RESEARCH ARTICLE

Influence of Different Treatment Protocols on Blood Biochemical Profile and Fertility in Crossbred Cows with Cystic Ovarian Degeneration

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Abstract

The present study was conducted under field conditions on >90 days postpartum HF crossbred cows with cystic ovaries (n=58). The diagnosis was confirmed by history and trans-rectal palpation and ultrasonographic examinations twice at weekly interval using linear array transducer with 5.0-7.5 MHz frequency. The animals with follicular cysts were randomly treated either with conventional Ovsynch or with Ovsynch + CIDR protocol (n=10 each), and those having luteal cysts were treated either with Double PG injections 11 days apart or with modified Ovsynch protocol (n=16 each) with fixed time AI. A group of six cows having ovarian cyst was kept as untreated Control. Estrus response and conception rates at induced estrus/ fixed time AI were recorded. Blood samples were collected simultaneous to hormonal treatments on four occasions, *i.e.,* on day 0 (day of first treatment), on day 7/11/14 (day of last $PGF_2\alpha$ inj.), on day of induced estrus/FTAI, and on day 12 post-AI. The estrus induction response noted within 48 to 96 hrs of last PG injection among cystic cows treated 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 in cows under these protocols were 50.00, 40.00, 50.00 and 43.75 %, respectively. The variations in blood glucose, plasma cortisol, total protein and creatinine concentrations between periods of treatment were, however, statistically non-significant in cows under all four protocols, except creatinine in Ovsynch protocol. Furthermore, the effect of treatment/ period was also statistically non-significant among conceived and non-conceived cows in all four treatment protocols, except blood glucose which was significantly higher in non-conceived cows.

Key words: Blood glucose, Crossbred cattle, Cystic ovarian degeneration, Plasma cortisol, Plasma biochemical profile, Treatment response.

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Introduction

Cystic ovarian degeneration (COD) with prevalence of 10-13% is a common and economically significant reproductive disorder affecting dairy cattle fertility (Opsomer *et al*., 1998; Johnson and Coates, 2004). It is more common in exotic and crossbred cattle than the zebu cattle and buffaloes. Etiology of ovarian cysts is multifactorial and it depends on the phenotypic, genetic and environmental factors. The most widely accepted hypothesis describing the formation of a cyst is that the pre-ovulatory LH-surge is either absent, insufficient or occurs at a wrong time during dominant follicle maturation (Lopez-Diaz and Bosu, 1992; Hamilton *et al.,* 1995). Ovarian cysts were associated with high serum cortisol levels in dairy cows (Mimoune *et al.,* 2017). Jafari Dehkordi *et al.* (2016) reported significantly increased cortisol profile together with blood urea nitrogen (BUN), beta-hydroxy butyrate (BHBA), non-esterified fatty acids (NEFA), and decreased insulin in cows with ovarian follicular cysts than the normal cyclic ones, and opined that increasing blood cortisol and decreasing blood insulin may play a major role in the formation of ovarian cyst. In contrast, Probo *et al.* (2011) and Bors *et al.* (2018) did not find such differences in corisol concentrations of cystic and normal cows and also before and after treatment. The work on these aspects on crossbred cattle in Indian context is very meager. Hence, this study was planned to know the blood biochemical and cortisol profile as well as response to various treatment protocols in cattle with ovarian cysts.

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Materials and Methods

The study was carried out on animals 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. The study covered

58 HF crossbred cows with cystic ovaries during >90 days postpartum period. Problem breeders 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 animals with follicular cysts were randomly treated either with conventional Ovsynch or with Ovsynch + CIDR protocols (n=10 each), and those having luteal cysts were treated either with Double PG injections 11 days apart or with modified Ovsynch protocols (n=16 each) with fixed time AI. Modified Ovsynch protocol consisted of simultaneous treatment with i/m Inj. 20 µg Buserelin acetate, a GnRH analogue (Receptal, 5 ml,) and Inj. 25 mg Dinoprost tromethamine, a $PGF_2\alpha$ analogue (Lutalyse, 5 ml) administered on day 0. On day 14, animals were treated with second i/m Inj. of 25 mg Dinoprost tromethamine and second Inj. of 20 µg GnRH was administered on day 16. FTAI was accomplished 24 hrs later. A group of six cystic cows was kept as untreated Control. The comparative efficacy of different protocols was evaluated in terms of estrus response and conception rate at fixed time AI.

Blood samples were collected simultaneously to hormonal treatments four occasions, *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 induced estrus/FTAI), and on day 12 post-AI. Plasma was separated and stored at -20°C to estimate cortisol levels using RIA technique and kit of Immunotech, France. Plasma total protein and creatinine concentration were determined by Biuret and modified Jaffe's kinetic method, respectively, using commercial kits procured from Crest Biosystems, Goa, India on biochemistry analyzer. Blood glucose was measured in fresh whole blood by using Glucometer (GlucoOne, Model No: BG-03, Morepen Lab. Ltd., Himachal Pradesh). The data were analyzed suitably using descriptive statistics for estrus response and conception rate, and ANOVA, DMRT and 't' test for blood biochemical profile (Snedecor and Cochran, 1994).

Results and Discussion

In the present study, 90.00, 100.00, 87.50 and 81.25% of cystic cows treated with Ovsynch, Ovsynch + CIDR, Modified Ovsynch and Double PG protocols responded with estrus signs within 48 to 96 hrs of last PG injection. The response was relatively poor in cows with luteal cysts as compared to those with follicular cysts. The conception rates at induced estrus with FTAI in cows under these protocols were 50.00, 40.00, 50.00 and 43.75%, respectively. Conception rates were better with Ovsynch and modified Ovsynch in cows with follicular and luteal cysts, respectively. None of the cows from control group conceived over next two month follow up. These conception rates were in line with several earlier reports, such as 60.00 and 71.40 % reported in cows with luteal cyst using Ovsynch like protocol by Nanda *et al*. (1989) and Zulu *et al.* (2003), respectively, and of 57.1 and 52.30 % reported in cows with ovarian cysts using Ovsynch + CIDR and CIDR-based TAI protocols by Amer and Badr (2006) and Kim *et al.* (2006), respectively. However, Lopez Gatius and Lopez Bezar (2002) and Stastna and Stastny (2012) found conception rates of 28.10 and 30.65 % at FTAI with Modified Ovsynch protocol, while Brito and Palmer (2004) and Luttgenau *et al.* (2016) documented conception rate of 66.0 and 63.3 % with double PG protocol in cystic cows.

Blood Glucose

The mean values of blood glucose levels in cows with COD under different treatment protocols on day 0, day of last PG Inj., day of FTAI and day 12 post-AI presented in Table 1 revealed that the variations between periods were statistically non-significant in cows under all treatment protocols. However, the mean values of blood glucose were higher in non-conceived than conceived cows in all protocols on almost all days studied, but differed significantly ($p<0.01$) only in Ovsynch protocol. The levels of blood glucose did not reveal any specific trend between periods in conceived and non-conceived cows under any of the treatment protocols.

Table 1: Mean (± SE) blood glucose levels on different days of various treatment protocols in crossbred cows with cystic ovaries

			Blood glucose (mg/dl) on days of treatment/sampling			
Protocol	*Status*	*No.*	*Day 0*	*Day of last PG Inj*	*Day of FTAI*	*D-12 post-AI*
Ovsynch	Conceived	5	63.20±3.97*	66.60±1.29**	65.60±1.94*	65.80±3.01
	Non-Conc	5	73.80±2.22	79.60±2.14	75.40±2.20	75.00±2.77
	Pooled	10	68.50±2.78	73.10±2.47	70.50±2.14	70.40±2.46
Ovysnch + CIDR	Conceived	4	71.25±1.80	75.00±3.24	79.00±2.48	71.75±3.71
	Non-Conc	6	78.00±2.70	71.00±2.13	75.00±2.92	75.50±2.53
	Pooled	10	75.30±2.02	72.60±1.83	76.60±2.02	74.00±2.08
Modified Ovsynch	Conceived	6	68.33±3.62	69.83±3.82	72.17±3.34	69.33±2.75
	Non-Conc	4	75.00±3.11	75.75±2.66	79.25±3.68	76.50±3.57
	Pooled	10	71.00±2.62	72.20±2.59	75.00±2.62	72.20±2.37
Double PG	Conceived	4	67.25±3.25	69.50±4.03	69.50±2.40	69.00±1.68
	Non-Conc	6	75.67±2.29	73.33±2.75	74.50±2.25	74.83±2.06
	Pooled	10	72.30±2.25	71.80±2.25	72.50±1.77	72.50±1.64

*$p < 0.05$ and **$p < 0.01$ between conceived and non conceived groups.

Similar non-significant variations in blood glucose were also noted by Mimoune *et al*. (2017) in treated cystic cows, although cows with ovarian cysts were associated with low serum concentrations of glucose, insulin and urea as compared to normal cyclic ones. Jafari Dehkordi *et al.* (2016) also failed to see significant variation in serum glucose between cows with ovarian cysts and normal cycling cows. However, no report could be seen in literature regarding change in blood glucose over days of hormonal treatment in cystic cows to support or deny the present results.

Plasma Cortisol

The variations in mean values of plasma cortisol levels in cows with COD on sampling days were found to be statistically non-significant (52.83±8.77 to 68.60±7.68 ng/ml) and also among conceived and non-conceived subgroups (54.83±5.74 to 63.00±4.20 ng/ml). The levels of cortisol did not reveal any specific trend between periods in conceived and non-conceived cows in any of the treatment protocols studied. Thus, the role of cortisol in causation and/ or regression of COD following different treatment could not be established with certainty.

Pushp *et al*. (2016) observed similar plasma cortisol levels before and after treatment with GnRH plus potassium iodine and Ovsynch protocols in dairy cattle with ovarian cysts. Although one of the earlier studies evidenced that the regular administration of ACTH induced cyst-like condition in dairy cows (Douthwaite and Dobson, 2000), in another study the plasma cortisol levels were non-significantly different in cows with clinical ovarian cysts from unaffected cows before and after therapy (Bros *et al.,* 2018). In one of the other studies, the cortisol concentration was reported a bit lower in cystic cows (3.5-16.2 ng/ml) compared to the cycling ones (4.9-20.5 ng/ml), but without significant differences between groups or within the group (Probo *et al.,* 2011). Jafari Dehkordi *et al.* (2016), however, found significantly increased cortisol profile together with BUN, BHBA, NEFA, and decreased insulin in cows with ovarian follicular cysts than the normal cyclic ones, and opined that increasing blood cortisol and decreasing blood insulin may play a major role in the formation of ovarian cyst. Mimoune *et al.* (2017) found that ovarian cysts were associated with high serum cortisol levels in dairy cows.

Plasma Total Proteins

The variations in plasma total protein concentrations between periods were found to be statistically non-significant in cows under all four treatment protocols (Table 2). Furthermore, the effect of treatment/period was statistically non-significant among conceived and non-conceived cows in all four treatment protocols, except that the non-conceived cows of Modified Ovsynch protocol had significantly lower value on day of last PG injection (6.88±0.18 g/dl) and higher value on day 12 post-AI (7.38±0.17 g/dl) with values of other two periods as intermediate ones. The levels of total protein did not reveal any specific trend between periods overall or in conceived and non-conceived subgroups in any of the treatment protocols studied.

The normal physiological range of total protein varies from 6.0 to 8.0 g/dl in bovines. The present results thus indicate that total protein may have no any role in pathogenesis of cyst formation or resolution of cyst. Thus total protein is not an indicator parameter for diagnosis of cystic ovary. Virmani *et al*. (2011) recorded significantly higher levels of serum total proteins and cholesterol in cows after CIDR and Ovsynch treatment protocols as compared to the levels before treatment.

Plasma Creatinine Concentration

The mean values of plasma creatinine levels were significantly ($p < 0.05$) different among 4 protocols on day 0 and on day of last PG injection, but with inconsistent trend, and cows under Modified Ovsynch protocol had low values ($p < 0.05$) at both these periods (Table 3). The variations in plasma creatinine concentrations between periods were found to be statistically non-significant in all treatment protocols of cystic ovarian degeneration, except Ovsynch protocol wherein the

Table 2: Mean (±SE) plasma total protein concentrations on different days of various treatment protocols in crossbred cows with cystic ovaries

			Plasma protein (g/dl) on days of treatment/sampling			
Protocol	*Status*	*No.*	*Day of 0*	*Day of last PG Inj*	*Day of FTAI*	*D-12 post-AI*
Ovsynch	Conceived	5	7.04±0.19	6.74±0.11	7.15±0.12	7.05±0.15
	Non-Conc	5	7.05±0.39	7.00±0.42	7.01±0.37	7.16±0.33
	Pooled	10	7.04±0.21	6.87±0.21	7.08±0.18	7.11±0.17
Ovysnch + CIDR	Conceived	4	6.94±0.22	6.94±0.19	6.97±0.21	6.96±0.20
	Non-Conc	6	7.39±0.09	7.38±0.04	7.43±0.04	7.45±0.04
	Pooled	10	7.21±0.12	7.20±0.10	7.25±0.11	7.25±0.11
Modified Ovsynch	Conceived	6	7.51±0.16	7.53±0.20	7.55±0.16	7.31±0.10
	Non-Conc	4	7.19±0.11^{pq}	6.88±0.18^{p}	7.14±0.12^{pq}	7.38±0.17^{q}
	Pooled	10	7.38±0.11	7.27±0.17	7.38±0.12	7.34±0.09
Double PG	Conceived	4	7.27±0.35	7.18±0.29	7.20±0.28	7.29±0.28
	Non-Conc	6	7.40±0.17	7.30±0.17	7.60±0.13	7.38±0.10
	Pooled	10	7.35±0.16	7.25±0.15	7.44±0.14	7.34±0.12

Means bearing uncommon superscripts within row (p, q) differ significantly ($p < 0.05$).

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

			Plasma creatinine (mg/dl) on days of treatment/sampling			
Protocol	*Status*	*No.*	*Day of 0*	*Day of last PG Inj*	*Day of FTAI*	*D-12 post-AI*
Ovsynch	Conceived	5	2.64±0.07	2.49±0.08	2.61±0.07	2.62±0.06
	Non-Conc	5	2.74±0.09	2.50±0.09	2.53±0.17	2.56±0.07
	Pooled	10	2.69^{c}±0.05^{q}	2.49^{a}±0.06^{p}	2.57±0.09^{pq}	2.59±0.05^{pq}
Ovysnch + CIDR	Conceived	4	2.53±0.04	2.59±0.04	2.61±0.05	2.56±0.04
	Non-Conc	6	2.68±0.09	2.66±0.07	2.71±0.08	2.68±0.07
	Pooled	10	2.62^{ab}±0.06	2.63^{ab}±0.05	2.67±0.05	2.63±0.04
Modified Ovsynch	Conceived	6	2.53±0.06	2.50±0.06	2.52±0.04	2.55±0.04
	Non-Conc	4	2.43±0.10	2.49±0.04	2.51±0.06	2.50±0.06
	Pooled	10	2.49^{a}±0.05	2.49^{a}±0.04	2.51±0.03	2.53±0.03
Double PG	Conceived	4	2.60±0.07	2.65±0.07	2.61±0.05	2.62±0.06
	Non-Conc	6	2.66±0.08	2.82±0.05	2.62±0.06	2.62±0.07
	Pooled	10	2.63^{ab}±0.05	2.75^{b}±0.05	2.61±0.04	2.62±0.05

Means bearing uncommon superscripts within the row (p, q) and within the column (a, b, c) differ significantly ($p < 0.05$).

value on day 0 was significantly higher than on day of last PG injection (2.69±0.05 vs 2.49±0.06 mg/dl). Furthermore, the effect of treatment/period was statistically non-significant among conceived and non-conceived cows in all four treatment protocols. The levels of plasma creatinine did not reveal any specific trend between periods and between conceived and non-conceived cows in any of the treatment protocols studied.

There is no report on creatinine profile of cows with cystic ovaries with or without treatment effect in the literature reviewed. However, Mimoune *et al.* (2017) found ovarian cysts to be associated with low serum concentrations of glucose and urea, while Jafari Dehkordi *et al.* (2016) found significantly increased BUN in cows with ovarian follicular cysts as compared to normal cows. Zulu *et al.* (2003) found statistically similar values of blood urea nitrogen and transaminases between the day of PRID insertion and removal, in animals with follicular and luteal cysts.

Thus, the study showed that blood glucose, plasma protein, creatinine and cortisol were not associated with COD and were not influenced by different hormonal treatments, and that Ovsynch in follicular cysts and modified Ovsynch in luteal cysts were better in resolving the cystic conditions in crossbred cows.

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