Effect of Insemination Time on Pregnancy Rate and Ovulatory Events of Nili Ravi Buffalo Heifers in 7 Day CIDR Co-Synch

Sajjad Haider
University of Veterinary and Animal Sciences

Ghazanfar Ali Chishti
University of Veterinary and Animal Sciences

Muhammad Usman Mehmood (✉️ usman.mehmood@uvas.edu.pk)
University of Veterinary and Animal Sciences  https://orcid.org/0000-0002-2998-7510

Muhammad Ameen Jamal
University of Veterinary and Animal Sciences

Khalid Mehmood
University of Veterinary and Animal Sciences

Muhammad Shahzad
Nuclear Institute for Agriculture and Biology

Muhammad Zahid Tahir
University of Veterinary and Animal Sciences

Research Article

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Abstract

The present study aimed to compare the pregnancy rates at the optimal time of artificial insemination in a 7-day CIDR Co-synch in Nili Ravi buffalo heifers. Forty Nili Ravi buffalo heifers were randomly separated into two treatments based on artificial insemination (A.I.) timing (72 vs 84 hours). All heifers were subjected to controlled internal drug release (CIDR), containing 1.38 grams of progesterone for 7 days. On CIDR removal, both treatments received 150 µg of prostaglandin intramuscularly. In 7-day CIDR Co-synch (n = 20), animals were injected 100 µg of GnRH intramuscularly and inseminated concurrently at 72 hours after CIDRs removal. The remaining half (n=20) were injected and inseminated concurrently at 84 hours of CIDRs removal. Pregnancy diagnosis was performed after 40 days of timed artificial insemination (TAI). The follicular growth rate in 7-day CIDR Co-synch was more significant at 84 than 72 hours. The interval from GnRH/A.I. to ovulation in 7-day CIDR Co-synch was significantly (P < 0.05) different between 72 and 84 hours. Pregnancy rates were considerably higher in buffalo heifers inseminated at 84 hours (65%) than 72 hours (25%) in 7-day CIDR Co-synch treatment. In conclusion, the buffalo heifers treated with CIDR Co-synch based protocols in relation to TAI at 84 hours improves follicular growth rate, interval from GnRH/A.I. to ovulation, and pregnancy rate.

2. Introduction

Domestic water buffalo (Bubalus bubalis) have an imperative role in the agricultural economy of developing countries. Possessing the unique capacity to reduce energy requirements, buffaloes gain more productive value to other ruminants in fodder shortage seasons (Campanile et al. 2010). Buffaloes have the potential of producing 30 liter of milk per day (Bhatti et al. 2010) and are considered the best milk producers with 6.5 % butterfat (Hussain et al. 2006). Irrespective of these advantages, buffaloes are regarded as the poor breeding animals due to weak estrus expression, and reduced fertility resulting in prolonged calving intervals (Drost 2007), which creates a significant hindrance to commercial farming of buffalo. To resolve breeding problems of buffalo, hormonal manipulations have been practiced for synchronization of estrus and ovulation.

In large commercial dairy farms, high producer animals have a negative energy balance that impacts animal reproduction, particularly weak estrus expression and fertility. Therefore, timed artificial insemination (TAI) with or without estrus detection nullify the weak/poor estrus expression issues. New reproductive strategies (Ovsynch, Cosynch, CIDR (Controlled Internal Drug Release) and G6G) based on TAI and synchronized ovulation has revolutionized the fertility status of buffaloes. Incorporation of intravaginal P₄ supplementation with Ovsynch has increased pregnancy rate, up to 40% at first A.I. in dairy cows (Stevenson et al. 2008, Stevenson et al. 2006). Similar improvement (up to 50%) in pregnancy rates were attained in dairy heifers when CIDR was used in combination with the Co-synch, and concurrent TAI was performed with last GnRH injection (Kasimanickam et al. 2014).

Since buffalo heifers have a problem with delayed ovulation relative to cattle, there is currently very little information available about the effective timing of concurrent A.I./GnRH in CIDR Co-synch protocol in
buffalo heifers. Therefore, we hypothesized that artificial insemination at 84h time interval in CIDR Co-synch would synchronize better with ovulation to achieve greater pregnancy rates. Therefore, the main objective was to evaluate the effect of 72 vs 84 hours timed artificial insemination (TAI) on pre-ovulatory follicular size, follicular growth rate, estrus intensity score, intervals from PGF$_{2\alpha}$/CIDR removal to ovulation, interval from GnRH/A.I. to ovulation, ovulation and pregnancy rate using CIDR Co-synch protocol in Nili Ravi buffalo heifers.

3. Materials And Methods

3.1. Experimental site, animals, and management:

This study was conducted at Military Dairy Farm, Okara (30° 48_29_N, 73° 26_45_E) Punjab, Pakistan, from 2016-17 (Jan to Feb). Forty Nili Ravi buffalo heifers of 2–3 years of age, body condition score (BCS) 3.6 ± 0.1 on a 1–5 scale (Ferguson et al. 1994), 300–450 kg of body weight were selected for this study. Animals were housed in a free-stall system with free access to water and fed on a standard formulated ration. All the animals were scanned before the synchronization protocol was initiated to assess the normality of the reproductive tract.

3.2. Experimental design:

A randomized controlled study was conducted in which animals were randomly selected and divided into two groups (72 vs 84 hours) based on TAI. The schematic diagram representing the whole study plan is also shown in Fig. 1. The animals received an intravaginal CIDR™ (1.38 g Progesterone Pfizer Co., USA) insert for 7 days. On the day of CIDR removal, PGF$_{2\alpha}$ (d-cloprostenol 150 µg; Dalmazin, Fatro®, Ozzano Emilia Italy; 2 ml) was injected intramuscularly (im). Animals were divided into two groups for different timing of timed artificial insemination (TAI). Half of the animals (n = 20) received GnRH (GnRH; 100 µg of lecirelin acetate, Dalmarlein™; Fatro®, Italy; 2 ml; i.m) simultaneously with TAI after 72 hours of CIDR removal/ PGF$_{2\alpha}$ administration, while the remaining half animals (n = 20) received GnRH and TAI after 84 hours of CIDR removal/ PGF$_{2\alpha}$ administration.

3.3. Estrus expression and timed artificial insemination (TAI):

All the animals were evaluated for estrus expression by using a scale of 1–5. Estrus expression was ranked by the presence of tone in the uterine horns, mucous discharge, edematous vulva, bellowing, restlessness, and other estrus signs (Yousuf et al. 2015). Fixed time artificial insemination (FTAI) was done according to the standard protocols by an experienced technician.

3.4. Ultrasonography, ovulation, and pregnancy diagnosis:

Trans-rectal ultrasound was used as a tool for the precise assessment of the reproductive status of experimental animals. At the start of the synchronization protocol, both ovaries and uterus of all the
heifers were scanned with a B-mode ultrasound console (Honda HS-1500 Tokyo, Japan) for presence or absence of fetus and C.L, follicle or any structural abnormality by using a 7.5MHz linear probe. Follicular dynamics and ovulation time was monitored ultrasonically after every 12 hours following CIDR removal/PGF$_{2\alpha}$ administration till ovulation. The ovulation criteria were based upon the disappearance of a large dominant follicle present during the last ultrasound scanning. Ovulation was further evidenced by ultrasonically visualizing the C.L on the respective ovary after 8 days of the disappearance of the dominant follicle.

3.5. Radioimmunoassay (RIA) for progesterone:

Blood samples were taken from 40 heifers (72 hours n = 20; 84 hours n = 20) at the time of GnRH/TAI, as previously mentioned. Briefly, blood was drawn from the jugular vein and centrifuged at 2000 r.p.m at 37 °C for 10 minutes to collect the serum, which was stored at -20º C until assayed for hormonal analysis. Progesterone concentrations were measured in a duplicate run using an RIA protocol (Waqas et al. 2016). The intra-assay coefficient of variation was 8.15%. The sensitivity of this assay was 0.03ng/ml.

4. Statistical Analysis

The normality of data was determined by using Kolmogorov-Smirnov and Shapiro Wilk test. The probability level of ($P< 0.05$) was considered significant. Data were presented as mean ± S.E. between treatment groups. Progesterone concentrations, pre-ovulatory follicular size, follicular growth rate, estrus intensity score, the interval from PGF$_{2\alpha}$/CIDR removal to ovulation, the interval from GnRH/TAI to ovulation, ovulation rate and pregnancy rate, were analyzed by MIXED or GLIMMIX procedure of SAS (SAS ver. 9.4 Institute, Inc., Cary, NC, USA). The model included the fixed effect of timing of artificial insemination (TAI) and the random effect of buffalo. Binary responses such as ovulation and pregnancy rates were analyzed by logistic regression using the GLIMMIX procedure of SAS.

5. Results

5.1 Effect of CIDR Co-synch on follicular dynamics:

The effects of timed artificial insemination (TAI) on the follicular growth rate, pre-ovulatory follicular size, the interval from PGF$_{2\alpha}$/CIDR removal and TAI/ GnRH administration to ovulation, ovulation and pregnancy rates in 7 days CIDR Co-synch in Nili Ravi buffalo heifers were presented in Table 1. The mean follicular growth rate (mm/day) was significantly higher (1.67 ± 0.08 vs 1.16 ± 0.08, $P= 0.01$) in 84 compared to 72 hours of timed artificial insemination in 7 days CIDR Co-synch treatment. Conversely, there was a non-significant difference in pre-ovulatory follicular size 72 vs 84 hours of timed artificial insemination in 7 day CIDR co-synch protocol. Likewise, the interval from PGF$_{2\alpha}$/CIDR removal to ovulation was non-significant (99.00 ± 1.37 vs 99.60 ± 1.37, $P= 0.01$) between 72 vs 84 hours of timed artificial insemination in 7 days CIDR Co-synch protocol. The interval from GnRH/TAI to Ovulation was significant (27.00 ± 1.37 vs. 15.60 ± 1.37, $P= 0.01$) between 72 and 84 hours of timed artificial
insemination in 7 day CIDR Co-synch protocol. Conversely, ovulation rate was non-significant between 72 vs. 84 hours of timed artificial insemination in 7 day CIDR Co-synch protocol. There was a significant (25 vs 65 %, P = 0.01) difference between 72 vs. 84 hours of timed artificial insemination in 7 day CIDR Co-synch protocol on pregnancy rate.

Table 1. Effect of timed artificial insemination (TAI) on follicular growth rate, pre-ovulatory follicular size, interval from PGF$_{2\alpha}$/CIDR removal and TAI/ GnRH administration to ovulation, ovulation rate and pregnancy rate in 7 day CIDR Co-synch in Nili Ravi buffalo heifers.

| Characteristics                          | Timing of Al (hrs) in 7 day CIDR-Cosynch |
|------------------------------------------|------------------------------------------|
|                                         | 72                                       |
|                                         | 84                                       |
| SEM                                      | 6.53                                     |
| P-value                                  | 0.23                                     |
| Initial Weight (kg)                      | 390                                      |
| Initial BCS                              | 3.54                                     |
| Pre-Ovulatory follicular size (mm)       | 11.3                                     |
| Follicular growth rate (mm/day)          | 1.16                                     |
| Estrus Intensity Score                   | 3.85                                     |
| Interval from PGF$_{2\alpha}$/CIDR removal to ovulation (hrs) | 99.0 |
| Interval from GnRH/TAI to Ovulation (hr) | 27.0                                     |
| Ovulation rate (%)                       | 85 (17/20)                               |
| Pregnancy rate (%)                       | 25 (5/20)                                |

CIDR, controlled internal drug release (1.38 g progesterone); PGF$_{2\alpha}$ Prostaglandin F$_{2\alpha}$

5.2 Progesterone concentration

Although, mean serum P4 concentration was compared between 72 vs. 84 hours; however, there was a non-significant difference between groups (P > 0.05). The majority of the heifers in both groups had low P4 concentration at the time of CIDR insertion (Table 1).

6. Discussion

In both dairy and beef cows and heifers, considerable research has been done using CIDR Co-synch protocol for improving pregnancy rates. There are very few studies reporting the efficacy of CIDR Co-synch in buffaloes. To the best of our knowledge, this is the first study to report appropriate timing for TAI in buffalo heifers under CIDR Co-synch protocol.
The timing of insemination has been the point of focus in all the CIDR Co-synch programme (Colazo and Mapletoft 2014). The outcomes of the present study indicated that the best time of TAI in Nili Ravi heifers using CIDR Co-synch was 84 hours (after CIDR removal), yielding 65% pregnancy rates and these results were in close agreement with the results of other studies in buffalo (Kumar et al. 2016). Animals inseminated at 84 hours had 12 more hours of follicle growth, which increased follicle maturation by increasing diameter and contributed significantly to optimal estrogen production (Palomares et al. 2015).

Low pregnancy rates at 72 hours in 7 days CIDR Co-synch might be the inadequate oocyte development when smaller follicles were subjected to ovulation. Oocytes collected from small follicles had inadequate developmental competence compared with oocytes collected from large follicles (Lonergan et al. 1994). Consequently, embryonic, fetal survival might be impaired when oocytes were derived from follicles that had not been utterly matured.

The fact that we found, in 7 day CIDR Co-synch, the animals inseminated 72 hours (TAI/GnRH administration) after CIDR removal could ovulate at 27 ± 1.4 hours differ significantly ($P < 0.05$) than animals inseminated at 84 hours (15 ± 1.2 hours). The higher pregnancy rates at 84 hours of both treatments were due to smaller/lesser intervals from TAI/GnRH to ovulation. The time interval between the introduction of spermatozoa in the uterus and ovulation was 15–16 hours at 84 hours from TAI/GnRH to ovulation of 7 days CIDR Co-synch. Whereas in the 72 hours, the spermatozoa were introduced in the uterus 21 to 27 hours before ovulation, the relatively longer interval from sperm introduction in the female tract to ovulation. Spermatozoa took 8 to 12 hours to arrive at the site of fertilization after capacitation and hyper-activation in the uterus (Wilmut and Hunter 1984), and the entire viable life span of spermatozoa in the female reproductive tract is 24 to 30 hours (Trimberger 1948). So it can be deduced from data of previous studies that after 8 to 12 hours of maturation, the spermatozoa can viably wait up to 22 hours for an ova to get fertilized. In the current experiment, ovulation at 84 hours in 7 CIDR Co-synch programme occurred within the viable life span of spermatozoa. The spermatozoa were prepared for fertilization when the oocyte arrived at the site of fertilization. Conversely, at 72 hours, the ovulation was too late that spermatozoon started to lose their fertilization capability (Dalton et al. 2001). This research would pave the path for other researchers to design the study to understand the mechanism and underlying phenomenon of sperm transportation in the reproductive tract of female buffalo.

Progesterone concentration was analyzed at the time of initiation of the protocol to assess the cyclicity of animals. Animals with progesterone concentrations higher than 1 ng/ml were considered cyclic, while other animals with progesterone concentration lower than 1 ng/ml were considered acyclic (Cartmill et al. 2001). Data analysis showed that there was a non-significant effect of progesterone concentration at protocol initiation. Progesterone concentration at the initiation of the CIDR Co-synch protocol does not affect the pregnancy/A.I. in dairy heifers (Spencer et al. 2018). Numerous studies have shown a positive effect of elevated progesterone concentration at the beginning of the protocol on pregnancy rates (Santos et al. 2004, Sterry et al. 2006). The concentration of P4 has been shown to influence the frequency of L.H. pulses (Adams et al. 1992), follicular growth during the luteal phase, and the
development of persistent follicles, which has been linked to low fertility (Kojima et al. 2003). Furthermore, follicles that grow under high P4 status are more receptive to the L.H. surge, resulting in standard luteal phase lengths.

A high percentage of heifers showed estrus intensity in both treatments of 7 days CIDR co-synch which could be an indicator of good estrus activity and had a positive effect on pregnancy rates under conventional type of farming system (Mehmood et al. 2017).

In conclusion, higher pregnancy rates were achieved in 7 days CIDR Co-synch when animals were inseminated after 84 hours interval of CIDR removal/PGF$_{2\alpha}$ administration. The results of this study suggest that a high pregnancy rate is primarily attributed to the significant follicular growth rate and short interval from timed artificial insemination to ovulation. This study provides a way out for a large dairy farmer to increase fertility with optimized management.

**Declarations**

7. Acknowledgement

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7.1 Contributions

SH and MUM conceived and design the research. SH conducted the experiments and KM assisted during the trial. MUM and GAC performed the statistical analysis. MS contributed hormone analysis. MUM, MAJ and GAC wrote the manuscript. ZT wrote and edited the research paper. All authors read and approved the manuscript.

8.1 Data availability

Data will be made available on reasonable request

8.2 Code availability

Not applicable

8.3 Ethics approval

The research and procedures conducted on the animals were approved by ethical committee of University of Veterinary and Animal Sciences Lahore.

8.4 Conflict of interest
Authors declare no conflict of interest.

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Figures

Figure 1

Schematic illustration of 7 day CIDR Co-synch synchronization protocol in relation to timed artificial insemination in nili ravi buffalo heifers. CIDR, controlled internal drug release; P-Test, pregnancy test; TAI,
timed artificial insemination; USG, ultrasonography; PG, prostaglandins F2α; GnRH, gonadotropin releasing hormone.