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Effect of Exogenous Progesterone on Success of Ovsynch Protocol in Dairy Cows with Ovarian Cyst

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ABSTRACT

***Background*:** It has been suggested that unresponsiveness of the hypothalamus to increasing estradiol may be an underlying mechanism in the development of ovarian cysts which may be treated with progesterone. Therefore, this study was designed to compare the effectiveness of Ovsynch and Ovsynch modified with progesterone releasing intravaginal device (PRID) for the treatment of ovarian cysts that cause important economic in Holstein dairy cows.

***Materials, Methods & Results*:** Cows with ovarian cysts (n = 33) were divided into randomly Ovsynch (Ov) (n = 9) and Modified Ovsynch (MOv) (n = 9) groups for follicular cysts and Ov (n = 7) and MOv (n = 8) groups for luteal cyst. Ov group were treated with im GnRH on Day 0, im $PGF_{2\alpha}$ on Day 7, im GnRH on Day 9 and then were time-inseminated 18 h later. MOv group were treated with Ov protocol combined with PRID from Day 0 to 7. Data obtained by transrectal ultrasonography on Day 0, 7, 10, 17, 31 and 45 and progesterone (P4) and 17β-estradiole (E2) concentrations in blood samples collected on Day 0, 7, 10, 17 and 31 were evaluated. The treatments resulted in regression, increased diameter or no changes in ultrasonic appearance in OV group in 2 (22%), 5 (56%) and 2 (22%) cows with follicular cyst and in 4 (57%), 2 (29%) and 1 (14%) cows with luteal cyst, respectively. MOv group showed regression, increased diameter or no changes in ultrasonic appearance in 4 (44%), 2 (23%) and 3 (33%) cows with follicular cyst, respectively, whereas 7 (88%) cows with luteal cyst displayed regression and one cow presented no changes in ultrasonic appearance. P4 on Day 0, 7, and 10 did not differ significantly in Ov group. In MOv group, P4 was higher on Day 7 than those detected on Day 0 and 10 ($P < 0.001$). The cows with ovarian luteal cyst in Ov group revealed P4 on Day 0 that was significantly higher than those on Day 10 ($P < 0.05$). In MOv group, P4 on Day 0 and 7 was significantly higher than those on Day 10 ($P < 0.05$). The mean diameter of ovulatory follicle of cows with follicular cyst was higher in MOv group than those in Ov group ($P < 0.05$). Return to cyclicity, conception and pregnancy rates of ovarian follicular and luteal cyst or overall results regardless of the type of the ovarian cyst in Ov and MOv groups did not show any significant difference.

***Discussion*:** The increasing diameter of follicular cyst in Ov protocol might be due to the luteinization process of cystic ovarian follicle by GnRH treatment and a too short recovery period during the prostaglandin administration on Day 7. Decreasing cyst diameter in response to MOv treatment in cows with ovarian follicular cyst (44%) may be associated with the administration of PRID because the fact that exogenous progesterone leads to suppress LH pulse frequency and regression of follicular cyst. Most of the cows with luteal cyst (88%) showed decreasing diameter in cyst in MOv group (57% in Ov group). The regression process of luteal cysts may be facilitated by increased ovarian blood flow after GnRH treatment (on Day 0) or increased LH pulse frequency following progesterone administration that may lead to faster decreasing in diameter. The presence of corpus luteum on Day 17 after the second GnRH injection occurred in Ov and MOv groups indicated that both treatment protocols had high return the cyclicity rates. However, the increased preovulatory follicle diameter in MOv protocol compared to Ov protocol (1.84 vs 1.60, $P < 0.05$) suggests that MOv protocol may be more effective on return to cyclicity rate than Ov treatment. In conclusion, it seems that MOv protocol has greater pregnancy rates for ovarian follicular cyst, however overall pregnancy rates are similar for both treatment models. It is suggested that new strategies are needed to enhance embryo survival due to possibility of late embryonic loss.

Keywords: PRID, follicular, luteal, conception rate, pregnancy rate.

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INTRODUCTION

Ovarian follicular cysts are much more common than luteal cyst [15,24] that approximately 18 to 29% of dairy cows develop ovarian follicular cysts [9,17,36] which increase the days open by 22-64 days [7]. Cows with ovarian cysts often respond to endocrine based therapy [38], leading to estrous about three weeks after treatment [6]. Recently, it has been indicated that ovulation synchronization protocol (Ovsynch) can be used for the treatment of cystic ovarian follicles [4]. This protocol contributes the luteinization of younger follicular cysts or the ovulation of a present dominant follicle by GnRH induced LH [39], the regression of the luteinized cyst or newly formed corpus luteum by injection of prostaglandin and synchronization of ovulation with timed artificial insemination by last GnRH injection [37].

The exact pathophysiology of cystic structures seems to involve the unresponsiveness of hypothalamus to increasing estradiol, which leads to inappropriate or lack of release of GnRH surge at the time of estrous that does not allow the ovulation [19]. It has been stated that a deficiency in the functional estrogen receptor α (ER α) in the lateral mediobasal hypothalamus could prevent the subsequent induction of a GnRH surge and progesterone could upregulate the ERα which will induce the GnRH/LH surge in response to follicular estrogen [18-20]. The hypothesis of this study was that Ovsynch with exogenous progesterone would have better results for the treatment of ovarian follicular cyst. Therefore, this study was designed to compare the effectiveness of Ovsynch with or without exogenous progesterone for the treatment of ovarian cysts in Holstein dairy cows.

MATERIALS AND METHODS

Animals

This study was conducted in a dairy farm (approximately 500 milking cows) in Afyonkarahisar province, Turkey. Cows were milked twice daily and were housed in semi open barns and fed a total mixed ration formulated to meet or exceed the requirements of the National Research Council. The voluntary waiting period used in this dairy herd was 45 days and the mean and range of days in milk (DIM) for cows were 225 days.

Ovarian cysts were identified in this dairy farm that was visited twice weekly to detect the lactating dairy cows 45-60 DIM with ovarian cyst. On the day of each visit, the examination of 30-40 cows was performed.

Experimental design

The diagnosis of ovarian cyst was based on transrectal ultrasonography1 of the ovaries. The criteria used on transrectal ultrasonography were the presence of large anovular follicle (≥ 25 mm) that persisted on the ovary for at least 10 days in the absence of a corpus luteum (CL) [24]. Moreover, on ultrasonographic examination of the ovaries, ovarian cysts were recognized by their hypoechodensity [34]. The absence of luteal tissue on either ovary implied that the cyst was functional at diagnosis and that normal cycles were not occurring [22]. However, cows presenting ovarian cyst in the breeding herd were visualized by transrectal ultrasonography at 10 days intervals for detection of the presence of ovarian cyst. Once the presence of ovarian cyst was clearly detected, the diameter of cyst wall was measured to differentiate the diagnosis between a follicular and a luteal cyst, whereby the thickness of the wall of a follicular cyst was ≤ 3 mm, and the thickness of the wall of a luteal cyst was > 3 mm [21].

Experimental protocols are summarized in Figure 1. Cows diagnosed as having ovarian cysts by transrectal ultrasonography were divided into randomly Ovsynch (Ov) (n = 9) and Modified Ovsynch (MOv) (n = 9) groups for follicular cysts and Ov (n = 7) and MOv (n = 8) groups for luteal cyst. Cows in the Ov group (n = 16) were treated with GnRH[2] on Day 0, $PGF_{2\alpha}$[3] on Day 7, GnRH (0.021 mg, im) on Day 9 and were timed inseminated 18 h later. Cows in the MOv group (n = 17) were treated with GnRH (0.021 mg, im) on Day 0 and PRID[4] on Day 0 for 7 days. On Day 7, the PRID was removed and cows were treated with $PGF_{2\alpha}$ (25 mg, im). GnRH (0.021 mg, im) was injected on Day 9 and animals were timed inseminated 18 h later.

The transrectal ultrasonography was performed to detect the diameter of ovarian follicular and luteal cysts on Day 0, 7 and seven days after (Day 17) artificial insemination (AI). Moreover, the diameter of dominant follicle on Day 0 and the diameter of ovulatory follicle during AI (Day 10) were recorded. The presence of CL after first GnRH injection was examined on Day 7, whereas ovulation was defined as disappearance of ovulatory follicle and the presence of CL seven days after AI.

Twenty one days after AI (Day 31), all cows in both groups were subjected to transrectal ultrasonography to detect the presence of CL and conceptus. Pregnancy was determined 35 days (Day 45) after AI using transrectal ultrasonography.

Blood sampling

Once the presence of an ovarian cyst was detected by transrectal ultrasonography, blood samples were collected into evacuated tubes via jugular venipuncture from all animals immediately before the first GnRH injection (Day 0), before $PGF_{2\alpha}$ injection (Day 7), at the time of artificial insemination (AI) (Day 10), seven days after AI (Day 17) and twenty one days after AI (Day 31). Samples were immediately transport from farm to the laboratory. Samples were centrifuged at 3000 x *g* for 30 min and serum was stored at –20ºC until assayed for progesterone (P4) and 17β-estradiole (E2). P4 and E2 concentrations were determined by electrochemiluminiscence immunoassay (ECLIA) with a commercial test kit5 according to the manufacturer's instructions, in an immunologic test analyser. The sensitivities of assay for P4 and E2 were 0.15 ng/mL and 12.00 pg/mL, respectively. The average intraassay and interassay coeffecients of variation were kept under 20% for all investigated hormones.

The concentration of P4 on the first day of the treatment was used for the confirmation of classification of cyst as follicular (< 1.0 ng/mL) and luteal (> 1 ng/mL). Functional regression of the luteal cyst were defined a decrement of serum P4 concentration at the time of AI as compared to P4 concentration on first day of the treatment, whereas the decrement of serum P4 concentration at the time of AI as compared to P4 concentration on seventh day of the treatment defined the functional regression of follicular cyst. Ovulation was defined as P4 concentration at the time of insemination, disappearance of ovulatory follicle and detection of CL seven days after AI. The presence of CL seven days after AI was used to determine the return the cyclicity rate in groups. Serum progesterone concentrations on Day 31 were used to determine the conception rate (> 1 ng/mL).

Statistical analysis

Data were analyzed by using statistic program SPSS version 16.0. All values are presented as mean ± standard deviation (SD). The differences in diameter of ovarian follicular or luteal cysts, E2 and P4 concentrations within groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey test. The diameter of ovarian follicular or luteal cysts, serum E2 and P4 concentrations and the diameter of dominant follicles between groups were analyzed by *t*-test. Chi-square test was applied to analyze the differences between return to cyclicity, conception and pregnancy rates. Group differences were considered significant at $P < 0.05$.

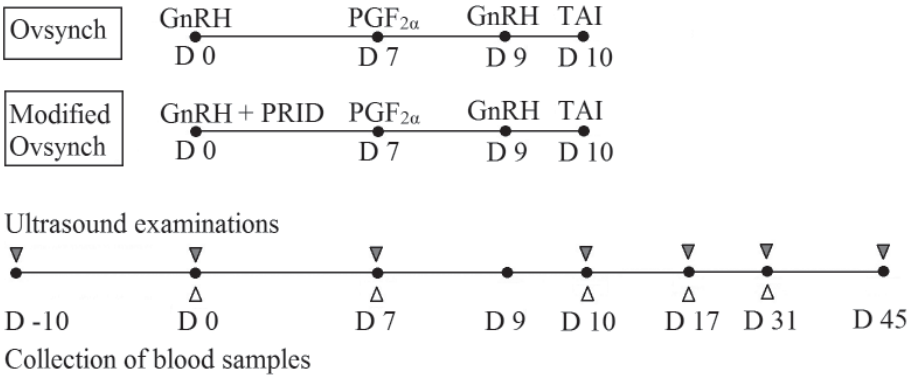

Figure 1. Schematic diagram of Ovsynch and Modified Ovsynch protocols used. Transrectal ultrasonography and collection of blood samples were performed as indicated in the figure. TAI: timed artificial insemination, D: Day.

RESULTS

The mean diameter of ovarian follicular and luteal cysts, E2 and P4 concentrations on Day 0, 7, 10 and 17 is given Table 1 and 2. Accordingly, in Ov group, nine cows were diagnosed as having ovarian follicular cyst (Day 0; E2: 11.48 ± 1.93 pg/mL; P4: 0.23 ± 0.07 ng/mL), whereas seven cows had luteal cyst (Day 0; E2: 8.79 ± 1.35 pg/mL; P4: 2.81 ± 0.47 ng/mL). MOv group had nine cows that were diagnosed as having ovarian follicular cyst (Day 0; E2: 10.48 ± 1.51 pg/mL; P4: 0.33 ± 0.06 ng/mL), whereas eight cows had luteal cyst (Day 0; E2: 16.54 ± 5.07 pg/mL; P4: 3.15 ± 0.48 ng/mL).

The treatments resulted in regression, increased diameter or no changes in ultrasonic appearance in 2 (22%), 5 (56%) and 2 (22%) cows with follicular cyst in Ov protocol and 4 (44%), 2 (23%) and 3 (33%) cows in MOv protocol, respectively. However, the mean diameter of ovarian follicular cysts throughout the examination days between Ov and MOv groups did not show any significant difference. It was observed that the diameter of follicular cyst slightly increased on Day 7 and Day 17 in both groups as compared to the diameter on Day 0 but the difference was not significant within groups [Table 1]. Similar to ovarian follicular cyst, the diameters of ovarian luteal cyst did not differ significantly between Ov and MOv groups. However, the diameter of ovarian luteal cyst on Day 7, 10 and 17 decreased as compared to diameter on Day 0 but the difference was not significant within the both groups [Table 2]. The treatments showed regression, increased diameter or no changes in ultrasonic appearance in 4 (57%), 2 (29%) and 1 (14%) cows with luteal cyst in Ov group, respectively, whereas 7 (88%) cows displayed regression and one cow presented no changes in ultrasonic appearance in MOv group.

It was observed that serum P4 concentration on Day 10 decreased as compared to serum P4 concentration on Day 7 in animals diagnosed as ovarian follicular cyst in both Ov and MOv groups (Table 1). Moreover, animals with ovarian luteal cyst showed a decreasing serum P4 concentration on Day 10 as compared to Day 0 in Ov and MOv groups [Table 2].

The cows with ovarian follicular cyst showed that the concentrations of E2 on Day 17 and P4 on Day 7 were significantly higher in MOv group than those in Ov group ($P < 0.05$). The concentrations of E2 did not show any significant difference within both groups throughout the examination days. However, the concentration of P4 on Day 17 was significantly higher than those on Day 0 and 10 in Ov group ($P < 0.001$), whereas it did not differ as compared to Day 7. In MOv group, the highest P4 concentration was detected on Day 17 ($P < 0.001$), whereas the concentration of P4 was higher on Day 7 than those detected on Day 0 and 10 ($P < 0.001$) [Table 1]. The cows with ovarian luteal cyst revealed that the concentrations of E2 did not significantly differ between groups on Day 0, 7 and 10, however E2 concentration on Day 17 was higher in MOv group than those in Ov group ($P < 0.05$) [Figure 2]. The concentrations of P4 showed no difference throughout the examination days between Ov and MOv groups, whereas P4 concentration significantly differed within the both groups. In Ov group, the concentrations of P4 on Day 0 and 17 was significantly higher than those on Day 10 ($P < 0.05$) but no significant difference was detected as compared to Day 7. In MOv group, the concentrations of P4 on Day 0 and 7 was significantly higher than those on Day 10 ($P < 0.05$), whereas there was no significant difference between Day 10 and Day 17 [Table 2].

The mean diameter of dominant follicle at first GnRH injection and the mean diameter of ovulatory follicle at artificial insemination are given in Table 3. The mean diameter of dominant follicle for cows with follicular cyst did not differ significantly, whereas the diameter of ovulatory follicle was higher in MOv group than those in Ov group ($P < 0.05$). In contrast, the mean diameter of dominant follicle for cows with luteal cyst in MOv group was higher than those in Ov group, whereas there was no significant difference according to the diameter of ovulatory follicles in both groups.

The presence of CL on the ovary was detected by transrectal ultrasonography of five cows with ovarian follicular cyst in Ov group and six cows in MOv group on Day. On Day 17, one cow did not have CL in Ov group, whereas all cows presented CL in MOv group. However, cows with ovarian luteal cyst showed that four cows had CL in Ov group and six cows in MOv group on Day 7, whereas five cows in Ov group and seven cows in MOv group presented CL on Day 17 (Table 4). The presence of CL on Day 17 revealed that return to cyclicity rate of ovarian follicular cyst in Ov and MOv groups was 89% (9/8) and 100% (9/9), respectively, whereas the return the cyclicity rate of ovarian luteal cyst was 71% (7/5) and 88% (8/7) in Ov and MOv groups,

respectively (Table 4). The overall results based on the two protocols regardless of the type of the ovarian cyst (follicular or luteal) are given in Table 4.

Conception rates for cows with follicular cyst in Ov and MOv group were 55% (9/5) and 89% (9/8), whereas these rates were 71% (7/5) and 63% (8/5) for cows with ovarian luteal cyst, respectively. Ultrasonography of genital tract on day 35 revealed that pregnancy rates for cows with follicular cyst in Ov and MOv group 44% (9/4) and 55% (9/5), whereas these rates were 43% (7/3) and 38% (8/3) for cows with ovarian luteal cyst, respectively (Table 4). Regardless of the type of the ovarian cyst (follicular or luteal), the conception and pregnancy rates are given on Table 4.

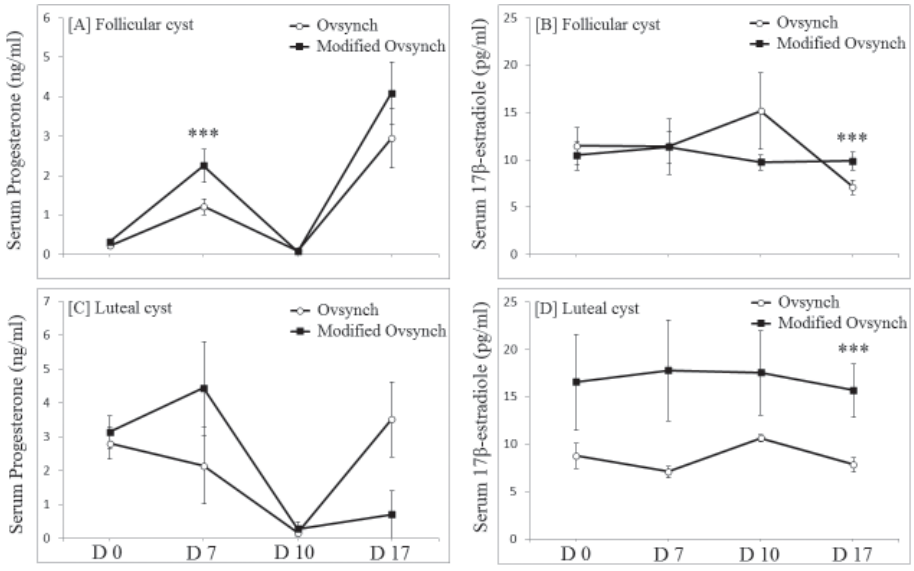

Figure 2. Serum progesterone (P4) (ng/mL) and 17β-estradiole (E2) (pg/mL) concentrations of cows having ovarian follicular and luteal cysts on Day 0, 7, 10 and 17 in Ovsynch and Modified Ovsynch groups. ***Indicates differences ($P < 0.05$) in 17β-estradiole and P4 concentrations between Ovsynch vs. Modified Ovsynch.

Table 1. The concentrations of 17β-estradiole (E2) (pg/mL) and progesterone (P4) (ng/mL) detected on Day (D) 0 (D 0), 7 (D 7) and 17 (D 17) and the diameter (cm) of ovarian follicular cyst measured on D 0, D7 and D 17 in Ovsynch and Modified Ovsynch protocols (mean ± SEM).

Ovarian Follicular Cyst						
Day	Ovsynch (n = 9)			Modified Ovsynch (n = 9)		
	E2	P4*	Diameter	E2	P4*	Diameter
D 0	11.48 ± 1.93	0.23 ± 0.07^{y}	3.24 ± 0.20	10.48 ± 1.51	0.33 ± 0.06^{z}	3.42 ± 0.07
D 7	11.43 ± 2.94	1.22 ± 0.20^{xy}	3.56 ± 0.28	11.38 ± 1.66	2.25 ± 0.42^{y}	3.61 ± 0.21
D 10	15.16 ± 4.04	0.10 ± 0.01^{y}		9.75 ± 0.80	0.08 ± 0.02^{z}	
D 17	7.13 ± 0.75	2.95 ± 0.75^{x}	4.33 ± 0.32	9.88 ± 0.96	4.09 ± 0.79^{x}	3.48 ± 0.40

^{x,y,z}Superscripts in the same column differ significantly, *$P < 0.001$

Table 2. The concentrations of 17β-estradiole (E2) (pg/mL) and progesterone (P4) (ng/mL) detected on Day (D) 0 (D 0), 7 (D 7) and 17 (D 17) and the diameter (cm) of ovarian luteal cyst measured on D 0, D7 and D 17 in Ovsynch and Modified Ovsynch protocols (mean ± SEM).

Ovarian Luteal Cyst						
Day	Ovsynch (n = 7)			Modified Ovsynch (n = 8)		
	E2	P4 *	Diameter	E2	P4 *	Diameter
D 0	8.79 ± 1.35	2.81 ± 0.47^{x}	3.19 ± 0.27	16.54 ± 5.07	3.15 ± 0.48^{x}	3.91 ± 0.20
D 7	7.12 ± 0.61	2.15 ± 1.13^{xy}	2.88 ± 0.42	17.78 ± 5.31	4.44 ± 1.39^{x}	3.81 ± 0.32
D 10	10.64 ± 0.40	0.16 ± 0.07^{y}		17.54 ± 4.45	0.28 ± 0.21^{y}	
D 17	7.87 ± 0.77	3.52 ± 1.10^{x}	2.78 ± 0.92	15.69 ± 2.82	2.46 ± 0.71^{xy}	3.53 ± 0.38

^{x,y}superscripts in the same column differ significantly, *$P < 0.05$.

Table 3. The diameter (cm) of dominant follicle detected at first GnRH injection time (Day 0) and the diameter (cm) of ovulatory follicle measured at artificial insemination time (Day 10) by transrectal ultrasonography of cows with ovarian follicular and luteal cysts treated with Ovsynch and Modified Ovsynch protocols (mean ± SEM).

	Diameter of dominant follicle (cm)		Diameter of ovulatory follicle (cm)	
Cyst type	Ovsynch	Modified Ovsynch	Ovsynch	Modified Ovsynch
Follicular	1.45 ± 0.12	1.35 ± 0.30	1.60 ± 0.30[b]	1.84 ± 0.07[a]
Luteal	1.34 ± 0.05[b]	1.79 ± 0.39[a]	1.91 ± 0.07	1.84 ± 0.05

[a,b]Superscripts in the same row differ significantly at $P < 0.05$.

Table 4. The presence of corpus luteum (CL) detected by transrectal ultrasonography on Day 7 (CL 7) and Day 17 (CL 17), conceptus (C) detected by transrectal ultrasonography and according to serum progesterone concentration (> 1 ng/mL) on Day 31(C 31) and pregnancy (P) detected by transrectal ultrasonography on Day 45 (P 45) in ovarian follicular [A] and luteal cyst [B] or ovarian cyst (regardless of the type of cyst, i.e. follicular, luteal) [C] in Ovsynch and Modified Ovsynch groups. No significant difference was observed between or within groups ($P > 0.05$).

[A]	Ovsynch (n = 9)				Modified Ovsynch (n = 9)			
	CL 7	CL 17	C 31	P 45	CL 7	CL 17	C 31	P 45
Rate	5/9	8/9	5/9	4/9	6/9	9/9	8/9	5/9
%	55	89	55	44	66	100	89	55
[B]	Ovsynch (n = 7)				Modified Ovsynch (n = 8)			
	CL 7	CL 17	C 31	P 45	CL 7	CL 17	C 31	P 45
Rate	4/7	5/7	5/7	3/7	6/8	7/8	5/8	3/8
%	57	71	71	43	75	88	63	38
[C]	Ovsynch (n = 16)				Modified Ovsynch (n = 17)			
	CL 7	CL 17	C 31	P 45	CL 7	CL 17	C 31	P 45
Rate	9/16	13/16	10/16	7/16	12/17	16/17	13/17	8/17
%	56	81	62	44	70	94	76	47

DISCUSSION

The Ov and MOv protocols did not significantly alter the mean diameter of cysts but gradually increased in cows with follicular cyst and decreased in cows with luteal cyst. However, individual evaluation of the data showed that Ov and MOv protocols had different responses for follicular or luteal cysts, such as decreasing or increasing in diameter or no ultrasonic changes in the appearance of the cyst which was in accordance with the previous findings of Douthwaite & Dobson [12]. The cows with follicular cysts responded to Ov treatment by increasing diameter of cyst (56%), whereas MOv protocol resulted in more cows (44%) with decreasing diameter of cyst. Nanda *et al*. [30] demonstrated that luteinized follicular cysts showed poor response to prostaglandins administered 7 days after GnRH treatment in cows with follicular cysts. The increasing diameter in Ov protocol might be due to the luteinization process of cystic ovarian follicle by GnRH treatment [15,39] and a too short recovery period during the prostaglandin administration on Day 7 [28]. It has been stated that ovarian follicular cysts are associated with high LH pulse frequency and administration of exogenous progesterone leads to suppress LH pulse frequency and consequently, regression of follicular cyst [22]. It is possible that decreasing cyst diameter in response to MOv treatment in cows with ovarian follicular cyst (44%) may be partially explained by the administration of PRID, since the mean P4 concentration detected on Day 7 was higher in MOv group as compared to Ov group ($P < 0.05$). On the other hand, in the present study, all cows would be expected to show regression. This might be related to the length of progesterone exposure [20] which requires daily administration for 10-14 days, implanting norgestomet for 14 days or treatment with a PRID for 9-14 days [32]. In the present study, most of the cows with luteal cyst (88%) showed decreasing diameter in cyst in MOv group (57% in Ov group). It has been

indicated that follicular and luteal cysts are the different forms of the same disorder [31] and luteal cysts are considered to be the later stages of follicular cysts [14]. Luteal cysts have increased blood flow compared with follicular cysts [29] and there is an association between administration of GnRH and ovarian blood flow [1]. It is known that subluteal concentrations of progesterone are capable of blocking the preovulatory LH surge but do not slow the frequency of LH pulses [27]. The regression process of luteal cysts may be facilitated by increased ovarian blood flow after GnRH treatment (on Day 0) or increased LH pulse frequency following progesterone administration that may lead to faster decreasing in diameter.

Silvia *et al*. [38] reported that there were some discrepancies about the definition of effectiveness of the treatment protocols in which either regression of the original cyst within 28 days regardless of subsequent ovarian activity or regression of cyst following by ovulation and formation of a normal corpus luteum within 15 days. In the present study, it was observed that a decline in the mean serum P4 concentration on Day 10 (TAI) was evident in cows with follicular and luteal cysts in Ov and MOv groups. It may speculate that the functional regression of ovarian follicular and luteal cysts in both groups was achieved. However, the individual data should be also evaluated. Accordingly, the percentage of detected corpus luteum on Day 7 in Ov and MOv groups was 55 and 66 for follicular cyst and 57 and 75 for luteal cyst, respectively. In the present study, the detection of CL on Day 7 indicates that there may be ovulation from dominant follicles co-existing with ovarian cyst following GnRH injection which was in accordance with several previous reports [3,16,26,33]. Cows with bovine ovarian cysts have ovarian follicular waves similar to those of cows which ovulate at the end of estrous [15]. It has been stated that a new follicle develops following first GnRH treatment within 7 days [2,11,25] and a newly developed follicle ovulated either in response to second GnRH treatment or spontaneously upon progesterone withdrawal, regardless of the type of ovarian cyst [2]. The observations in the present study that the presence of CL on Day 17 after the second GnRH injection (on Day 9) occurred in Ov and MOv groups based on the follicular (89% (9/8) and 100% (9/9), respectively) and luteal cysts (71% (7/5) and 88% (8/7), respectively) or regardless of the type of the cyst (81% (16/13) and 94% (17/16), respectively) indicated that both treatment protocols had high return the cyclicity rates. Return to cyclicity rates in cows with ovarian cysts treated with different protocols have been reported to be range from 73 to 83% for Ovsynch [2,10,13] and 61.5 to 100 for various progesterone treatments [2,8,10,23]. This variation may be due to the variable number of cows used and the application of different protocols. However, the increased preovulatory follicle diameter in MOv protocol compared to Ov protocol (1.84 vs 1.60, $P < 0.05$) suggests that MOv protocol may be more effective on return to cyclicity rate than Ov treatment.

Various conception and pregnancy rates have been also reported in cows with ovarian cysts subjected to the Ovsynch or progesterone included protocols. However, pregnancy rate is an important parameter to evaluate the success of treatment protocols in cows with ovarian cyst. In the present study, Ov protocol showed higher conception rates in cows with follicular (55%) and luteal cysts (71%) than those observed by Probo *et al*. [35] who found 40% for follicular and 40.7% for luteal using single GnRH infection. This difference is likely due to the fact that cows in that study were observed for estrous 30 days. Nevertheless, in the present study, Ov protocol displayed similar pregnancy rates for either follicular (44%) and luteal cysts (43%) or for overall ovarian cysts (44%) which were similar to Ambrose *et al*. [2] who detected as 41%. In progesterone included protocols, the conception rate varies 18% for follicular and 28% for luteal cysts [12] and 20% for ovarian cyst using PRID inserted for 12 days [40] and 27.3% [5] and 23.1% [10] for ovarian cyst using CIDR inserted for 7 days. However, the conception rates are 52.3% for follicular cyst using Ovsynch protocol combined with CIDR [26], whereas pregnancy rates vary 53.3% for follicular [25] cyst and 37.5% ovarian cyst using Ovsynch protocol combined with CIDR [5]. In the present study, although conception rates were found to be as higher than above mentioned studies for follicular (89%) and luteal (63%) or overall ovarian cysts (76%), the pregnancy rates were similar as other studies.

CONCLUSIONS

In conclusion, administration of Ovsynch protocol or Ovsynch protocol combined with exogenous progesterone resulted in the ovulation of follicle following either in response to second GnRH injection

or following GnRH injection and PRID removal. It seems that Ovsynch treatment combined with PRID inserted for 7 days has greater pregnancy rates for ovarian follicular cyst, however overall pregnancy rates are similar for both treatment models. It is suggested that new strategies are needed to enhance embryo survival due to possibility of late embryonic loss.

SOURCES AND MANUFACTURERS

[1]6.0-8.0 MHz, Falco Vet 100, Maastrich, The Netherlands.

[2]0.021 mg buserelin acetate, im; Receptal; Intervet, Turkey.

[3]526 µg cloprosterol sodium, im; Estrumate; Intervet, Turkey.

[4]1.55 g progesterone, intravaginal insert; PRID-DELTA; CEVA, Turkey.

[5]Elecsys Progesterone II, cobas e, Roche Diagnostics GmbH, Germany.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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