EFFECT OF CIDR AND OVSYNCH PROTOCOLS ON ESTRUS RESPONSE, FERTILITY AND PLASMA PROGESTERONE AND BIOCHEMICAL PROFILE IN TRUE ANOESTRUS CROSSBRED COWS*

K. R. PATEL[1], A. J. DHAMI[2], K. K. HADIYA[3], K. K. SAVALIA[4] AND N. P. SARVAIYA[5]
Department of Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry
Anand Agricultural University, Anand-388 001, Gujarat, India

ABSTRACT

A study was carried out in the AMUL milk shed area on 30 postpartum crossbred cows, comprising 20 true anoestrus and 10 normal cyclic cows to evaluate clinical response to oestrus induction protocols and plasma progesterone and biochemical profile at different time intervals post treatment/pot-AI. The oestrus induction response obtained with CIDR and Ovsynch protocol in anoestrus cows (10 each) was 100% and 90% with oestrus induction intervals of 6.00 ± 3.22 and 86.67 ± 3.33 hrs from PG injection, respectively. The conception rates at induced/first oestrus and overall of 3 cycles were 50 and 80% in CIDR 30 and 60% in Ovsynch protocol: and 80% in normal cyclic control cows. The plasma progesterone levels in CIDR protocol on day 0, 7, 9/10 (AI) and day 21 post-AI were 0.65 ± 0.23, 3.48 ± 0.54, 0.48 ± 0.13 and 10.44 ± 1.77 ng/ml in conceived and 0.70 ± 0.18, 3.61 ± 1.05, 0.32 ± 0.09 and 1.42 ± 0.38 ng/ml in non conceived cows. The corresponding P_4 levels in Ovsynch protocol were 0.28 ± 0.06, 2.98 ± 0.93, 0.48 ± 0.19 and 8.06 ± 1.16 ng/ml in conceived and 0.32 ± 0.07, 3.82 ± 1.35, 0.52 ± 0.30 and 1.24 ± 0.71 ng/ml in non-conceived cows. There was significant cariation in P4 profile between periods in both the protocols (P<0.05). The P4 levels in conceived and non-conceived cows of normal cyclic control group on day 0(AI) were 0.24 ± 0.04 and 0.41 ± 0.15ng/ml, and on day 21 post-AI 10.48 ± 1.34 nd 0.63 ± 0.11 ng/ml (P<0.01), respectively. The plasma total cholestrol and total protein concentrations in conceived and non-conceived cows of CIDR protocol were 173.62 ± 13.28 and 186.43 ± 8.94 mg/dl, and 12.59 ± 0.38 and 11.07 ± 0.06 g/dl, respectively, without significant variation between sampling days. In Ovsynch protocol, the corresponding calues of cholestrol and protein were 128.88 ± 3.74 and 213.11 ± 5.73 mg/dl, and $11.83+0.22$ and $12.14+0.13$ gdl. The plasma total cholestrol levels varied significantly between sampling days in non-conceived animals (P<0.05). There was significant variation between conceoved and non-conceived animals in total cholestrol profile inCIDR protocol and in total proterin in Ovsynch protocol (P<0.01). In normal cyclic control animals, the choestrol concentrations on day of AI and day 21 post-AI were $219.47+22.15$ and $192.80+16.79$ mg/dl and total protein levels $12.35+0.36$ and $12.76+0.15$ g/dl without significant variation (P0.05) between days and between pregnancy status. It is concluded that both the treatment protocols induce fertile/ovulatory oestrus and improve conception rates and plasma P_4 profile in true anoestrus cows, though there was no significant infuence on plasma protein and cholestrol profile.

Key words: Crossbred cows, Anoestrus, Oestrus induction/synchronization protocols, Fertility, Progesterone, Metabolites, Conceived and non-conceived groups.

Reproductie inefficiency in lactating dairy cows is a source of frustation to dairy producers and their consultants and it reduces dairy farm profability. Anoestrus forms the major condition constituting about 2/3rd of the infertility problems

occuring in crossbred catte[17]. Ovaries of animals suffering from true anoestrus are smooth, small and inactive with absence of oestrus and functional structure[1]. Now a day, time-artificial insemination (TAI) is a common practice in dairy herds to improve reproductive efficiency since all cows are inseminated independent management to enhance the reproductive performance of cattle and is currently applied routinely in the reproductive management programs on farms[5]. Timed AI protocols such as Ovsynch/Cosynch, which are composed of GnRH, PGF2a and GnRH injections, and progesterone (P$_4$) based regimens (CIDR, PRID) have been successfully used in the past in bringing acyclic animals to normal cyclicity, inducing synchronized oestrus and in getting better conception rate, shorter service period and thereby ideal inter-calving interval7,14. The present study was planned to evaluate the effect of CIDRand Ovsynch protocols of oestrus induction/ synchronization on conception rate the plasma profile of progesterone, protein and cholesterol in anoestrus crossbred cows under field conditions.

MATERIAL AND METHODS

The study was undertaken on 30 crossbred cows, 20 true anoestrus and 10 normal cyclic/ fertile (control) ones at farmers' door in villages around Anand during the months of November 2012 to March, 2013. Problem breeders were confirmed by ractal palpation twice 10 days aart, while 10 cows exhibiting oestrus spontaneously within 3 months postpartum with normal healthy genitalia and inseminated without any treatment served as normal cyclic/fertile control. All the animals identified were dewormed using Invermectin 70 mg/ sc (Inh. Ivectin @ 7 ml, IIL) and by providing medicated concentrate mixture of AMul (fenbendazole 3 g/kg feed). Owners of these ear-marked animals were also supplied with 1 kg mineral mixture (Amul brand) for supplementing to their animals @25-30g/day. Animals once inseminated were followed for recurrence of oestrus regularly for 3 cycles and in non-return cases pregnancy was confirmed per rectum 60 days post -AI.

Twenty true anoestrus crossbred cows identified of average BCS (2.75 to 3.50) without visible and palpable genital abnormalities were treated initially once i/m with sodium acid phosphate 2g (Inj, Alphos-40, 10 ml, Pfizer) and multivitamins AD$_3$E (Inj. Vetacept, 10ml, Concept Pharma). Ten cows each were then randomly subjected to CIDR and Ovsynch protocol. In Group-I (n=10), a CIDR (containing 1.38 g of progesterone in silastic coil, Pfizer Animal Health) was inserted intra-vaginally on day 0, removed on day 7 together with i/m Inj. of PGF$_{2a}$25 mg (Inj. Lutalyse, 5 ml, Pfizer) and fixed time AI (FTAI) was done on day 9 with i/m Inj. of GnRH 10 ug (Inj. Receptal @ 2.5 ml, Intervet). In Group-II (n=10), Ovsynch protocol, 10 true anoestrus cows were administered with i/m Inj. of GnRH 20 ug (Inj. Receptal, 5ml) on day 0, Inj. PGF$_{2a}$25mg (Inj. Lutalyse, 5ml, Pfizer) on day 7, and second Inj. of GnRH 10 ug (Inj. Receptal, 2.5 ml) on day 9 followed by FTAI 24 hrs later, i.e., on day 10. Oestrus response to treatment and rectal palpation findings of each animal were recorded initialy and at AI/FTAI.

The jugular blood samples were collected in heparinized vacutainers four times from true anoestrus animals, i.e. on day0-just before treatment, on day 7 at the time of PGF$_{2a}$ administration, on day 9/10 induced oestrus/AI and on day 21 post-AI. In normal cyclic group, blood sampling was done on day AI and day 21 post-AI. The blood samples were centrifuged at 3000 rpm for 15 minutes, and plasma separated out was stored deep frozen at 20^{0}C with a drop of merthiolate (0.1%) until analyzed. Plasma progesterone concentrations were estimated by employing standar RIA technique[15]. Labelled

antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech, France. The sensivity of assay was 0.1 ng/ml. The co-efficients of variation for intra-and inter-assay were 5.4 and 9.1% rspectively. Plasma total cholesterol was estimated by CHOD/PAP method and total plasma protein by Biuret method using standard procedures and asay kits procured from Crest Bio-systems, Goa with the help of Chemistry Analyzer (Mindray, BS 120).

Animals conceived at induced/first oestrus were taken pregnant/conceived group and the rests as non-pregnant/non-conceived group. The observations/data on oestrus response, conception rates and blood profiles of plasma P_4, cholesterol and protein were analyzed statistically using standar procedures (CRD, t-test) within and between groups for effect of period and pregnancy status.

RESULTS AND DISCUSSION

Oestrus Induction Response and Conception Rate :

The oestrus induction response with CIDR protocol was 100% with 8 animals exhibiting prominent oestrus and 2 with moderate oestrus, while Ovsynch protocol, the oestrus induction response was 90% with 6 animals exhibiting prominent oestrus, 2 with moderate oestrus and 1 with weak oestrus response. The findings with CIDR protocol are in accordance with the earlier reports in anoestrus cows[2,7,11,13]. Similarly, the oestrus response obtained with Ovsynch protocol in crossbred cows is in agreement with earlier reports. ... in Holstein cows (85.70%) and in crossbred cattle (95.56 and 80%, respectively).

The interval from PGF_{2a} injection to induced oestrus was 66.00±3.22 hrs (n=10) in CIDR treated animals and 86.67±3.33 hrs (n=9) in Ovsynch protocol. Further the conception rates obtained in induced/first, second, third cycle and overall of 3 cycles were 50.00, 40.00, 33.33 and 80% (Table 1, Fig.1). It was reported conception rates of 50.00 and 53.30% at induced oestrus in anoestrus crossbred and HF cows, respectively 18.8. Earlier workers 7 reported 33.33% first service conception rate in Kankrej cows. The possible reasons for variation could be the reproductive, nutritional, managerical, lactational status, drug source, age and such other factors. The results revealed that the application of CIDR protocol is the best tool for induction of ovulatory oestrus and enhancement of conception rate in true anoestrus crossbred cows followed by Ovsynch potocol.

Plasma Progesterone Profile:

The overall mean plasma progesterone (P4) levels on day 0,7,9 (AI) of treament and on day 21 post-AI in cows subjected to CIDR protocol were 0.68±0.14, 3.55±0.56, 0.40±0.08 and 5.93±1.73 ng/ml respectively. The corresponding P4 values for Ovsynch protocol were 0.31±0.05, 3.57±0.96, 0.51±0.21 and 3.29±1.19 ng/ml respectively. The mean plasma P_4 level on day 21 post-AI in conceived cows of CIDR group was significantly (P<0.01) higher than that on day 7 (10.44±1.77 vs. 3.48±0.54 ng/ml), though it did not differ at earlier stages. This indicated better ovulatory oestrus and luteal function for maintenance of pregnancy. AMong non-conceived cows under CIDR, the mean plasma progesterone level on day 21 post-AI was 1.42±0.38 ng/ml. This low P4 level might be due to luteolysis of CL and repeat to next oestrus in some of the cows at variable intervals. The plasma progesterone concentration was significantly higher (P<0.01) on day 21 post-AI in conceived than non-conceived (10.44±1.77 vs 1.42±0.38 ng/ml) group (Table 2, Gig.2). The present observations are in close agreement with the earlier reports[2,6,7], wherein significantly (P<0.05) higher plasma P_4 concentrations were found on day 7 for CIDR protocol as compared to the values on day 0.9(AI) and on day 20 post-AI, with higher (P<0.05) mean

values of plasma progesterone on day 20 post-AI in conceived than the non-conceived Gir and Kankrej cows, which concurred with the present findings.

The plasma P4 value for Ovsynch treatment on day 21 post-AI was significantly (P<0.01) higher in conceived cows than non-conceived ones (8.06+1.16 vs 1.24+0.71 ng/ml). The overall progesterone values (ng/ml) on day 7(3.57+0.96) and day 21 post-AI (3.29+1.19) were significantly higher than the values on day 0 (0.31+0.05) and day 10 (0.51+0.21) (P<0.05). Similar results were also obtained by earlier researcher[2,13] on day 0,7 and 9 of treatment and on day 21 post-AI in conceived and non-conceived groups of Gir cattle reported similar plasma progesterone pattern with significant higher (P<0.05) mean values on day 20 post-AI in conceived than non-conceived Kankrej was reported[6,7]. In normal cyclic control group, the mean progesterone concentrations on day 0(AI) and day 21 pos-AI were 0.24+0.04 and 10.48+1.34 ng/ml for conceived animals and 0.41+0.15 and 0.63+0.11 ng/ml for non-conceived cows, respectively. The P_4 value on day 21 post-AI was significantly (P<0.01) higher in conceived than non-conceived group.

Plasma Total Cholesterol Profile:

The mean plasma total cholesterol concentrations in anoestrus cows under CIDR and Ovsynch protocols did not vary significantly between days/periods. The overall pooled mean value of total cholesterol was 173.62+13.28 mg/dl for conceived and 186.43+8.94 mg/dl for non-conceived animals with a grand mean of 180.03+7.96 mg/dl in CIDR group. However in Ovsynch group, the plasma total cholesterol concentration was significantly (P<0.01) higher in non-conceived than conceived cows at all periods including the overall pooled means (213.11+5.73 vs 128.88+3.74 mg/dl), with a grand value of 187.84+8.37 mg/dl (Table3). The present findings of non-significant variations between days were in close agreement with earlier report in Kankrej cows[6]. It was observed comparable findings in Gir cows under CIDR and Ovsynch protocols[3]. Further, in normal cyclic control group, the overall mean plasma total cholesterol value was 206.12+13.87 mg/dl, which did not vary significantly between days and between conceived and non-conceived cows, though the levels were apparently higher in conceived than non-conceived cows (219.47+22.15.15 vs. 192.80+16.79 mg/dl). Further, the pooled mean plasma total cholesterol concentrations in cyclic control and anoestrus groups did not differ significantly, though the value was higher in normal cyclic group. Like present finding, earlier workers[12] also reported lower plasma total cholesterol values in anoestrus than the normal cyclic cows.

Plasma Total Protein Profile:

The plasma total protein values on different days of CIDR and Ovsynch protocols were more or less constant. The values were higher in conceived than non-conceived cows at each interval with significant (P<0.01) difference in pooled means of CIDR group (12.59+0.38 vs. 11.07+0.06 g/dl), but not in Ovsynch group (11.83+0.22 vs 12.14+0.13 g/dl; Table4). These findings are in close agreement with the previous reports[6,10], wherein significantly higher level of plasma protein has been documented in pregnant than non-pregnant cows. The non-significant variations observed in protein with Ovsynch protocol also corroborated with the findings of earlier workers[20]. In normal cyclic control cows also the values of total protein did not vary significantly between groups or days. Further, the pooled mean plasma total protein concentrations in cyclic control was insignificantly higher as compared to anoestrus groups. The plasma total protein levels observed both in anoestrus and normal cyclic animals under the present study were higher than those reported by earlier researchers[3,9,16].

CONCLUSION

The study inferred that both the treatment protocols induced ovulatory oestrus and improved conception rates and plasma P_4 profile in true anoestrus cows, but they did not influence the plasma protein and cholesterol profile.

Table 1: Effect of CIDR and Ovsynch protocols on oestrus induction response and conception rates to FTAI at induced oestrus and overall of three cycles in anoestrus crossbred cows.

Treatment Group	No. of Cows	Percent Oestrus Response	PG Injection to Oestrus Interval (hrs)	Conception Rate (%)			
				Induced/First Cycle	Second Cycle	Third Cycle	Overall of 3 Cycles
CIDR Protocol	10	100.00% (10/10)	66.00+3.22 (n=10)	50.00% (5/10)	40.00% (2/5)	33.33% (1/3)	80.00% (8/10)
Ovsynch Protocol	10	90.00% (9/10)	86.67+3.33 (n=9)	30.00% (3/10)	42.86% (3/7)	0 % (0/4)	60.00% (6/10)
Normal Cyclic Control	10	100.00%	--	50.00%	60.00%	0 %	80.00%

Figures in parentheses indicate number of cows.

Table 2: Plasma progesterone concentration (ng/ml) in anoestrus crossbred cows on different days of treatment/AI under various oestrus induction protocols.

Treatment Group	Status	No.	Days from Treatment/AI				Overall
			D-0	D-7	D-9/10(AI)	D-21 post-AI	
CIDR Protocol	Conceived	5	0.65+0.23	3.48+0.54	0.48+0.13	10.44+1.77	3.76+1.02
	Non-Conc	5	0.70+0.18	3.61+1.05	0.32+0.90	1.42+0.38	1.52+0.39
	Overall	10	0.68+0.14a	3.55+0.56b	0.40+0.80a	5.93+1.73b	2.64+0.57
Ovsynch Protocol	Conceived	3	0.28+0.06	2.98+0.93	0.48+0.19	8.06+1.16**	2.95+0.99
	Non-conc	7	0.32+0.07	3.82+1.35	0.52+0.30	1.24+0.71	1.48+0.46
	Overall	10	0.31+0.05a	3.57+96b	0.51+0.21a	3.29+1.19b	1.92+0.44
Normal Cyclic Control	Conceived	5	-	-	0.24+0.04	10.48+1.34**	5.36+1.82*
	Non-conc	5	-	-	0.41+0.15	0.63+0.11	0.52+0.10
	Overall	10	-	-	0.32+0.08a	5.55+1.76b	2.93+1.05

*P<0.05, **PP<0.01 between conceived and non-conceived status within the protocol/group.
Means bearing uncommon surperscripts within the row differ significantly (P<0.05).
Day 0-Day of starting the treatment, Day 7-Day of administration of PG.
Day9/10-Fixed Time AI 9Day 9 in CIDR, Day 10 in Ovsynch) Day 21-21 Days post-AI.

Table 3 : Plasma total cholesterol concentration (mg/dl) in anoestrus crossbred cows on different days of treatment/AI under various oestrus induction protocols.

Treatment Group	Status	No.	Days from Treatment/AI				Overall
			D-0	D-7	D-9/10(AI)	D-21 post-AI	
CIDR Protocol	C	5	181.10±30.97	179.32±30.03	169.91±26.73	164.17±26.94	173.62±13.28
	N C	5	187.11±22.50	190.73±17.49	184.93±15.73	182.96±21.19	186.43±8.94
	Av	10	184.10±18.08	185.03±15.49	177.42±14.83	173.56±16.46	180.03±7.96
Ovsynch Protocol	C	3	132.32±31.09	129.51±34.90	126.98±32.69	126.72±29.82	128.88±3.74
	N C	7	211.99±12.73*	216.10±10.81**	218.82±13.52**	205.53±10.64**	213.11±5.73**
	Av	10	188.09±16.98	190.12±17.63	191.27±18.80	181.89±16.03	187.84±8.37
Normal Cyclic Control	C	5	-	-	218.48±36.08	220.41±30.09	219.47±22.15
	N C	5	-	-	200.08±25.17	185.51±24.68	192.80±16.79
	Av	10	-	-	209.28±20.96	202.96±19.25	206.12±13.87

*P<0.05, **PP<0.01 between conceived and non-conceived status within the protocol/group.
Day 0-Day of starting the treatment, Day 7-Day of administration of PG.
Day9/10-Fixed Time AI 9Day 9 in CIDR, Day 10 in Ovsynch) Day 21-21 Days post-AI.

Table 4 : Plasma total protein concentration(g/dl) in anoestrus crossbred cows on different days of treatment/AI under various estrus induction protocols

Treatment Group	Status	No.	Days from Treatment/AI				Overall
			D-0	D-7	D-9/10(AI)	D-21 post-AI	
CIDR Protocol	C	5	12.71±0.89	12.72±0.84	12.60±0.86	12.31±0.65	12.59±0.3888
	N C	5	11.00±0.03	11.15±0.08	10.97±0.21	11.14±0.07	11.07±0.06
	Av	10	11.85±0.51	11.94±0.48	11.78±0.50	11.73±0.36	11.83±0.22
Ovsynch Protocol	C	3	11.89±0.42	11.85±0.59	12.17±0.58	11.41±0.31	11.83±0.22
	N C	7	12.06±0.23	12.23±0.31	12.16±0.29	12.09±0.25	12.14±0.13
	Av	10	12.01±0.19	12.11±0.27	12.16±0.25	11.89±0.22	12.04±0.11
Normal Cyclic Control	C	5	-	-	12.07±0.33	12.63±0.65	12.35±0.36
	N C	5	-	-	12.60±0.22	12.92±0.21	12.76±0.15
	Av	10	-	-	12.33±0.20	12.77±0.33	12.55±0.19

*P<0.05, **PP<0.01 between conceived and non-conceived status within the protocol/group.
Day 0-Day of starting the treatment, Day 7-Day of administration of PG.
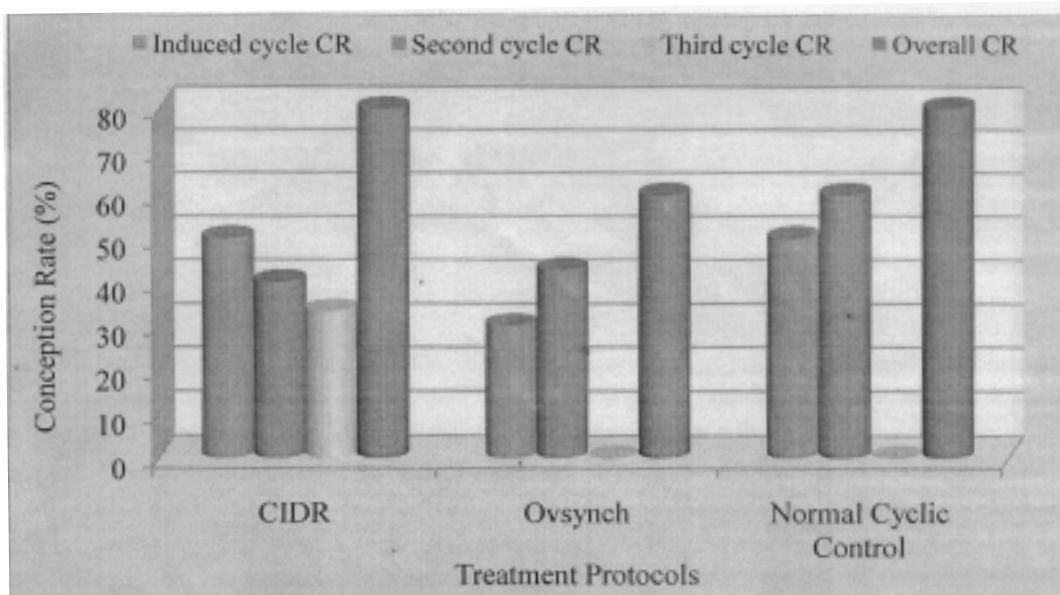Day9/10-Fixed Time AI 9Day 9 in CIDR, Day 10 in Ovsynch) Day 21-21 Days post-AI.

Figure 1. Conception rate in true anoestrus crossbred cows under different oestrus induction protocols and in normal cyclic control group
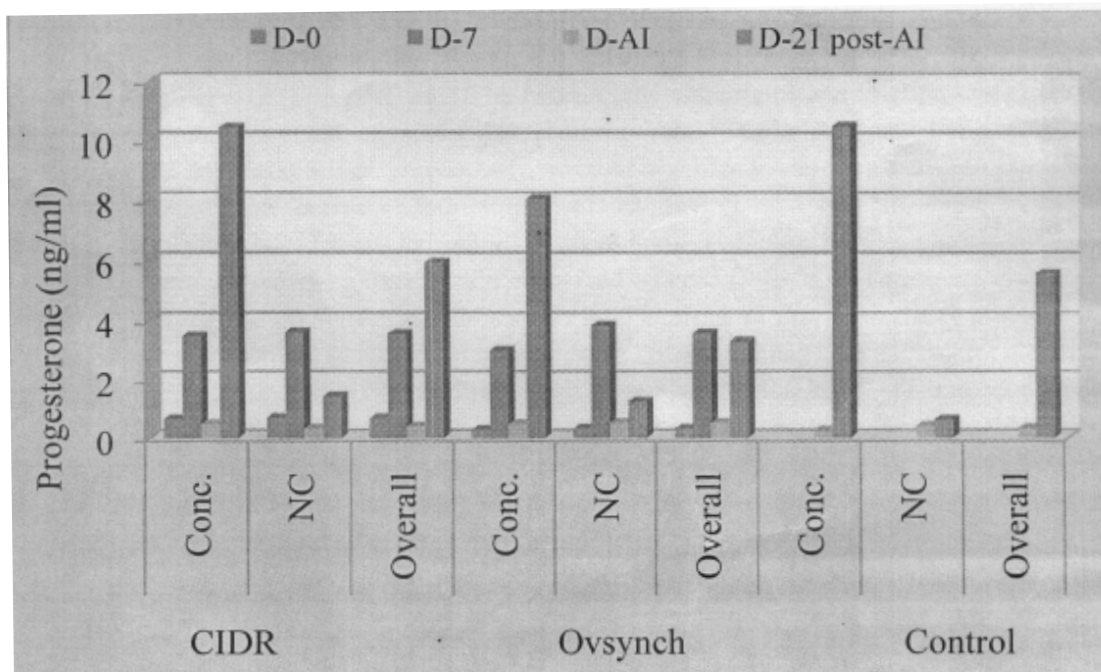

Figure 2. Plasma Progesterone profile of conceived and non-conceived anoestrus cows under different oestrus induction protocols and in normal cyclic control group

REFERENCES

1. Agarwal, S.K., Shankar, U. and Ansari, M.R. 2004. *Laboratory Manual on Gynaecology*. Published by Communication Center, IVRI, Izatnagar, India.

2. Ammu, R. Dhami, A.J. Naikoo, M., Parmar, B.C. and Divekar, B.S.2012. Estrus induction and fertility response in postpartum anoestrus Gir cows. Indian J.Anim. Reprod.., 33(1):37-42.

3. Ammu, R., Dhami, A.J. Naikoo, M., Patel, S.B. and Savaliya, F.P. 2012. Effect of puerperium, estrus synchronization and post-breeding periods on plasma profile of total protein, cholesterol and triglycerides in Gir cows. Indian J. Field Vets., 7(4):11-17.

4. Bartolome, J.A., Santos, J.E.P., Pancarci, S.M., Melendez, P., Arteche, A.C.M.m Hernandez, O., Archbald, L.F., Trigg, T. and Thatcher, W.W. 2004. Induction of ovulation in nonlactating dairy cows and heifers using different doses of a deslorelin implant. Theriogenology, 61:407-419.

5. Baruselli, P.S. Sales, J.N.S., Sala R.V., Vieira, L.N. and Sa Filho, M.F. (2012). History, evolution and prspectives of timed artificial insemination programs in Brazil. Anim, Reprod., 9(3):139-152.

6. Bhoraniya, H.L. Dhami, A.J. and Killedar, A. 2012. Influence of estrus synchronization protocols on fertility, plasma progesterone and biochemical constituents in Kankrej cows. Indian J.Anim, Reprod., 33(2):14-18.

7. Bhoraniya, H.L., Dhami, A.J., Naikoo, M.m Parmar, B.C. and Sarvaiya, N.P. 2012. Effect of oestrus synchronization protocols on plasma progesterone profile and fertility in postpartum anoestrus Kankrej cows. Trop. Animn. Health Prod., 44(6):1191-1197.

8. Cevik, 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 Faf Derg., 16:85-89.

9. Dhami, A.J., patel, P.M. Savalia, F.P. and Kavani, F.S. 2007. Biweekly plasma protein profile in anoestrus and suboestrus HF cows without and with GnRH and PGF2a therapy on day 49 postpartum. Int. J. Cow Sci., 3(1&2):44-47.

10. Dhoble, R.L. Mane, S.D., Sawale, Ag.G and Ingawale, M.V. 2004. Variation in bloof biochemical constituents during postpartum period in crossbred cows. Indian J. ANim,. Reprod., 25(1):14-16.

11. Ghuman, S.P.S., Honparkhe, M., Singh, J. and Gandotra, V.K.2011. Ovsynch plus CIDR based fixed time AI protocol as a therapeutic strategy in repeat-breeder crossbred dairy cattle. India J.Anim.Sci., 81(3):257-259.

12. Kavani, F.S., Sharma, V.K. Siddiquee, G.M. and Vadodaria, V.P. 1987. Serum proteins, ascorbic acid and total cholesterol in anoestrus Kankrej heifers. Indian J. ANim. Reprod., 8(2): 148-150.

13. Khade, N.B. Patel, D.M. Naikoo, M., Dhami, A.J., Sarvaiya, N.P. and Gohel, M.M. 2011. Oestrus induction in pubertal anoestrus Gir heifers using different hormone protocols. Indian J. Field Vets., 7(1):4-8.

14. Kim, U., Suh, G., Hur, T., Kang, S., Kang, H., Park, S., KIm, H. and Kim, I. 2007. Comparison of two types of CIDR based artificial insemination protocols for repeat breeder dairy cows. J. Reprod. Dev., 53(3):639-645.

15. 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.

16. Kumar Rajesh, Butani, M.G., Dhami, A.J., Kavani, F.S. and Shah, R.G. 2009. Effects of different therapies on fertility and serum progesterone metabolites and minerals profile in repeat breeding crossbred cows. Indian J. Field Vets., 5(2):1-8.

17. Kutty, C.I.and Ramachandran , K. 2003. Bovine infertility a field oriented categorization based on investigation among crossbred cattle in a district of Kerela. Indian J. Anim. Sci., 73(2):35-37.

18. 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.

19. Mahour, S.S., Nema, S.P. Shukla, S.P., Mehta, H.K., and Gupta, V.K. 2012. Effiency of PGF_{2a}, GnRH and Ovsynch protocol in postpartum anoestrus crossbred cows. Indian J. Field Vets., 8(1):65-68.

20. patel, J.,A., Dhami, A.J., Kavani, F.S. and Sarvaiya, N.P. 2005. Effect of hormonal therapies at breeding on plasma progesterone profile and fertility in repeat breeding Holstein Friesian cows. Int. J. Co Sci.,1(1):52-59.

21. Pawshe, C.H., Ingawale, M.V. Deshmukh, S.G., Munde, V.K. and Pawshe, M.D. 2011. Estrus synchronization in bovine: present status and future perspective Lead Paper in Proc: National symposium and XXVII Annual convention of ISSAR, Mizoram, India, 27-29 Sept., P.78-82.

22. Takahasi, J. Osawa. T. Sugama, K., Nakataubo, T. Matsubara, K. and Miyake, Y.I. 2009. Effects of the interval between PGF2a and 2nd GnRH injections in Ovsynch protocol on the ovulation synchronization rate, embryo survival rate, and pregnancy rate after time AI in Holsetin cows. Japanese J. Vet CLin. 32(1):1-7.

★ ★ ★