# EFFICACY OF DECREASING THE DOSE OF GnRH USED FOR SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION IN ANESTRUS LACTATING DAIRY COWS

**By**

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## ABSTRACT

To estimate the efficacy of reducing the dosage of GnRH used for synchronization of ovulation with timed-AI, 20 anestrus lactating Holstein cows were randomly assigned to 1 of 2 treatment groups. Additional 10 cows were used as control, without hormonal treatment ($G_1$). Ovulation was synchronized for cows in the second group using intramuscular injection of GnRH and $PGF_{2\alpha}$ as follows: Day 0, 100 μg GnRH; day 7, 500 μg $PGF_{2\alpha}$; day 9, 100 μg GnRH. Ovulation was synchronized in the third group of cows using the same manner but only 50 μg GnRH was injected. Pregnancy was diagnosed at 28 and at 65 days post AI by assessment of progesterone concentration in serum and by rectal palpation, respectively. For all cows, synchronization rate was 80%. Conception rates calculated using all cows receiving 100 μg GnRH were 75% at 28d and 50.0% at 65 days post AI. The corresponding values for cows receiving 50 μg GnRH were 100% and 71.4%, respectively. Pregnancy loss from 28 to 65d post AI was 33.3% in 100 μ GnRH treated group, while it was 28.6% in 50 μg GnRH treatment. Estimated saving in hormone costs using 50 instead of 100 μg GnRH per injection for synchronization ovulation were LE 7 per cow and LE 20.25 per pregnancy. The present study indicated that, decreasing the dosage of GnRH used for synchronization of ovulation and timed AI in anestrus lactating dairy cows reduces pregnancy loss and synchronization costs without compromising the efficacy of the synchronization protocol. Thus, Ovsynch appears to be an effective method for establishing pregnancy in anestrus lactating dairy cows.

## INTRODUCTION

A sequence of GnRH and $PGF_{2\alpha}$ injections or repeated $PGF_{2\alpha}$ injections often are recommended for treatment of several reproductive disorders (Risco *et al.*, 1995; Pursley *et al.*, 1998; Mialot *et al.*, 1999 and Lemaster *et al.*, 2001). Recent studies have incorporated GnRH into a synchronization protocol (Pursley *et al.*, 1995; Stevenson *et al.*, 1996; Burke *et al.*, 1996 and LeBlanc *et al.*, 1998) that involves a series of hormone administrations. The timed AI (artificial insemination) program involves administration of GnRH, followed by $PGF_{2\alpha}$ 7 d later and a second administration of GnRH 36 to 48h after $PGF_{2\alpha}$ (Pursley *et al.*, 1995). The first administration of GnRH is given at a random stage of the estrous cycle, causing either luteinization or ovulation of the largest follicle (Pursley *et al.*, 1997a) in approximately 85% of cows. Administration of $PGF_{2\alpha}$ regresses the corpus luteum or the luteinized follicle induced by GnRH. A new dominant follicle forms and is available for ovulation at the time of the second GnRH administration, given 36 to 48 h after $PGF_{2\alpha}$. The second administration of GnRH has been shown to synchronize ovulation rather than estrus (Nebel and Jobst, 1998). This protocol allowed for effective management of AI in lactating dairy cows, without estrus detection and provided similar pregnancy rates per AI when compared with those of classical reproductive management system based on estrus detection and hormonal therapy when necessary (Pursley *et al.*, 1997-a and Pursley *et al.*, 1997b).

The aim of this study was to compare the effectiveness of decreasing the GnRH dose (100 vs 50 μg) used for synchronizing ovulation & conception rate and pregnancy loss after timed-artificial insemination (AI) in anestrus lactating dairy cows.

## MATERIALS AND METHODS

**Animals and managements:**

Thirty primiparous and multiparous lactating Holstein cows 60 to 90 d post-partum located at Sakha Experimental Station, Ministry of Agriculture were used for this study, beginning in March and ending in June 2003. The cows were carefully selected to be subestrous, as defined by the absence of detectable estrus from calving and by the presence of an abnormal uterine involution and ovarian cysts detected by rectal palpation for the uterus and ovaries. Cows were considered cystic if there were no corpus luteum (CL) and multiple follicles (> 20 mm in diameter) were present on one or both ovaries in the absence of uterine tonicity (Archbald *et al.*, 1991 and Garverick, 1999). All cows were free from signs of mastitis. Animals were fed rations to meet their maintenance and milk production requirements according to the standard allowances recommended by N.R.C. (1988).

**Synchronization of ovulation (Ovsynch):**

Cows were distributed by parity, days in milk and were assigned randomly to one of three experimental groups for synchronization of ovulation. The first group served as control without any treatment. The other two groups were hormonally treated. Synchronization of ovulation was initiated at a random stage of the estrous cycle using intramuscular injection of GnRH (Fertagyl; Intervet, Millsboro, DE) and $PGF_{2\alpha}$ (Estrumate, Coopers Tierazneimittel GmbH, Burghwedel, W. Germany) as follows: Day 0, GnRH; Day 7, $PGF_{2\alpha}$; day 9, GnRH Fig. (1). Ovulation was synchronized with 500 µg (Cloprostenol) $PGF_{2\alpha}$ analogue plus either 100 µg (n = 10 Group 2) or 50 µg (n = 10; Group 3) GnRH per injection.

All cows underwent a timed AI within 8 to 20 h after the second GnRH injection (Fig. 1). Animals in the first group were visually observed for estrus and were artificially inseminated within 14 h after onset of the first spontaneously occurring estrous.

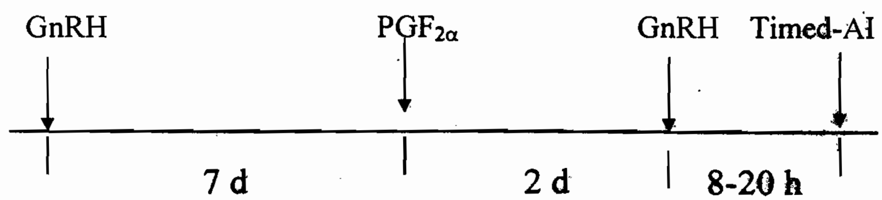


**Figure (1):** The protocol used for the synchronization of ovulation and timed AI in lactating dairy cows using GnRH + $PGF_{2\alpha}$ + GnRH.

**Pregnancy diagnosis:**

Pregnancy was determined by either assessed of serum progesterone concentration using RIA method at day 28 and by rectal palpation of the uterus approximately 65d after artificial insemination. Pregnancy at 28 d post AI was confirmed when progesterone concentration in the serum was $\geq 1.0$ ng/ml. For each treatment group, the per pregnancy costs were calculated as the total cost of hormone used for synchronization divided by the number of cows diagnosed pregnant at 65 days post AI.

**Statistical analysis:**

The obtained data were statistically analysed using (Chi-square test) General Linear Models Procedure Adapted by SPSS (1997) for Use's Guide.

## RESULTS AND DISCUSSION

None of the untreated abnormal cows (control) observed in estrus throughout the experimental period. Results presented in Table (1) indicated that after rectal palpation (65 post AI), one cow from cows treated with full-dose of GnRH (100 µg) was excluded from the calculation of conception rate at 28 and 65d post AI and pregnancy loss because it had uterine infection and uterine fibrosis. The percentage of cows return to estrus was 10% (1 out of 10) and 30% (3 out of 10) in cows treated with either 100 µg and 50 µg GnRH, respectively (Table, 1). The synchronization rate for all cows (80%) in the present study appears to be lower than that reported by Vasconcelos *et al.* (1997, 87%) but it was similar to that of Fricke *et al.* (1998, 84%) using Ovysnch. This protocol synchronizes the growth of a new follicular wave (first injection of GnRH), synchronizes luteal function (first injection of GnRH and $PGF_{2\alpha}$ injection), and synchronizes the time of ovulation into an 8-h period (second injection of GnRH, Pursley *et al.*, 1998). However, aggressive use of Ovsynch to initiate the first postpartum AI and manage the voluntary waiting periods results in improved reproductive efficiency compared with relaying on visual estrus detection alone for AI (Pursley *et al.*, 1997-b).

**Table (1):** Synchronization rate, conception rates and pregnancy losses in anestrus lactating dairy cows in response to timed AI after synchronization of ovulation using either 100 µg or 50 µg GnRH.

| Items | Control | GnRH-treatments | | | | | |
|---|---|---|---|---|---|---|---|
| | | Full-dose $G_2$ (100 µg) | | Half-dose $G_3$ (50 µg) | | Total | |
| | $(G_1)$ | No. | % | No. | % | No. | % |
| No. of cows | 10 | 10 | - | 10 | - | 20 | - |
| Return to estrus | - | 1 | 10 a | 3 | 30 b | 4 | 20 |
| Synchronized rate | - | 9 | 90 | 7 | 70 | 16 | 80 |
| Excluded animals | - | 1 | 10 | - | - | 1 | 5 |
| Healthy synchronized cows | - | 8 | - | 7 | - | 15 | - |
| Conception at 28d-post AI | - | 6 | 75 | 7 | 100 | 13 | 86.7 |
| Conception at 65 d post-AI | - | 4 | 50.0 | 5 | 71.4 | 9 | 60.0 |
| Pregnancy loss | - | 2 | 33.3 | 2 | 28.6 | 4 | 30.7 |
| Progesterone concentration ng/ml at 28 d post AI | 0.087 ±0.017 | 2.219 ± 0.21 | | 1.767 ± 0.08 | | 1.993 ± 0.066 | |

a, b: Mean in row with different superscripts significantly differ ($P < 0.05$)

Conception rate at 28 d post AI was 100% and 75% for cows treated with the half and the full-dose of GnRH, receptively, with overall mean -86.7%. The present finding was markedly higher than that previously reported by Pursley *et al.* (1997a and b) and Fricke *et al.* (1998) using Ovsynch and timed AI in lactating dairy cows.

The percentage of cows diagnosed pregnant by rectal palpation at 65d after timed insemination was 50.0 and 71.4% for cows treated with 100 and 50 µg GnRH, respectively. The conception rate for all cows was 60.0%, which was higher than that reported by Pursley *et al.* (1998) and Moreira *et al.* (2001). Pregnancy loss from 28 to 65 d post AI was lower (28.6%) in the half-dose treatment group compared with the full-dose treatment group (33.3%). For all cows, pregnancy loss from 28d to 65 d post AI was 30.7%, resulting in an attrition rate of 0.829% per day. This rate of pregnancy loss was higher than that reported by Smith and Stevenson (1995, 19%), Vasconcelos *et al.* (1997, 16.8%), Pursley *et al.* (1998, 20%) and Fricke *et al.* (1998, 13.5%) when ultrasound was used for pregnancy diagnoses at 28 to 30 d after AI. The physiological mechanisms responsible for pregnancy loss in lactating dairy cows are not known, but may include lactational stress associated with increased milk production (Nebel and

McGilliard, 1993 and Jobst *et al.*, 2000), negative energy balance (Butler and Smith, 1989),toxic effects of urea and nitrogen (Butler *et al.*, 1995) or reduced ability to respond to increased environmental temperature (Hansen *et al.*, 1992). Pursley *et al.* (1998) found that the timed of AI in relation to ovulation affected pregnancy losses. Also, they reported that, when spermatozoa were introduced into the uterus at any time from -28 h perior to ovulation, until just before ovulation pregnancy rates per AI were similar. Thus, the age of the oocyte at fertilization appears to affect pregnancy rate/AI and pregnancy loss.

Per pregnancy cost analysis were based on 4 pregnancies from 9 cows received the full-dose treatment compared with 5 pregnancies from 10 cows for the half dose treatment (Table 2). Per cow cost for GnRH was LE 7 less for cows in the half-vs the full-dose treatment, whereas per cow cost for $PGF_{2\alpha}$ was identical for both treatment groups. Per pregnancy costs for GnRH and $PGF_{2\alpha}$ were LE 17.5 and LE 2.75 less, respectively, for cows in the half-vs the full-dose treatment. Total cost of hormones per cow required for synchronization of ovulation was LE 25 vs LE 18 for cows in the full-vs the half dose treatment, respectively. However, the total cost of hormones per pregnancy was LE 56.25 vs LE 36 for cows in the full-vs the half dose treatment, respectively (Table, 2).

The 100 µg dose of GnRH used to improve the synchronization of ovulation (Ovsynch) was based on the manufacturer's recommendation for treating cystic follicles. However, a 50 µg dose of GnRH induced a slightly higher response compared to that of a 100 µg dose, indicating that 50% of the recommended dose of GnRH may effectively ovulate normally growing, non-cystic follicles during the synchronization of ovulation protocol (Fricke *et al.*, 1998), improve conception rate at 28 and 65 days post AI and reduced pregnancy loss from 28 to 65 d post AI in the present work. Previous studies (Britt and Gaska, 1998 Nebel and Jobst, 1998 and Fricke *et al.*, 1998) reported applying the cost savings associated with reducing the dose of GnRH required for synchronization of ovulation by 50%, the estimated cost per pregnancy could be decreased by \$15.92 to \$30.2 & by \$11.76 to \$31.56 and \$29.61 to \$46.10, respectively. Thus, decreasing the dosage of GnRH for synchronization of ovulation and timed AI in lactating dairy cows reduces synchronization costs per cow and per pregnancy without compromising the efficacy of the protocol. It was concluded that the benefit of using timed-insemination in subestrus lactating dairy cows lies in the fact that these cows do not have to be detected in estrus for insemination and achieving pregnancy. In addition, decreasing the dosage of GnRH used for synchronization of ovulation and timed AI in anestrus lactating dairy cows reduces pregnancy loss and synchronization costs per cow and per pregnancy without compromising the efficacy of the synchronization protocol.

**Table (2):** Cost analysis of synchronization of ovulation in anestrus lactating dairy cows using either 100 µg (full-dose) or 50 µg (half-dose) of GnRH.

| Item | GnRH-treatments | |
|---|---|---|
| | 100 µg (full dose) | 50 µg (half-dose) |
| No. of cows | 9 (10-1) | 10 |
| No. of pregnancies [a] | 4 | 5 |
| Cost of GnRH [b] | | |
| 1- LE/cow | 14 | 7 |
| 2- LE/pregnancy [c] | 31.50 A | 14 B |
| Cost of $PGF_{2\alpha}$ [b] | | |
| 1- LE/cow | 11 | 11 |
| 2- LE/pregnancy [c] | 24.75 | 22 |
| Total cost of hormones | | |
| 1- LE/cow | 25.0 | 18 |
| 2- LE/pregnancy [c] | 56.25 A | 36 B |

[a] For each treatment group, number of cows pregnant at 65 days post AI

[b] Mean hormone costs was LE 7.0 per 100 µg GnRH and LE 11 per 500 µg $PGF_{2\alpha}$

[c] Per pregnancy costs for each treatment group were calculated as the total cost of hormones divided by the number of cows diagnosed pregnant at 65 days post AI.

A, B: Values in row with different superscripts significantly differ ($P < 0.05$)

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# فعالية خفض جرعة الهرمون المحرر للجونادوترفينات المستخدم لتنظيم التبويض والتلقيح الصناعى عند وقت محدد فى أبقار اللبن الحلابة والخاملة جنسيا

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## الملخص العربى

لتقدير فعالية انخفاض جرعة الهرمون المنبه لتحرير الهرمونات الجونادوترفيه والمستخدم فى تنظيم التبويض مع التلقيح الصناعى المحدد بوقت ، ٢٠ بقرة حلابة فريزيان خاملة جنسيا وزعت عشوائيا إلى معاملتين. هذا بالاضافة الى مجموعة مقارنة مكونه من ١٠ بقرات تركت بدون معامله هرمونيه (المجموعة الاولى). نظم التبويض فى أبقار المجموعة الثانية باستخدام الحقن العضلى للهرمون المنبه لتحرير الهرمونات الجونادوترفيه والبروستاجلاندين بحيث ان الحيوانات فى اليوم صفر تعطى ١٠٠ ميكروجرام GnRH ، اليوم السابع تعطى ٥٠٠ ميكروجرام بروستاجلاندين واليوم التاسع تعطى ١٠٠ ميكروجرام GnRH. ينظم التبويض فى المجموعة الثالثة بنفس برنامج الحقن وجرعة البروستاجلاندين ولكن تحقن بـ ٥٠ ميكروجرام GnRH. تم تلقيح كل الأبقار صناعيا عند ٨-٢٠ ساعة بعد الحقن الثانى للـ GnRH ولقد تم تشخيص الحمل عند اليوم ٢٨ ، ٦٥ بعد التلقيح الصناعى عن طريق تقدير تركيز هرمون البروجسترون فى سيرم الدم وعن طريق الجس المهبلى على التوالى. أظهرت النتائج ان معدل تنظيم التبويض فى الابقار المعاملة هرمونيا ٨٠%. النسب المئوية للخصوبة للأبقار المعاملة بـ ١٠٠ ميكروجرام GnRH هى ٧٥% عند اليوم ٢٨ ، ٥٠% عند ٦٥ يوم من التلقيح الصناعى. القيم المقابلة للأبقار المعاملة بـ ٥٠ ميكروجرام GnRH هى ١٠٠% ، ٧١,٤% على التوالى. نسبة فقد الحمل من اليوم ٢٨ إلى اليوم ٦٥ بعد التلقيح الصناعى كانت نسبته ٣٣,٣% فى الأبقار المعاملة بـ ١٠٠ ميكروجرام GnRH بينما هى ٢٨,٦% فى الأبقار المعاملة بـ ٥٠ ميكروجرام GnRH. قدر التوفير فى تكاليف الهرمون باستخدام ٥٠ ميكروجرام مفضلا ذلك على ١٠٠ ميكروجرام GnRH لكلى حقن لتنظيم التبويض بمقدار ٧ جنيهات لكل بقرة ، ٢٠,٢٥ جنيها لكل حمل.

اتضح من التجربة أن خفض جرعة هرمون الـ GnRH المستخدم لتنظيم التبويض والتلقيح الصناعى عند وقت محدد فى أبقار اللبن الخاملة جنسيا أدى إلى انخفاض نسبة فقد الحمل وكذا قلل من تكاليف التنظيم لكلى بقرة ولكل حمل بدون انخفاض فى فعالية برنامج التنظيم. علاوة على ذلك فإن تنظيم التبويض يبدو أنها طريقة فعالة لتحقيق الحمل فى حيوانات اللبن الحلابة الخاملة جنسيا.