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Hormonal profiles associated with treatment of cystic ovarian disease with GnRH and PGF2α with and without CIDR in dairy cows

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Citation

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Abstract

This study aimed to investigate the ovarian and endocrine responses associated with the treatment of cystic ovarian follicles with gonadotropin (GnRH) and prostaglandin (PGF2α) +/-Progesterone-CIDR placement; and to determine pregnancy establishment following synchronization and timed artificial insemination in cows suffering from cystic ovarian follicles. The ovarian cysts were typed based on the rectal and ultrasonic examination (B-mode System; Pie-Medical Scanner-240 with 6-8 MHz linear probe)into follicular, luteinized and persistent cysts. 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 PGF2α administered 7 days after 1st GnRH. 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 PGF2α 7 days later. The CIDR was removed 2 days after PGF2α. The cows were inseminated 16 hours after 2nd GnRH dose. Blood samples were collected from the jugular vein during the course of 2 schedules to assay the progesterone and estradiol. In schedule-A, a percentage of 57.1%, 33.3% and 40% of follicular, luteinized and persistent cysts were ovulated after the 1st GnRH injection, while a total of 100%, 66.7% and 100% were ovulated after the 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 significantly higher at 1st GnRH injection, then decreased after PGF2α injection 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, 12/14 (85.7%) 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. Plasma progesterone decreased after CIDR removal, but increased again at the 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 ovarian follicles with GnRH followed by PGF2α 7 days later resulted in recruitment of a new healthy follicles, synchronization of ovulation and resulted in a marked pregnancy rate with than without CIDR intra vaginal placement.

INTRODUCTION

Bovine ovarian cysts are follicles that fail to ovulate at the time of oestrus. Cystic ovaries is a major cause of infertility and economic loss to the dairy industry, and occurred in the dairy cows with 10-13% (Ganverick, 1999). From the predisposing factors leading to the occurrence of cystic ovarian follicles (COF) are high milk yield, season, stress and negative energy balance (Seguin, 1998; Lopez-Gatius et al., 2002). 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 (Gumen and Wiltbank, 2002). The relationship between circulating progesterone and COF has been docomented by Hatler et al. (2003). Moreover, the presence of COF is detrimental to fertility and some practitioners may resort to manual rupture of the cystic structures, because this is still a documented form of treatment (Seguin, 1998). Some hormones such as GnRH, hCG, LH and progesterone are frequently used to treat this condition (Thatcher et al., 1993), 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 2 nd GnRH injection yields pregnancy rates of about 25% in cows suffering from COF (Fricke and Wiltbank, 1999; Bartolome et al., 2000; Meyer, etal., 2007). The response to treatment with GnRH, PGF2 and GnRH were not fully documented. Diagnosis of a COF was based on the palpation per rectum of an abnormally large (≥20 mm) follicle and no corpus luteum, verified by transrectal ultrasonography (Bartolome et al., 2000). After the first GnRH injection, another existing follicle ovulated in 4 cows, and a new follicle developed in all cows then ovulated in all cows within 48 h of the 2 nd GnRH injection, and the development of a CL was confirmed in all cases.

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 an intra vaginal progesterone releasing device (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, 4-8 years old and milked three times daily. A total of 29 Holstein dairy cows suffering from cystic ovarian follicles (one or more spontaneously-developed COFs) 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 (Ganverick, 1999) based on rectal and ultrasonographic scanning (B-mode System; Pie-Medical Scanner-240 with 6-8 MHz linear rectal probe as performed by Yoshioka et al. (1996);

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.

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.

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 PGF2 7 days later and a 2 nd dose of GnRH after 2 days. The cows were artificially inseminated approximately 16 hours after the 2 nd 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 PGF2. 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 –20C until hormonal assay:

1-In schedule-A, progesterone was assayed by RIA (Knickerbocker et al., 1986) at day –10 (1 st GnRH injection), day –3 (PGF2 injection), day 0 (at AI) and 7 days after AI.

2-In schedule-B, progesterone and estradiol were assayed in peripheral plasma by RIA (Knickerbocker et al., 1986 and Badinga et al., 1992, respectively). 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 design analysis of variance (Little et al., 1991) 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. Least squares to the estradiol and progesterone of the pretreatment period were estimated by least squares analysis of variance (SAS, 1988).

RESULTS

SCHEDULE-A

The examination revealed 15 cows suffering from cystic ovarian follicles, 7, 3 and 5 cows were diagnosed as having 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 progesterone concentration of the three types of cysts is presented in Fig. 1. The concentrations were significantly higher at 1 st GnRH injection, then decreased after PGF2α injection (13 day). At the 0 day (day of insemination, the concentrations were not variable between types of cysts, 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 determined. All the cows developed a new follicle after 1 st 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 1 st GnRH treatment in all cows. After the 2 nd 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).

Figure 1

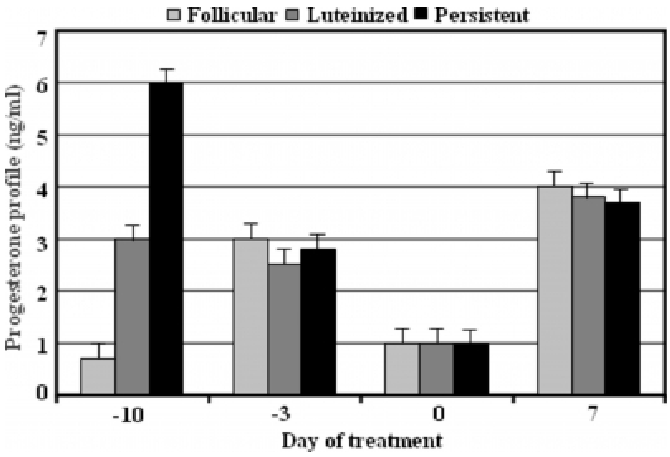
Table 1: The effect of treatment with GnRH and PGF2α (Schedule-A) in dairy cows suffering from follicular, luteinized and persistent ovarian cysts.

Type of cyst	Reproductive observations					
	Ovulation after 1st GnRH	New follicles after 1st GnRH	Ovulation after 1st GnRH	Ovulation after 2nd 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 2

Figure 1: Progesterone profiles (Mean +/- SEM) of cows classified as having follicular cyst, luteinized cyst or persistent cyst in schedule-A at the 1 GnRH injection (day Â– 10), at PGF2? (day Â– 3), at artificial insemination (day 0) and at 7 days after artificial insemination (day 7)



SCHEDULE-B

On the basis of hormonal level (Fig. 2), 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) were categorized as persistent cysts (Table 2).

Although none of the cystic ovarian follicles ovulated after 1 st 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 (Fig. 3).

Figure 3

Figure 2: Plasma concentrations (Mean±SEM) of progesterone and estradiol in the pretreatment period in dairy cows suffering from follicular, luteinized and persistent ovarian cysts (schedule-B).

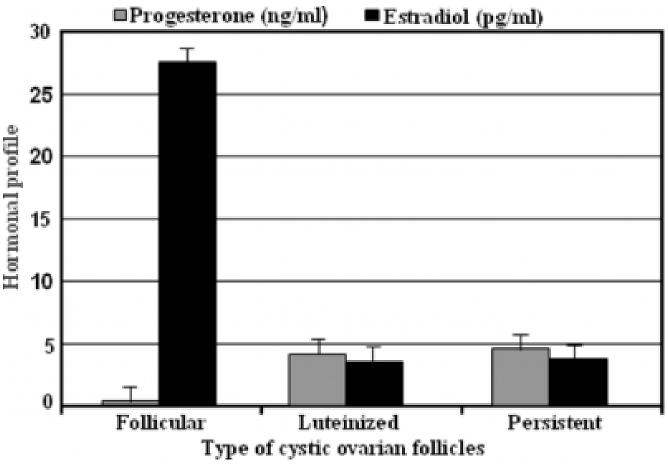


Figure 4

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

Type of cyst	Reproductive observations					
	Ovulation after 1st GnRH	New follicles after 1st GnRH	Ovulation after 1st 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

The percentages of new developed CL as well as the pregnancy rate were higher in the cows of schedule-B (after 2 nd GnRH injection or CIDR removal) compared to these cows in schedule-A (after 2 nd GnRH injection alone), as shown in Fig. 4. In the cows affected with the follicular and persistent cysts, the percentages of CL and pregnancy were higher in the schedule-B than schedule-A

Figure 5

Figure 3: Plasma progesterone and estradiol profiles (Mean±SEM) in the cow classified as having follicular cyst in schedule-B. the hormonal profile measured at days related to the GnRH and CIDR placement.

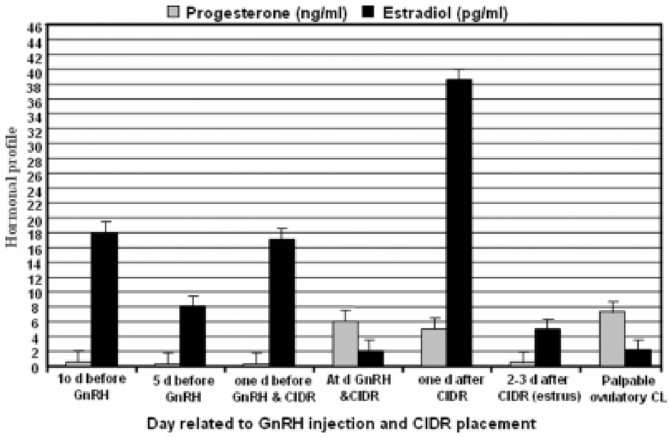
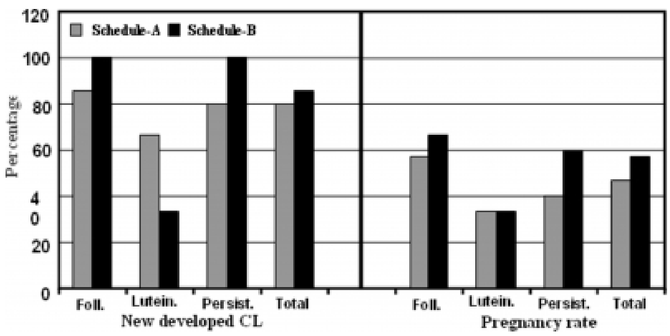


Figure 6

Figure 4: The new developed CL and pregnancy rates, when schedule-A (after 2 GnRH injection) compared to schedule-B (after 2 GnRH injection and CIDR removal) in the cows affected with follicular, luteinized and persistent ovarian cysts.



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 (Pursley et al., 1997; Stevenson et al., 1999). In a previous study [Bartolome et al., 2000], the Ovsynch protocol was effective in cows with ovarian cysts. In the present study, pregnancy rates were higher 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 (Yoshioka et al., 1996); therefore, the dominant follicle may or may not respond to GnRH treatment [Kesler et al., 1981]. In the GnRH+Ovsynch protocol, cows received two doses of GnRH and may be more likely to have a CL at the time of PGF2a. 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 ovulated a cystic ovarian follicles, 57.1% of the cows ovulated a follicle other than the cystic ovarian follicles that were present at the time og 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 (Garverick, 1999; Fricke and Wiltbank, 1999; Wiltbank et al., 2002). However, luteinization of the cystic ovarian follicle following GnRH-treatment has been reported (Garverick, 1999; Wiltbank et al., 2002; Stevenson et al., 2007).

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 (Pursley et al., 1995, Meyer et al., 2007). The effectiveness of the ovsynch and timed artificial insemination protocol in lactating dairy cows diagnosed with ovarian cysts has been reported by Fricke and Wiltbank (1999); Bartolome et al. (2000), 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 (Garverick, 1999; Noble et al., 2000), 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 (Calder et al., 1999). Increased LH concentrations favor the development and persistence of large ovarian follicles (Taft et al., 1996), whereas treatment with exogenous progesterone induces atresia of persistent follicle (Manikkam and Rajamahendran, 1997; McDowell et al., 1998). 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- (Peter, 1998; Bicalho et al., 2007) or 14- (Todoroki et al., 2001) day periods. The results in this study and previous studies (Calder et al., 1999; Todoroki et al., 2001), 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.

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. However, the observations that 100% of the cystic cows developed a new follicle after GnRH injection, and 46.7% (schedule-A) or 57.1% (schedule-B) of the inseminated cows conceived following ovsynch and timed artificial insemination; and in many of these cows; the cystic ovarian follicles persisted with no approach detrimental influence on the establishment or maintenance of pregnancy.

This study concluded that, the cystic cows administered GnRH (day 0), followed by PGF2α (day 7), +/- CIDR; resulted in the recruitment of a new healthy ovarian follicle.

The the majority of the cases developed new follicles either in response to a 2 nd 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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