

Hormonal Profiles Associated with Treatment of Cystic Ovarian Disease with GnRH and PGF2 α with and without CIDR in Dairy Cows

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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 (PGF2 α) +/-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 μ g GnRH (9 days interval) with 25 mg PGF2 α 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 PGF2 α 7 days later. The CIDR was removed 2 days after PGF2 α . The cows were inseminated 16 h 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 PGF2 α 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). So, treatment of cows with cystic ovarian follicles with GnRH followed by PGF2 α 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.

Key words: Cystic ovaries, estrus, synchronization, pregnancy, CIDR

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% (Garverick, 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 documented by Hatler *et al.* (2003). 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 (Seguin, 1998). Some hormones such as GnRH, hCG, LH and progesterone are frequently used to treat

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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 2nd GnRH injection yields pregnancy rates of about 25% in cows suffering from COF (Fricke and Wiltbank, 1999; Bartolome *et al.*, 2000; Meyer *et al.*, 2007). The response to treatment with GnRH, PGF2 α 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 2nd GnRH injection and the development of a CL was confirmed in all cases.

This study aimed to, 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 determine the pregnancy rates following ovsynch and timed AI in dairy cows affected with cystic ovarian follicles.

MATERIALS 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 3 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 (Garverick, 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 $^{-1}$ 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 $^{-1}$) 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 $^{-1}$) 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 μ g of GnRH (Fertagyl, Intervet), 25 mg of PGF2 α 7 days later and a 2nd dose of GnRH after 2 days. The cows were artificially inseminated approximately 16 h 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 μ g 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 α . 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 h by centrifugation and stored at -20°C until hormonal assay:

- In schedule-A, progesterone was assayed by RIA (Knickerbocker *et al.*, 1986) at day -10 (1st GnRH injection), day -3 (PGF2 α injection), day 0 (at AI) and 7 days after AI.
- In schedule-B, progesterone and estradiol were assayed in peripheral plasma by RIA (Knickerbocker *et al.*, 1986; 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 (Littel *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 1st GnRH injection, then decreased after PGF2 α 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 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 (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).

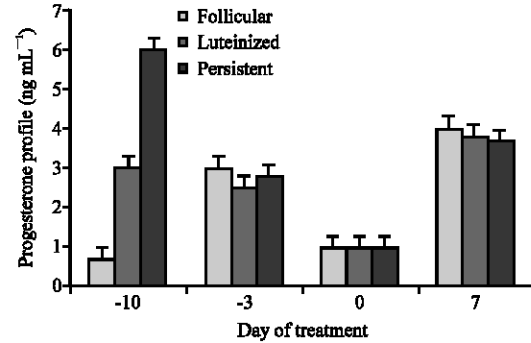


Fig. 1: Progesterone profiles (Mean \pm SEM) of cows classified as having a follicular cyst, luteinized cyst or persistent cyst in schedule-A at the 1st GnRH injection (day -10), at PGF2 α (day -3), at artificial insemination (day 0) and at 7 days after artificial insemination (day 7)

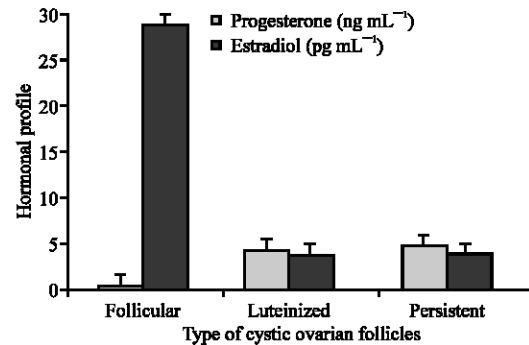


Fig. 2: Plasma concentrations (Mean \pm SEM) of progesterone and estradiol in the pretreatment period in dairy cows suffering from follicular luteinized and persistent ovarian cysts (schedule- B)

Table 1: The effect of treatment with GnRH and PGF2 α (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

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

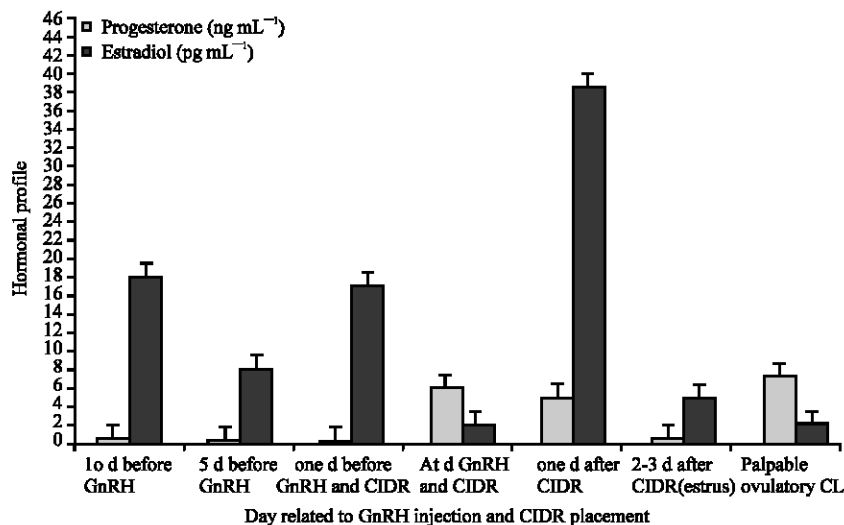


Fig. 3: Plasma progesterone and estradiol profiles (Mean±SEM) in the cow classified as having follicular cyst in schedule-B. the hormonal profile measured at days related to the GnRH and CIDR placement

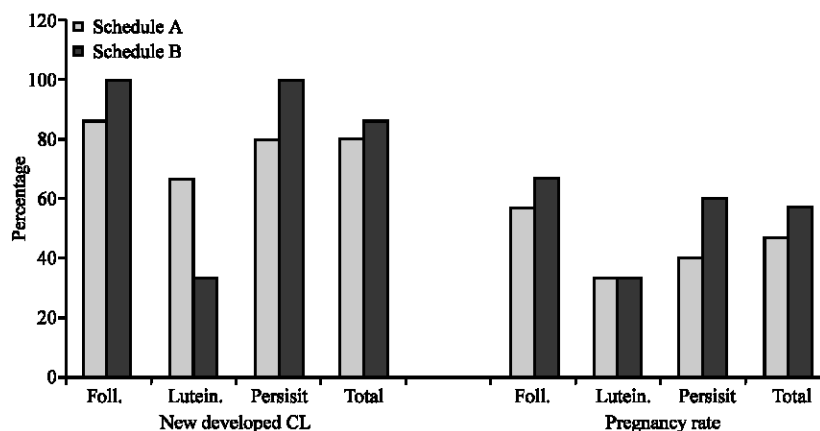


Fig. 4: The new developed CL and pregnancy rates, when schedule-A (after 2nd GnRH injection) compared to schedule-B (after 2nd GnRH injection and CIDR removal) in the cows affected with follicular, luteinized and persistent ovarian cysts

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 (Fig. 3).

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 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.

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 of GnRH injection. Ovulation of a cystic ovarian follicles 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 follicles 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 (Peter, 1998; Bicalho *et al.*, 2007; 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.

CONCLUSION

This study concluded that, 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 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 devices 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.

REFERENCES

- Badinga, L., M.A. Driancourt and J.D. Savio, 1992. Endocrine and ovarian responses associated with the first wave dominant follicle in cattle. *Biol. Reprod.*, 47: 871-883.
- Bartolome, J.A., L.F. Archbold and P. Morrese, 2000. Comparison of synchronization of ovulation and induction of oestrus as therapeutic strategies for bovine ovarian cysts in the dairy cows. *Theriogenology*, 53: 815-825.
- Bicalho, R.C., S.H. Cheong, L.D. Warnick and C.L. Guard, 2007. Evaluation of progesterone supplementation in a prostaglandin F2 (alpha)-based presynchronization protocol before timed insemination. *J. Dairy Sci.*, 90: 1193-1200.
- Calder, M.D., B.E. Salfen, B. Bao, R.S. Youngquist and H.H. Garverick, 1999. Administration of progesterone to cows with ovarian follicular cysts results in a reduction in mean LH and LH pulse frequency and initiates ovulatory follicular growth. *J. Anim. Sci.*, 77: 3037-3042.
- Fricke, P.M. and M.C. Wiltbank, 1999. Effect of milk production on the incidence of double ovulation in dairy cows. *Theriogenology*, 52: 1133-1143.
- Garverick, H.H., 1999. Ovarian Follicular Dynamics and Endocrine Profiles in Cows with Ovarian Follicular Cysts. In: *Current Veterinary Therapy, Food Animal Practice*. Howard, J.L. and R.A. Smith (Eds.). Philadelphia WB, Saunders, pp: 577-580.
- Gumen, A. and M.C. Wiltbank, 2002. An alteration in the hypothalamic action of estradiol due to lack of progesterone exposure can cause follicular cysts in cattle. *Biol. Reprod.*, 66: 1689-1695.
- Hatler, T.B., S.H. Hayes, L.F. Laranja-de-Fonseca and W.J. Silva, 2003. Relationship between endogenous progesterone and follicular dynamics in lactating dairy cows with ovarian follicular cysts. *Biol. Reprod.*, 69: 218-223.
- Kesler, D.J., R.G. Elmore, E.M. Brown and H.A. Garverick, 1981. Gonadotropin releasing hormone treatment of dairy cows with ovarian cysts. I. Gross ovarian morphology and endocrinology. *Theriogenology*, 16: 207-217.
- Knickerbocker, J.J., W.W. Thatcher and F.W. Bazer, 1986. Proteins secreted by day 16 to 18 conceptuses extend corpus luteum functioning in cows. *J. Reprod. Fert.*, 77: 381-391.
- Littel, R.C., J.F. Freund and P.C. Spector, 1991. SAS system for linear models, 3rd Edn., SAS Series in Statistical Applications. SAS Institute Inc., Cary, NC, USA.
- Lopez-Gatius, F., P. Santolaria, J. Yaniz, M. Fenech, M. Lopez-Bejar, 2002. Risk factors for postpartum ovarian cysts and their spontaneous recovery or persistence in lactating dairy cows. *Theriogenology*, 58: 1623-1632.
- Manikkam, M. and R. Rajamahendran, 1997. Progesterone-induced atresia of the proestrus dominant follicle in the bovine ovary: Changes in diameter, insulin-like growth factor system, aromatase activity, steroid hormones and apoptotic index. *Biol. Reprod.*, 57: 580-587.
- McDowell, C.M., L.H. Anderson, J.E. Kinder and M.L. Day, 1998. Duration of treatment with progesterone and regression of persistent ovarian follicles in cattle. *J. Anim. Sci.*, 76: 850-855.

- Meyer, J.P., R.P. Radeliff, M.L. Rhoads, J.F. Bader, C.N. Murphy and M.C. Lucy, 2007. Timed artificial insemination of 2 consecutive services in dairy cows using prostaglandin F2-alpha and gonadotropin-releasing hormone. *J. Dairy Sci.*, 90: 691-698.
- Nobel, K.M., J.E. Tobble, D. Harvey and H. Dobson, 2000. Ultrasonography and hormone profiles of persistent ovarian follicles (cysts) induced with low doses of progesterone in cattle. *J. Reprod. Fert.*, 120: 361-366.
- Peter, A.T., 1998. Infertility Due to Abnormalities of the Ovaries. In: *Current Therapy in Large Animal*. Youngquist, R.S., (Ed.), Theriogenology, Philadelphia: WB Saunders, pp: 349-354.
- Pursley, J.R., M.O. Mee and M.C. Wiltbank, 1995. Synchronization of ovulation in dairy cows using PGF2 α and GnRH. *Theriogenology*, 44: 915-923.
- Pursley, J.R., M.C. Wiltbank, J.S. Stevenson, J.S. Ottobre, H.A. Garverick and L.L. Anderson, 1997. Pregnancy rates in cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.*, 80: 295-300.
- Schmitt, E.J., M. Drost, T. Diaz, C. Roomes and W.W. Thatcher, 1996. Effect of a gonadotropin-releasing hormone on follicle recruitment and pregnancy rate in cattle. *J. Anim. Sci.*, 74: 154-161.
- Seguin, B.E., 1998. Follicular Cystic Ovary Disease: Overview. In: *The Merck Vet. Manual*. Aiello, S.E., A. Mays (Eds.), White-House Station: Merck and Company, pp: 1005-1007.
- Statistical Analysis System, SAS., 1988. SAS online Doc, Version 8. Cary, North Carolina: SAS Institute.
- Stevenson, J.S., Y. Kobayashi and K.E. Thompson, 1999. Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin F2. *J. Dairy Sci.*, 99: 506-515.
- Stevenson, J.S., M.A. Portaluppi and D.E. Tenhouse, 2007. Ovarian traits after gonadotropin-releasing hormone-induced ovulation and subsequent delay of induced luteolysis in an ovsynch protocol. *J. Dairy Sci.*, 90: 1281-1288.
- Taft, R., N. Ahmad and E.K. Inskeep, 1996. Exogenous pulses of luteinizing hormone causes persistence of the largest bovine ovarian follicle. *J. Anim. Sci.*, 74: 2985-2991.
- Thatcher, W.W., M. Drost and J.D. Savio, 1993. New clinical uses of GnRH and its analogues in cattle. *Anim. Reprod. Sci.*, 33: 27-49.
- Todoroki, J., H. Yamakuchi and K. Mizoshita, 2001. Restoring ovulation in beef donor cows with ovarian cysts by progesterone-releasing intra vaginal plastic devices. *Theriogenology*, 55: 1919-1932.
- Wiltbank, M.C., A. Gumen and R. Sartori, 2002. Physiological classification of anovulatory conditions in cattle. *Theriogenology*, 57: 21-52.
- Yoshioka, K., S. Iwamura and H. Kamomae, 1996. Ultrasonic observations on the turnover of ovarian follicular cysts and associated changes of plasma LH, FSH, progesterone and estradiol-17b in cows. *Res. Vet. Sci.*, 61: 240-244.