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Ovarian Dynamics, Plasma Endocrine Profile and Fertility Response Following Synchronization Protocols in Crossbred Cows with Cystic Ovaries

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ABSTRACT

Background: Cystic ovarian degeneration (COD) with prevalence of 10-13% is a common and economically important condition in dairy cattle affecting fertility. Predisposing factors and aetiology of ovarian cysts are multifaceted. The most widely accepted cause is either absent, insufficient or wrong time occurrence of pre-ovulatory LH-surge. The accuracy of differential diagnosis of cyst per rectum can be increased by simultaneous use of USG and/or plasma progesterone assay and therapeutic success depends on type and duration of cystic condition. The literature on these aspects in crossbred cows is meagre, hence was planned to differentially diagnose the ovarian follicular and luteal cysts based on clinical, sonographic and endocrine findings and to study the estrus response and conception rates following different treatment modalities of COD in crossbred cattle.

Methods: This study was conducted on >90 days postpartum HF crossbred cows with cystic ovaries (n=58) confirmed by per rectal palpation and ultrasonography. The animals with follicular cysts were randomly treated with either conventional Ovsynch or Ovsynch + CIDR protocol (n=10 each) and those with luteal cysts with either Double PG injections 11 days apart or modified Ovsynch protocol (n=16 each) with fixed time AI. A group of six cystic cows was kept as untreated control. Blood samples were collected in heparinized vacutainers, together with trans-rectal ultrasonography, on day 0 (just before initiation of treatment), on day of last $PGF_2\alpha$ injection, on day of induced estrus/FTAI and on day 12 post-AI for determination of plasma progesterone and estradiol-17β by RIA technique.

Result: The mean diameters and wall thickness of ovarian cysts regressed significantly (p<0.01) among responded cows of all four protocols. The estrus induction response within 48-96 hrs of last PG injection with Ovsynch, Ovsynch + CIDR, Modified Ovsynch and Double PG protocols was 90.00, 100.00, 87.50 and 81.25%, respectively. The conception rates at induced estrus with FTAI for these protocols were 50.00, 40.00, 50.00 and 43.75%, respectively and the corresponding overall conception rates for 3 cycles' post-treatment were 60.00, 70.00, 68.75 and 56.25%, respectively, compared to zero result of control group. The mean plasma progesterone concentration varied highly significantly (p<0.01) between periods of treatment in all protocols, the values were lower (p<0.01) on day 0 and on day of FTAI than on the day of last PG injection and on day 12 post-AI. The values on day 12 post-AI were significantly higher in conceived than non-conceived cows. The trend of estradiol-17β profile on day of last PG injection was reversed than on day 0. The higher levels of estradiol-17β on day of FTAI were associated with regression of cystic structures and development of new dominant follicles with estrogenic activity concomitant to induced estrus and ovulatory LH surge in most of the animals as evident from USG monitoring, behavioural signs and conception rates.

Key words: Confirmatory diagnosis, Crossbred cows, Cystic ovary, Fertility response, Steroids profile, Synchronization protocols, Ultrasound.

INTRODUCTION

One of the most common ovarian dysfunctions during the postpartum period is formation of ovarian cyst following ovulation failure (Opsomer *et al*., 1998). Cystic ovarian degeneration (COD) with prevalence of 10-13% is a common and economically significant condition of dairy cattle affecting their fertility (Lopez-Diaz and Bosu, 1992; Johnson and Coates, 2004). The exotic and crossbred animals are highly susceptible to COD. The predisposing factors like higher parity, high milk production, season, stress and negative energy status are attributed to occurrence of COD (Lopez-Diaz and Bosu, 1992). Etiology of ovarian cysts is multifactorial and it depends on the phenotypic, genetic and environmental factors, yet the most widely accepted one is that the pre-ovulatory LH-surge is either absent, insufficient or occurs at a wrong time during maturation of dominant follicle (Lopez-Diaz and Bosu, 1992; Hamilton *et al.,* 1995). In determining the type of cyst, the accuracy of palpation per rectum can be increased by simultaneous use of USG

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and/or plasma progesterone assay (Youngquist and Threlfall, 2007). The success rate of different treatment varies greatly depending upon the type and duration of cystic condition (Zulu *et al.,* 2003; Amer and Badr, 2006; Lüttgenau *et al.,* 2016). The literature on these aspects in crossbred cows is meagre. Therefore, this study was planned to differentially diagnose the ovarian follicular and luteal cysts based on clinical, sonographic and endocrine findings and to study the estrus response and conception rates following different treatment modalities of COD in crossbred cattle.

MATERIALS AND METHODS

The study was carried out on 58 Holstein Friesian crossbred cows with cystic ovaries during >90 days postpartum selected from the field as well as from the organized private dairy farms under the Amul milk shed area in and around Anand, Gujarat, during September, 2018 to May, 2019. Cystic cows were confirmed by history, trans-rectal palpation and ultrasonographic examinations twice at weekly interval using linear array transducer with 5.0-7.5 MHz frequency. The diameters and wall thickness of ovarian cysts were recorded in millimetre and ovarian cysts were classified as follicular and luteal cysts as per standard criteria (Youngquist and Threlfall, 2007).

The animals with follicular cysts were randomly treated either with conventional Ovsynch/ GPG or Ovsynch + CIDR protocols with fixed time AI (n=10 each) and those with luteal cysts either with Double PG injections 11 days apart or with modified Ovsynch protocols (n=16 each). In modified Ovsynch protocol, Inj. 20 µg Buserelin acetate and 25 mg Dinoprost tromethamine were given i/m simultaneously on day 0. On day 14, animals were treated with second i/m Inj. of 25 mg Dinoprost and on day 16, second Inj. of 20 µg Buserelin. Fixed time AI was done on day 17. A group of six cystic cows was kept as untreated control. The animals returning to estrus were re-inseminated for two more cycles. The estrus response and conception rate to fixed time AI as well as overall of three cycles were recorded by palpating per rectum the non-return cows at day 60 of last AI.

Blood samples were collected in heparinized vacutainers simultaneously to hormonal treatment and ultrasound examinations of ovaries of each case on four occasion, *i.e.,* on day 0 (day of first treatment), on day 7/11/14 (day of last $PGF_2\alpha$ inj.), on day 10/14/17 (day of estrus/FTAI) and on day 12 post-AI. Samples were centrifuged at 3000 rpm for 10 min and plasma stored at -20°C with a drop of merthiolate. The plasma progesterone and estradiol-17β levels were determined using RIA techniques using kits procured from Immunotech-SAS, Marseille Cedex, France to confirm the ovarian findings retrospectively. The sensitivity of the progesterone and estradiol assays was 0.1 ng/ml and 9.58 pg/ml, respectively. The intra- and inter-assay coefficients of variation were 5.4 and 9.1% for progesterone and 14.4 and 14.5% for estradiol 17β, respectively.

The comparative efficacy of different protocols was evaluated in terms of estrus response and conception rate at FTAI and overall of three cycles including ovarian and endocrine changes. The data were analyzed using descriptive statistics for estrus response and conception rates and those of ovarian dynamics and plasma endocrine profile using ANOVA, DMRT and 't' test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Ultrasonographic evaluation of ovarian response to treatment

The ultrasonographic monitoring of ovarian cysts made at the beginning and on day of last PG Inj. as well as on day of estrus/FTAI revealed highly significant (p<0.01) variation in the mean diameters and wall thickness of cysts between periods of treatment in all four protocols with resolution of cystic structures after all treatment protocols (Table 1).

In each group of treatment, the responded cows showed regression of cystic conditions as recorded by rectal palpation and ultrasonographic findings of structural dimensions (Table 1, Fig 1) and they also showed induced estrus within 48-96 hrs of last/2nd PG injection in most of the cases. The reduction in diameter and thickness of cyst wall observed with progress of treatment corroborated with the earlier results of Lopaz-Gatius and Lopez-Bejar (2002), Ambrose *et al.* (2004) and Enginler *et al.* (2012). Successful resolution of ovarian cysts with development of new ovulatory follicles following various treatment protocols have also been

Table 1: Gross changes in diameters and wall thickness of ovarian cysts in cows at different time intervals of various treatment protocols.

Protocol	Cyst characteristics	Periods/days of trans-rectal USG monitoring		
		Day of first Trt (Day 0)	Day of last PG Inj.	*Day of last GnRH Inj./AI
Ovsynch	Diameter (mm)	24.17±0.79^a	21.33±0.99^b	13.08±0.37^c
	Wall thickness (mm)	1.85±0.10^a	2.05±0.06^a	0.78±0.07^b
Ovsynch + CIDR	Diameter (mm)	24.00±0.58^a	23.00±0.53^a	13.42±0.49^b
	Wall thickness (mm)	2.70±0.49^a	2.83±0.46^a	0.86±0.11^b
Modified Ovsynch	Diameter (mm)	21.25±0.56^a	12.08±0.58^b	10.75±0.31^c
	Wall thickness (mm)	3.37±0.11^a	1.93±0.05^b	0.79±0.05^c
Double PG Inj.	Diameter (mm)	22.17±0.31^a	10.75±0.44^b	9.67±0.42^c
	Wall thickness (mm)	3.70±0.21^a	2.52±0.13^b	1.02±0.08^c

Means bearing uncommon superscripts within the row differ significantly (p<0.05).*New follicle.

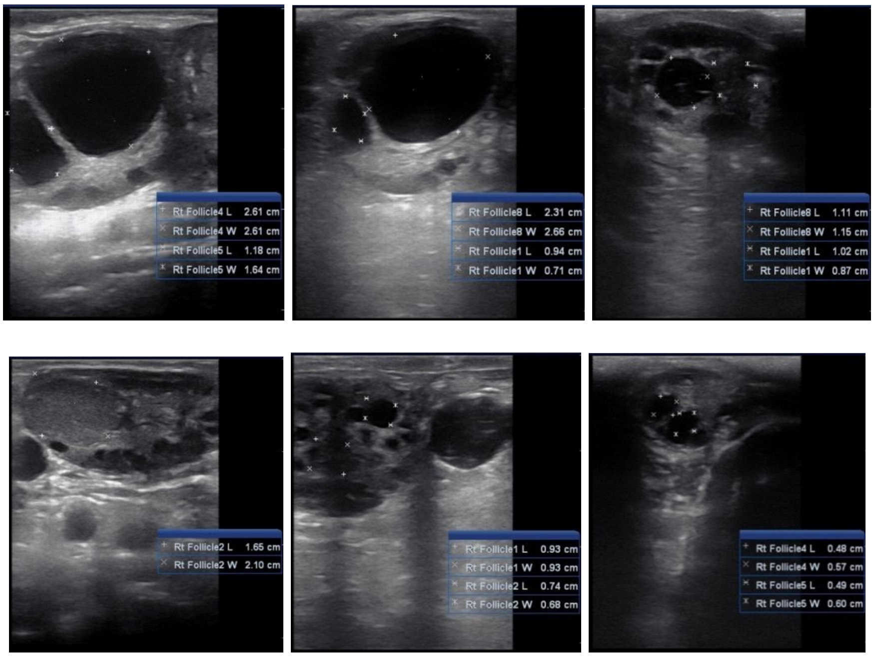

Fig 1: Sonograms of crossbred cows with follicular and luteal cysts (before, during and after treatment in a row).

documented in dairy cows by Honparkhe *et al.* (2011), Rudowska *et al.* (2015) and Gundling *et al.* (2015). In the present study, all the protocols were found to be effective in resolving the cystic ovarian problem in crossbred cows, though their efficacy varied as per the type of hormone and protocol used.

Estrus response and conception rates with different protocols

The findings on estrus response and conception rates obtained at induced estrus with FTAI, followed by 2nd and 3rd post-treatment cycles and overall of three cycles in cystic crossbred cows treated with different approaches are presented in Table 2 and Fig 2. Out of 10, 10, 16 and 16 cystic cows treated with Ovsynch, Ovsynch + CIDR, modified Ovsynch and Double PG protocols, 90.00, 100.00, 87.50 and 81.25% cows responded with estrus signs within 48 to 96 hrs of last PG injection. The response was relatively poor in cows with luteal cysts treated using Modified Ovsynch and Double PG protocols as compared to those with follicular cysts and treated using Ovsynch and Ovsynch + CIDR protocols. The conception rate at induced estrus with FTAI in cows under Ovsynch protocol was 50.00% and one more cow conceived at second cycle post-treatment giving overall conception rate of 60.00 (6/10)% for 3 cycles. This result corroborated with Rudowska *et al.* (2015), who recorded 62.00% conception with GnRH + $PGF_2\alpha$ treatment.

In the Ovsynch + CIDR group, 40.00% cystic cows conceived at FTAI and overall 70.00 (7/10)% conceived within 3 cycles post-treatment. This result closely corroborated with the findings of Zulu *et al.* (2003), who reported conception rate of 71.40% in luteal cystic cows, while Amer and Badr (2006) found 57.1% conception rate in cows with ovarian cysts using same treatment protocol. However, Brito and Palmer (2004) reported only 18-28% conception rates in cows with cystic ovaries.

The conception rates in cystic cows treated with modified Ovsynch protocol were 50.00, 28.57 and 20.00% at induced, second and third estrus post-treatment, respectively, giving an overall conception rate of 68.75 (11/16)% for 3 cycles (Table 2, Fig 2). This result of 50.00% conception rate at FTAI was higher than 28.10% and 30.65% reported by Lopez Gatius and Lopez Bezar (2002) and Šťastná and Šťastný (2012) with Modified Ovsynch protocol. The present conception rates at FTAI and overall of three cycles with this protocol were much higher than previous reports in exotic cows, perhaps due to mixed type of follicular and luteal cysts cases included in this group and the

Table 2: Estrus response and conception rates in crossbred cows with ovarian cysts after use of various treatment protocols.

Protocol	No. treated	No. (%) responded with estrus	Conception rate (%)			
			First estrus / FTAI	Second estrus	Third estrus	Total conceived
Ovsynch	10	9 (90.00)	5/10 (50.00)	1/4 (25.00)	0/3 (0.00)	6/10 (60.00)
Ovsynch + CIDR	10	10 (100.0)	4/10 (40.00)	2/6 (33.33)	1/4 (25.00)	7/10 (70.00)
Modified Ovsynch	16	14 (87.50)	8/16 (50.00)	2/7 (28.57)	1/5 (20.00)	11/16 (68.75)
Double PG	16	13 (81.25)	7/16 (43.75)	1/8 (12.50)	1/6 (16.66)	9/16 (56.25)
Overall Pooled	52	46 (88.46)	24/52 (46.15)	6/25 (24.00)	3/18 (16.66)	33/52 (63.46)

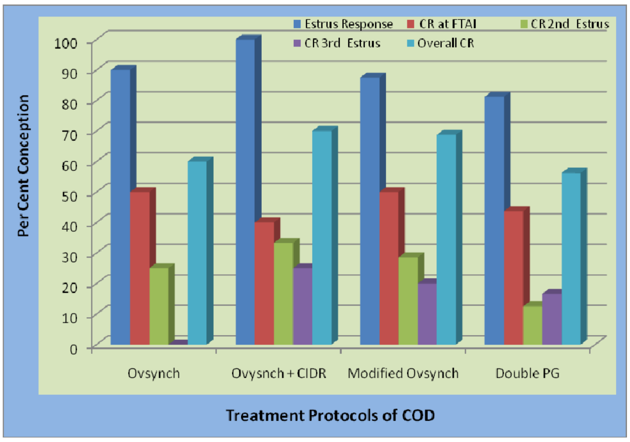

Fig 2: Estrus response and conception rates at induced estrus and overall of three cycles in crossbred cows with COD following different treatment protocols.

beneficial effect of simultaneous Inj. of GnRH and prostaglandin for resolution of cystic condition leading to development of new ovulatory follicle at timed AI yielding better conception rate.

In the Double PG treatment group, 9 out of 16 luteal cystic animals conceived within 3 cycles' post-treatment giving 56.25% conception rate. In this group the conception rates at induced/ first, second and third estrus/cycle were 43.75, 12.50 and 16.66%, respectively. The earlier workers reported comparable conception rate of 66.0% (Brito and Palmer, 2004) and 63.3% (Lüttgenau *et al.,* 2016). However, the present conception rate of 56.25% achieved was much lower than 87.5 and 91.0% reported by Leslie and Bosu (1983) and Tebble *et al.* (2001), respectively. The present results perhaps could have been further increased with the use of ovulatory drug GnRH/hCG at FTAI, though we did not test it. The preset results with all 4 protocols were quite satisfactory and economically viable when compared with zero result of untreated control group of 6 cystic cows, wherein five cows were cycling but none conceived over next 2 months follow up.

Plasma progesterone profile in different treatment protocols

The mean plasma progesterone concentrations varied highly significantly ($p<0.01$) between periods of treatment in all four protocols. The values were lower ($p<0.01$) on day 0 as well as on day of FTAI than on the day of last PG injection and on day 12 post-AI in animals under Ovsynch and Ovsynch + CIDR protocols, while in Modified Ovsynch and Double PG protocols, the plasma progesterone levels on the day of FTAI were significantly ($p<0.01$) lower than other three periods of sampling which were statistically same. Very similar and statistically significant trends of plasma progesterone over periods were also noticed in conceived and non-conceived subgroups under each protocol (Table 3).

Significantly ($p<0.01$) higher levels of plasma progesterone on day 0 in animals treated with Modified Ovsynch and Double PG protocols (6.05±0.5 and 6.16 ±0.67 ng/ml) as compared to Ovsynch and Ovsynch + CIDR protocols (1.41±0.35 and 2.70±0.56 ng/ml) were mainly due to inclusion of most cases of luteal cysts and presence of more amount of luteal tissues in the animals of the former two treatment protocols and follicular cysts cases in later two protocols. However, the plasma progesterone profile on day of last PG injection was almost same in all four protocols due to the effect of initial injections of GnRH and/ or $PGF_2\alpha$ leading to lutenization of cyst wall and/or ovulation and formation of CL from same or new follicle following lysis of existing cyst/luteal tissues and also as a direct effect of intra-vaginal CIDR implant in the particular group. Further, on the day of induced estrus / FTAI, the plasma levels were statistically similar between groups and significantly lower than either side of sampling days in each protocol due to instant luteolysis of CL / regression of luteal tissues by the last $PGF_2\alpha$ injection and/or withdrawal of CIDR 2 days before

Table 3: Mean (±SE) plasma progesterone concentrations on different days of various treatment protocols in crossbred cows with cystic ovaries.

Protocol	Status	No.	Plasma progesterone (ng/ml) on days of treatment/sampling			
			Day 0	Day of last PG Inj.	Day of FTAI	Day 12 post-AI
Ovsynch	Conceived	5	1.77 ± 0.53^{p}	10.14 ± 1.56^{q}	1.19 ± 0.23^{p}	$11.56 \pm 1.80^{q*}$
	Non-Conc	5	1.05 ± 0.23^{p}	8.60 ± 0.81^{r}	3.16 ± 1.22^{pq}	4.80 ± 1.51^{q}
	Pooled	10	$1.41^{a} \pm 0.35^{p}$	9.37 ± 0.62^{q}	2.18 ± 0.67^{p}	8.18 ± 1.46^{q}
Ovysnch + CIDR	Conceived	4	$1.15 \pm 0.24^{p**}$	10.79 ± 0.74^{q}	1.80 ± 0.18^{p}	$10.43 \pm 1.27^{q*}$
	Non-Conc	6	3.73 ± 0.61^{p}	9.37 ± 0.76^{r}	2.02 ± 0.52^{p}	5.95 ± 0.69^{q}
	Pooled	10	$2.70^{a} \pm 0.56^{p}$	9.94 ± 0.57^{r}	1.93 ± 0.31^{p}	7.74 ± 0.95^{q}
Modified Ovsynch	Conceived	6	6.42 ± 0.79^{q}	8.66 ± 0.89^{r}	1.40 ± 0.24^{p}	$9.95 \pm 0.37^{r**}$
	Non-Conc	4	5.50 ± 0.83^{qr}	7.28 ± 1.37^{r}	1.67 ± 0.38^{p}	3.75 ± 1.11^{pq}
	Pooled	10	$6.05^{b} \pm 0.57^{q}$	8.10 ± 0.75^{q}	1.51 ± 0.20^{p}	7.47 ± 1.11^{q}
Double PG	Conceived	4	5.00 ± 0.26^{q}	8.15 ± 0.27^{r}	1.65 ± 0.24^{p}	$9.40 \pm 0.27^{**}$
	Non-Conc	6	6.93 ± 1.00^{q}	7.88 ± 1.46^{q}	1.48 ± 0.15^{p}	4.80 ± 0.98^{q}
	Pooled	10	$6.16^{b} \pm 0.67^{q}$	7.99 ± 0.85^{q}	1.55 ± 0.13^{p}	6.64 ± 0.94^{q}

Means bearing uncommon superscripts within row (p,q,r) and within column (a,b) differ significantly.

*p<0.05 and **p<0.01, between conceived and non conceived subgroups within the protocol.

in respective groups, with development of new dominant follicle and expression of estrus. Similarly on day 12 post-AI, the group difference was statistically non-significant, however, the concentrations were significantly ($p<0.05$, $p<0.01$) higher in conceived than non-conceived cows. These differences could be attributed to ovulatory response and formation of pregnancy CL in conceived animals and anovulatory response or total failure of response to therapy in some of the non-conceived animals in each subgroup (Table 3). The higher levels of plasma progesterone on day 12 post-AI in Ovsynch and Ovsynch + CIDR treatment protocols compared to Modified Ovsynch and Double PG treatment protocols can be attributed to difference in the initial ovarian structure as well as the interval for last PG Inj./blood collection making difference in the receptor sites and their sensitivity to subsequent treatment coupled with other associated ovarian changes.

The present trend of steep and significant rise in plasma progesterone from day 0 to day 7 (day of PG injection) and then again steep drop on the day of FTAI observed on day 10 following $PGF_2\alpha$ injection and PRID removal on day 7 in cows under Ovsynch and Ovsynch + CIDR protocols concurred well with the previous reports of Ribadu *et al*. (1994), Amer and Badr (2006) and Honparkhe *et al*. (2011). The trend of mean plasma progesterone observed in crossbred cows on day 0 and day 11*, i.e.*, between two PG Injections 11 days apart and then on day of FTAI and day 12 post-AI in Double PG treatment protocol were also in line with Amer and Badr (2006) and Honparakhe *et al*. (2011). However, the influence of Modified Ovsynch protocol on progesterone profile could not be seen in the literature reviewed. Ribadu *et al*. (1994) found elevated progesterone in all cows with ovarian cysts by day 7 after Ovsynch + CIDR treatment mainly due to progesterone containing CIDR insert as well as luteal cysts and luteinized follicles as evidenced by positive correlation of plasma progesterone with thickness of luteal cyst walls (Douthwaite and Dobson 2000).

Plasma estradiol-17β concentration in different treatment protocols

The mean plasma estradiol-17β concentrations in all four groups were significantly ($p<0.05$) lower on day of last PG injection and on day 12 post-AI as compared to values on day of FTAI and/or day of start of treatment (day 0) (Table 4). Further, significantly ($p<0.05$) lower level of estradiol-17β found on day 0 in Double PG treated animals as compared to those in other three protocols may be due to selection of animals with luteal cysts and the presence of more amount of luteal tissues rather than granulosa cells on the ovaries of these animals. However, the trend of estradiol-17β profile on day of last PG injection was reversed than on day 0 among these protocols with significantly lower estradiol-17β in Ovsynch followed by Ovsynch + CIDR and Double PG protocols and higher in Modified Ovsynch treated animals. The higher levels of plasma estradiol-17β in Modified Ovsynch treatment protocol compared to other three protocols may be attributed to difference in interval of last PG Inj./blood collection and the effect of simultaneous injections of GnRH and $PGF_2\alpha$ on day 0. The higher levels of estradiol-17β on day of FTAI were due to regression of cystic structures and development of new dominant follicles with high estrogenic activity concomitant with induced estrus and ovulatory LH surge in most of the animals (Lopez-Gatis and Lopez-Bezar, 2002).

The mean plasma estradiol-17β (pg/ml) concentrations on day 0, *i.e.*, on the day of initiation of treatment, on day of last PG injection and on day of FTAI among conceived and non-conceived animals under all four protocols were statistically similar, except on day 0 in Double PG protocol (64.00±4.76 vs 110.33±16.47 pg/ml), on day of last PG injection in Ovsynch + CIDR protocol (87.50±5.87 vs

Table 4: Mean (±SE) plasma estradiol concentrations on different days of various treatment protocols in crossbred cows with cystic ovaries.

Protocol	Status	No.	Plasma estradiol-17β (pg/ml) on days of treatment/sampling			
			Day 0	Day of last PG Inj.	Day of FTAI	Day 12 post-AI
Ovsynch	Conceived	5	133.80±26.35	88.80±15.73	170.00±32.71	95.60±23.44
	Non-Conc	5	$145.00{\pm}7.42^{q}$	$89.80{\pm}21.79^{p}$	$149.00{\pm}6.78^{q}$	$101.00{\pm}12.88^{p}$
	Pooled	10	$139.40^{b}{\pm}13.04^{q}$	$89.30^{a}{\pm}12.67^{p}$	$159.50^{a}{\pm}16.13^{q}$	$98.30a{\pm}12.64^{p}$
Ovysnch + CIDR	Conceived	4	$157.50{\pm}25.62^{q}$	$87.50{\pm}5.87^{p*}$	$182.50{\pm}25.29^{q}$	$94.50{\pm}8.58^{p**}$
	Non-Conc	6	168.33±19.90	126.83±13.24	175.83±17.44	154.50±9.98
	Pooled	10	$164.00^{b}{\pm}14.92^{qr}$	$111.10^{ab}{\pm}10.21^{p}$	$178.50^{ab}{\pm}13.70^{r}$	$130.50^{bc}{\pm}11.79^{pq}$
Modified Ovsynch	Conceived	6	$131.67{\pm}11.01^{p}$	$137.17{\pm}13.37^{p}$	$208.33{\pm}14.24^{q}$	$148.83{\pm}5.41^{p}$
	Non-Conc	4	$123.25{\pm}6.10^{p}$	$138.75{\pm}5.15^{p}$	$190.00{\pm}4.08^{q}$	$155.00{\pm}12.33^{pq}$
	Pooled	10	$128.30^{b}{\pm}6.87^{p}$	$137.80^{b}{\pm}7.95^{p}$	$201.00^{b}{\pm}8.88^{q}$	$151.30^{c}{\pm}9.81^{p}$
Double PG	Conceived	4	$64.00{\pm}4.76^{p*}$	$86.25{\pm}8.51^{p*}$	$148.50{\pm}18.48^{q}$	$93.50{\pm}2.36^{p**}$
	Non-Conc	6	$110.33{\pm}16.47^{p}$	$131.67{\pm}12.56^{pq}$	$159.17{\pm}11.14^{q}$	$130.00{\pm}8.56^{pq}$
	Pooled	10	$91.80^{a}{\pm}12.28^{p}$	$113.50^{ab}{\pm}13.87^{p}$	$154.90^{a}{\pm}9.48^{q}$	$115.40^{ab}{\pm}7.79^{p}$

Means bearing uncommon superscripts within row (pqr) and within column (abc) differ significantly.

*p<0.05 and **p<0.01, between conceived and non conceived subgroups within the protocol.

126.83±13.24 pg/ml) and in Double PG protocol (86.25±8.51 vs 131.67±12.56 pg/ml), where these were significantly ($p<0.01$) higher in non-conceived than the conceived cows (Table 4). The non-significantly higher mean plasma estradiol-17β concentrations found on day of FTAI in conceived than non-conceived cows in all protocols, except Double PG protocol ($p<0.05$), could be attributed to presence of dominant follicle of induced estrus phase in the responded cows (Douthwaite and Dobson (2000).

The mean plasma estradiol-17β (pg/ml) concentrations on day 12 post-AI in conceived cows under Ovsynch + CIDR, Modified Ovsynch and Double PG protocols were significantly ($p<0.05$) lower than in non-conceived cows, but in Ovsynch protocol the difference was non-significant (Table 4). These findings show that during pregnancy also the follicular and estrogenic activities in the ovaries continue in presence of functional CL to provide balance mount of circulatory progesterone and estrogen for the fetal growth and maintenance of pregnancy.

Meager literature on the influence of different protocols we adopted for treatment of cystic cows on plasma estradiol-17β profile was available. Douthwaite and Dobson (2000) using CIDR implant reported the mean estradiol levels of 7.9 and 24.2 pg/ml ($p=0.002$) in cows with follicular cysts but with and without other follicles greater than 5 mm in diameter, respectively. Amer and Badr (2006) observed decline in plasma estradiol following Ovsynch + CIDR protocol, but it was markedly increased one day after removal of CIDR in cystic dairy cows. Enginler *et al.* (2011) reported similar serum estradiol concentration before and after Ovsynch protocol (141.337 vs 131.067 pg/ml) in cows with follicular cyst, although three days after $PGF_2\alpha$ administration, the USG revealed lysis of the luteinized cyst. Plasma estradiol-17β concentrations on days 0, 7 and 9, in cystic cows under Ovsynch and Ovsynch + CIDR treatment groups, which did not differ significantly at any day between the groups of two types of cystic ovary (Kawate *et al.,* 2011). In cows with follicular cysts, the estradiol-17β concentration decreased ($p<0.05$) from day 0 to day 7, but did not change ($p>0.1$) during the same period in cows with luteinized cysts/ cystic CLs. Similarly, the estradiol-17β concentrations increased ($p<0.005$) from day 7 to day 9 in the Ovsynch + CIDR group, but did not change significantly during the same period in the Ovsynch group in both types of cystic ovary (Kawate *et al.,* 2011). The present findings concurred well with these reports particularly Ovsynch + CIDR protocol. However, it was hard to find any report on plasma estradiol concentrations in cattle with luteal cysts to debate on the present findings in luteal cystic cows treated with Modified Ovsynch and Double PG protocols.

CONCLUSION

The findings of the present study conducted on 58 crossbred cows with cystic ovaries revealed significant ($p<0.01$) variations in the mean diameters and wall thickness of cysts between periods of all four synchronization treatment with regression of cystic conditions in responded cows. A very good estrus induction response and conception rates of 50.00, 40.00, 50.00 and 43.75% at induced estrus with FTAI and overall of 60.00, 70.00, 68.75 and 56.25%, respectively, recorded with Ovsynch, Ovsynch + CIDR, Modified Ovsynch and Double PG protocols were quite satisfactory and economically viable when compared with zero result of untreated control group of 6 cystic cows. The mean plasma progesterone and estradiol 17β concentrations in cows with COD varied highly significantly ($p<0.01$) between periods of treatment accordingly to ovarian structures and products used. The higher levels of estradiol-17β on day of FTAI were associated with regression of cystic structures and development of new dominant follicles with estrogenic activity concomitant to induced estrus and ovulatory LH surge in most of the animals as evident from USG monitoring, behavioural signs and conception rates. The results showed that Ovsynch or Ovsynch + CIDR for cows with follicular cysts and Modified Ovsynch protocol for cows with luteal cysts could be the right choice for treatment of such cows.

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