Theriogenology Insight: An International Journal of Reproduction of Animals
Citation: Theriogenology Insight: **13**(01): 25-31, June 2023
DOI: 10.30954/2277-3371.01.2023.5
Peer-reviewed Journal

Research Paper

Comparative Study of Different Estrus Synchronization Protocols in Pubertal Anoestrus Gir Heifers (*Bos indicus*)

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Received: 20-01-2023 **Revised:** 23-05-2023 **Accepted:** 02-06-2023

ABSTRACT

The study was carried out to investigate the various estrus synchronization protocols for induction of estrus in pubertal anestrous Gir heifers. A total of 32 pubertal heifers that failed to exhibit estrus were selected and randomly divided into four groups of 08 heifers each. Three estrus induction/synchronization protocols viz. CIDR +PG (G1), Ovsynch (G2) and Cosynch (G3) along with one control group (G4) were evaluated for efficacy of estrus induction and synchronization. Estrus induction responses of 87.50%, 75.00%, and 75.00 % were observed in G1, G2, and G3, respectively; whereas, subsequent fertility response in terms of conception rates were obtained as 50.00 %, 37.50 % and 25.00 % in G1, G2, and G3, respectively. However, in control group (G4) no heifer showed sign of estrus during the experiment period. It can be concluded that induction of estrus and improvement in conception is possible with the use of different hormone protocols, viz. CIDR+PG, Ovsynch and Cosynch in pubertal anestrous Gir heifer. For treating pubertal anoestrus as well as for subsequent fertility, CIDR+PG was found to be most effective out of the three hormonal protocols followed by ovsynch and cosynch.

Keywords: CIDR, ovsynch, cosynch

Gir cattle is famous milch breed reared by farmers in southern Rajasthan. Gir cattle the famous Indian milch breed (Kumar, P. and Singhal, L.K. 2006). The native tract of the breed is Gir forests of Kathiawar including Junagadh, Bhavnagar, Rajkot and Amreli districts of Gujarat. The total cattle population of Saurashtra region i.e. breeding tract of Gir cattle is 2.5 million. Adult body weight, height at withers, body length and heart Girth in cows averaged 313 kg, 120 cm, 125 cm and 160 cm, respectively (Gaur *et al*. 2003, FAO). The prevalence of delayed puberty in cow heifers is 20.81 per cent (Bhattacharya *et al*. 2009). Delay in puberty may be related to many factors, such as inadequate body weight due to poor nutrition or inadequate gonadotropin release. Thus, there is a need that heifers should be of optimum body weight i.e., at least 250 kg (breed variation) for attending puberty. Puberty occurs when the heifer responds to the estrogen from the growing follicle by expressing estrus (heat) and producing an LH surge. The LH surge causes ovulation, which begins the heifer's first cycle (Hall *et al*. 2009). However, Synchronization of estrous implies the manipulation of estrous cycle or induction of estrous to bring a large percentage of a group of females into estrous at a short, predetermined time

How to cite this article: Pargi, K.K., Gaur, M., Jhamb, D., Juneja, R., Satish and Nirwan, S.S. (2023). Comparative Study of Different Estrus Synchronization Protocols in Pubertal Anoestrus Gir Heifers (*Bos indicus*). *Theriogenology Insight: An Int. J. Reprod. of Anim.*, **13**(01): 25-31.

Source of Support: None; **Conflict of Interest:** None

(Odde, 1990). Improving estrus synchronization, progestogens will initiate estrus and ovulation in a percentage of prepubertal heifers and anoestrous cows (Anderson *et al.* 1996; Fike *et al.* 1997; Imwalle *et al.* 1998). Progesterone supplementation in the form of injection, implants or intravaginal devices in non-cyclic heifers can be used to induce artificial luteal phase. This enables accumulation of gonadotropins and when progesterone source is withdrawn suddenly gonadotropins are released followed by LH peak and ovulation (Cetin *et al.* 2007). Combinations of hormones are also used to mimic the hormonal changes that occur at the time of puberty. Use of CIDR in an Ovsynch/Co-synch program improved pregnancy rates in heifers (Martinez *et al.* 2002). Therefore, the present study has been designed to evaluate the comparative study of different estrus synchronization protocol in pubertal anoestrus.

MATERIALS METHODS

Present study was undertaken on mature and healthy anestrous Gir heifers in Gir Cattle Breeding Farm of College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (RAJUVAS). Anestrous Gir heifer cows were supplemented with mineral mixture in their ration for a period of 30 days. This study was conducted from September 2018 to December 2018.

Selection of heifers the research work was carried out on 32 heifers that have not shown signs of estrus after attaining their pubertal age. Heifers (Gir) with optimum body weight of ≥250 kg and more than 30 months of age. All the heifers were fed mineral mixture continuously for 30 days along with balanced feed, all animals were observed for the signs of estrus. Heifers not showing any sign of estrus were treated with various hormonal regimens for induction of estrus. Fixed time artificial insemination was performed as per the protocols.

Experimental

Group-I (CIDR +PG 08) treated with control internal drug release insert into vagina (progesterone 1.38 gm, Pfizer India Ltd.). cloprostenol 500 µg i/m (Pragma, Intas pharmaceuticals Ltd) on day 7, while removing the CIDR and FTAI was performed on day 9, 48 hrs after PG injection.

Group II: Another group of 8 animals were administered an intramuscular injection Receptal 20µg i/m (GnRH) on day 0, followed by an injection Pragma 500µg i/m (PGF2α) (Intas pharmaceuticals Ltd., as 2 ml presentation.) on day 7 and a second injection Receptal 20µg i/m (GnRH) on day 9. Fixed time AI was done on day 10.

Group III (Cosynch, 08) treated administered an intramuscular injection GnRH 20µg (Receptal), followed cloprostenol 500µg (Pragma, Intas pharmaceuticals Ltd) on day 7 and fixed time AI was performed on day 9 together with injection GnRH 20µg i/m (Receptal Intas pharmaceuticals Ltd).

Group- IV (Control Group) 08 pubertal Gir heifers were taken as control group This group of heifers were not treated with any hormones however all heifers were observed for signs of estrus.

Blood sampling

Blood samples for all the treatment groups were taken on day 0, at the time of AI and 21 days post AI from jugular vein in sterilized collecting tubes. Collecting tube was placed in slanting position for clot formation at room temperature and serum was separated by centrifugation (1500 rpm for 15 min). The serum sample was immediately placed in deep refrigerator at -20 °C until quantification of progesterone by ELISA.

Progesterone assay

Assay for blood serum progesterone was done by solid phase enzyme immunoassay using progesterone kit (Cal biotech., Life Science). Each kit was having micro plates for 96 tests. The kits were stored at 2-5 ˚C till use. The level of hormone progesterone was estimated in blood serum of the heifers.

Conception Rate

Pregnancy diagnosis in all experimental animals was carried out after 60 days post-insemination by per-rectal examinations. Calculation of conception rate, pregnancy rate and establishment of cyclicity was carried out by using following formula (Deshmukh, 2010).

Print ISSN: 2249-6610

Conception Rate (%) =

$$\frac{\text{Number of conceived animals}}{\text{Number of animal bred}} \times 100$$

STATISTICAL ANALYSIS

Statistical analysis was done by using one-way ANOVA with post hoc Turkey HSD (Honestly significant difference) calculator.

RESULTS AND DISCUSSION

Estrus induction and conception rate

Effect of CIDR +PG (G-I 08): The estrus induction response and conception rate in Gir heifer under different hormonal protocol, In this group estrus induction rate of 87.50 percent achieved with the result of Van Cleef *et al*. (1996) reporting 85.1% estrus detection in dairy heifers however, estrus induction rate of 85.00, 83.33 percent were reported by Ryan *et al*. (1995), Naikoo *et al*. (2016), in postpartum anoestrus cows respectively. Whereas, higher estrus induction rate of 100 percent is reported by, Bhoraniya *et al.* (2010), Buhecha *et al.* (2015) in postpartum anoestrus cow. Lower value of estrus induction rate as 66.7 and 60.00 percent, reported by Cevik *et al.* (2010) and Dhami *et al.* (2015) respectively.

The conception rate of 50.00 percent observed in the present study concurred with the findings of Larson *et al*. (2004) as well as Ramakrishnan *et al.* (2012) reporting 50.00 percent in anoestrus cows, However higher conception rate of 53.3, 71.4, 59.00, 55.6 and 76.00 percent were obtained by Cevik *et al.* (2010), Ahmadzadeh *et al.* (2015), Ambrose *et al*. (2008), Martinez *et al*. (2000), respectively. Lower value of conception rate than present study as 41.66, 42.74, 46.66, 46.5 and 33.00 percent reported by Hadiya *et al*. (2015), Sathiamoorthy and Kathirchelvan (2010), Ryan *et al*. (1995), Van Cleef *et al*. (1996) and Buhecha *et al.* (2015) respectively. The prolonged exogenous progesterone priming from CIDR device might have caused negative feedback effect on hypothalamo-hypophyseal-gonadal axis and increased receptors for gonadotropins on the ovaries followed by rebound on its sudden withdrawal causing stimulated FSH secretion, folliculogenesis and ovulation the possible reasons for variation in results etc. Different results could be the stage of ovarian cycle at the beginning of the protocol, apart from variations in different environmental, management and genetic factors like nutritional status, parity, stage of lactation, suckling stimulus, season/climate, drug source, age, breed, and species of animal.

Effect of Ovsynch protocol (G-II, 08)

Ovsynch protocol was second most effective protocol in which induction estrus rate was 75.00% and subsequently 3 out of 8 cattle conceived, resulting in conception rate of 37.50%, Findings are in close agreement with De Jarnette *et al.* (2004) reporting estrus induction rate as 75.00 percent in cow heifers. Higher estrus response of 82.00 per cent was reported by Ghuman *et al.* (2009) in anoestrus buffalo heifers as compared to the findings of the present study which may be due to species variation and different source of hormones. Estrus induction rate of 100 percent was also reported by Vijayaranjan *et al.* (2009) in cyclic crossbred heifers whereas estrus induction rate of 29.41 percent in both cyclic and acyclic dairy heifers reported by Nak *et al.* (2005) was lower than the present study, which may be due to individual variations. The conception rate of 37.50 percent of the present study concurred with the findings of Moriera *et al*. (2000) reporting a conception rate of 37.50 percent, (Table: 3, 10) (Fig: 1, 2). Lower pregnancy rates of 35.1, 30.00, 33.00 and 26.30 percent were reported by parsley *et al.* (1997), Dejernette *et al*. (2001), Kumar *et al*. (2015) and Nak *et al.* (2011), respectively. Higher pregnancy rates

Table 1: Estrus induction and conception rate of different estrus synchronization protocols

Sl. No.	Group No. of animals	Estrus Induction	Conceived	Non-conceived	Conception rate
1	CIDR + PG (8)	87.5 0% (7/8)	4	4	50.00% (4/8)
2	Ovsynch (8)	75.00% (6/8)	3	5	37.50% (3/8)
3	Cosynch (8)	75.00% (6/8)	2	6	25.00% (2/8)
4	Control group (8)	0%	—	—	0

of 41.66, 50.00, 58.28, 60.00 and 62.50 percent were reported by Hadiya *et al.* (2015), Ramakrishnan *et al.* (2012), Nake *et al*. (2005), Amle *et al*. (2015) and Derar *et al.* (2012) respectively. Differences between the results of these studies might occur due to many factors such as, climate, timing of artificial insemination, quality of semen, and region.

Effect of Cosynch protocol (G-III, 08)

During the present study, Cosynch protocol was initiated in eight Gir pubertal anoestrus Gir heifers, resulting in expression of behavioural estrus signs in 75.00 percent heifers and 2 out of 8 heifers conceived with resulting conception rate of 25.00 percent. The conception rate in present study was similar to Ahuja *et al*. (2005) reporting 28 percent in cross breed cows. The conception rate reported by Larson *et al.* (2004) in anoestrus cow 38.00 percent and cyclic cow 44.00 percent, Ramakrishnan *et al.* (2012) reported 33 percent in postpartum Gir cow. Higher conception rates 47.00 to 83.00 percent were noted in several other studies on pluriparous cows (Geary and Whittier, 1998; Geary *et al.* 2001a, b; Lamb *et al.* 2001; Filho *et al.* 2009), and heifers (Colazo e*t al*. 2004; Oricun *et al.* 2006).

Control Group- In the control group, none of the heifer exhibited any sign of estrus, hence, nil conception rate recorded.

Overall comparison of three estrus synchronization protocols

The comparative success rate of three estrus induction/synchronization protocols, viz., CIDR, Ovsynch, and Cosynch used eight Gir cows each revealed that though percent, the highest estrus induction rate and conception rate as 87.00 and 50.00 per cent respectively, was recorded for heifers in G1 (CIDR+PG protocol) (Table 2). Estrus induction rate in different protocols were observed as 87.00, 75.00, 75.00 percent in G1 (CIDR), G2 (Ovsynch), and G3 (Cosynch) respectively. Further, conception rate of 50.00, 37.50, and 25.00, percent was observed in protocols G1, G2 and G3, respectively. However, in control group (G4) no heifer showed signs of estrus.

Aali *et al*. (2008) used Ovsynch and CIDR in ovulation synchronization/TAI protocols, and obtained conception rates of 31.00 and 41.00 percent respectively. Dhami *et al.,* (2015) observed that CIDR and Ovsynch protocols resulted in 100 percent estrus induction with conception rates at induced estrus of 60.00 and 50.00 percent, respectively in anoestrus crossbreed cows. Naikoo and Patel (2009) obtained estrus induction response of 100 percent with conception rates of 66.66 and 50.00 percent with CIDR and Ovsynch protocol, respectively, in anoestrus Mehsana buffaloes. Bhoraniya *et al.* (2010) recorded 100 per cent estrus induction/

Table 2: Mean serum progesterone concentration ng/ml (Mean± SE) different estrus synchronization protocols (Conceived and Non-Conceived Groups)

Group and No. of Animals	Status	Day 0	Day AI	21-day post AI
CIDR + PG (8)	Conceived (4)	0.58±0.03^{b}	0.49±0.02^{b}	*4.12±0.22^{a}
	Non-conceived (4)	0.57±0.02^{b}	0.53±0.03^{b}	1.43±0.10^{a}
	Overall	0.57±0.02^{b}	0.51±0.02^{b}	2.77±0.52^{a}
OVSYNCH (8)	Conceived (3)	0.56±0.04^{b}	0.52±0.03^{b}	*4.52±0.17^{a}
	Non-conceived (5)	0.69±0.04^{b}	0.57±0.04^{b}	1.38±0.09^{a}
	Overall	6.44±0.06	0.55±0.02	2.56±0.77
COSYNCH (8)	Conceived (2)	0.61±0.03	0.52±01	*4.37±0.11
	Non-conceived (6)	0.61±0.02	0.52±0.02	1.51±0.20^{a}
	Overall	0.61±0.02	0.52±0.02	2.23±0.47^{a}
CONTROL (8)	No observed estrus	0.56 ± 0.04	—	—

*Different superscripts in small letters indicate difference between the means within column at P<0.05 level of significance on the basis of One-way ANOVA. Value with * superscripts differ significantly between the row at P<0.05 level of significance on the basis of students t- test.*

Print ISSN: 2249-6610

synchronization response in postpartum anoestrus Kankrej cow by Ovsynch and CIDR protocols. However, the conception rates in Ovsynch and CIDR protocol were 33.33 and 66.66 percent, respectively. Sathiamoorthy and Kathirchelvan (2010) observed induction and conception rate in CIDR as 83.20 and 42.74 percent, while in Ovsynch protocol it was found to be 67.50 and 55.55 percent respectively, in postpartum crossbred cows. Ramakrishnan *et al.* (2012) reported the estrus induction response as 83.33, 83.33 and 100.00% of cows under CIDR, Ovsynch and Co-synch protocols, respectively. The conception rates (FTAI) in CIDR, Ovsynch and Cosynch protocol were 50.00, 50.00 and 33.33%, respectively, in postpartum anoestrous Gir cow. Hadiya *et al*. (2015) observed that the conception rates of 12 sub fertile cows, each subjected to CIDR and Ovsynch treatment protocols were 41.66 and 41.66 % respectively, The varied conception rate reported by different authors for heifers could be due to effect of several factors such as age, nutrition, cycling status, BCS, heat stress, stage of estrous cycle at which Ovsynch is initiated and embryonic losses. It is noteworthy that increased embryonic losses following TAI relative to AI at detected estrus have been reported in some studies. Induced ovulation in TAI protocols may result in ovulation of small (less mature) follicles, which may not form a robust CL contributing to increased pregnancy losses (Kantharaj, 2015). The explanation for better estrus response and conception rate in CIDR group could be due to progesterone priming through CIDR, establishment of proper endocrine harmony/ synchrony and FTAI (Ramakrishnan *et al*. 2012).

Serum progesterone profile under different synchronization protocols

Serum progesterone (ng/ml) concentrations were low toward basal values on day 0 (CIDR 0.57±0.02 ng/ml; Ovsynch 6.44±0.06 ng/ml; Cosynch 0.61±0.02 ng/ml; Control 0.56 ± 0.04 ng/ml). Progesterone concentration levels were non-significantly ($p<0.05$) decreased on day of AI (CIDR 0.51±0.02 ng/ml; Ovsynch 0.55±0.02 ng/ml; Cosynch 0.52±0.02 ng/ml).

Serum progesterone concentrations in conceived and non-conceived groups in all three treatment protocols and control group were found to be similar on day 0, and day AI, but on day 21 post-AI, the conceived cows had significantly ($P<0.05$) higher mean plasma progesterone concentrations as compared to non-conceived heifers as in CIDR (4.12±0.22 vs. 1.43±0.10 ng/ml), Ovsynch (4.52±0.17 vs. 1.38±0.09 ng/ml) and Cosynch (4.37±0.11 ng/ml vs 1.51±0.20 ng/ml) protocols (Table-9). In control group, the mean serum progesterone concentration was the lowest on day 0 (0.56±0.04 ng/ml). Dhami *et al*. (2015) and Buchecha *et al*. (2015) observed similar findings for plasma progesterone concentrations at the day 21 post-AI with the conceived cows having significantly ($P<0.05$) higher mean plasma progesterone concentrations as compared to non-conceived cows (4.36±0.12 vs. 1.65±0.82 ng/ml) and (4.85±0.62 vs 1.59±0.34 ng/ml) respectively. Bhoraniya *et al*. (2012) reported that plasma progesterone (ng/ml) concentrations at the day 21 post AI was significantly ($P<0.05$) higher in conceived cows in CIDR (3.20±0.10 vs 2.04±1.07) and Ovsynch (3.45±0.17 vs 1.60±0.50) treated post-partum cattle. One possible explanation for higher conception rate in CIDR-treated cows is the progesterone via the CIDR that helped the cows to become slightly more fertile. Progesterone could possibly be affecting the oocyte quality or the environment in the uterus and its secretions. Although the specific action of progesterone to improve fertility in timed AI programmes remains poorly defined, results of the current study and others (Melendez *et al*. 2006; Stevenson *et al*. 2006) strongly suggest that exogenous progesterone may in some way enhance the quality of the uterine environment and improve the likelihood of successful pregnancy. For treating pubertal anoestrus in Gir heifers, CIDR+PG was found to be most effective out of the three hormonal protocols followed by Ovsynch and Cosynch, respectively.

ACKNOWLEDGEMENTS

The authors are thankful to authorities of College of Veterinary and Animal Science, Navania, Vallabhnagar Udaipur for providing the necessary facilities of research.

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