Iranian Journal of Veterinary
Science and Technology

Received: 2021- Apr- 20
Accepted after revision: 2021-May- 29
Published online: 2021- Jul- 21

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2021.69741.1034

Evaluation of hormonal treatments for different scenarios of cystic ovarian follicles in dairy cattle

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ABSTRACT

The present study aimed to evaluate the efficacy of different hormonal interventions in the treatment of cystic ovarian follicles (COF) based on different scenarios, including the size of the cyst and the presence of other follicles on the ovaries of dairy cows. A total of 199 Holstein cows with COF in the first 100 days postpartum were enrolled in the study. These cows were randomly assigned to the four following groups: 1) GnRH (G) group: intramuscular (IM) injections of 100 µg gonadorelin acetate on day 0 and 150 µg d-cloprostenol 7-12 days later, 2) double GnRH (DG) group: two IM injections of 100 µg gonadorelin acetate at 6 h intervals on day 0 and d-cloprostenol 7-12 days later, 3) intravaginal progesterone device (IPD) group: insertion of PRID Delta for 7-12 days and injection of d-cloprostenol on the withdrawal of PRID Delta, and 4) control group: IM injection of 2 mL sterile saline on day 0 and 7-12 days later. The cure rate of COF significantly improved in the G and DG groups, in comparison with the IPD and control groups. There was no significant difference between the cows in the G and DG groups. In the control group, animals with ovarian cysts smaller than 2.5 cm had a significantly greater self-cure rate, compared to the other cows. In conclusion, this field study demonstrated a good clinical cure in the groups of cows treated by GnRH. However, no improvement was observed in the reproductive performance of these animals.

Keywords

dairy cattle; GnRH; progesterone; cystic ovarian follicles

Number of Figures: 0
Number of Tables: 6
Number of References: 35
Number of Pages: 7

Abbreviations

COF: Cystic ovarian follicle
GnRH: gonadotropin-releasing hormone
LH: luteinizing hormone
P4: progesterone
PGF2α: Prostaglandin F2α
IPD: Intravaginal progesterone device
BCS: body condition score
DIM: Days in milk.

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Introduction

Cystic ovarian follicles (COFs) have been defined as follicles of at least 2 cm in diameter on one or both ovaries without any active luteal tissue that disrupts normal ovarian function. The COF, as a reproductive disorder, is a serious economic problem with a high incidence rate ranging from 16.3% to 30.3% during the first 9-10 weeks post-partum in dairy cows worldwide [1, 2, 3]. In a study by Sakaguchi et al., 80% of COF cases were detected before the first ovulation and 20% before the second or third ovulation [4, 5].

Cysts developing from pre-ovulatory follicles, which are unable to ovulate, persist and interfere with normal ovarian function [6]. The COF results from a "hormonal imbalance" within the hypothalamic-pituitary-gonadal axis. The defective surge of gonadotropin-releasing hormone (GnRH) by the hypothalamus occurs due to impaired response to follicular estradiol [6]. This failure in GnRH surge leads to the decreased stimulation of the anterior pituitary gland for surging pre-ovulatory luteinizing hormone (LH). This hypothalamic insensitivity to estradiol may result from the intermediate concentration of progesterone (P4), which is common in cows with COF [7, 8, 9, 10, 11, 12]. Moreover, it has been shown that higher milk production in dairy cattle is associated with an increased risk of ovarian cysts [4]. Several hormonal and metabolic modifications that are associated with negative energy balance can affect the function of the ovaries. However, the exact underlying mechanism of ovarian cysts is still unclear [2].

Although the efficacy of different hormonal treatments has been evaluated in various studies, no criteria have been considered for treatment decisions. Therefore, given the high treatment costs, it seems necessary to adopt targeted selective treatments. It seems that the concurrent ultrasonographic examination of ovaries with conventional therapies, such as GnRH administration, intravaginal P4 devices, and hormonal combinations may eliminate some of the ambiguity in findings.

Under field conditions, GnRH injection is the treatment of choice. The GnRH and its analogs have been considered for COF treatment with variable success rates irrespective of the type of the cyst, lack of antigenic effects, and cost of the treatment [6,13,14]. Following GnRH treatment, LH level reached a peak in 2 h and remained high in the blood for 6 h, while normally, the LH concentration remains elevated for 12 h [15]. In general, three weeks after GnRH administration, a resumption of the normal estrous cycle is observed in 60%-95% of the treated cows followed by the pregnancy rates of 60%-85%. Furthermore, COF can be treated by intravaginal P4-releasing devices. The success of this method is high, as 70%-85% of cystic cows recover within two weeks [16]. Prostaglandin F2α (PGF2α) or its analogs are used to treat luteal cysts [17]. In addition, this treatment can be combined with GnRH/hCG to treat follicular cysts and reduce the duration of the luteal phase [18]. Various sources have provided information on the risks following the use of hormonal products in livestock and their impact on health, fertility, and the livestock economy. Consequently, a proper and minimal selection of hormones is required for treating various conditions, such as ovarian cysts [19].

With this background, this study aimed to evaluate the success of different treatments, including GnRH and intravaginal P4 devices for ovarian cysts based on distinct ovarian structures and the size of ovarian cysts by using ultrasound examination. Moreover, we compared the efficacy of conventional treatments with two doses of GnRH injected at an interval of 6 h.

Results

Out of 221 Holstein cows, 199 cows were included in the present study. Twenty-two cows were excluded from the study, due to high progesterone concentration (> 1 ng/ml) at the first ultrasonographic exam. At the beginning of the study there were no significant differences in the DIM, parity, milk yield, and BCS of different groups. Moreover, the treatment groups did not have a significant difference in terms of the size of the cyst and follicle.

The cure rate was significantly different between the test and control groups ($p = 0.0001$). The cure rate of the ovarian cyst was significantly higher in the G and DG groups, compared to the PRID Delta group ($p < 0.05$) (Table 1). No significant difference was found between cows that received single or double injections of GnRH.

Table 1.
Total cure rates in treatment groups.

Group	N	%Cure rate (n)
G	49	83.67 (41)^{a}
DG	50	82.00 (41)^{a}
IPD	48	64.58 (31)^{a,b}
C	52	46.15 (24)^{c}

G: GnRH, DG: Double GnRH, IPD: PRID Delta, C: Control; different superscripts in columns indicate significant differences ($p < 0.05$).

Cyst size significantly affected treatment outcomes in all test groups ($p < 0.05$) (Table 2). The results showed that 53 out of 68 cows (77.94%) with a COF diameter between 2 to 2.5 cm, and 84 out of 131 animals (64.12%) with a COF diameter >2.5 cm were treated.

Table 2.
Total cure rates based on cyst size.

Cyst size (cm)	N	%Cure rate (n)
≤2.5	68	77.94 (53)^{a}
>2.5	131	64.12 (84)^{b}

Different superscripts in columns indicate significant differences ($p < 0.05$).

In cases with cysts greater than 2.5 cm in diameter, there was no statistically significant difference among treatment groups. However, in the DG group, the cure rate was numerically higher. The cure rate of cysts ≤2.5 and >2.5 cm in diameter was not significantly different between treatment groups. In the control group, cows with ovarian cysts ≤2.5 cm had a significantly higher self-cure rate compared to other cows ($p < 0.05$) (Table 3).

There was no significant difference between the groups, regarding cure rate and follicular size. In the control group, the cure rate was significantly greater in cows with follicular size ≥1 cm in diameter, compared to animals with smaller follicles ($p < 0.05$) (Table 4).

Table 3.
Percent of cure rates based on cyst size in different groups.

Group	N	Cure rate (%)	
		Cyst size ≤ 2.5cm	Cyst size >2.5cm
G	49	21/23 (91.30%)	20/26 (76.92%)^{a}
DG	50	11/13 (84.62%)	30/37 (81.88%)^{a}
IPD	48	10/16 (62.50%)	21/32 (65.63%)^{a}
C	52	11/16 (68.75%)	13/36 (36.11%)^{b}

G: GnRH, DG: double GnRH, IPD: PRID Delta, C: control; different superscripts in columns show significant differences ($p < 0.05$).

Overall, the difference in the treatment response, including the ovulation of the cyst, ovulation of the follicle, and luteinization or regression of the cysts was significant among treatment groups, ($p < 0.05$) (Table 5). The first service conception rate and the percentage of pregnant cows on 120 DIM were not significantly different among the groups (Table 6).

Table 4.
Total cure rates based on follicular size.

Group	N	Cure rate (%)	
		Follicular size < 1^{cm}	Follicular size ≥1^{cm}
G	49	19/22 (86.36%)	22/27 (81.84%)
DG	50	15/19 (78.95%)	26/31 (83.87%)
IPD	48	19/26 (73.08%)	12/22 (54.55%)
C	52	4/20 (20.00%)^{a}	20/32 (62.50%)^{b}

G: GnRH, DG: double GnRH, IPD: PRID Delta, C: control; different superscripts in the rows show significant differences ($p < 0.05$).

Discussion

In the present study, different hormonal interventions were evaluated for treating COF based on varying scenarios that occurred on the ovaries of dairy cows. Cyst wall diameter evaluated by ultrasound was highly correlated with milk P4 concentration with an accuracy range of 75%–95% as reported by various authors [20, 16, 21, 22]. The cows in G, DG, and IDP groups showed significantly better results than the control group. The overall treatment results in the G and DG groups were significantly better than the IPD group. GnRH is used to induce a new follicular wave, luteinize the cyst, and/or ovulate the follicle at the same time [23]. GnRH induces LH release leading to the highest plasma LH concentration in 90-150 min, which initiates the formation of active luteal tissue as shown by elevated serum P4 levels 7 days post-treatment [24, 25]. The concentration of blood LH depends on the dose of injected GnRH and reaches the peak level in 2 h after injection. The LH concentration decreases after 4 h. Based on reports, the range of LH surge is directly related to the success of treatment [25].

A success rate of 72% was observed by applying GnRH as the treatment, while the average cyst diameter was 2.5 cm and the cysts did not ovulate after GnRH injection [6]. In another study, ovarian cysts were treated with the Ovsynch program causing a new follicular wave in 100% of the cases due to GnRH injection, and a dominant follicle formed and ovulated in 83% of cases [1]. However, the efficacy of GnRH treatments is controversial in different studies and in some studies, there was no difference in ovarian response between treated and untreated animals [26].

Dinsmore et al. showed that the concomitant injection of GnRH and PGF2α have no advantage over GnRH alone [27], while another study demonstrated that cows received GnRH alone, experienced a higher rate of adverse effects [21].

In a study by Kawate (2011), the simultaneous

Table 5.
Treatment response (ovulation of the cyst, ovulation of the follicle, and luteinization or regression of the cysts) in different treatment groups.

Group	Cured (n)	% Ovulation of the cyst (n)	% Ovulation of the follicle (n)	% Luteinization or regression (n)
G	41	58.54[a] (24)	24.39[a] (10)	17.07[a] (7)
DG	41	43.90[a] (18)	41.46[a] (17)	14.63[a] (6)
IPD	31	22.58[b] (7)	35.48[a] (11)	41.93 (13)
C	24	16.67[b] (4)	66.67[b] (16)	16.67[a] (4)

G: GnRH, DG: double GnRH, IPD: PRID Delta, C: control; different superscripts show significant differences ($p < 0.05$).

Table 6.
First service conception rate and percentage of pregnant cows on 120 DIM in different treatment groups.

Group	N	FSCR % (n)	% Pregnant cows on 120 DIM (n)
G	46	36.96 (17)	58.69 (27)
DG	48	37.50 (18)	56.25 (27)
IPD	47	44.68 (21)	61.70 (29)
C	50	34.00 (17)	56.00 (28)

G: GnRH, DG: double GnRH, IPD: PRID Delta, C: control

application of controlled internal drug release with GnRH was evaluated in a 9-day protocol. The results of this study showed no advantage for the combination of intravaginal P4 and GnRH in treating ovarian cysts, compared to GnRH alone as an Ovsynch protocol [20].

The cure rate of the cysts using GnRH in this study was similar to other studies. In most studies, the effect of GnRH on the treatment of ovarian cysts was assessed based on two criteria, ovulation of a follicle in the presence of the cyst, and/or luteinization of the cyst wall. In this study in addition to these criteria, another therapeutic response was observed. In 53 cases out of 137 treated cows (38.68%), the cyst ovulated. In 40 out of 53 cases, cyst diameter at the time of treatment was ≤ 2.5 cm and ovulation of the cysts was significantly higher in cases with a cyst diameter of ≤2.5 cm. The ovulation of COF was observed in all treatment groups and was the most observed treatment response in G and DG groups (58.54% and 43.9%, respectively). The difference between the results of this study and those of others might be due to the lack of the ultrasonographic evaluation of ovarian structure during treatment and follow-up in some studies, the difference in therapeutic responses, and the variations in ovarian cyst definition.

Ovarian cysts previously were defined as follicular structures >2.5 cm in diameter, while in recent definitions, cysts are known as follicles with diameters >2 cm, even >1.7 cm [3, 28].

A study on the effect of PRID Delta in the treatment of ovarian cysts found that 83% of cows with follicular cysts responded to treatment within 14 days of starting treatment and developed a corpus luteum [29]. In the current study, the cure rate after treatment with PRID Delta was 64% which was significantly higher than the control group and lower than the G and DG groups, and ovulation of the follicle was the most treatment response in this group.

The P4 implant treatment may disrupt the endocrine environment required for maintaining COFs leading to their regression [30]. The P4 acts against follicular cysts by restoring hypothalamic responsiveness to estradiol-positive feedback resulting in normal estrus and ovulation seven days after implant removal.

This study aimed to make more specific therapies based on the characteristics of the ovarian structures, such as cyst and follicular size at the time of treatment. A higher cure rate was observed in G and DG groups, in comparison with the animals in PRID and control groups irrespective of the cyst size and presence or absence of follicle >1 cm in diameter. It was shown that GnRH was the most effective and the best choice for the treatment of cysts of any size. The cure rate for cysts ≤2.5 cm in diameter was higher in all groups. In the control group, the self-cure rate of cystic structures was 46.15% (24/52) and the self-cure rate of ovarian cysts ≤2.5 cm in diameter was significantly higher than that of ovarian cysts >2.5 cm in diameter. So it was better not to treat the cows with cysts ≤2.5 cm in diameter prior to 50 DIM, which concomitantly had follicle(s) >1 cm on their ovaries because the self-cure rate was very high in these cases (63.63%). It may be due to the inactivity of follicular cysts, which do not produce estrogen and the new follicle was allowed to grow up to 1 cm in diameter.

Morrow et al. (1969) showed that the self-cure rate of the cysts that developed within 45 days postpartum was about 50% [31]. In a study by López-Gatius (2002), 80% of cows with ovarian cysts in their first lactation were spontaneously cured, while this rate was 30% for older cows [21]. In the present study, the spontaneous cure rate was not significantly different between the primiparous and multiparous dairy cows (40% and 47.61%, respectively).

No significant improvement in reproductive performance (first service conception rate and percentage of pregnant cows on 120 DIM) was observed among different groups. This may be due to various individual and environmental factors that affect the success of different hormonal therapy and fertility [32, 33, 14].

Materials & Methods

Animals and Study Design

The experiment was conducted in a dairy herd with approximately 900 lactating Holstein cows in Mashhad, northeast of Iran. The sample size was calculated based on the study performed by Kim et al. [35], Cows were milked thrice daily with average daily milk production of about 38 kg. The animals were housed in freestall barns with sand bedding and were fed a total mixed ration. The voluntary waiting period in this herd was 45 days. A total of 199 Holstein cows up to 100 days in milk (DIM) with ovarian cysts were enrolled in the present randomized controlled trial.

Cystic cows were identified after the first ultrasound examination (real-time linear array, 7.5 MHz rectal probes, Easi-Scan, Scotland, UK). In order to confirm the diagnosis, a second ultrasound scan was performed 7-12 days after the first one. Cows with follicles larger than 2 cm in diameter in the absence of corpus luteum in both examinations were identified as COF. Finally, the treatments were carried out for 199 cows. The pattern of ovarian structure, including the size and number of cysts and follicles in the ovaries, was assessed in the second ultrasound scan. Afterwards, the cows were randomly assigned to the four following groups: 1) GnRH (G) group: an IM injection of 100 μg gonadorelin acetate (Gonasyl; Syva, Spain) on day 0 and 150 μg d-cloprostenol (Luteosyl; Syva, Spain) 7-12 days later, 2) double GnRH (DG) group: two IM injections of 100 μg gonadorelin acetate with 6 h interval on day 0 and d-cloprostenol 7-12 days later, 3) intravaginal progesterone device (IPD) group: (PRID Delta, Ceva, France) insertion for 7-12 days followed by PRID removal and simultaneous d-cloprostenol injection, and 4) control group: two IM injections of 2 mL sterile saline on days 0 and 7-12.

The third ultrasound examination was performed 7-12 days after the initiation of treatment in G, DG, and control groups. In the IPD group, the third examination was carried out 7-12 days after PRID removal. In all examinations, changes at the ovarian level were recorded in detail. The cases were considered as treated based on a set of criteria, including the presence of corpus luteum, cyst wall luteinization, cyst regression, and estrus following PRID Delta removal.

Other inclusion criteria in the current study entailed not being affected by concomitant diseases, such as lameness, endometritis and the lack of other concurrent ovarian problems, like adhesion and tumor. At the time of enrolment, the body condition score (BCS) of cows was recorded by a single evaluator based on a scale of 1 to 5 in increments of 0.25 [34]. Data on milk yield was utilized to assess the effects of milk yield on different treatments.

Milk Samples and Laboratory Analysis

Milk samples were collected into 9-mL evacuated tubes at the time of enrolment (on the day of cyst diagnosis) and on the day of PGF2α injection in the 1st and 2nd treatment groups and 7-12 days after PRID removal in the 3rd group and on the day of the last injection in the control group. The milk samples were chilled on ice packs immediately after collection, frozen at -20°C within 2 h, and delivered to the laboratory for further analysis.

Progesterone concentration of milk samples was measured using a solid-phase radioimmunoassay commercial kit (Progesterone RIA KIT; Institute of Isotopes Co. Ltd.; Hungary) by a RALS Gamma Counter instrument (Dream Gamma-10; Shinjin Medics Inc.; South Korea) according to the guidelines of the manufacturer [7]. Cases with milk P4 concentration of 2 ng/mL were considered to have active luteal tissue. Ultrasound scan and P4 concentration were used to assess therapeutic response on the day of PGF2α injection. Presence of a corpus luteum and luteinization or regression of the cyst are considered as criteria for successful treatment. The presence of luteal tissue was confirmed by measuring P4 concentration in milk concurrent with the third ultrasonic examination in G, DG, and control groups. However, the measurement of P4 concentration in the IPD group was performed 7-12 days after PRID removal.

Data Management and Statistical Analysis

All analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). The relationship between treatment regimens and different outcomes, including cure rate, percentage of pregnant cows by 120 DIM, first service conception rate, cyst size, and follicular size were analyzed by using Chi-square test with PROC FREQ. Parity, BCS, DIM, and milk production were considered as covariates in the models. The variables were removed by manual backward stepwise elimination if $p > 0.2$. Finally, the relationships between the factors were assessed using multivariable logistic regression as PROC LOGISTIC modeling through a backward selection model. Cows that had a BCS of <3 were classified as thin, a BCS of 3.25 or 3.5 as ideal, and a BCS of > 3.75 as fat. Based on parity, the cows were classified into two groups of primiparous and multiparous cows. Dummy variables were created for DIM (1: DIM ≤ 45 and 2: DIM > 45), milk yield (1: ≤ 40 and 2: > 40), cyst size (1: cysts of ≤ 2.5 cm and 2: cysts of

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DOI:10.22067/ijvst.2021.69741.1034

> 2.5 cm), and follicular size (1: follicles of ≤1 cm and 2: follicles of > 1 cm). To determine the degree of relationship between the risk factors and outcome variables, the odds ratio, and 95% confidence interval were calculated. For all statistical analyses, $p < 0.05$ was considered significant.

Authors' Contributions

N.F., H.S. and M.H. conceived and planned the experiments. M.H. and N.F. carried out the experiments. H.S., M.H. and N.F. contributed to the interpretation of the results. M.H. and N.F took the lead in writing the manuscript.

Acknowledgments

We would like to thank the manager and all employees of Quds Razavi Industrial Livestock Institute (Keneh Bist Dairy Farm), especially Dr. Mostafa Sekhavati for providing excellent technical help during this study. This study was funded by Ferdowsi University of Mashhad (Project No. 3/39636).

Competing Interests

The authors declare that there is no conflict of interest.

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How to cite this article
Haddadi, M, Seifi, HA, Farzaneh, N (2021). Evaluation of hormonal treatments for different scenarios of cystic ovarian follicles in dairy cattle. *Iran J Vet Sci Technol.* 13(1): 48-54.
DOI: 10.22067/ijvst.2021.69741.1034
URL: https://ijvst.um.ac.ir/article_40079.html