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Ovarian Cysts in Dairy Cows: Treatment Trials in Relation to Changes in Hematological, Biochemical and Hormonal Parameters

[1]Tamer S. Allam, [2]Emad M. Abd El-Razek and [1]Nahed S. Saleh

[1]Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Sadat City, Egypt
[2]Department of Theriogenology, Faculty of Veterinary Medicine, University of Sadat City, Egypt

Abstract: The present study was carried out to compare between two different protocols, Ovsynch TAI and CIDR synch for the treatment of ovarian cysts in dairy cows in relation to the changes in hematological, biochemical and hormonal parameters. Hundred Holstein cows affected with cystic ovaries were used in this study. The diagnosis was based on gynecological ultrasonographic examination. The affected cows were randomly divided into two equal groups each. The groups were assigned to two treatment protocols, Ovsynch protocol (50 cows) and CIDR-synch protocol (50 cows). Another 10 animals were gynecologically normal and served as control group and received no treatment. Blood samples were collected from each animal at four times, Day 0 (day of the diagnosis of the cyst, positive group for cystic ovaries), Day 3 following the first dose of Receptal or Receptal+CIDR, Day 8 following the administration of Estrumate and finally Day 10 after the second treatment with Receptal before FTAI. The results demonstrated a significant decrease in RBCs, Hb, PCV, serum total protein, albumin, glucose, calcium values and significant increases in TLC, estradiol and FSH in cystic ovary-affected animals compared to the non-treated control. A significant increase in serum malondialdehyde concentration and significant decrease in serum catalase activity were observed in affected animals as well as over the sample collection times in the two treatment protocols. The conception rate in cows with cystic ovaries and treated with Ovsynch was 48% while that of cows treated with CIDR-synch was 46%. There was a non significant difference between Ovsynch and CIDR-synch therapeutic protocols for treatment of cows with cystic ovaries. On the basis of the present results it can be concluded that, the use of ultrasonography appears to be very helpful to get a rapid and sound diagnosis of cystic ovaries in dairy cows. Changes in several hematological and biochemical components were evidenced in cystic ovaries animals which signifying the physiological role of these parameters and their clinical importance in characterizing reproductive problems. Finally, ovsynch protocol might be recommended as a therapeutic approach for treatment of cystic ovary in lactating Holstein cows whereas it is economically cheaper than the CIDR-synch.

Key words: Dairy cows · Cystic ovaries · Ovsynch · CIDR · Hematology · Hormones

INTRODUCTION

Ovarian cysts are defined as non-ovulating liquid-filled follicular structures that are larger than 2.5 cm in diameter and that persist for at least 10 days in the absence of corpus luteum [1]. Etiology of ovarian cysts is multi-factorial and depends on the phenotypic, genetic and environmental factors. High milk yield, hot season, stress and negative energy balance may contribute for the development of cystic ovarian follicles through the metabolic adaptations that occur to sustain the animal's high level of production [2, 3]. The effect of nutrition and/or energy reserves on reproductive function has long been suspected to be mediated by metabolic signal(s) and metabolic profile analysis of farm animals could identify the reasons behind differential fertility [4]. Minerals such as phosphorus and calcium play an important role in the improvement of action of hormones at sub cellular levels in an integrated fashion which will regulate functions of reproduction and production of domestic animals [4-7]. The prevailing follicle fate is dependent on the Luteinizing hormone (LH). The LH secretion is governed by combination of progesterone and estradiol, as higher progesterone inhibited the LH pulse frequency, whereas,

Corresponding Author: Tamer S. Allam, Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Sadat City, P.O. Box 32511, Egypt.

the higher estradiol inhibited the LH pulse amplitude [8]. Further, higher incidence of progesterone deficiency was also observed during early phase of estrus cycle [9]. The incidence of ovarian cysts in dairy cows ranged from 5.6 -18.8% with an average occurrence estimated to be 10% [10]. Cystic ovarian follicles are one of the main factors that cause subfertility in dairy cattle, as they prolonged the calving interval [1, 11]. Follicular cysts may be single or multiple on one or both ovaries with a thin wall (≤3mm) and the follicular fluid is uniformly anechogenic, while luteal cysts have a thicker wall (□3mm) with echogenic rim. Clinical signs of cystic ovarian follicles were variable including the anestrus which occurs frequently during the postpartum period that was seen in both with follicular and luteal cysts, irregular estrus intervals, nymphomania and relaxation of the pelvic ligaments [12]. These cysts produce estrogen in the absence of follicles which can cease a variable time period [10]. The primary therapies for bovine follicular cysts include either the use of gonadotropic releasing hormone (GnRH) analogues or exogenous progesterone [13]. Synthetic GnRH has been used successfully for the treatment of ovarian cyst either by induction of lutenization of cystic structures or initiating ovulation of other follicles from the cystic ovary [14-17].

However, the success of a single GnRH treatment had been determined case by case whereas a larger proportion (25-39%) of cows with ovarian cysts treated with GnRH did not respond [18]. Recently, the Ovsynch-based timed artificial insemination (TAI) protocol has been successfully applied for the treatment of ovarian cysts in lactating dairy cows and produced an acceptable conception rate [19, 20]. Also, the controlled internal drug release (CIDR) device has become a well-accepted protocol worldwide to treat some diseases including cystic ovaries.

The aim of the present study was to compare between two different protocols, Ovsynch TAI and CIDR synch for treatment of ovarian cysts in dairy cows in relation to the changes in the hematological, the biochemical and the hormonal parameters, thus, the investigated parameters may be a potential in characterizing the problem, aiding in diagnosis and suggesting a proper suitable treatment.

MATERIALS AND METHODS

Animals: This study was carried out in a well-managed private Holstein herd located in Northwestern Egypt. The herd was characterized by mean annual milk yield of 10405 kg per cow (9150-11660 kg), with 3.8% fat (3.7-3.75%) and 3.5% proteins (3.3-3.7%). This dairy herd was on a reproductive herd health program and well-computerized reproductive health and management records. Cows were routinely vaccinated against bovine virus diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR), Bovine respiratory syncytial virus, Parainfluenza, Leptospirosis, Campylobacteriosis and Colistridiosis.

Gynecological Examination: A hundred Holstein cows were evaluated and considered cystic ovaries cows based on the clinical examination which was performed once a week from the 4th week after calving by both rectal palpation and ultrasonographic examination equipped with real time linear array B-mode 7.5 MHz rectal probe, (Sonoscape, China). Cows bearing ovarian follicular structures with a diameter > 25mm were considered affected with ovarian cysts. A non-cystic10 animals were gynecologically normal received no treatment and served as control group.

Treatment Protocols: All animals diagnosed with follicular cysts were randomly assigned to one of the following treatment protocols:

Ovsynch-protocol Group (Group A): Animals in this group (n = 50) received an intramuscular (IM) injection of 2.5 ml Receptal® (each 1 ml contains 4 µg buserelin, Intervet international GmbH-Germany) on Day 0 (day of diagnosis). On Day 7, the animals received an IM injection of 2 ml Estrumate® (synthetic prostaglandin analogue each 1 ml contains 250 µg Cloprostenol, Schering-Plough Animal Health). Then 56 hours later, each cow was administered another IM injection of 2.5 ml Receptal®. This was followed by a timed artificial insemination (TAI) 16 hours after the second injection of Receptal®.

Cidr-Synch Protocol Group (Group B): Animals in this group (n = 50) received an IM injection of 2.5 ml Receptal® with application of CIDR on Day 0. On Day 7, cows received an IM injection of 2 ml Estrumate® with the removal of CIDR. Then, 56 hours later, the animals were received another IM dose of 2.5 ml Receptal® followed by TAI 16 hours after the second injection of Receptal®.

Estrus Return Rate and Pregnancy Diagnosis: All animals were visually observed for determination of the cows that return to estrus for 30 days after insemination (for determination of return rate). Animals that returned to estrus within 30 days were examined with trans-rectal

ultrasonography to evaluate the status of the ovaries and determine the animals that showed response to treatment. At 35 days, the animals that did not return to estrus were examined with trans-rectal ultrasonography for detecting early pregnancy followed by second ultrasonographic examination 55-60 days after TAI for the confirmation of pregnancy.

Blood Samples: Blood samples were collected from each animal by jugular vein-puncture. Blood samples were collected on Day 0 (day of diagnosis of the cyst, positive group for cystic ovaries), Day 3 following the first dose of Receptal/ Receptal plus CIDR), Day 8 following administration of Estrumate and finally day 10 after the second treatment with Receptal and before TAI. Blood samples were divided into two portions. The first portion (1ml) was collected on disodium ethylene diaminetetracetic acid (EDTA) for hemogram. The second portion (8ml) was placed in plain centrifuge tubes for separation of serum. Serum samples were divided into aliquots in Eppendorf tubes so that each was used for one time and the tubes were stored at -20°C until assayed for the rest biochemical parameters and hormonal assay.

Hemogram: The evaluated hematological parameters included estimation of red blood cells count (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total leukocytic(TLC) and differential leukocytic counts (DLC). These parameters were performed according to the routine hematological procedures adopted by Feldman *et al.* [22].

Serum Biochemistry: The evaluated biochemical parameters in the study included estimation of serum concentrations of total proteins (TP), albumin (Alb), glucose (Glu), total cholesterol (Ch), triglyceride (TG), blood urea (U), creatinine (Cr), calcium (Ca), inorganic phosphorus (iP), sodium (Na), potassium (K) and serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).These parameters were assessed by spectrophotometric method using commercial diagnostic kits supplied by Bio-diagnostic (Egypt) and following manufacturer's instructions. Oxidant-antioxidant status was evaluated by spectrophotometric measurement of serum levels of malondialdehyde (MDA) and serum enzymatic activity of catalase (CAT) were assayed colorimetrically using commercial kits supplied by Spinreact (GIRONA, Spain) according to the manufacturer's instructions. Serum globulin (Glob) was estimated by subtraction of serum albumin from serum total protein and then A/G ratio was estimated by dividing albumin on globulin.

Hormonal Assay: Serum levels of estradiol (E2), follicular stimulating hormone (FSH), luteinizing hormone (LH) and progesterone (PRO) were assayed by ELIS Amicro-well technique using kits from Bioneovan. Co. (China) and following manufacture instructions.

Statistical Analysis: Data were subjected to statistical analysis using one-way analysis of variance (ANOVA). All data were presented as mean±standard error (SE). Means were compared by the Duncan test at $P<0.05$ level of probability [23].

RESULTS

Ultrasonographic Examination: Trans-rectal ultrasound examination of animals revealed that, one or both ovaries have either one or more large anechoic structure(s) of more than 25 mm in diameter with very thin wall of less than 3 mm in thickness called follicular cysts (Fig. 1).

Hemogram: Data shown in Tables 1, 2 revealed that compared to the control, there was a significant ($P\leq0.05$) decrease in RBCs count, Hb and PCV values in the cystic ovary-affected animals before as well as at 3 days following application of both treatment protocols. MCV, MCH and MCHC values did not show significant differences between groups. With regard to total and differential leukocytic counts, there were significant ($P\leq0.05$) increase in TLC indicated by marked increases in neutrophil count when compared to the control animals (Tables 1, 2).

Serum Biochemistry: The data of serum biochemical parameters presented in Tables 3, 4 implicated that the mean values of serum total protein, albumin and AG ratio were significantly ($P\leq0.05$) low in the cystic ovary-affected cows and at 3 days following application of both treatment protocols. Serum concentrations of glucose and calcium showed significant decreases in cystic ovary animals compared to control which continued along the whole periods of ovsynch or CIDR treatment protocols. The mean values of serum sodium levels demonstrated significant decreases at 8 and 10 days in ovsynch group and at 8 days in CIDR group (Table 3, 4). Evaluation of oxidant and antioxidant status

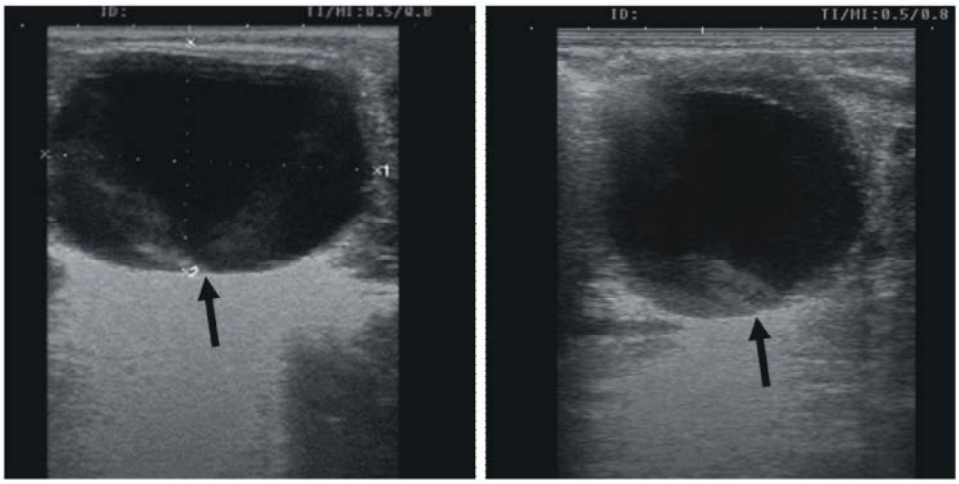

Fig. 1: Ultrasonographic characteristics of the ovaries with follicular cyst (black arrows). Follicular cyst > 25 mm in diameter with very thin wall < 3 mm in thickness.

Table 1: Hematological parameters in cystic ovary-affected cows before and at different time intervals during application of Ovsynch protocol. Values are means±SE

	Groups				
Parameters	Control (N=10)	Cystic Ovary (N=30)	3 days (N=20)	8 days (N=20)	10 days (N=20)
RBCs (×106/µl)	7.59±0.06a	5.86±0.23b	6.11±0.10 b	6.94±0.06 a	6.96±0.03 a
PCV (%)	43.33±1.51 a	33.00±2.64c	36.13±1.21 b	41.03±1.00 a	40.15±1.71 a
Hb (g/dl)	14.52±0.53 a	11.32±0.76b	13.10±0.23 a	12.88±0.43 a	13.58±0.13 a
MCV(fl)	57.36±3.64	56.32±2.15	59.22±2.64	59.06±2.24	57.46±3.12
MCH (Pg)	20.03±2.06	21.39±3.74	21.61±2.06	19.83±2.06	19.37±1.06
MCHC (%)	33.08±2.56	33.14±4.12	35.08±1.51	32.08±2.36	33.81±2.55
TLC (×103/µl))	7.15±1.5b	9.28±0.65a	9.06±0.35a	7.22±0.15b	7.31±0.65b
Neutrophil (×103/µl)	3.85±0.55b	5.99±2.04 a	5.79±2.01 a	3.91±2.04 b	4.01±1.04 b
Eosinophil (×103/µl)	0.22±0.12	0.22±0.17	0.22±0.17	0.22±0.17	0.22±0.11
Basophil (×103/µl)	0.02±0.04	0.01±0.06	0.01±0.01	0.01±0.02	0.01±0.06
Lymphocyte (×103/µl)	2.68±0.21	2.69±0.56	2.67±0.56	2.69±0.26	2.69±0.56
Monocyte (×103/µl)	0.38±0.12	0.37±0.15	0.37±0.13	0.39±0.15	0.38±0.15

Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

Table 2: Hematological parameters in cystic ovary-affected cows before and at different time intervals during application of CIDR protocol. Values are means±SE

	Groups				
Parameters	Control N=10	Cystic Ovary N=30	3 days N=20	8 days N=20	10 days N=20
RBCs ($\times10^6$/µl)	7.59±0.06^{a}	5.86±0.23^{b}	6.20±0.06^{b}	6.73±0.06^{a}	7.13±0.10^{a}
PCV (%)	43.33±1.51^{a}	33.00±2.64^{c}	39.10±1.11^{ab}	39.03±0.80^{ab}	40.01±1.51^{ab}
Hb (g/dl)	14.52±0.53^{a}	11.32±0.76^{b}	12.10±0.23^{bc}	12.59±0.33^{abc}	13.18±0.13^{ab}
MCV(fl)	57.36±3.64	56.32±2.15	62.02±1.14	57.93±1.74	56.86±2.11
MCH (Pg)	20.03±2.06	21.39±3.74	19.61±1.26	18.87±2.01	18.87±1.03
MCHC (%)	33.08±2.56	33.14±4.12	31.94±1.51	32.28±2.06	32.94±2.05
TLC ($\times10^3$/µl))	7.15±1.5^{b}	9.28±0.65^{a}	7.96±0.13^{ab}	7.91±0.15^{ab}	7.93±0.25^{ab}
Neutrophil ($\times10^3$/µl)	3.85±0.55^{b}	5.99±2.04^{a}	4.65±1.61^{ab}	4.63±2.04^{ab}	4.63±1.07^{ab}
Eosinophil ($\times10^3$/µl)	0.22±0.12	0.22±0.17	0.22±0.14	0.22±0.17	0.22±0.10
Basophil ($\times10^3$/µl)	0.02±0.04	0.01±0.06	0.01±0.01	0.01±0.02	0.01±0.06
Lymphocyte ($\times10^3$/µl)	2.68±0.21	2.69±0.56	2.70±0.36	2.68±0.21	2.69±0.16
Monocyte ($\times10^3$/µl)	0.38±0.12	0.37±0.15	0.38±0.11	0.37±0.05	0.38±0.17

Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

Table 3: Serum biochemical parameters in cystic ovary-affected cows before and at different time intervals during application of Ovsynch protocol. Values are means±SE

	Groups				
Parameters	Control (N=10)	Cystic Ovary (N=30)	3 days (N=20)	8 days (N=20)	10 days (N=20)
TP (g/dl)	7.16±0.77[a]	5.56±0.24[b]	5.82±0.24[b]	6.62±0.24[ab]	6.86±0.24[a]
Alb (g/dl)	4.60±0.32[a]	2.88±0.26[b]	3.21±0.16[b]	3.73±0.26[ab]	3.98±0.26[ab]
Glob (g/dl)	2.66±0.21	2.48±0.23	2.61±0.13	2.89±0.11	2.88±0.17
A/G ratio	1.72±0.18[a]	1.17±0.20[b]	1.22±0.08[b]	1.29±0.11[ab]	1.38±0.14[a]
Glucose (mg/dl)	100.77±8.73[a]	49.71±5.63[b]	61.31±4.63[b]	66.11±3.44[b]	86.77±4.73
Ch (mg/dl)	188±5.33	198±10.98	192±6.98	183±4.91	179±4.67
TG (mg/dl)	55.80±5.37	61.00±4.39	63.00±1.35	59.02±4.22	59.00±3.55
U (mg/dl)	45.89±3.72	45.50±6.19	50.30±4.19	47.10±4.11	45.32±4.09
Cr (mg/dl)	1.72±0.10	1.66±0.02	1.64±0.01	1.62±0.11	1.68±0.04
ALT (U/L)	55.80±5.34	40.60±2.22	50.10±3.12	40.60±2.22	45.13±2.34
AST (U/L)	80.30±4.92	75.80±5.15	71.40±3.15	75.80±5.15	81.30±3.45
Ca (mg/dl)	11.55±1.12[a]	5.89±0.52[b]	5.64±0.22[b]	6.19±0.42[b]	6.32±0.32[b]
iP (mg/dl)	4.84±0.32	3.98±0.21	4.63±0.01	4.71±0.17	4.28±0.25
Na (mmol/L)	122±0.82[a]	126±0.57[a]	129±0.22[a]	98±0.21[b]	103±0.72[b]
K (mmol/L)	4.55±0.51	4.60±0.45	4.51±0.31	4.33±0.04	4.49±0.71
MDA (mmol/ml)	1.94±0.09[b]	3.41±0.27[a]	3.11±0.20[a]	4.31±0.17[a]	2.21±0.21[b]
CAT (U/ml)	2.18±0.40[a]	1.48±0.31[b]	1.28±0.31[b]	1.08±0.31[c]	1.32±0.31[b]

Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

Table 4: Serum biochemical parameters in cystic ovary cows before and at different time intervals during application of CIDR-synch protocol. Values are means±SE

	Groups				
Parameters	Control (N=10)	Cystic Ovary (N=30)	3 days (N=20)	8 days (N=20)	10 days (N=20)
TP (g/dl)	7.16±0.77[a]	5.56±0.24[b]	5.42±0.20[b]	6.22±0.14[ab]	6.77±0.07[a]
Alb (g/dl)	4.60±0.32[a]	2.88±0.26[b]	3.11±0.18[b]	3.32±0.46[ab]	3.86±0.06[ab]
Glob (g/dl)	2.66±0.21	2.48±0.23	2.31±0.18	2.92±0.10	2.91±0.13
A/G ratio	1.72±0.18[a]	1.17±0.20[b]	1.34±0.02[ab]	1.13±0.11[b]	1.32±0.11[ab]
Glucose (mg/dl)	100.77±8.73[a]	49.71±5.63[b]	57.31±4.63[b]	61.11±3.41[b]	66.17±3.70[b]
Ch (mg/dl)	188±5.33	198±10.98	190±5.18	187±2.91	189±3.67
TG (mg/dl)	55.80±5.37	61.00±4.39	60.00±1.11	62.02±2.12	59.00±1.53
U (mg/dl)	45.89±3.72	45.50±6.19	49.40±2.21	47.30±1.31	50.12±3.14
Cr (mg/dl)	1.72±0.10	1.66±0.02	1.61±0.00	1.62±0.07	1.70±0.02
ALT (U/L)	55.80±5.34	40.60±2.22	51.11±1.12	44.30±2.22	48.12±3.34
AST (U/L)	80.30±4.92	75.80±5.15	73.15±2.15	74.80±4.20	78.20±1.35
Ca (mg/dl)	11.55±1.12[a]	5.89±0.52[b]	5.44±0.36[b]	5.69±0.12[b]	6.15±0.12[b]
iP (mg/dl)	4.84±0.32	3.98±0.21	4.16±0.01	4.11±0.13	4.09±0.27
Na (mmol/L)	122±0.82[a]	126±0.57[a]	121±0.22[a]	101±2.21[b]	126±0.32[a]
K (mmol/L)	4.55±0.51	4.60±0.45	4.21±0.11	4.73±0.06	4.19±0.44
MDA (mmol/ml)	1.94±0.09[b]	3.41±0.27[a]	3.18±0.10[a]	3.31±0.19[a]	3.21±0.21[a]
CAT (U/ml)	2.18±0.40[a]	1.48±0.31[b]	1.18±0.31[b]	1.23±0.21[b]	1.22±0.21[b]

Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

has shown significant ($P \leq 0.05$) increases in serum levels of malondialdehyde and significant ($P \leq 0.05$) decreases in serum catalase activity in cystic ovary animals and over the periods of sample collection in both treated groups. No significant changes were noticed in other serum biochemical markers evaluated in this study.

Hormonal Assay: In the ovsynch group, the data in Table 5 revealed that there was a significant ($P \leq 0.05$) increase in estradiol in cystic ovary-affected animals and at 10 days of the treatment protocol following the second dose of Receptal. The increase in serum levels of FSH was evidenced in cystic ovary group and at 3 days following the first dose of GnRH while serum LH showed significant ($P \leq 0.05$) increases at 3 and 10 days of Ovsynch treatment protocol. Serum progesterone markedly decreased at 8 days following Estrumate administration (Table 5).

In CIDR group, estradiol levels were significantly ($P \leq 0.05$) higher in cystic ovary animals but they showed a significant ($P \leq 0.05$) decrease at 3 days of the treatment protocol following administration of Receptal+CIDR then increased again at 10 days of the protocol when compared

Table 5: Serum hormonal profile in cystic ovary-affected cows before and at different time intervals during application of Ovsynch protocol. Values are means±SE

	Groups				
Hormone	Control (N=10)	Cystic Ovary (N=30)	3 days (N=20)	8 days (N=20)	10 days (N=20)
Estradiol (pg/ml)	15.44±0.11 [b]	24.13±0.08 [a]	17.64±0.05 [b]	13.74±0.11 [b]	29.14±0.10 [a]
FSH (ng/ml)	4.45±0.17 [b]	6.65±0.04[a]	5.89±0.04 [a]	3.92±0.19 [b]	4.35±0.21 [b]
LH (ng/ml)	3.05±0.09 [b]	2.95±0.13 [b]	4.67±0.11 [a]	3.55±0.11 [b]	4.55±0.11 [a]
Progesterone (ng/ml)	2.66±0.84[a]	2.31±0.21[a]	3.01±0.10 [a]	1.13±0.04[b]	2.38±0.21[a]

Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

Table 6: Serum hormonal profile in cystic ovary cows before and at different time intervals during application of CIDR-synch protocol. Values are means±SE.

	Groups				
Hormone	Control (N=10)	Cystic Ovary (N=30)	3 days (N=20)	8 days (N=20)	10 days (N=20)
Estradiol (pg/ml)	15.44±0.11[b]	24.13±0.08[a]	10.14±0.15[c]	16.53±0.14 [b]	26.10±0.20 [a]
FSH (ng/ml)	4.45±0.17[c]	6.65±0.04[a]	4.71±0.14[c]	4.52±0.16[c]	5.47±0.21[ab]
LH (ng/ml)	3.05±0.09[b]	2.95±0.13[b]	2.02±0.13[c]	2.84±0.05[b]	3.85±0.01 [a]
Progesterone (ng/ml)	2.66±0.84[b]	2.11±0.21[b]	5.21±0.10 [a]	2.13±0.04[b]	2.19±0.31 [b]

Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

Table 7: Estrus return, treatment response and pregnancy rates of Ovsynch and CIDR-synch treatment protocols

		Estrus return rate				Treatment response rate				Pregnancy rate			
		Returned		Not returned		Responded		Not responded		Pregnant		Not pregnant	
Protocol	No	No	%	No	%	No	%	No	%	No	%	No	%
Ovsynch	50	20	40	30	60	45	90	5	10	24	48	6	12
CIDR-synch	50	19	38	31	62	46	92	4	8	23	46	8	16

to control (Table 6). After application of CIDR protocol, FSH levels significantly ($P \leq 0.05$) increased at 10 days. With respect to LH levels, the results in Table 6 implicated significant decreases at 3 days following administration of Receptal+ CIDR and significant increases in its levels at 10 days of the protocol. On the other hand, serum concentrations of progesterone showed significant increase at 3 days of protocol to decrease to the control levels at 8 days of the treatment protocol (Table 6).

Response for Treatment Protocols, Estrus Return Rate and Pregnancy Diagnosis

Ovsynch Protocol (Group A): All animals treated with ovsynch protocol were followed for determination of estrus return rate and the data clarified that 20 of the 50 cows (40%) returned to estrus within 20-30 days after TAI. Based on the re-examination with trans-rectal ultrasonography, 5 of the 20 cows (10%) did not respond to treatment as evidenced by the presence of cyst (s) on the same ovary while the other 15 cows (30%) were in true estrus by detecting Graafian follicle on the ovaries.

After 35 days of TAI, the other 30 cows (non-return cows; 60%) were examined with trans-rectal ultrasonography for pregnancy diagnosis whereas 24 cows (48%) get pregnant and 6 cows (12%) were non pregnant. After insemination (55-60 days), the pregnant cows were re-examined again with trans-rectal ultrasonography to confirm the pregnancy status (Table 7).

CIDR-Synch Protocol (Group B): Animals treated with CIDR-synch protocol have shown that 19 cows (38%) were in estrus within 20-30 days after TAI. By trans-rectal ultrasonographic examination, 4 of those 19 cows (8%) did not respond to treatment as indicated by the presence of cyst (s) on the same ovary while the other 15 cows (30%) were in true estrus and their ovaries carried Graafian follicle. A 35 days following TAI, all the non-return 31 cows (62%) were examined with trans-rectal ultrasonography for pregnancy diagnosis and the results revealed that only 23 cows (46%) get pregnant and 8 cows (16%) were non pregnant. After 55-60 days of TAI, the

non-return cows were re-examined with trans-rectal ultrasonography to confirm pregnancy diagnosis (Table 7).

DISCUSSION

The basic causes of the reproductive problems in a herd are multiple and include managemental, nutritional and pathological factors. The endocrine and metabolic changes associated with pregnancy, parturition and postpartum period are usually neglected during disease diagnosis. In addition, the stressful conditions during transition period or postpartum period and the decreased food intake with drop in the concentration of different blood constituents makes it very important in the animal's life. Poor management during transition period might result in impairment of reproductive performance and resulting in considerable economic losses to dairy farmers [24, 25].

Cystic ovarian follicles are one of the main factors that cause subfertility in dairy cattle, as they prolong the calving interval [11].Various types of hematological changes could be observed in association with reproductive disorders. Among the predominant hematological variations seen in this study, there was a significant decrease in RBCs count, Hb and PCV values in cystic ovary- affected animals compared to control. Contrary, the estrus cows had higher metabolic rate, which causes increased production of higher number of RBC and this leads to increased values of other hematological parameters [26]. Others stated that lack of sufficient quantities of Hb in blood is responsible for reduced oxygen transport to the vital tissues that causes reduced oxidation of nutrients, which in turn affects the whole cellular metabolism in gonadal cells which is metabolically more active and in turn affects the cyclicity [27, 28].

In the present study, total leucocytic count was significantly higher in animals with cystic ovaries compared to control with marked increases in neutrophil count which could probably related to bacterial infection in the affected cows [29]. Stress to which the animals are exposed during the postpartum period could be also a reasonable cause.

The considerable increase in the incidence of reproductive abnormalities and falling pregnancy rates have promoted a focus on the role of nutrition and energy balance in preservation normal reproductive function particularly in the postpartum period. Previous reports have established the physiological role of nutrients in the reproductive development and provided their prospective mechanisms impairing fertility in farm animals [30, 31].

In this regard consistent with the previous reports and data obtained from the present study implicated significantly lower values of serum total protein, albumin and albumin globulin ratio in cystic ovary-affected animals when compared to control. This could be allied with deficiency of certain amino acids needed for the biosynthesis of gonadal hormones and gonadotropins and consequence disturbances of reproductive hormones leading to repeat breeding [32, 33].

On the other hand, some authors did not record significant variations in serum protein levels between normally cycling and repeat breeding cows [34]. These differences between studies could be due to differences in breeds, environment and level of nutrition.

Monitoring serum glucose concentrations revealed a significant hypoglycemia in animals with cystic ovaries compared to control which continued for 8 days of application of the treatment protocols. Glucose is one of the key nutrients affecting fertility and cyclicity in farm animals which at lower level was postulated as the cause for decreased fertility of the animal [35]. Serum glucose was found to be higher in normally cyclic cows compared to non-cyclic cows which could possibly explained by either instability between peripheral uptake of the glucose and hepatic output or alterations in the endocrine regulatory mechanisms, which control these processes [36- 38]. Abnormal performance of hormone-producing organs might also affect glucose levels [35]. Saleh *et al.* [39] found significant positive correlations between the mean values of glucose and estradiol suggesting a role for glucose in the regulation and resumption of estrous cycling after parturition.

The trend of lower calcium concentration in cystic ovaries group observed in this study was in agreement with the previous studies [4,40]. Calcium appeared to play a major role in maintaining the normal reproductive efficiency in animals indirectly. It influences the animal's ability to use other trace elements thus leading to a disruption of reproductive efficiency [7]. In addition, calcium was reported to play a key part in improving the number and size of ovarian preovulatory follicles and the ovulation rate [5]. Serum sodium concentrations showed significant decreases at 8 and 10 days in Ovsynch group and at 8 days in CIDR group following IM injection of Estrumate which can be attributed to the diuretic and natriuretic effects of prostaglandin F2α with the resultant increase in urinary sodium excretion [41].

The current findings of oxidant-antioxidant status demonstrated significant increases in serum values of malondialdehyde and significant decreases in serum catalase activity in cystic ovary group when compared to the control cows indicating the existence of oxidative stress in this group. A relationship between the physiological changes associated with periparturient period and a loss in overall antioxidant potential was supported by various studies conducted either in vivo or in vitro [42-44]. The changes in oxidant-antioxidant biomarkers were observed over the periods of sample collection in both treated groups particularly at Day 8. Interestingly, reactive oxygen species (ROS) including hydrogen peroxide, superoxide anion and hydroxyl radical have been implicated in the luteolytic effect of prostaglandin F2α probably through their ability to decrease progesterone biosynthesis (functional luteolysis) followed by tissue degeneration apoptosis in luteal endothelial cells (structural luteolysis) [45]. Therefore, the increase in MDA levels and the reduction of CAT activity following injection of prostaglandin F2α indicated enhanced oxidative stress process for the promotion of structural luteolysis [46].

Regarding hormonal profile, serum estradiol levels increased significantly in cystic ovary group as well as at 10 days following the second dose of GnRH in Ovsynch group. In CIDR group, estradiol showed a significant decrease at 3 days to rise again at 10 days of the protocol. Ovarian cysts produce estrogen in the absence of follicles which can cease a variable time period [10]. Similarly, Kim *et al.* [47] used the CIDR protocol in lactating dairy cows and found that plasma estradiol declined following intra-vaginal placement of CIDR, but markedly increased one day after removal. FSH was significantly increased in cystic ovary group and at 3 days in Ovsynch protocol and at 10 days in CIDR group. Pituitary FSH is essential for development and maintenance of ovarian follicles in single and multiple ovulating species [48]. GnRH causes the release of FSH and LH from the anterior pituitary and initiates normal cyclicity FSH and LH directly act on the ovary to stimulate follicular growth and maturation leading to estrus [49]. Serum concentrations of LH did not show significant differences between the cystic ovary and control groups. The findings were corroborated with previous reports which demonstrate that there is no interference with the ability of the pituitary gland to release luteinizing hormone in cystic animals, but the function of the hypothalamic-pituitary-ovarian axis would be disrupted and the pre-ovulatory LH surge that normally induces ovulation does not occur [39]. Significant increases in LH were noticed at 3 and 10 days in ovsynch group probably due to the effect of GnRH of Receptal. In CIDR treated group, LH demonstrated significant decreases at 3 days to increase above control levels at 10 days. CIDR causes the animal's blood progesterone concentrations to rise rapidly. Progesterone at suprabasal concentrations blocks the LH-surge, thereby inhibiting ovulation, but increases the LH pulse frequency [49]. Revah and Butler [50] claimed that a high level of progesterone in the follicular phase causes the reduction of LH secretion, which improves the quality of oocytes. The mean values of serum progesterone showed significant decrease at 8 days following Estrumate in ovsynch group. Estrumate is a synthetic prostaglandin analogue structurally related to prostaglandin F2α. Prostaglandin F2α was found to act on the corpus luteum to cause luteolysis, forming a corpus albicans and stopping the production of progesterone an action which is dependent on the number of receptors on the corpus luteum membrane [47, 51]. In CIDR group, serum concentrations of progesterone showed significant increases at 3 days of protocol to decrease to the control levels at 8 days of the treatment protocol. Revah and Butler [50] indicated that CIDR causes the animal's blood progesterone concentrations to rise rapidly and those concentrations were maintained at a relatively constant level during the next seven days. Upon removal, progesterone concentrations were quickly eliminated. Similar findings were documented by Kim *et al.* [47] who observed significant decreases in plasma progesterone after CIDR removal, but increased again at the presence of palpable corpus luteum.

Detection of heat in cows by observation was a traditional and time-consuming method. In addition, there were many factors that have been documented to shorten the period of heat and producing weak behavioral signs that express heat. Among these factors, imbalanced ration, increased milk yield as well as different stressful conditions. Many hormonal treatment protocols have been widely used to make heat detection more effective and thus improve the insemination success rate by allowing synchronization between heat and ovulation in combination with timed artificial insemination without the need for detection of signs of heat [52]. Ovsynch is an injection protocol that can lead to the equalization of follicle development causing ovulation and enabling artificial insemination. The principle of this protocol depends on that the first injection of GnRH induces ovulation of the ovarian follicle, which leads to formation

of the corpus luteum. The effectiveness of this step is determined by the maturation stage of the follicles at the time of treatment [53]. The aim of injection of Estrumate (PGF2α) on the seventh day is to induce luteolysis and thus the dominant follicle of the next wave develops. This dominant follicle will be estimated to ovulate by the second GnRH injection and insemination should be performed blindly 16 hours later [51].

In the present study, the conception rate (CR) recorded in cows with cystic ovaries treated with Ovsynch was 48% which was higher than that reported by previous studies [19, 53, 54].

CIDR is an intravaginal device constructed of a progesterone-impregnated medical silicone elastomere molded into a T-shape. It is labeled for estrus synchronization in beef and dairy cattle. When the follicle is not capable of ovulation, this can render the hypothalamus unresponsive to the feedback mechanism of estradiol which results in the formation of ovarian cysts. To restore the feedback mechanism, the hypothalamus needs to be exposed to progesterone [49]. Conception rate in cows treated with CIDR-synch was 46% which was nearly similar to that observed by Bartolome *et al.* [53] but lower than that observed by Johnson and Ulberg [55]. Kim *et al.*[47] recorded an acceptable conception rate of 52.3% with the CIDR-based TAI protocol in lactating dairy cows diagnosed with follicular cysts.

The differences between CR in Ovsynch and CIDR-synch groups might be due to differences of the patents used, differences between the scheduled protocols and overall variations in farm management or levels of milk production. Regardless of the treatment protocol adopted, no factors significantly influenced treatment response in treated cows. Crane *et al.* [19] stated that the level of milk production on the day of cyst diagnosis and treatment might be a predicting variable for treatment success in dairy cows. The authors added that primiparous cows were more likely to become pregnant than multiparous cows. Discrepancy could be explained by management practices involving primiparous cows. Age at calving, nutrition, stress and housing environment differences between herds may partially explain the variable response to treatment and fertility among primiparous cows in different studies [19].

In conclusion, use of ultrasonography appears to be very helpful to get a rapid and sound diagnosis of cystic ovaries in dairy cows. Changes in several hematological and biochemical components were evidenced in cystic ovaries animals which signifying the physiological role of these parameters and their clinical importance in characterizing reproductive problems. Interestingly, there was no significant difference between ovsynch and CIDR-synch therapeutic protocols for treatment of cows with cystic ovaries. Ovsynch protocol might be recommended as a therapeutic approach for treatment of cystic ovary in lactating Holstein cows where it is economically cheaper than the CIDR-synch.

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