholistani cow is one of the 15 Zebu cattle breeds in Pakistan[2]. As a famous breed from the Cholistan desert,[4] contributes 250 tons of milk per day and accounts for up to 50.00% of the total beef production in the Punjab province.[5] Genetically superior animals can yield 15.00 - 18.00 L of milk daily.[4] However, the milk production of Cholistani cows is significantly lower compared with distinct milk breeds like Sahiwal and Gir.

Reproductive management can be improved by a better understanding of follicular dynamics and the proper regulation of follicular waves.[6] Recent reports have showed the effectiveness of using sonography to study ovarian follicular dynamics during the estrous cycle in *Bos taurus* and some *Bos indicus* breeds.[7] By considering the sonographic information of the reproductive cycle in *Bos taurus* cattle,[8] various manipulation techniques have been developed to improve conception rates, including estrus synchronization[9] and fixed-timed artificial insemination.[10]

***Correspondence:**

Corresponding author: Nasim Ahmad. DVM, MSc, PhD
Department of Theriogenology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan
E-mail: nasimahmad@uvas.edu.pk

Accurate control of the estrous cycle depends on follicular waves and the lifespan of the corpus luteum (CL).[11] Synchronization of follicular development and the timing of luteal regression can be achieved by administering gonadotropin-releasing hormone (GnRH) followed by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) seven days apart.[12] Additional GnRH administration may be necessary to induce estrus and ovulation at regular intervals, especially in anestrus cows.[13] The Ovsynch (OVS) has been commonly used for reproductive management in *Bos taurus*[14] and buffaloes[15] but its use in *Bos indicus* breeds in Pakistan has been limited.[16] To our knowledge, no study has been conducted on the controlled internal drug release (CIDR)-GnRH combination in *Bos indicus*, particularly in Cholistani cows. There is limited information available on the efficacy of OVS and CIDR-GnRH in Cholistani cows. This study aimed to characterize follicular and luteal dynamics, understand the basic reproductive physiology and evaluate the effects of estrus synchronization (OVS and CIDR-GnRH) on reproductive responses in *Bos indicus* dairy cows.

Materials and Methods

This study was performed at the Government Livestock Farm Jugait Peer, District Bahawalpur, Punjab, Pakistan. The project was approved by the ethical review committee of the University of Veterinary and Animal Sciences, Lahore (Reference No. DR/303). Multiparous, cyclic, lactating, Cholistani cows (n = 69) of mixed parity 3.20 ± 1.30 (mean ± SD) with days in milk 70.20 ± 18.60 were selected during the breeding season (March to July) for the experiments. B-mode ultrasonography (Honda HS-1600; Tokyo, Japan), with 7.50 MHz linear array trans-rectal probe, was performed to screen reproductive tract and cows without any ovarian or uterine abnormalities (cystic ovarian disease, para-ovarian cyst, pyometra, metritis, or endometritis) were enrolled. Cows with a body condition score (BCS) ≥ 3.00 were included in the study. The BCS was assessed at the beginning of the study using a 5.00-point scale: 1.00 = emaciated to 5.00 = obese.[17] Cows were kept under the same management conditions.

Experiment 1. Cyclic cows (n = 6) were subjected to a teaser bull twice daily for estrus detection. Animals showing signs of estrus were examined via ultrasonography from the first estrus to the subsequent estrus. Both ovaries of each cow were scanned twice daily on alternate days to monitor the number and size of follicles and the timing of ovulation from first to subsequent ovulation. Ultrasonography was performed at 8:00 am and 8:00 pm by an experienced sonographer. Ovulation time was recorded from the onset of standing heat to the disappearance of the dominant follicle (DF). This was considered day 0 of the estrous cycle, later firmed by the appearance of CL on the same ovary. The behavioral signs and size of the DF and CL were measured and recorded at each examination. The day of wave emergence was declared when a cohort of small antral follicles was found on the ovaries. The follicle that grew to the maximum diameter in the follicular wave was considered dominant.

Experiment 2. Cholistani cows (n = 63) were randomly selected regardless of the stage of the estrous cycle and were divided into two groups: controlled internal drug release (CIDR)-GnRH (n = 31) or OVS (n = 32) methods. In the CIDR-GnRH group, each cow received an intravaginal device (CIDR™; 1.38 g progesterone Eazi-breed™; InterAg, Hamilton, New Zealand) on a random day of the estrous cycle, considered day 0 of treatment. The CIDR was removed on day 7, and on day 6, cows were injected with a luteolytic dose of $PGF_{2\alpha}$ (d-cloprostenol 0.15 mg; Fatro, Bologna, Italy) intramuscularly (IM). Additionally, a GnRH injection (Lecirelin acetate; 50.00 µg Fatro; IM) was given 36 hr after CIDR removal followed by Artificial insemination (AI) 12 and 24 hr after the GnRH injection. In the OVS group, each cow received an i.m injection of GnRH after screening both ovaries (Lecirelin acetate; 50.00 µg of a GnRH analog; 2.00 mL; IM, day 0). On day 7 these cows were treated with $PGF_{2\alpha}$ (d-cloprostenol 0.15 mg IM) followed by a second GnRH injection on day 9. AI was performed 12 and 24 hr after the second GnRH injection. The experimental design evaluating the effect of synchronization methods (CIDR-GnRH *vs.* OVS) in *Bos indicus* dairy cows is presented in Figure 1.

Descriptive statistics were used to evaluate all parameters, including DF size, day of follicular wave emergence, CL emergence, regression, and ovulation time. The data's normality was determined using Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Follicular growth rate, pre-ovulatory follicle (POF) size, and ovulation timing were compared using an independent *t*-test. Estrus expression, ovulation rate, and conception rate data were compared using the Chi-square test between the two synchronization methods. All the data was analyzed with SPSS Software (version 17.0; IBM Corp., Armonk, USA). A significance level of $p \leq 0.05$ was applied.

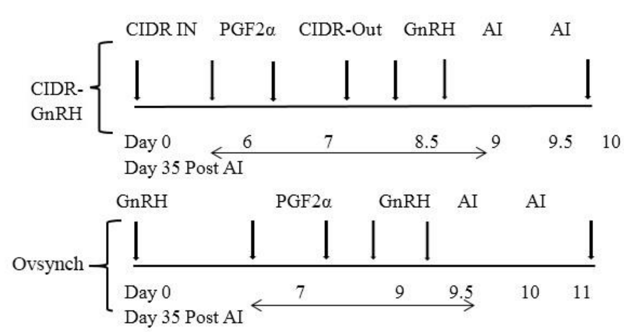

Fig.1. Experimental design to evaluate the effect of synchronization methods (CIDR-GnRH *vs.* Ovsynch) in *Bos indicus* dairy cows.

Results

The characteristics of the estrous cycle (mean ± SD) in Cholistani cows are presented in Table 1.

In experiment 2, we determined the effect of two different estrus synchronization methods (CIDR-GnRH *vs.* OVS) on follicular dynamics and estrus response in Cholistani cows (Table 2). The ovulation and conception rates were higher in CIDR-GnRH than OVS in Cholistani cows (Fig. 2).

Table 1. Descriptive statistics of characteristics of estrous cycle, follicular and luteal dynamics, and standing estrus in *Bos indicus* dairy cows (n = 6) in Experiment 1.

Variables	Values
Characteristics of estrous cycle	
Length (day)	19.81 ± 0.56
Follicular waves, (N)[1]	2.05 ± 0.21
First follicular wave, (day)[2]	0.75 ± 0.54
Second follicular wave, (day)[2]	7.42 ± 0.92
Follicular and luteal dynamics	
Pre-ovulatory follicle (mm)[3]	13.83 ± 2.37
Growth rate of growing POF (mm)[4]	0.78 ± 0.24
Ovulation day	20.80 ± 0.52
CL size (mm)	17.65 ± 1.64
CL emergence day	4.33 ± 1.12
CL regression day	13.55 ± 2.57
Characteristics of standing estrus[5]	
Pre-standing estrus duration (hr)	8.51 ± 1.46
Standing estrus duration (hr)	23.66 ± 6.37
Standing estrus to ovulation (hr)	31.07 ± 6.32

N: number of follicular waves, CL: Corpus luteum

[1] The wave emergence was characterized by the sudden appearance of a cohort of follicles ≥ 3.00 mm of which one or two follicles reached a size ≥ 5.00 mm within the next 48 hr.

[2] Day of wave emergence of first and second follicular waves.

[3] The largest dominant follicle before ovulation was considered the pre-ovulatory follicle and its sudden disappearance on the subsequent ovarian ultrasound scan was defined as ovulation.

[4] B-mode ultrasonography was performed daily to map the ovarian follicular dynamics throughout the estrous cycle, and the growth rate was calculated of the ovulatory follicular wave.

[5] Pre-standing estrus was referred to when a cow was displaying signs of estrus. However, when a cow displayed stands to be mounted and stays in that posture for 3 - 4 sec, it is termed as standing estrus.

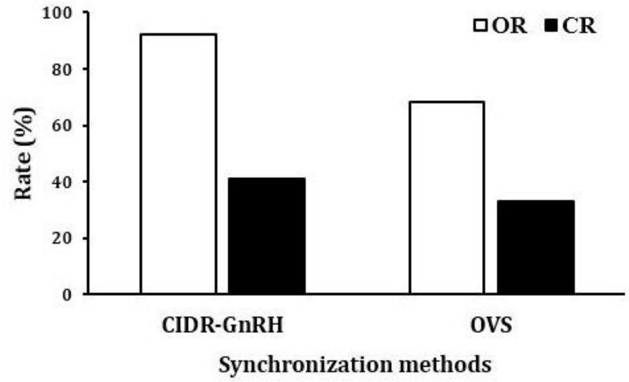

Fig. 2. The effect of synchronization methods (controlled internal drug release gonadotropin-releasing hormone (CIDR-GnRH *vs.* Ovsynch) on ovulation rate (OR) and conception rate (CR) in *Bos indicus* dairy cows. No significant differences were observed ($p < 0.05$).

Discussion

The estrus cycle is the interval between subsequent estruses, while the inter-ovulatory interval is the gap between two successive ovulations. The mean estrous cycle, number of follicular waves, and inter-ovulatory interval of Cholistani cows are similar to the Sahiwal cows.[7] However, four follicular waves per cycle were reported to be prevalent in European[18] and Zebu cows.[19] This difference may be associated with the variable climatic conditions, seasonal patterns influencing luteinizing hormone release,[20] and physiological differences among the cow breeds.[21] Previous studies have reported[1] a positive correlation between the inter-ovulatory period and the number of follicular waves per cycle. In Cholistani cows, the emergence of the first follicular waves was almost similar to the days observed in Brahman cattle, while[22] the second follicular wave emerged earlier at 7.42 ± 0.92 days compared to the Nellore cattle, which was 12.00 ± 0.91 days of the cycle.[23] This indicates that Cholistani cows have similar reproductive parameters to other breeds of *Bos indicus*. Based on these findings, we can improve the reproductive performance of these cows by adopting the reproductive biotechnology used in other *Bos indicus* breeds.

Table 2. Effect of synchronization methods (Controlled internal drug release - gonadotropin-releasing hormone (CIDR-GnRH) *vs.* Ovsynch) on follicular dynamics, timing of ovulation and estrus response in *Bos indicus* dairy cows. Values are mean ± SEM.

Variables	CIDR-GnRH	Ovsynch	*p*-Value
Follicle growth rate (mm per hr)[P] from $PGF_{2\alpha}$ to 60 hr	0.15 ± 0.02[b]	0.20 ± 0.12[a]	0.01
Size of dominant follicle (mm) just before ovulation	12.94 ± 0.76	13.42 ± 0.63	0.77
Timing of ovulation* (hr)	27.40 ± 6.12	26.01 ± 8.48	0.34
Estrus response (%)	94.54	84.37	0.15

*With reference to the last GnRH

[P] The follicle growth rate statistically differs between treatment groups calculated as the size of the dominant follicle at 60 hr minus initial size at the time of $PGF_{2\alpha}$ administration

Values with different superscripts within rows are significantly different ($p < 0.05$).

As the cow reaches puberty, it shows repeated cycles of reproductive events throughout its productive life unless the cow is pregnant or experiencing a negative energy balance.[24] These recurring events begin with one estrus and end at the subsequent estrus, indicating estrous cycles that are entirely controlled by ovarian structures such as follicles and the CL. Throughout the estrous cycle, growth and regression of follicles and CL[25] take place and last for 21 days.[21]

The mean diameter of the POF observed in Cholistani cows was similar to that of *Bos indicus* and *Bos taurus* cows.[26] However, the maximum diameter of the DF observed was greater than that of the Nellore breed[23] but smaller than that of the Brahman breed.[22] A recent report suggested that these differences in the diameter and growth rate of the POFs among the different cow breeds may be associated with variations in genetic profile, liver metabolism, gonadal steroid (Progesterone and Estrogen) concentration, regulation, and hemodynamics of the genital organs.[7] The circulating concentrations of the P_4 are effective in expressing reproductive parameters like estrus expression, follicular size, growth rate, and oocyte quality. The small diameter of CL in Cholistani cows may be associated with photoperiod, but the mechanism by which photoperiod exerts its effect needs is not clearly understood. Therefore, further research is needed to investigate the effect of photoperiod on the ovarian dynamics of Cholistani cows.

In Cholistani cows, the estrus response in OVS cows was consistent with an earlier study in Sahiwal cows.[16] However, the CIDR-GnRH protocol resulted in a higher estrus response than OVS. The difference in responses may be attributed to the presence or absence of the ovulatory follicle on the ovaries or the cycle stage at the time of the first GnRH administration.

In this study, the low ovulation rates in OVS cows were reflected in the lower conception rate compared to CIDR-GnRH cows. The conception rate of OVS cows was in close agreement with the outcomes of another study performed on Sahiwal cows.[16] The lack of significance may be associated with the smaller number of animals in each group. In CIDR-GnRH cows, the increase in the ovulation rate led to a higher conception rate. However, this increase was not as significant as expected. The limited improvement in the reproductive performance of CIDR-GnRH cows may be attributed to the smaller size of the ovulatory follicle, which could have impacted oocyte quality. Numerous factors influence the conception rate.

It is concluded that i) Cholistani cows have two follicular waves and the same size of ovulatory follicles as other breeds of *Bos indicus* species, ii) the use of CIDR-GnRH results in a better estrus response, higher ovulation rate, and subsequently greater conception rate than OVS in Cholistani cows.

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Conflict of Interest

The authors declare no conflict of interest.

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