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COMPARATIVE EFFICACY OF MEDROXYPROGESTERONE ACETATE (PROGESTIN) SPONGES WITH CO-SYNCH PROTOCOL FOR ESTRUS SYNCHRONIZATION IN HOLSTEIN FRIESIAN CATTLE

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ABSTRACT

The study involved forty non-pregnant, first-parity Holstein Friesian cows housed under semi-intensive management conditions. They were divided into four groups: Group A received a 7-day CIDR followed by a PGF2α injection, Group B received a 12-day CIDR followed by a PGF2α injection, Group C underwent a 10-day Co-Synch protocol, and Group D served as the control. Estrus symptoms were weak in all groups, while strong signs of standing heat were observed. Group B showed the highest estrus response, earliest onset of estrus, longest duration, and highest conception rate, resulting in positive pregnancies. In contrast, Group D had no pregnancies. These findings suggest that the extended CIDR treatment in Group B led to improved reproductive outcomes, indicating its potential as an effective method for estrus synchronization in Holstein Friesian cattle.

1. INTRODUCTION

The livestock sector is the backbone of agriculture in Pakistan. It engages about 30 to 35 million people in rural areas, who derive more than 40% of their incomes from livestock sector (Faisal et al., 2021; Memon et al., 2022). Milk is the most important commodity derived from livestock sector. The estimated annual milk production in 2021-2022 was 63 million tons (GOP, 2021). But the demand of milk is increasing rapidly due to increase in human population and urbanization. Most of the indigenous animals are non-descriptive type having low productivity compared with developed countries.

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Pakistan has three times more animals but have low yields than Germany, New Zealand's, and USA. They are producing 94.5 billion litters of milk annually from 3.4 million animals representing a significant loss in economic and social value (Tahir et al., 2019).

The increase in milk production in Pakistan was 3%, while the demand was projected 5% leaving a significant gap in the supply and demand chain (Shabir et al., 2021). This gap was tried to be met by the import of Holstein Friesian cows which are well known worldwide for their high milk yield and reproductive performance. Holstein Friesian cow produces 10,000-12,000 litres milk per lactation period. The Holstein Friesian calf gets mature within two years and start producing milk, while local breeds take three years (Wondossen et al., 2018).

The milk demand in summer (April to July) is higher in Pakistan than its production (Ayaz et al., 2011; Channo et al., 2022). To address this issue, reproductive cycle is manipulated using various estrus synchronization protocols in order to get animal conceived and thus flow of milk supply may be maintained (Jelani et al., 2022). Reproductive cycle of the animals play important role in productivity of the animals in terms of milk yield and meat. Earlier attempts were focused on the control of follicle growth and luteal span (Hryciuk et al., 2021; Channo et al., 2022). There are two methods of manipulating corpus luteum (CL) using either Progestins or injecting with luteolytic agents. Several hormones have been used to synchronize animals such as progestins, prostaglandins, gonadotropins and their analogues (Szymanska&Blitek, 2020; Iqbal et al., 2022).

Commercially, available intra-vaginal devices are expensive and are not easily approachable for dairy farmers. A local prototype prepared by Nuclear Institute of Agriculture and Biology (NIAB) from polyurethane sponges were purchased that was impregnated with medroxyprogesterone acetate (Rihana et al., 2013). Moreover, little work on Holstein Friesian cattle using progesterone-based protocols in local environment were reported. Therefore, the present study was designed to compare efficacy of medroxyprogesterone acetate (Progestin) sponges with co-synch protocol for estrussynchronization and their effect on conception rate in Holstein Friesian cattle.

2. MATERIALS AND METHODS

Animals and management

A total of forty (n=40) non-pregnant 1st parity and 60-120 days post-parturition Holstein Friesian cows were housed under semi-intensive management condition at National Dairy Farm, Kotri near Hyderabad. The routine farm practices were followed as recommended by farm policies. Duration of experiment was 2 months (February to March, 2022).

Experimental design

The all animals were randomly allocated into four different groups.

Group - A Progestin sponges group with 7th Day Prostaglandin n=10:

In this group, animals (*n* = *10*) were inserted intra vaginal with Progestin sponges. Polyurethane sponges (diameter 6 cm & length 10 cm) were impregnated with 250 mg Progestin (Depo Provera; The Pfizer, Belgium). The prepared Progestin sponges were dusted with streptomycin, packed, and sealed in sterilized polythene bags. The prepared sponges were purchased form Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Punjab. The Progestin sponges were deposited in cranial part of the vagina for 7 days with the help of sterilized applicator lubricated with paraffin oil. On day 7 all the animals were given an injection of PGF2α (Dalmazin, cloprostenol sodium, 250 µg, Fatro Laboratories, Italy), and progestin-based sponges were removed. The experimental group was monitored for onset of estrus within 2-5 days after prostaglandin injection (Singh, 2003). The estrus was confirmed through behavioural signs, and examination of reproductive organs per rectum. Estrus cows were inseminated with Ghazi brother's imported semen (0.2ml) of Holstein Friesian bull.

Group - B Progestin sponges group with 12th Day Prostaglandin n=10:

In this group, animals (*n* = *10*) Progestin sponges were inserted on day 0. The prepared sponges were placed in vagina for 12 days. On the day 12 of Progestin sponges removed and an injection of prostaglandin analogue were given. The experimental group was monitored for onset of estrus within 2-5 days after prostaglandin injection (Singh, 2003). The estrus was confirmed through behavioural signs, examination of reproductive organs per rectum and then inseminated using Ghazi brother's imported semen of Holstein Friesian bull.

Group - C Ovsynchprotocol n=10:

In this group, animals (*n* = *10*) co-synch protocol were used. In this protocol, non-pregnant animals an injection of GnRH, Dalmarlin (Burserelin acetate 0.0042 mg, Fatro Laboratories Italy) were given on day 0, and an injection prostaglandin analogue, Dalmazin, (cloprostenol sodium, 250 µg, Fatro Laboratories Italy) were given on day 7. Second dose of GnRH injected on day 10, Darmarlin (Burserelin acetate 0.0042 mg, Fatro Laboratories Italy) and Fixted time AI were followed using Ghazi brother's imported semen of Holstein Friesian bull.

Group - D Control Group n=10

The control group consist of (n= 10) animals they were injected with 2ml Intra muscular normal saline (FDL Pakistan) on days 0, 7 and **10** of the experiment. The estrus was confirmed through behavioural signs and examination of reproductive organs per rectum Later on inseminated using Ghazi brother's imported semen of Holstein Friesian bull.

Behavioural signs of estrus

Sign of estrus were recorded three times a day and night (4 AM, 12 PM and 8 PM). The estrus behaviour was recorded according the scale developed by Van Eerdenburg et al., (1996) (Table-3.2). Each time signs were observed and if the sum of the points exceeded 100 during a whole day, estrus response was considered strong. If the sum of the points is below 100, the estrus response was considered weak. *Onset of estrus.* The females of all groups were watched closely after 2nd injection of PGF2α for behavioural signs of heat. The animals were artificially inseminated two times at 12 and 24 hours, after the last injection of GnRH in group A and C. However, females of B and D group were artificially inseminated at 72 and 96 hours after the last injection of $PGF_{2\alpha}$ with frozen-thawed semen.

Duration of estrus

Duration of estrus was recorded by observing estrus signs visually from onset of estrus signs till cessation of estrus signs.

Fertility/ Pregnancy rate

The pregnancy diagnosis was performed on day 30th of post artificial insemination through ultrasonography.

Statistical Analysis

Graph pad instate 3.05 versions of statistical package was used to analyse the data. Analysis of variance (ANOVA). To determine significant difference between means of groups chi square test was used.

3. RESULTS AND DISCUSSION

Behavioural signs of estrus

Results on the behavioural signs of estrus in Holstein Friesian cows administered with different protocols is mentioned in Table 4.1. Data indicates that estrus signs were observed as weak in all groups except group B, while standing heat was observed as strong in all groups. The observed overall chi-square between the groups was 61.91 and the estimated p-value among the groups were 0.0001, which indicates significant difference (P<0.05).

Estrus response

Results on the estrus response in Holstein Friesian cows administered with different protocols is mentioned in Table 4.2. This data indicates that the maximum estrus response was recorded in group B followed by A and C, while the minimum estrus response was recorded in group D. Significant (P<0.05) variation in estrus response were analyzed among the groups.

Onset of estrus (hrs)

Results on the onset of estrus in Holstein Friesian cows administered with different protocols is mentioned in Table 4.3. This data indicates that minimum time for onset of estrus was recorded in group A followed by B and C, while maximum time for onset of estrus was recorded in group D.Significant (P<0.05) variation in onset of estrus were analyzed among the groups.

Duration of estrus

Results on the duration of estrus in Holstein Friesian cows administered with different protocols is mentioned in Table 4.4. This data indicates that maximum time for duration of estrus was recorded in group C followed by B and A, while minimum time for duration of estrus was recorded in group D. Significant (P<0.05) variation in duration of estrus was analyzed among the groups.

Artificial insemination & Pregnancy diagnosis

Results on the artificial insemination and pregnancy diagnosis in Holstein Friesian cows administered with different protocols is mentioned in Table 4.5. Pregnancy was found positive six6 animals in group A, four4 animals in group B, and two2 animals in group C respectively. Pregnancy was found negative in all cows of group D.

Conception rate

Results on the conception rate in Holstein Friesian cows administered with different protocols is mentioned in Table 4.6. Data indicates that the conception rate of 75.00%, 66.66% and 50.00% was recorded in group A (CIDR for 7 days), group B (CIDR for 12 days) and group C (Co-Synch). Zero percent conception rate was observed in group D (Control).

To get more of females into heat at once, estrus synchronisation entails manipulating the estrous cycle. For dairy cows, estrus synchronisation is a crucial reproductive control strategy. This method aids in determining the optimal time for mating and timing the time of delivery when the environment is most conducive to the development and survival of the infants. Higher fertility is attained using these reproductive techniques throughout both breeding and non-breeding seasons. Estrus synchronisation increases animal productivity and reproductive effectiveness, which has greater economic advantages (Prusley et al., 2004). Bos indicus cows often have a lower rate of estrus detection than Bos taurus cows (Mukasa-Mugerwa, 1989). As a result, estrus induction therapies that provide either a TAI chance or insemination after estrus detection have a great deal of promise for increasing the reproductive effectiveness of both dairy and meat cattle.

In the current investigation, standing heat was noted to be strong in each of the groups (A, B, C, and D). According to Deshmukh et al. (2015), who observed the incidence of strong, moderate, and mild estrus to be 40%, 40%, and 20%, the current results of percent intensity of estrus are consistent with their observations. Similar results were reported by Bhoraniya et al. (2012), who reported intensity levels of 50, 33.33, and 16.66% high, medium, and mild, respectively. The results of the double Prostaglandin (PG) technique show that the strength of estrus is comparable to those from Makode (1990), who determined that the percentages of intense, moderate, and weak estrus were 37.5, 37.5, and 25.0%, respectively. Maximum estrus response in this research was substantially reported in group B (Co-Synch), followed by groups A (CIDR for 12 days) and C (CIDR for 7 days), and smallest estrus response in group D. (control). Variation in estrus response might be associated with the estrus synchronisation protocol used in different groups. Results are consistent with those of Shahid et al. (2021), who found that the estrus response in Sahiwal cows was 87% (40/46), while Hassan et al. (2017) found that the estrus response in nondescript indigenous cows was 95.45% when recorded with Ovsynch therapy in the field. The outcomes of the CIDR CO-Synch treatments for estrus response are consistent with those of Haider et al. (2017), who found 82.5% and 90% estrus responses in CIDR alone and CIDR combined with GnRH treated nondescript cows of Punjab (Pakistan). The breed, breeding season, genetic make-up, and environmental factors may all affect how an estrus responds differently. In the research, the smallest time for the onset of estrus (CIDR for 7 days) was recorded in group B, followed by groups A (CIDR for 12 days) and C (Co-Synch), while the highest time for the onset of estrus (CIDR for 14 days) was reported in group D. (control). The breed, breeding season, genetic make-up, and environmental factors may all have a role in the variation in estrus onset.

The findings of Shahid et al. (2021); Shah et al. (2023) and Hassan et al. (2017), which demonstrated that the beginning of estrus response was greater in the CIDR protocol compared to Ovsynch therapy, are consistent with the results. The results of the present research show that group B (Co-Synch) recorded the longest time for estrus duration, followed by groups A (CIDR for 12 days) and C (CIDR for 7 days), while group D (CIDR for 7 days) recorded the least time for estrus duration (control). The procedure utilised and duration are related to the difference in duration. The findings of Shahid et al. (2021) and Hassan et al. (2017), which demonstrated that the beginning of estrus response was greater in the CIDR protocol compared to Ovsynch therapy, are consistent with the results. In this investigation, the pregnant status of 6, 4 and 2 cows from groups B (CIDR for 7 days) and A (CIDR for 12 days) and Group C (Co-Synch), respectively, was determined to be positive. None of the cows in groups D were pregnant (control). Results are consistent with Shahid et al. (2021), who found that the 7-day CIDR procedure had a greater pregnancy rate. When heifers were given the CO-Synch+CIDR treatment, Estrada et al. (2002) saw a 60% pregnancy rate. Kim et al. (2005) showed that Holstein cows treated with CIDR plus GnRH had a conception rate of 52.3-53.9%. The different pregnancy rates might be the result of different protocols. In our research, group A (CIDR for 7 days) and group B both had conception rates of 75.00% and 66.66%, respectively (CIDR for 12 days). 50.00% conception rate was recorded in group C (Co-Synch) and in group D (Control) Zero percent conception rate was recorded.

The findings of the current investigation correspond to the findings published by Hassan et al. (2017) revealed that the pregnancy rate in Sahiwal cows was 43%

(17/40), however Shahid et al. (2021) stated that conception rate/AI (50%) with Ovsynch therapy in the unremarkable indigenous cows was higher. The results of the CIDR CO-Synch treatments' conception rate are consistent with those of Haider et al. (2017), who reported 52% and 58% in CIDR alone and CIDR along with GnRH treated nondescript cows of Punjab (Pakistan). Similarly, Naikoo et al. (2016) observed that 33.33% of cows who had CIDR therapy became pregnant. However, Sathiamoorty and Kathirchelvan (2010) observed significantly lower conception rates of 26.00 to 42.74 with CIDR, while greater conception rates of 50 to 80% have also been recorded in crossbred cows by Savalia et al. (2014) and Dhami et al. (2015). According to Pursley et al. (1997a), the Ovsynch protocol increased the rate of conception. The early timing of AI may be the cause of the reduced conception rates in the CIDR implanted groups. Therefore, more precise insemination time may lead to higher conception rates. The effectiveness of CIDR treatments may be impacted by a number of variables, including parity, suckling, breed composition, postpartum interval, variations in pasture and food, BCS, and location (Lamb et al., 2001). The variation in conception rate may be influenced by the mating season, breed, genetic make-up, and environmental factors.

4. CONCLUSION

The current study revealed that the treatment of Group B with CIDR + 12 days (Prostaglandin) resulted in the development of strong signs of estrus, including standing heat. Furthermore, this group exhibited an early onset of estrus, the longest duration of estrus, and a higher conception rate compared to other treatment groups.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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REFERENCES

Ayyaz S., Badar H., & Ghafoor, A. (2011). Level and determinants of consumers' perception of packed milk in Pakistan. *Journal of Business & Economics*, *3*(1), 60.

Bhoraniya H.L., Dhami A.J., Naikoo M., Parmar B.C., &Sarvaiya N.P. (2012). Effect of estrus synchronization protocols on plasma progesterone profile and fertility in postpartum anestrousKankrej cows. *Tropical animal health and production*, *44*(6), 1191-1197.

Channo A., Kaka A., Kalwar Q., Jamali I., Jelani G., Bukhsh M., Dahri G.N &Goil J.P. (2022). An Overview of Bovine Cystic Ovarian Disease. *Pakistan Journal of Zoology*, *54*(5): 2437-2444.

Deshmukh S.G, Bakshi S.A., Ingawale M.V., &Chepte S.D. (2015). Ovulation Synchronization for improving fertility in post-partum dairy cows. *Indian Journal Veterinary Science Biotech.* 11(2): 11-14.

Dhami A.J., Nakrani B.B., Hadiya K.K., Patel J.A., & Shah R.G. (2015). Comparative efficacy of different estrus synchronization protocols on estrus induction response, fertility and plasma progesterone and biochemical profile in crossbred anestrus cows. *Veterinary World*, *8*(11): 1310-1316.

Estrada A.T., Walton J., Bateman K., & Johnson W.H., (2002). The effect of estradiol benzoate or GnRH on CIDR-B based synchronization protocols in beef cattle. Proc. Soc. *Theriogenology*. p. 1.

Faisal M., Abbas A., Xia C., Raza M.H., Akhtar S., Ajmal M.A., & Cai Y. (2021). Assessing small livestock herders' adaptation to climate variability and its impact on livestock losses and poverty. *Climate Risk Management*, *34*, 100358.

GOP (Govt. of Pakistan). Economic Survey of Pakistan (2020-21); pp 18. Government of Pakistan. Finance Division. *Economic Advisor's Wing, Islamabad.* 17-44.

Haider M.S., Bilal M., Ahmed H., Anwar M., Sattar A., & Andrabi S.M.H. (2017). Effect of cidr with or without gnrh and double pgf 2α based estrus synchronization protocols on estrus response and pregnancy per ai in non-descript cows of the punjab. *JAPS: Journal of Animal & Plant Sciences*, *27*(4).

Hassan M., Husnain A., Naveed M.I., Riaz U., & Ahmad N. (2017). Effect of ovsynch versus prostaglandin F2α protocol on estrus response, ovulation rate, timing of ovulation and pregnancy per artificial insemination in Sahiwal cows. *Journal of Animal Science*. 88(3): 445-450.

Hryciuk M.M., Jewgenow K., & Braun, B.C. (2021). Luteinizing hormone effect on luteal cells is dependent on the corpus luteum stage in felids. *Animals*, *11*(1), 179.

Iqbal T., Yousaf A., BiBi N., Kumar L., Tunio S., Farooq M.M., Channo A., Wakeel A., Lanjar Z., Panhwar M.I &Soomro A.G. (2022). Ultrasonographic biometry of the ovaries and follicles in cyclic and non-cyclic Kundhibuffaloe. *Multidisciplinary Science Journal*, *4*, e2022005-e2022005.4(1).

Jelani G., Kalwar Q., Kaka A., Channo A., Ahmed A., Soomro M.H., Soomro H., Bhutani D.K &Jalbani Y.M. (2022). Historical background and significance of embryo transfer technology in cattle with its relevant applications. *Pakistan Journal of Zoology*.1-15.https:// doi. org/10.17582/JOURNAL. PJZ/20220, 403060427.

Kim U.H., Suh G.H., Nam H.W., Kang H.G., & KimI.H. (2005). Follicular wave emergence, luteal function and synchrony of ovulation following GnRH or estradiol benzoate in a CIDR-treated, lactating Holstein cows. *Theriogenology*. 63(1): 260-268.

Lamb G.C., Stevenson J.S., Kesler D.J., Garverick H.A., Brown D.R., & Salfen B.E. (2001). Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. *Journal of Animal Science*, *79*(9), 2253-2259.

Makode P.T. (1990). Synchronisation of oestrus and fertility in PGF 2 (Dinoprost) treated repeat breeder cows, M.V.Sc. thesis submitted to PunjabraoKrishiVidyapeeth, Akola.

MEMON A.S., KAKA A., CHANNO A., BAKHSH M., KALWAR Q., & SETHAR A. (2022). Effect of Soybean Milk and Tris Egg Yolk Based Extender on Semen Quality Parameters of Tharparkar Bull. *University of Sindh Journal of Animal Sciences (USJAS)*, *6*(03).

Mukasa-Mugerwa E. (1989). A review of a reproductive performance of female *Bos indicus* (zebu) cattle, In: ILCA Monograph 6. Ethiopia, ILCA, Addis Ababa

Naikoo M., Dhami A.J., &Ramakrishnan A. (2016). Effect of estrus synchronization on plasma progesterone profile and fertility response in postpartum suckled anestrousKankrej cows. *Indian Journal of Animal Research, 50*(4): 460-465

Prusley J.R., Mee M.O., Brown M.D., &Wiltbank M.C. (2004). Reproductive outcomes of dairy heifers treated with combinations of prostaglandin F2α, norgestomet, and gonadotropinreleasing hormone. *Journal of Dairy Sci*ence, 77: 230.

Pursley J.R., Kosorok M.R., &Wiltbank M.C. (1997a). Reproductive management of lactating dairy cows using synchronization of ovulation. *Journal Dairy Science*. 80: 301-306.3

Rihana S., Heddy Y.S., &Maghfoer M.D. (2013). Growth And Yield of Common Bean (Phaseolus vulgaris L.) On Various Goat Manure Dosage And Concentration of Plant Growth Regulators Dekamon. *JurnalProduksiTanaman*, *1*(4), 369-377.

Sathiamoorty T., &Kathirchelvan M. (2010). Efficacy of PGF2α, CIDR and Ovsynch treatment on estrus response and fertility rate in crossbred cows. *Indian Journal of Animal Reproduction*. 31: 43-45

Savalia K.K., Dhami A.J., Hadiya K.K., Patel K.R., &Sarvaiya N.P. (2014). Influence of controlled breeding techniques on fertility and plasma progesterone, protein and cholesterol profile in true anestrus and repeat breeding buffaloes. *Veterinary World*, *7*(9).

Shabir G., Iqbal S., Riaz S., Safdar G., & Javed M.N. (2021). Government Media Campaign for Agriculture Perfection in Punjab, Pakistan. *Journal of Agricultural Research*, *59*(1), 89-94.

Shah S.H., Memon A.A., Kaka A., Tunio A.N., Channo A., Kalwar Q., Kalwar Q., Panhwar M.I., Malak K.A & Rahoo, B. A. (2023). Evaluation of Ovsynch Estrus Synchronization in Holstein

Friesian and Holstein Friesian-Jersey Crossbred Cows at District Thatta, Sindh, Pakistan.

Shahid B., Khan M.I., Andrabi S.M.H., & Khan M.N. (2021). Efficacy of estrus synchronization protocols in non-descript cattle of azadjammu and kashmir during non-breeding and breeding seasons. *JAPS: Journal of Animal & Plant Sciences*, *31*(3).

Singh M. (2003). Circulating PGF2α during oestrus cycle among buffaloes. *Proceedings of the 1st World Buffalo Congress, Cario Egypt, 8*: 569-571.

Szymanska M., &Blitek A. (2020). In vivo response of the corpus luteum to progesterone treatment of gilts during early gestation. *Animal Reproduction Science*, *221*, 106583.

Tahir M.N., Riaz R., Bilal M., & Nouman H.M. (2019). Current standing and future challenges of dairying in Pakistan: a status update. *Milk production, processing and marketing*.

Van Eerdenburg F.J.C.M., Loeffler S.H., & Van Vliet J.H. (1996). Detection of oetrus in dairy cows: A new approach to an old problem. *Veterinary Quarantine, 18*: 52-54.

Wondossen A., Mohammed A., & Negussie E. (2018). Reproductive performance of Holstein Friesian dairy cows in a tropical highland environment. *Journal of Advances in Dairy Research*, *6*(2),203.

Table 1. Behavioral Signs of Estrus in Different Treatment Groups

Estrus signs	Group A (CIDR for 7 days)	Group B (CIDR for 12 days)	Group C (Co-Synch for 10 days)	Group D (Control)	Chi-square (p-value)
Mucous vulvular discharge	3%	10%	5%	3%	61.91 (0.0001)
Flehmen	3%	5%	5%	3%	
Restlessness	5%	15%	10%	5%	
Sniffing the vagina of another cow	10%	20%	15%	5%	
Chin resting	15%	30%	20%	10%	
Mounted but not standing	35%	40%	30%	5%	
Mounting (or attempt) other cows	45%	60%	50%	10%	
Standing heat	100%	100%	100%	100%	

Overall Chi-Square 61.91, *P-Value* 0.0001, Degrees of Freedom 21

Table 2. Estrus Response in Different Treatment Groups

Group (10 animals in each)	No. of animals	No. of animals showing estrus response	Percentage (%)
A (CIDR for 7 days)	10	6	60(%)
B (CIDR for 12 days)	10	8	80(%)
C (Co-Synch for 10 days)	10	4	40(%)
D (Control)	10	2	20(%)

Overall P-value = 0.0305, a,b,c,d= indicates significant ($p<0.05$) difference among groups.

Table 3. Onset of Estrus (hours) in Different Treatment Groups

Group (10 animals in each)	No. of animals exhibited estrus	Onset of estrus (hrs)
A (CIDR for 7 days)	6	52.30 ± 3.55^{d}
B (CIDR for 12 days)	8	66.41 ± 5.44^{a}
C (Co-Synch)	4	63.88 ± 3.61^{b}
D (Control)	2	56.54 ± 2.88^{c}

P-value = 0.0004, ^{a,b,c,d} indicates significant ($p<0.05$) difference among the groups.

Table 4. Duration of Estrus (hours) in Different Treatment Groups

Group (10 animals in each)	No. of animals exhibited estrus	Duration of estrus (hrs)
A (CIDR for 7 days)	6	21.14±2.10[c]
B (CIDR for 12 days)	8	25.33±2.18[a]
C (Co-Synch)	4	23.20±1.41[b]
D (Control)	2	16.54±1.24[d]

Overall P-value = 0.0014, [a,b,c,d] indicates significant ($p<0.05$) difference among the groups.

Table 5. Artificial Insemination and Pregnancy Diagnosis in Different Treatment Groups

Group (10 animals in each)	No of animals comes in heat	AI performed animals	Pregnant animals
A (CIDR for 7 days) 10	6	6	4[a]
B(CIDR for 12 days) 10	8	8	6[b]
C (Co-Synch) 10	4	4	2[c]
D (Control) 10	2	2	0[d]

Overall P-value = 0.0001, [a,b,c,d] indicates significant ($p<0.05$) difference among the groups.

Table 6. Conception Rate (%) in Different Treatment Groups

Group	No. of animals conceived	Conception rate (%)
A (CIDR for 7 days)	4	66.66%
B (CIDR for 12 days)	6	75.00%
C (Co-Synch)	2	50.00%
D (Control)	0	0%

P-value = 0.0001, [a,b,c,d] indicates significant ($p<0.05$) difference among the groups.