

Hormonal Profiles Associated with Treatment of Cystic Ovaries with GnRH and PGF2 α with and Without CIDR in Dairy Cows

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Abstract

This study aimed to investigate ovarian and endocrine responses associated with treatment of cystic ovaries with GnRH, PGF2 α , and +/-Progesterone-CIDR placement; and to determine pregnancy establishment following synchronization and timed artificial insemination in cows suffering from cystic ovaries. Ovarian cysts were typed rectally and ultrasonographically into follicular, luteinized and persistent types. Two schedules of treatment were designed, in Schedule-A, 15 cows with follicular (n=7), luteinized (n=3) and persistent (n=5) cysts were used. The cows received 2 injections of 100 ug GnRH (9 days interval) with 25 mg PGF2 α administered 7 days after 1st GnRH dose. In Schedule-B, 14 cows with follicular (n=6), luteinized (n=3) and persistent (n=5) cysts were used. The cows administered GnRH and CIDR concurrently, then 25 mg PGF2 α 7 days later. The CIDR was removed 2 days after PGF2 α . Cows were inseminated 16 hours after 2nd GnRH dose. Blood samples were collected to assay progesterone and estradiol levels. In schedule-A, 57.1%, 33.3% and 40% of follicular, luteinized and persistent cysts were ovulated after 1st GnRH injection, while a total of 100%, 66.7% and 100% were ovulated after 2nd GnRH dose. The cows with follicular cysts appeared the highest rate of pregnancy (57.1%) followed by the luteinized and persistent cysts (33.3% and 40%). Of a total 15 cystic-treated cows, 7 were confirmed pregnant (46.7%). The concentration of progesterone was significantly (P<0.05) higher at 1st GnRH injection, then decreased after PGF2 α injection and then increased again at 7 day after insemination. In schedule-B, after 1st GnRH dose, 66.7% of follicular, 0% of luteinized cysts and 80% of persistent were ovulated; with a 57.1% total ovulation rate. Following CIDR removal, 85.7% (12/14) cows ovulated the recruited follicles and developed a corpus luteum. Of a total 14 cystic-treated cows, 8 were confirmed pregnant (57.1%). Plasma estradiol declined following intra vaginal placement of CIDR, but markedly increased one day after removal. Progesterone decreased after CIDR removal, but increased again at presence of palpable corpus luteum. The developed corpus luteum and pregnancy rate were higher in schedule-B (+CIDR) than in schedule-A (-CIDR). *In conclusion*, treatment of cows with cystic ovaries with GnRH followed by PGF2 α 7 days later resulted in recruitment of new healthy follicles, synchronization of ovulation and a marked pregnancy rate (with than without CIDR).

Key words: Cystic, Ovaries, Estrus, follicles, Pregnancy, Synchronization.

INTRODUCTION

Bovine ovarian cysts are follicles that fail to ovulate at the time of oestrus. Cystic ovaries are a major cause of infertility and economic loss to the dairy industry, and occurred in 10-13% of the dairy cows [6]. The predisposing factors leading to the occurrence of cystic ovarian follicles (COF) are: high milk yield, hot season, stress and negative energy balance [12, 21]. The absence of the preovulatory surge of GnRH and LH are responsible for the development of COF. This phenomenon may be the result of hypothalamic insensitivity to the estradiol surge caused by inadequate exposure to progesterone [7]. The relationship between circulating progesterone and COF has been documented [8]. Moreover, the presence of COF is detrimental to fertility and some practioners may resort to manual rupture of the cystic structures, because this is still a documented form of treatment [21]. Some hormones such as GnRH, hCG, LH and progesterone are frequently used to treat this condition [26], but the outcomes are variable. Some recent protocols for synchronization of ovulation, commonly referred as ovsynch, followed by timed artificial insemination (AI) performed 16-20 h after the 2nd GnRH injection yields pregnancy rates of

about 25% in cows suffering from COF [2, 5, 15]. The response to treatment with GnRH, PGF2 α and GnRH were not fully documented. Diagnosis of a COF is based on the palpation per rectum of an abnormally large (≥ 20 mm) follicle and no corpus luteum, verified by transrectal ultrasonography [2]. The previous authors found that after the 1st GnRH injection, new existing follicle ovulated, and new follicles developed in cows then ovulated within 48 h of the 2nd GnRH injection, confirmed by the development of a CL.

This study aimed to, (a) assess the ovarian and endocrine responses in dairy cows affected with cystic ovarian follicles when a standard ovsynch protocol or coordinated sequence of treatments with GnRH were used in the presence of controlled internal drug release (CIDR), and (b) determine the pregnancy rates following ovsynch and timed AI in dairy cows affected with cystic ovarian follicles.

MATERIAL AND METHODS

Animals

The animals used in this study belonging to a private large dairy farm at Dakahlia Governorate, they were 4-8 years old

and were milked three times daily. A total of 29 Holstein dairy cows suffering from cystic ovarian follicles (one or more spontaneously-developed COF) were used. The cows were divided into 2 groups based on the type of treatment. The first group included 15 cows (Schedule-A), while the second included 14 cows (Schedule-B).

Classification of cows according to the type of ovarian cysts

The cows were classified into 3 types of ovarian follicular cysts according to hormonal profiles and accompanied ovarian structures [6] based on rectal and ultrasonographic scanning (B-mode System; Pie-Medical Scanner-240 with 6-8 MHz linear rectal probe [29];

1. Cows with at least one large follicular structure (≥ 20 mm), no CL, low progesterone (< 1 ng/ml at day -10) and high estradiol concentrations were classified as having follicular cyst.
2. Cows with at least one large follicular structure with luteinization texture, no CL, high progesterone (> 1 ng/ml) and low estradiol concentrations were classified as having luteinized cyst.
3. Cows with at least one large follicular structure and detectable CL with high progesterone (> 1 ng/ml) and low estradiol concentrations were classified as having persistent cyst.

Schedule-A

In this experiment, 15 cystic Holstein dairy cows were used; including 7 follicular, 3 luteinized and 5 persistent cysts. The cows received 100 ug of GnRH (Fertagyl, Intervet), 25 mg of PGF 2α 7 days later and a 2nd dose of GnRH after 2 days. The cows were artificially inseminated approximately 16 hours after the 2nd GnRH treatment without oestrus detection. The ovaries were palpated rectally to confirm the presence of CL (at 7 days after AI). Pregnancy was diagnosed per rectum and confirmed ultrasonographically 40-45 days after AI.

Schedule-B

In this experiment, 14 cystic Holstein dairy cows were used. At the end of the pretreatment period, cows received 8 ug of GnRH-agonist, Buserelin acetate (Receptal, Hoechst AG, Frankfurt, Germany); a progesterone releasing device CIDR, 1.9 g (Carter-Holt-Harvey Plastic Products Group, Hamilton, New Zealand) was placed intra vaginally. Seven days later, cows

received 25 mg of PGF 2α . At the end of the 9-day treatment period, the CIDR device was removed and cows were observed for oestrus twice daily. Pregnancy was diagnosed per rectum and confirmed ultrasonographically 40-45 days after AI.

Blood samples and hormonal assay: Blood samples were collected from the jugular vein in venipuncture tubes. Plasma was separated within 6 hours by centrifugation and stored at -20°C until hormonal assay:

1. In schedule-A, progesterone was assayed by RIA [10] at day -10 (1st GnRH injection), day -3 (PGF 2α injection), day 0 (at AI) and 7 days after AI.
2. In schedule-B, progesterone and estradiol were assayed in peripheral plasma by RIA [1, 10]. The samples were collected pretreatment 10 days before GnRH, 5 days before GnRH, one day before GnRH or CIDR placement, at day of GnRH or CIDR placement, one day after CIDR, 2-3 days (oestrus) after CIDR and when palpable CL can be detected.

Statistical analysis

Statistical analysis system (SAS) was used to perform a repeated measures analysis of variance [11] for progesterone. The analysis included the type of cysts, time of sampling as a repeated effect, and the interaction of type of cyst and time of sampling.

RESULTS

Schedule-A

The rectal examination revealed 15 cows suffering from cystic ovarian follicles, 7, 3 and 5 cows were diagnosed with follicular, luteinized and persistent cysts, respectively (Table 1). The progesterone concentration was influenced by the time of sampling ($P < 0.01$) and the interactions between type of cystic ovarian follicle and time of sampling ($P < 0.01$). The concentration of progesterone was significantly higher at 1st GnRH injection, and then decreased after PGF 2α injection (13 day). At the 0 day (day of insemination), the concentrations were not variable between types of cysts, and then increased again at 7 day (after insemination).

The ovarian responses to the ovsynch treatment and pregnancy outcome are presented in Table 1. Although none of the cystic ovarian follicles ovulated, by 7 days after GnRH treatment, the luteinized structures resembled a large CL were

Table 1. The effect of treatment with GnRH and PGF 2α (Schedule-A) in dairy cows suffering from follicular, luteinized and persistent ovarian cysts.

Type of cyst	Reproductive observations					
	Ovulation after 1 st GnRH	New follicles after 1 st GnRH	Ovulation after 1 st GnRH	Ovulation after 2 nd GnRH	New developed CL	Pregnancy 40-45 days after AI
Follicular n=7	0/7 (0.0)	7/7 (100)	4/7 (57.1)	7/7 (100)	6/7 (85.7)	4/7 (57.1)
Luteinized n=3	0/3 (0.0)	3/3 (100)	1/3 (33.3)	2/3 (66.7)	2/3 (66.7)	1/3 (33.3)
Persistent n=5	0/5 (0.0)	5/5 (100)	2/5 (40.0)	5/5 (100)	4/5 (80.0)	2/5 (40.0)
Total n=15	0/15 (0.0)	15/15 (100)	7/15 (46.7)	14/15 (93.3)	12/15 (80.0)	7/15 (46.7)

GnRH-Gonadotropin-Releasing Hormones; CL-Corpus Luteum; AI-Artificial Insemination

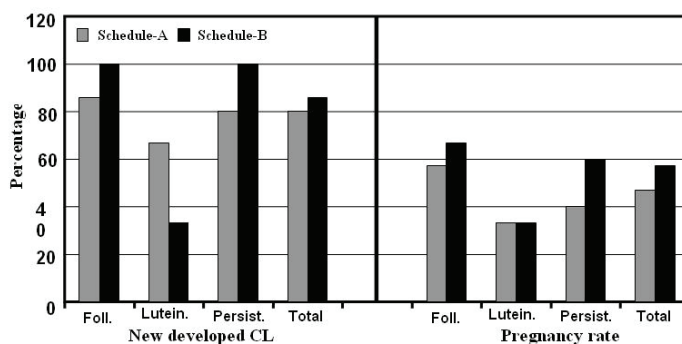


Figure 1. The new developed CL and pregnancy rates, when schedule-A (after 2nd GnRH injection) compared to schedule-B (after 2nd GnRH injection and CIDR removal) in the cows affected with follicular, luteinized and persistent ovarian cysts.

determined. All the cows developed a new follicle after 1st GnRH injection, from which 57.1%, 33.3% and 40% of follicular, luteinized and persistent cyst were ovulated, respectively. A new dominant follicle developed following 1st GnRH treatment in all cows. After the 2nd GnRH treatment, a total of 100%, 66.7% and 100% of the new follicles in cows with follicular, luteinized or persistent cysts were ovulated.

A new CL was confirmed 7 days after AI. Of the 15 cows that were inseminated, 12 (80%) developed a new CL and 7 (46.7%) were confirmed pregnant. The cows with follicular cysts appeared the highest rate of pregnancy (57.1%) followed by the luteinized and persistent cysts (33.3% and 40%, respectively).

Schedule-B

On the basis of hormonal level, of 14 cows in this trial, 6 were determined to have a follicular cyst (based on a high estradiol and a low progesterone profiles and the absence of any luteal tissue), 3 cows had a luteinized cysts (based on a low estradiol, and a high progesterone profile, and no luteal tissues) and 5 cows had a large CL cyst-like structure (based on a low estradiol and a high progesterone profiles) categorized as persistent cysts (Table 2).

Although none of the cystic ovarian follicles ovulated after 1st GnRH injection, 8 of the 14 cows ovulated after existing a new follicles, where 4/6 (66.7%) for follicular and 4/5 (80%) for persistent and 0% for luteinized cysts were ovulated. Additionally, all cows developed new follicles in response to GnRH injection. Following to CIDR removal, 12 of 14 cows ovulated the recruited follicles and developed

a CL, where all the cows with follicular and persistent cysts and none with luteinized cysts were ovulated. Of the 14 cows that were inseminated, 8 (57.1%) were confirmed pregnant. The cows with follicular cysts showed the highest rate (66.7%) of pregnancy followed by persistent cysts (60%) with low rate (33.3%) detected for luteinized cysts.

There was marked increase in the concentration of progesterone at the day or one day after GnRH injection or CIDR placement, then decreased at oestrus (2-3 days after CIDR). At the presence of palpable CL, the concentration elevated again. On the other hand, the concentration of estradiol decreased at the day of GnRH injection or CIDR placement but markedly increased one day after removal of CIDR, at oestrus (2-3 days after removal), the concentration was still higher than at the presence of a palpable CL.

The percentages of new developed CL as well as the pregnancy rate were higher in the cows of schedule-B (after 2nd GnRH injection or CIDR removal) compared to these cows in schedule-A (after 2nd GnRH injection alone) as illustrated in Fig. 1.

DISCUSSION

Reproductive efficiency in dairy herds increases by inseminating all the cows shortly after the end of the voluntary waiting period, obtaining high pregnancy rate to first service enhancing embryonic and fetal survival [19, 23]. In a previous study [2], the Ovsynch protocol was effective in cows with ovarian cysts. In the present study, pregnancy rates were higher

Table 2. The effect of treatment with GnRH and PGF2 α with CIDR placement (schedule-B) in dairy cows suffering from follicular, luteinized and persistent ovarian cysts.

Type of cyst	Reproductive observations					
	Ovulation after 1 st GnRH	New follicles after 1 st GnRH	Ovulation after 1 st GnRH	Ovulated new follicle after CIDR removal	New CL after CIDR removal	Pregnancy 40-45 days after AI
Follicular n=6	0/6 (0.0)	6/6 (100)	4/6 (66.7)	6/6 (100)	6/6 (100)	4/6 (66.7)
Luteinized n=3	0/3 (0.0)	3/3 (100)	0/3 (0.0)	1/3 (33.3)	1/3 (33.3)	1/3 (33.3)
Persistent n=5	0/5 (0.0)	5/5 (100)	4/5 (80.0)	5/5 (100)	5/5 (100)	3/5 (60)
Total n=14	0/14 (0.0)	14/14 (100)	8/14 (57.1)	12/14 (85.7)	12/14 (85.7)	8/14 (57.1)

GnRH-Gonadotropin-Releasing Hormones; CIDR-Controlled Intravaginal Device; AI-Artificial Insemination

for cows with ovarian cysts in the GnRH+Ovsynch protocol compared to cows in the Ovsynch protocol. Cows with ovarian cysts continue to have follicular waves [29]; therefore, the dominant follicle may or may not respond to GnRH treatment [9]. In the GnRH+Ovsynch protocol, cows received two doses of GnRH and may be more likely to have a CL at the time of PGF2 α . In the present investigation in which 2 different schedules were used, documented the recovery of cows diagnosed with cystic ovarian follicles. Even though none of the cows were ovulated a cystic ovarian follicle, 57.1% of the cows ovulated a follicle other than the cystic ovarian follicles that were present at the time of GnRH injection. Ovulation of a cystic ovarian follicle in response to GnRH treatment does not usually occur and has not been observed in other investigations [5, 6, 28]. However, luteinization of the cystic ovarian follicle following GnRH-treatment has been reported [6, 24, 28].

In the present study, development of a new follicle following GnRH treatment occurred in all cows in both schedules; this response is well documented for normal cycling cows that are treated with the ovsynch protocol [15, 18]. The effectiveness of the ovsynch and timed artificial insemination protocol in lactating dairy cows diagnosed with ovarian cysts has been reported [2, 5] with pregnancy rates of 26.9% and 23.6% (calculated based on a reported conception rate). The ovarian responses to the ovsynch treatment were either partially recorded or not recorded in these reports. By using a protocol similar to that used by the above authors, we have determined that all cows developed a new follicle in response to the 1st GnRH treatment and that a majority of these newly recruited follicles, subsequently ovulated. In the cows that were subjected to ovsynch and timed artificial insemination, a 46.7% (in schedule-A without CIDR) and 57.1% (in schedule-B with CIDR) pregnancy rates were achieved in the present study. The persistence of cystic ovarian follicles for prolonged periods has been recorded previously [6, 16], and it is highly unlikely that these have any negative influence on a new follicular development or pregnancy establishment.

Regardless the type of ovarian cyst, a new follicle developed following GnRH treatment in 100% of the cows examined. In a high percentage of cases, the newly developed follicle ovulated either in response to a 2nd GnRH treatments (93.3%; schedule-A) or spontaneously (85.7%; schedule-B) depending upon progesterone withdrawal. Cows with cystic ovarian follicles are known to have high plasma LH concentrations (Garverick, 1999) and increased LH pulse frequency [4]. Increased LH concentrations favor the development and persistence of large ovarian follicles [25], whereas treatment with exogenous progesterone induces atresia of persistent follicle [13, 14]. The later approach has been used successfully to reduce LH pulse frequency and induce turnover of cystic ovarian follicles in cattle by placing intra vaginal P4-releasing devices for either 9- [3, 17] or 14- [27] day periods. The results in this study and previous studies [4, 27] showed that the cystic ovarian follicles declined in size following insertion of CIDR. Associated with the insertion of a CIDR device and GnRH injection were an increase in progesterone and a concomitant decline in circulating estradiol concentrations. After 9 days of the CIDR removal, estradiol concentrations increased rapidly and in schedule-B all

cows come into oestrus within 3 days, and 12/14 (85.7%) cows ovulated spontaneously.

Base on the results of this study and those of others, we recommended that treatment of cystic ovarian follicles must focus on altering the endocrine milieu, such that it allows recruitment of a new follicle (by using GnRH) and induces the turnover of the cystic ovarian follicle (by using a progesterone device), preferably within an ovsynch-type protocol. The importance of ovsynch protocol in the treatment of cystic ovarian follicles is emphasized by the ability of GnRH given after CIDR withdrawal to induce ovulation of a newly recruited follicle in a follicular cystic cow that failed to ovulate spontaneously after CIDR withdrawal in a previous instance. The observation that 100% of the cystic cows developed a new follicle after GnRH injection, and 46.7% (without CIDR) or 57.1% (with CIDR) of cows conceived, indicating that the cystic ovarian follicles without approach influence the establishment of pregnancy.

In conclusion, the cystic cows administered GnRH (day 0), followed by PGF2 α (day 7), +/- CIDR; resulted in the recruitment of a new healthy ovarian follicle. The majority of the cases developed new follicles either in response to a 2nd GnRH treatment or spontaneously following CIDR withdrawal are ovulated resulting in 46.7% (schedule-A) or 57.1% (schedule-B) pregnancy rates. Thus, the incorporation of an intra vaginal progesterone-releasing device such as CIDR into ovsynch and timed artificial insemination program is recommended to increase the chances to treat the cases of cystic ovaries in dairy cows.

CONCLUSION

The cystic cows administered GnRH (day 0), followed by PGF2 α (day 7), +/- CIDR; resulted in the recruitment of a new healthy ovarian follicle. The majority of the cases developed new follicles either in response to a 2nd GnRH treatment or spontaneously following CIDR withdrawal are ovulated resulting in 46.7% (schedule-A) or 57.1% (schedule-B) pregnancy rates. Thus, the incorporation of an intra vaginal progesterone-releasing device such as CIDR into ovsynch and timed artificial insemination program is recommended to increase the chances to treat the cases of cystic ovaries in dairy cows.

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