ESTRUS INDUCTION AND FERTILITY RESPONSE IN POSTPARTUM ANESTRUS GIR COWS*

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ABSTRACT

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The study was aimed at induction/synchronization of estrus in suckler postpartum anoestrus/ suboestrus Gir cows (n=18) of an organized farm. The anoestrus/suboestrus cows were divided into 3 equal groups each of 6 animals, and a group of 6 normal cyclic cows was kept as control. Three standard synchronization protocols initiated around day 90 to 92 postpartum were CIDR, Ovsynch, and Cosynch, considering the day of first GnRH injection or CIDR insertion as day 0. The animals were bred by fixed-time AI (FTAI). Pregnancy was confirmed per rectum on day 45 post-AI. Plasma progesterone was estimated by RIA on day 0 (day of first treatment), 7 (day of PG injection), 9/10 (day of estrus/AI) and on day 10, 20, 30 and 40 post-AI. The estrus induction response as noted by uterine tone and mucus discharge was 100% in all 3 groups, but the behavioural signs were observed in 83.33, 83.33 and 100.00 % of cows under CIDR, Ovsynch and Co-synch protocols, respectively. The conception rates at induced estrus (FTAI) in CIDR, Ovsynch and Cosynch protocol were 50.00, 50.00 and 33.33 %, respectively. The corresponding overall conception rates of 2 cycles post-treatment were 83.33 (5/6), 66.66 (4/6), and 83.33 (5/6) %. In control group, the first service and overall conception rates were 33.33 and 66.66 (4/6) %, respectively. The results were the best with CIDR, followed by Cosynch and Ovsynch protocol. The plasma progesterone (ng/ml) concentrations were significantly ($P<0.05$) higher on day 7 in CIDR (6.65±2.43 ng/ml), Ovsynch (3.51 ng/ml) and Cosynch (6.27±0.92 ng/ml) protocols as compared to the corresponding values obtained on day 0 and 9/10 (AI) for the same protocols. However, the progesterone values on day 10, 20, 30 and 40 post-AI did not differ significantly between these groups and even from control group. Mean (± SE) values of plasma progesterone (ng/ml) on day 20 and 30 post-AI were higher ($P<0.05$) in conceived cows than the non-conceived cows. These results suggest that use of CIDR and Cosynch protocols may serve as an excellent tool for induction/synchronization of estrus and improvement of conception rate in postpartum anestrus/subestrus Gir cows.

Key words: Gir cows, Estrus synchronization, CIDR, Ovsynch and Cosynch, Plasma progesterone

INTRODUCTION

Gir cattle are amongst the hardiest of high yielders in the world (Kumar and Singhal, 2006). However, they are slow breeders and have extended post-pubertal and postpartum anestrus periods, compared to their temperate counterparts exposed to similar environment and management. Anestrus is the main factor that negatively affects reproductive performance of these

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animals. Several factors affect postpartum anestrus/ subestrus, including suckling and maternal-offspring bond, and pre- and postpartum nutritional status. Most hormonal treatments used to alter reproductive pattern of such cattle are based on progesterone/progestogen (P_4) releasing devices, $PGF_{2\alpha}$, or a combination of GnRH-$PGF_{2\alpha}$-GnRH. The choice of approaches for controlling cycle length are: 1) to regress or "kill" the corpus luteum (CL) of the animal before the time of natural luteolysis, by administration of a $PGF_{2\alpha}$ and thereby shorten the cycle, or 2) to administer exogenous progestins to delay the time of estrus following natural or induced luteolysis that may extend the length of the estrous cycle, or 3) to select the ovulatory follicle by an injection of GnRH, which should cause premature ovulation of that follicle. Using these concepts, researchers have made tremendous strides in developing numerous systems / protocols such as Ovsynch, Cosynch, Heatsynch, CIDR etc. to control the estrous cycle and improve reproductive efficiency (Lucy *et al.*, 2004). The present study was, therefore, aimed to evaluate the comparative efficacy of three estrus induction and synchronization protocols, viz, CIDR, Ovsynch and Cosynch in postpartum anestrus/subestrus Gir cows.

MATERIALS AND METHOD

Twenty four postpartum suckled Gir cows of 2^{nd} - 4^{th} parity were obtained from organized herd of Livestock Research Station, AAU, Anand. The reproductive/ovarian status in cows not expressing behavioural estrus till day 70 postpartum were assessed by per rectal palpation of the genitalia on three occasions, each at 10 days interval. Based on the reproductive/ovarian status, the cows (n=18) confirmed as anestrus and subestrus were randomly distributed at day 90-92 postpartum into following 3 groups of estrus induction/ synchronization protocols, each with 6 animals. Six normal cyclic cows that exhibiting first estrus within 90 days postpartum and inseminated served as control group.

A group of 6 animals, three each with subestrus and true anestrus, were inserted with CIDR (1.38 g progesterone in elastic rubber molded over a nylon spine, Pfizer India Ltd.) per vaginum on day 90-92 postpartum. They were treated with single i/m injection of 500µg (2 ml) of $PGF_{2\alpha}$ (Cyclix, Intervet) on day 7 while removing CIDR, and FTAI was performed on day 9, 48 hrs after PG injection.

Another six animals, 4 subestrus and 2 anestrus, were included in Ovsynch protocol with administration of an i/m injection of 20 µg (5 ml) of GnRH (Receptal, Intervet) on day 0, followed by an injection of 500 µg (2 ml) PGF_2á (Cyclix, Intervèt) on day 7 and a second GnRH injection of 20 µg (5 ml) on day 9. Fixed time AI was performed 22 hrs after second GnRH injection.

Third group of 6 animals comprising 4 subestrus and 2 anestrus cows (Cosynch group) were treated with the single i/m injection of 20 µg (5 ml) GnRH, followed by an injection of 500 µg (2 ml) of $PGF_{2\alpha}$ on day 7 and fixed time AI was performed 48 hours after PG injection together with single i/m injection of 20 µg (5 ml) of GnRH.

Blood samples were collected from jugular vein on the day of first treatment (day 0), day of $PGF_{2\alpha}$ injection (Day 7), day of induced estrus (day 9/10, AI), and then on day 10, 20, 30 and 40 post-AI. The plasma samples stored at -20°C were used to determine progesterone profile by Radio-Immuno-Assay technique of Kubasic *et al.* (1984). The data were analyzed statistically. The variation between groups in estrus response and conception was compared by chi-square test, and the variation in plasma progesterone levels between groups/ periods was tested by CRD and Duncan's NMRT using SAS system of statistical analysis.

RESULTS AND DISCUSSION

Among six cows of CIDR group, none exhibited behavioural signs of estrus within 7 days of treatment. However, all the animals showed induced estrus clinically on day 9, i.e. 48 hrs after $PGF_{2\alpha}$ injection. One of the 6 cows in this group showed silent estrus as confirmed by presence of a mature Graafian follicle on the ovary without clinical signs at the time of FTAI. The results of CIDR protocol were better than the normal cyclic/fertile (control) group, where though 100 % animals exhibited varying degrees of heat signs, only

33.33 and 50.00 % cows conceived at first and second cycle, with overall conception rate of 66.66 %. 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.

Estrus synchronization rate of 100 % achieved with CIDR was in harmony with the results of Zabeel *et al.* (2009) and Bhoraniya *et al.* (2012) in buffaloes and Kankrej cows, respectively. However, lower values of synchronization rate of 43 to 83 % were reported in cyclic and/or acyclic cows by Tauck *et al.* (2007) and Cevik *et al.* (2010). The conception results of the present study concurred with the findings (60 %) of Martinez *et al.* (1998), whereas Cevik *et al.* (2010) reported 53.3 % conception rate. Contrary to this, higher conception rates of 69 to 80 % were obtained in cows by Tauck *et al.* (2007) and Aali *et al.* (2008), and 67.00 to 83.33 % in buffaloes by Zabeel *et al.* (2009).

Among six Gir cows of Ovsynch group, none exhibited behavioural signs of estrus before the last GnRH injection. Five cows showed synchronized estrus on day 10 between 10 and 20 hrs after the last GnRH injection, and one cow was found in silent estrus on per rectal palpation. The estrus signs were prominent in three animals, and moderate, weak and silent in one animal each . The conception rates obtained during the induced estrus and second service/cycle following treatment were 50.00 and 33.33 %, respectively, with an overall conception rate of 66.66 (4/6) % for two cycles.

Estrus induced in all 6 cows of this group was ovulatory as confirmed by presence of CL on the ovary 12 days later. Similar ovulatory response was reported with Ovsynch protocol by Ansari *et al.* (2008), Dagli *et al.* (2008), and Vijayarajan *et al.* (2009) in crossbred cows, while lower ovulatory response was recorded by Keskin *et al.* (2011) in HF (88.4%) and Swedish Red cattle (88.5%). The conception rate obtained with Ovsynch protocol was similar to that (46.66 to 55.55%) reported in other breeds of cows elsewhere (Pursley *et al.*, 1995; Geary *et al.*, 1998; and Sathiamoorthy and Kathirchelvan, 2010). However, relatively higher conception rates of 61.00 to 90.00 % were also noted in cows by Ansari *et al.* (2008) and Muneer *et al.* (2009). The possible reason for variation could be the reproductive status or stage of estrous cycle at the beginning of the protocol, besides nutritional, managerial, lactation, drug source, age and breed and variations in geographical locations.

The results of Cosynch protocol showed that all the six animals exhibited behavioural signs of estrus within 48 hrs after $PGF_{2\alpha}$ injection. However, the signs of estrus were prominent in only two animals and moderate in four animals. The conception rate was 33.33 % at induced estrus and 75.00 % in second cycle, with an overall conception rate of 83.33 % for two cycles. The conception rate obtained at first service of this study was similar (28-38%) to that reported previously in other breeds of anestrus cows (Larson *et al.*, 2004; Ahuja *et al.*, 2005).

The comparative success rate of three estrus induction/synchronization protocols, viz., CIDR, Ovsynch, and Cosynch used on six anestrus/ suboestrus Gir cows each revealed that though cent % cows expressed synchronized estrus clinically, with some 17 % having silent estrus in first two protocols, the first service CR varied between them with values of 50.00, 50.00 and 33.33 %, respectively, as compared to 33.33 % first service conception rate in normal cyclic control cows. The corresponding second service conception rates were 66.66. 33.33, 75.00 and 50.00 % in three treated and control group. The overall conception rates of two cycles over the 25 days period following induced estrus with CIDR, Ovsynch and Cosynch protocols were 83.33, 66.66 and 83.33 %, respectively, as against 66.66 % in normal cyclic group. The CIDR and Cosynch protocols were superior over Ovsynch protocol and normal cyclic -control- group.

The plasma P_4 levels around day 90 postpartum and on the day of initiation of treatment indicated that at least a few cows in each group were in cyclic/ subestrus stage. The mean progesterone concentration of all the groups remained at the lowest or basal level

on the day of AI, irrespective of whether they exhibited induced or spontaneous estrus .

In control group, 10 days after AI the mean progesterone level increased significantly and then remained at that elevated level till day 40 post-AI in conceived animals, whereas in non-conceived animals the level dropped significantly (P<0.05) on day 20 post-AI, and thereafter showed cyclic pattern upto day 40 post-AI. Out of six animals, two cows conceived by first service at 70-80 days postpartum.

In CIDR insert group, the plasma P_4 levels showed a steep and significant increase by day 7 in all animals. The level was also relatively higher on the day of AI probably due to latent effect of exogenous progesterone given through CIDR. The levels came down by day 9 due to removal of CIDR and PG injection given on day 7. On day 10 post-AI the mean values of progesterone again increased significantly and remained high in conceived group, but in non-conceived group the values decreased thereafter at day 20 and then showed a cyclic pattern due to failure of conception and return to next estrus at varying intervals post-AI. Almost similar were the observations in Ovsysnch a nd Cosynch groups.

These findings inn CIDR and Ovsynch groups are in agreement with the reports of Bhoraniya *et al.* (2012) and Naikoo *et al.* (2010). Martinez (2002) reported that plasma progesterone concentrations following CIDR insertion in cows were near to luteal level (5 to 7 ng/ml) by 24 hrs, remained static until CIDR removal on day 7, and then declined by 12 hrs after CIDR removal. Willard *et al.* (2003) observed that serum progesterone was increased (P<0.05) after administration of GnRH as compared to control cows. The present findings thus clearly support their views.

Bhoraniya *et al.* (2012) recorded significantly (P<0.05) higher plasma progesterone (ng/ml) concentrations in Kankrej cows on day 7 in Ovsynch (5.727 ± 1.26) and CIDR (4.37 ± 0.66) protocols as compared to the corresponding values obtained on day of initiation of treatment, day of estrus/AI and on day 20 post-AI for the same protocols. Mean values of plasma progesterone (ng/ml) on day 20 post-AI were higher (P<0.05) in conceived than the non-conceived cows of both the treatment protocols. Chander *et al.* (2002) also reported higher values of plasma P_4 in pregnant than non-pregnant cows, indicating luteal insufficiency or anovulation in non-pregnant animals. Komoto *et al.* (1991) observed that the plasma P_4 concentration on day 6 after AI was higher in conceived than in non-conceived heifers, but the difference was significant only after 10 days of AI.

It is concluded that induction of estrus/cyclicity, estrus synchronization and improvement in conception is possible with the use of different hormone protocols, viz. CIDR, Ovsynch and Cosynch in postpartum anestrus/subestrus Gir cows. Although estrus induction was cent % with all hormone protocols, their relative efficacy varied in expressivity of behavioural signs. CIDR and Cosynch protocols showed good estrus expression and fertility, and thus, reduce the maintenance cost of dry animals, which in fact is of economic importance to the farmers.

REFERENCES

Aali, M., Pretheeban, T., Giritharan, G. and Rajamahendran, R. (2008). Pregnancy rates and peripheral progesterone levels following Ovsynch or CIDR ovulation synchronization/ timed artificial insemination protocols in postpartum dairy cows. *Canadian J. Anim. Sci.*, **88**: 457-461.

Ahuja, C., Montiel, F., Canseco, R., Silva, E. and Mapes, G. (2005). Pregnancy rate following GnRH+PGF_2á treatment of low body condition, anestrous *Bos taurus* by *Bos indicus* crossbred cows during the summer months in a tropical environment. *Anim. Reprod. Sci.,* **87**(3): 203-213.

Ansari, S.M.A., Rao, K.S. and Raju, K.G.S. (2008). Studies on post-partum anoestrus with special emphasis on induction of oestrus in crossbred cows. *Proc. XXIV Annual Convention of ISSAR and National Symposium, KVAFSU, Bangalore*, 11-13 Dec., p.23.

Bhoraniya, H.L., Dhami, A.J., Naikoo, M., Parmar, B.C. and Sarvaiya, N.P (2012). Effect of estrus synchronization protocols on plasma progesterone profile and fertility in postpartum anestrous Kankrej cows. *Trop. Anim. Health Prod.*, **44**(3): online, DOI 10.1007/s11250-011-0057-1.

Çevik, M., Selcuk, M. and Dogan, S.(2010). Comparison of pregnancy rates after timed artificial insemination in Ovsynch, Heatsynch and CIDR-based synchronization protocol in dairy cows. *Kafkas. Univ. Vet. Fak. Derg.*, **16**(1): 85-89.

Chander, S., Sangwan, N., Malik, C.P., Barak, R. and Garg, S.L. (2002). Circulating sex steroid hormones profile in repeat breeding cows. *9th Int. Cong. on Biotechnology in Anim. Reprod.*, Dec, 2002, p. 229.

Dagli, N.R., Gulawane, S.V. and Bakshi, S.A. (2008). Use of Ovsynch an effective reproductive managemental tool for a dairy farm. *Proc. XXIV Annual Convention of ISSAR and National Symposium held at KVAFSU, Bangalore*,11-13 Dec., p.62.

Geary, T.W., Whittier, J.C., Downing, E.R., LeFever, D.G., Silcox, R.W., Holland, M. D., Nett, T.M. and Niswender, G.D. (1998). Pregnancy rates of postpartum beef cows that were synchronized using Syncro-Mate-B or the Ovsynch protocol. *J. Anim. Sci.*, **76**:1523-1527

Keskin, A., Yilmazbas-Mecitoglu, G., Gumen, A., Karakaya, E., Celik, Y., Okut, H. and Wiltbank, M.C.(2011). Comparison of responses to Ovsynch between Holstein-Friesian and Swedish Red cows. *J. Dairy Sci.*, **94**(4): 1784-1789.

Komoto, H., Hoshino, K. and Mori, Y. (1991). Distribution pattern of concentrations of ovarian hormones in peripheral blood in cows. *Jap. J. Anim. Reprod.*, **37**(3): 213-118.

Kubasic, N.P., Hallauer, G.D. and Brodows, R.G. (1984). Evaluation of direct solid-phase RIA for progesterone, useful for monitoring luteal function. *Clin. Chem.*, **30**(2): 284-286.

Kumar, P. and Singhal, L.K. (2006). Gir: An important milch cattle of Western India. *The Indian Cow*, **1**: 67-68.

Larson, J.E., Lamb, G.C., Geary, T.W., Stevenson, J.S., Johnson, S.K., Day, M.L., Kesler, D.J., DeJarnette, J.M. and Landblom, D. (2004). Synchronization of estrus in replacement beef heifers using GnRH, prostaglandin F (PG), and progesterone (CIDR): a multi-location study. *J. Anim. Sci.*, **82**(1): 368.

Lucy, M.C., McDougall, S. and Nation, D.P. (2004). The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management systems. *Anim. Reprod. Sci.*, **82–83**: 495–512.

Martinez, M.F. (2002). Synchronization of follicular wave dynamic and ovulation for fixed-time artificial insemination in cattle. *Ph.D. thesis, University of Saskatchewan*, Chapter-5.

Martinez, M.F., Kastelic, J.P., Adams, G.P., Janzen, E., Olson, W. and Mapletoft, R.J. (1998). Alternative methods of synchronizing estrus and ovulation for fixed time insemination in cattle. *Theriogenolgy*, **50**: 350 (Abstr.).

Muneer, S., Rao, K.S. and Raju, K.G.S. (2009). Efficacy of GnRH-PGF2á-GnRH, PMSG and PMSG+ hCG in postpartum anestrous crossbred cows. *Indian J. Anim Reprod.*,**30**(1):7-9.

Naikoo, M., Patel, D.M., Sarvaiya, N.P. and Killader, A. (2010). Estrus synchronization in postpartum anestrous Mehsana buffaloes using different hormone protocols. *Indian J. Field Vets.*, **6**(2):1-4.

Pursley, J.R., Mee, M.O. and Wiltbank, M.C. (1995). Synchronization of ovulation in dairy cows using PGF and GnRH. *Theriogenology*, **44**(7): 915-923.

Sathiamoorthy, T. and Kathirchelvan, M. (2010). Efficacy of $PGF_{2\alpha}$, CIDR and Ovsynch treatment on estrus response and fertility rate in crossbred cows. *Indian J. Anim. Reprod.*, **31**(2): 43-45.

Tauck, S.A., Wilkinson, J.R.C., Olsen, J.R. and Berardinelli, J.G. (2007). Comparison of using 7- or 14-d CIDR treatments in an estrous synchronization protocol that included PGF2á, timed A I and GnRH in primiparous, suckled beef cows. *Proc. Western Section, Am. Soc. Anim. Sci.*, **58**: 271-273.

Vijayarajan, A., Chandrahasan, C. and Napolean, R.E. (2009). Synchronization of ovulation in repeat breeding crossbred cows. *Indian J. Field Vet.*, **5**(1): 57-58.

Willard, S., Gandy, S., Bowers, S., Graves, K., Elias, A. and Whisnant, C. (2003). The effects of GnRH administration post-insemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. *J. Anim. Sci.*, **59**:1799-1810.

Zabeel, S.M., Hegab, A.O., Montasser, A.E. and El-Sheikh, H. (2009). Reproductive performance of anestrous buffaloes treated with CIDR. *Anim. Reprod.*, **6**(3): 460-464.