

**EFFECT OF COMBINED USE OF GnRH AND PGF₂α
ON REPRODUCTIVE EFFICIENCY IN DAIRY COWS
WITH PUERPERAL ENDOMETRITIS**

(With 2 Tables)

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تأثير استخدام الجمع بين محفز الهرمون الحاث للغدة المنسلية
والبروستاجلاندين (ف₂ ألفا) على الكفاءة التناسلية في الأبقار الحلوب
التي تعاني من التهابات رحمية خلال فترة النفاس

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تهدف هذه الدراسة إلى معرفة تأثير الحان المتوالي بالهرمون الحاث للغدة المنسلية (Cystorelin – GnRH) والبروستاجلاندين ف₂ ألفا (اللوناليز) بالإضافة إلى العلاج الرحمي باستخدام الأوكسيتتراسيكلين في الأبقار الحلوب التي تعاني من التهابات رحمية بعد الولادة. إجراء الفحص التناسلي للأبقار التي ولدت حديثاً عند الزيارات المعتادة للقطعان. تم اختيار عدد ٥٥ بقرة حلوب واللاتي تعاني من التهابات رحمية عند اليوم ١٢ - ١٤ (اليوم الأول لبداية التجربة) بعد الولادة. تم علاج هذه الأبقار بـ ١٥٠٠ ملي جرام من الأوكسيتتراسيكلين داخل الرحم والذي تم تكراره مرة أخرى بعد ١٠ أيام من العلاج الأول. تم تقسيم هذه الحيوانات إلى مجموعتين - عند إجراء العلاج الرحمي عند اليوم ١٢-١٤ بعد الولادة - المجموعة الأولى علاجية وعددها ٢١ بقرة والتي تم حقنها بـ ١٠٠ ميكروجرام من الهرمون الحاث للغدة المنسلية (GnRH) وفي اليوم العاشر من بداية التجربة تم إعطائها ٢٥ ملي جرام من البروستاجلاندين ف₂ ألفا. بينما كانت المجموعة الثانية ضابطة لم تعالج بالهرمون. تم إجراء الفحص التناسلي (الشرجي والمهبلي) لهذه الأبقار أسبوعياً بداية من اليوم ١٢ - ١٤ بعد الولادة حتى اكتمال رجوع الرحم إلى حالة ما قبل الحمل تقريباً. تم اخذ عينات من اللبن لقياس مستوى هرمون البروجيستيرون مرتين أسبوعياً حتى اليوم ٦٠ بعد الولادة لمتابعة حدوث التيويض ونشاط الجسم الأصفر في هذه الأبقار. تم ملاحظة حدوث الشبق في هذه الحيوانات من أجل تلقيحها. قد أظهرت نتائج هذه التجربة أن العلاج بالهرمون له تأثير على الفترة ما بين الولادة وحدث أول ارتفاع في مستوى هرمون البروجيستيرون في اللبن حيث أن هذه الفترة كانت أقصر في المجموعة المعالجة بالهرمون (٦,٢٢±٢٣,١٧ يوم) مقارنة بالمجموعة الضابطة (٧,٧٧±٢٩,٥٠ يوم). وكذلك وجد أن العلاج له تأثير على رجوع الرحم إلى حالة ما قبل حدوث الحمل وعلى نسبة شفاء من التهابات خلال فترة ٤٥ يوم بعد الولادة. حيث كانت هذه النسبة عالية معنوياً

في المجموعة المعالجة بالهرمون (٩٠,٥%) عنها في المجموعة الضابطة (٦٤,٧%) كما لوحظ أيضاً أنه بالرغم من عدم وجود اختلاف في الفترة بين الولادة وحدث أول نتيج بين المجموعتين المعالجة والضابطة أن هناك تأثير للعلاج بالهرمون على نسبة حدوث الحمل حيث كانت هذه النسبة أعلى معنوياً في المجموعة المعالجة بالهرمون (٥٧,٩%) عنها في المجموعة الضابطة (٢٧,٣%) وكذلك وجد أن هناك اختلافاً معنوياً في الفترة ما بين الولادة وحدث الحمل حيث كانت ٣٢,٦٨+٩٠,٧٩ يوم في المجموعة المعالجة بالهرمون و ٣٣,٨٣+١١٠,٢٣ يوم في المجموعة الضابطة. قد أظهرت هذه النتائج أن استخدام طريقة الحقن المتوالي بالهرمون الحاث للغدة المنبؤية والبروستاجلاندين ف٢ ألفا إضافة للعلاج الرحمي بالاكسيتيترايسيكلين له تأثير في استعادة المبيض لنشاطه وسرعة رجوع الرحم إلى حالة ما قبل حدوث الحمل والذي أدى إلى تحسن الكفاءة التناسلية لهذه الأبقار التي تعاني من التهابات رحمية خلال فترة النفاس.

SUMMARY

The objective of this study was to determine the effect of sequential injection of GnRH (Cystorelin) and PGF₂ α (Lutalyse) in combination with intrauterine infusion of oxytetracycline (OTC) on ovarian activity, uterine involution and reproductive efficiency in dairy cows with puerperal endometritis. At regular herd visits, the cows (n = 55) at days 12 - 14 after calving (day 0) with puerperal endometritis were treated with intrauterine infusion of 1500 mg OTC. The treatment was repeated after 10 days. The animals were divided into two groups. In addition to intrauterine infusion, 21 cows (treated group) were administered 100 μ g of GnRH at day 0 followed by 25 mg PGF₂ α 10 days later. Thirty four cows were left without hormonal treatment but with OTC and served as a control group. Gynecological examination was performed weekly beginning at day 12-14 postpartum (pp) and continued until completion of uterine involution. Milk samples for progesterone (P₄) determination were collected twice weekly until day 60 pp to monitor ovulation and CL activity. The animals were observed for estrus and artificially inseminated and then pregnancy was diagnosed at 45-60 days post-insemination. The first rise of P₄ level occurred significantly earlier in treated group than control (23.62 \pm 6.29 vs. 29.50 \pm 7.77 days; P < 0.05). Uterine involution was improved clinically and recovery rate within 45 days pp was significantly (P < 0.05) higher in treated group (90.5%) than in control group (64.7%). However, no significant differences were observed in interval from calving to first AI. First AI conception rate was significantly (P < 0.05) better in treated than control cows (57.9% vs. 27.3%). Likewise, interval from calving to conception was shorter (90.79 \pm 33.68 days) in treated group than in control cows (110.23 \pm

33.83 days, $P < 0.05$). The conception rate within 150 days pp showed no significant difference between the two groups. These results indicate that sequential administration of GnRH and PGF₂α in addition to intrauterine infusion with OTC was effective in restoring ovarian activity and enhancing the uterine involution with a consequent improving reproductive efficiency of dairy cows with puerperal endometritis.

Key words: Puerperium, GnRH, PGF₂α, Endometritis, Dairy cows.

INTRODUCTION

Reproductive efficiency is one of the key components of a profitable dairy system (Tenhagen *et al.*, 1998). The postpartum period is an important period in the reproductive life of the cow because of its enormous effect on future fertility (Oltencu *et al.*, 1983). Recent studies on postpartum reproductive physiology and pathology have indicated that delay in ovarian activity and uterine involution may directly or indirectly cause reproductive disorders (Slama *et al.*, 1991; Kamimura *et al.*, 1993; EL-Din Zain *et al.*, 1995 and Seals *et al.*, 2002). These pathophysiological findings have encouraged the routine examination of genital organs during postpartum period and the early treatment of any abnormal condition of the uterus and the ovaries. Königsson *et al.* (2002) reported that puerperal endometritis is a common problem and had a great impact on fertility in dairy cows.

Many antibiotics and antiseptic have been used to enhance the uterine involution and for treatment of postpartum uterine infections (Paisley *et al.*, 1986; Thurmond *et al.*, 1995 and Smith *et al.*, 1998). However, the effects of various treatments on subsequent reproductive efficiency have not been consistent, and there has been little evidence of beneficial effects of postpartum treatments. (Paisley *et al.*, 1986 and Hoedemaker, 1998).

Various hormonal therapies (GnRH and PGF₂α) administered sequentially in postpartum dairy cows for improvement the reproductive efficiency in dairy cows were reported by Murray *et al.* (1990); Randel *et al.* (1996) and Tallam *et al.* (2001). The administration of GnRH between 10 and 14 days postpartum induced an LH surge and ovulation, thus initiating resumption of ovarian activity and accelerated uterine involution (Etherington *et al.*, 1984 and Tallam *et al.*, 2001). The injection of PGF₂α between days 14 and 28 postpartum positively influenced uterine involution and induced estrus (Nakao *et al.*, 1997 and

Knutti *et al.*, 2000). The associated benefits of increased uterine activity have been shown to result in higher first service conception rates (Nash *et al.*, 1980) and a significant reduction in interval from calving to conception (Young *et al.*, 1984). Other studies have, however, reported no effect of GnRH or PGF₂α on fertility, when used in the early postpartum in dairy cows at risk for low fertility after calving (Etherington *et al.*, 1984; De Kruif, 1994 and Bostdt *et al.*, 1999). The use of combination of antibiotic and both GnRH and PGF₂α in treatment of puerperal endometritis has not been investigated. The purpose of our study was to determine the effectiveness of combined application of GnRH and PGF₂α in the treatment of puerperal endometritis in dairy cows pretreated with intrauterine OTC infusion.

MATERIALS and METHODS

Three Commercial dairy herds consisting of approximately 100 to 180 Holstein Friesian cows, each with an annual average milk yield of about 4500 to 6000 kg were visited regularly every 1 to 2 weeks for postpartum reproductive check. The animals were kept in an outdoors paddock with access to an open-sided shelter, and milked twice daily. A balanced nutritional diet including green fodder, dry fodder and concentrate mixture were fed to these animals. The cows that had calved in three months period and with reproductive disorders during the first 10 days after calving were included in this study. At the first examination, day 12 to 14 after calving (day 0), each cow's identity, lactation number, peri-parturient disease, difficulty of calving (unassisted or assisted) and whether the fetal membranes had been retained for more than 24 hours after calving were recorded. The examination included rectal palpation and vaginoscopy of the reproductive organs as described by Del Vecchio *et al.*, (1994). Briefly, the uterus was palpated per rectum to determine whether detectable amount of fluid had been accumulated. At the same time, the size and consistency of the uterine horns were determined as well as the presence of ovarian follicles and corpora lutea were recorded. The vestibule of vagina and the cervical os were examined through a vaginal speculum to determine whether purulent discharge was present. Enlarged size, decreased tone and accumulation of fluid in the uterus as well as presence of abnormal vaginal discharge were indicated as signs of puerperal endometritis.

A total of 55 cows (3-8 years of age) diagnosed as suffering from puerperal endometritis were selected and treated with intrauterine infusion of 1500 mg of oxytetracycline hydrochloride solution (OTC, 1500 mg Metrijct ®, Intervit, UK). The intrauterine treatment was repeated after 10 days. The animals were randomly assigned to either treated or control groups. The treated group included 21 cows which received 100 µg of GnRH intramuscularly (Cystorelin ®; Merial Canada, Montreal, Quebec, Canada) on day of first intrauterine infusion (day 0 of the study) followed by 25 mg of PGF_{2α} (5 ml of Lutalyse ®; Upjohn, USA) 10 days later. The control group included 34 animals which were treated twice only with intrauterine antibiotics. The rectal and vaginal examinations were weekly performed and continued for 2 months to monitor uterine involution and ovarian activity. The criteria adopted to determine completion of the uterine involution (recovery state) were the return of the uterus to its normal uterine tone and normal almost equal size uterine horns (Morrow *et al.*, 1966). Estrus was detected by a teaser, in addition to visual observation. Milk samples were collected twice a week until day 60 pp to assess the luteal activity (Milk P₄ level ≥ 1ng/ml was considered indicative of a functional CL (Bulman and Lamming, 1978). Ovulation was judged to occur third or fourth day retrospective to appearance of P₄ concentrations of 1.0 ng/ml or greater (Stevenson and Britt, 1980). At evening milking, after-milk (10 ml) was collected in test tube containing 100 mg potassium dichromate, then centrifuged (3000 rpm for 20 min). The cream layer was discarded and the remaining skim milk was transferred to another test tube and kept frozen (- 20 °C) until assayed (Coat-A-Count, Diagnostic Products Corporation, DPC, Los Angeles, CA). The resumption of ovarian activity, completion of uterine involution and absence of abnormal vaginal discharge were defined as clinical recovery. The recovered cows were artificially inseminated and pregnancy was diagnosed 45-60 days post-insemination.

The reproductive performance in both treated groups was compared by means of the recovery rate within 45 days pp, first service conception rate and overall conception rate within 150 days pp. Also, average intervals (mean ± SD) from calving to first rise of P₄, first insemination and conception were compared between treated groups. The statistical significance of difference was tested by Chi square analysis and Student's t-test.

RESULTS

The responses to hormonal treatment regime in dairy cows affected with puerperal endometritis are summarized in table 1. Injection of GnRH between days 12 to 14 pp was associated with increase in milk P₄ concentrations, which occurred earlier in the treated group. The mean interval to first rise of milk P₄ level were 23.62 ± 6.29 days and 29.62 ± 7.77 days for treated and control groups, respectively, and the difference was significant (P < 0.05) between groups (Table 1). The percentage of cows that showed completely clinical recovery within 45 days pp was significantly higher (p < 0.05) in treated group (90.5%) than in control group (64.7%). After GnRH and PGF₂α treatment, the cows showed shorter interval (71.57 ± 8.35 days) to first insemination in comparison with those treated by antibiotics only (75.50 ± 12.07 days), but the difference was not significant (Table 1).

Table 1: Effect of combine using of GnRH and PGF₂α on recovery rate and days (mean±SD) to resumption of ovarian activity and first AI in dairy cows affected with puerperal endometritis.

	No. of Cows	Recovery rate ¹ within 45 days (%)	Days to first rise of milk ² P ₄	Days to 1 st AI
Treated	21	90.5 ^a (19)	23.62 ± 6.29 ^a	71.57 ± 8.35 ^a
Control	34	64.7 ^b (22)	29.50 ± 7.77 ^b	75.50 ± 12.07 ^a

¹: % of cows with complete uterine involution, absence of abnormal vaginal discharge and resume ovarian activity at day 45 postpartum. (): Number of cows

²: Milk P₄ level above 1.0 ng/ml.

^{a,b}: Significant difference (P < 0.05).

P₄: progesterone.

Reproductive performance in treated cows with hormonal therapy and in control animals are shown in table 2. Favorable effect of GnRH and PGF₂α injections in dairy cows affected with puerperal endometritis was observed. The first AI conception rate was 57.9% for treated group and was significantly higher (p < 0.05) than those (27.3%) of the control group. Although, the treatment tended to improve conception rate within 150 days pp (78.9% v.s.63.6%), the difference was not significant (p < 0.10, Table 2). Moreover, interval from calving to conception showed significant (p < 0.05) improvement in cows that had been treated with hormones (90.79 ± 33.68 days) when compared with the control cows (110.23 ± 33.83).

Table 2: Effect of combine using of GnRH and PGF₂α on reproductive performance of dairy cows affected with puerperal endometritis.

	Reproductive performance			
	No. of Cows recovered ¹	1 st AI conception rate (No.)	Conception rate within 150 days pp (NO.)	Days from calving to conception ²
Treated	19	57.9 ^a (11)	78.9 ^a (15)	90.79 ± 33.68 ^a
Control	22	27.3 ^b (6)	63.6 ^a (14)	110.23 ± 33.83 ^b

¹: % of cows with complete uterine involution, absence of abnormal vaginal discharge and resume ovarian activity at day 45 postpartum.

²: Mean ± SD.

^{a,b}: Significant difference (P < 0.05).

DISCUSSION

Reproductive efficiency affects the productivity of dairy cows. The causes of reproductive failure are common and very complicated. Most authors share the opinion that puerperal complications play an important role in bovine infertility (Oltenucu *et al.*, 1983; EL-Din Zain *et al.*, 1995 and Konigsson *et al.*, 2002). The incidence of uterine infection ranges from approximately 10% - 40% of the postpartum cows in dairy herds (Lewis, 1997).

Puerperal diseases have long been implicated in delaying uterine involution complications (Bosu *et al.*, 1988 and Hussain and Daniel, 1991) and in causing deleterious deviations in ovarian activity (Oltenucu *et al.*, 1983 and EL-Din Zain *et al.*, 1995). In addition, Bosu *et al.* (1988) found that postpartum infection is associated with abnormal suppression of the hypothalamic release of gonadotropin releasing hormone consequently affecting folliculogenesis. Administration of GnRH or its analogues at about two week's pp has been reported to be effective to facilitate the resumption of ovarian activity in cows that had puerperal complications (Benmrad and Stevenson 1986 and Bosu *et al.*, 1988). Lopez-Gatiús *et al.* (2001) found that selection of first dominant follicle occurred between 7 to 15 days after calving and may persist as a result of failure to resume ovarian activity in the postpartum period. Previous works by Yamada *et al.* (1998) reported that this dominant follicle disappeared either by atresia or lutenization after administration of GnRH. The result of the present study indicates that interval from

calving to first rise of milk P_4 was shorter in treated group than in control ones. This may have been due to ovulation or lutenization of ovarian follicles after administration of GnRH.

Earlier re-establishment of ovarian activity and improved uterine involution after GnRH treatment in dairy cows with puerperal complications have been reported (Leslie *et al.*, 1984). This is in agreement with our results that the recovery rate within 45 days pp was higher for treated cows than in control ones. This may be due to the direct effect of GnRH or LH on the uterus. Recently, Shemesh (2001) reported that endometrial GnRH and LH receptors and its mRNA were greatest during luteal phase. LH induced a rapid increase in endometrial cAMP and inositol phosphate production (Shemesh *et al.*, 2001). This increased amount of second messenger was associated with increased concentrations of endometrial cyclooxygenase (COX-2) and PGF metabolite. This could explain the improvement in uterine involution rate of dairy cows in the present study. Since, increase in PGFM was associated with rapid involution of uterus (Fredriksson *et al.*, 1985).

Previous work by Etherington *et al.* (1984) found an increased incidence of pyometra in GnRH treated cows. This may be related to increase in P_4 levels. Elevated P_4 concentrations in blood resulted in more severe course and longer persistence of bacteria in infectious diseases and decreased clearance of antibody-coated erythrocytes in vivo (Scheibl and Zerbe, 2000). Moreover, the cyclical pattern of steroid hormones concentration regulates the potential pathogenicity of microorganisms that contaminate the postpartum uterus (Dhaliwal *et al.*, 2001). The endometrium is more susceptible to infection under P_4 than estrogen (E_2) dominance. In agreement with the previous reports (Leslie *et al.*, 1984 and Nakao *et al.*, 1997), the administration of $PGF_{2\alpha}$ 10 days later appeared to overcome the negative effect of GnRH on the incidence of pyometra and improved the rate of recovery within 45 days pp. This may be related to its luteolytic effect, thereby removing the inhibitory effect of P_4 on the host's defense mechanisms or positive influence on uterine involution. Previous work by Nakao *et al.* (1997) reported that duration of elevated plasma PGFM concentrations in cows with abnormal puerperium was shorter than that of the normal cows. Therefore, administration of $PGF_{2\alpha}$ may extend the high PGFM levels duration to its level at normal puerperium and enhance the rate of uterine involution through its effect on immune functions of uterus. In vitro experiments showed that $PGF_{2\alpha}$ and other arachidonic acid metabolites were chemoattractant and increased the ability of neutrophils to ingest

Staphylococcus aureus (Hoedemaker, *et al.*, 1992). These aspects of neutrophils functions are important to overcome the uterine infection and self clearance to accelerate the rate of uterine involution.

The reproductive performance of dairy cows treated by sequential GnRH and PGF₂ α application, in addition to intrauterine infusion by OTC, was improved in comparison with control animals treated only by OTC. There is some evidence that the early treatment of endometritis can be beneficial; for example, Sheldon and Noakes (1998) found that pregnancy rate to first service was higher for cows treated before 40 days pp. In this trial all cows were treated between days 12-14 pp. The conception rate after first AI was better in treated cows than in control ones. This finding was similar to that reported by Nakao *et al.* (1997). The interval from calving to conception is a useful overall measure of reproductive performance in dairy cows. In the present study, the treated cows by combined GnRH and PGF₂ α had shorter intervals to conception than control ones (P< 0.05). This is in agreement with previous works by Nakao *et al.* (1997) and Kristula and Bartholomew (1998) on cows with puerperal complications.

Thus, it can be concluded that sequential administration of GnRH and PGF₂ α in addition to intrauterine infusion with OTC is effective in restoring ovarian activity and enhances uterine involution resulting in improved reproductive efficiency in dairy cows with puerperal endometritis. Further studies are needed on large scales of animals to confirm the clinical usefulness of this hormonal regimen in combination with intrauterine antibiotics in the treatment of puerperal endometritis in dairy cows.

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