EVALUATION OF DIFFERENT PROTOCOLS FOR SYNCHRONIZATION OF ESTRUS AND OVULATION IN POSTPARTUM SUBESTRUS LACTATING DAIRY COWS

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ABSTRACT

Thirty three Friesian cows in postpartum subestrus were used in this study to compare two protocols for synchronization of estrus and ovulation with that of standard, 1 or 2 injections of $PGF_{2\alpha}$. Cows were divided randomly into three treatment groups as follows: Group 1(OvSynch): GnRH (Day 0, 50μμg im), $PGF_{2\alpha}$ (Day 7, 500 μμg im), GnRH (Day 9, 50 μμg im), TAI 8-20h later; Group 2 (GnRH- $PGF_{2\alpha}$): cows were synchronized using the same manner however, the second injection of GnRH was eliminated, AI 12h after exhibiting estrus or at 80-96 h for cows which not displayed estrus; Group 3 ($PGF_{2\alpha}$): (Day 0, 500 μμg im), AI 12 h after detection of estrus or, if estrus not observed, cows received a second $PGF_{2\alpha}$ injection on Day 14, and AI 12 h after observed estrus or, at 80-96 h when estrus was absent. The results showed that, conception rates at d 35 post AI was significantly ($P < 0.05$) higher for cows in Group 2 (77. 8%) than in Group 1 (63.6%) and Group 3 (50%). Similarly, Group 2 had a higher pregnancy rate (66.7; $P<0.05$) at d 65 post AI than Group 1 (54.6 %) and Group 3 (40%). However, pregnancy losses did not differ among treated groups. Estimated saving in hormone costs required for synchronizing estrus and ovulation was LE 4.17 and LE 15.75 per pregnancy for Group1 and 2, respectively. It could be concluded that GnRH- $PGF_{2\alpha}$ or GnRH- $PGF_{2\alpha}$-GnRH treatment was effective method for synchronizing a fertile estrus in postpartum subestrus lactating dairy cows. But, the better reproductive performance was observed in cows treated with GnRH- $PGF_{2\alpha}$ protocol.

INTRODUCTION

Postpartum subestrus considered as a major factor limiting economic success of many dairy operation, leading to economic losses (Jobst *et al.*, 2000) by reducing reproductive efficiency

(Tefera *et al.*, 1991 and Nebel and Jobst, 1998) or, infertility (Short *et al.*, 1990). Recently, a new methods to synchronization estrus and ovulation have been developed using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and / or gonadotropin-releasing hormone (GnRH) in an attempt to overcome the effect of poor estrus detection on reproductive efficiency (Pursley *et al.*, 1995, $1997_{a,b}$,1998 and El-Shama'a, 2003) and treatment of various disorders (Benmard and Stevenson, 1988; Risco *et al.*, 1995; Bartolome *et al.*, 2000; and López-Gatius and López-Béjar, 2002). For such a program, to receive widespread adoption, it must be cost and labor efficient. It must also effectively synchronize estrus and ovulation at definable time. Fricke *et al.*(1998) reported that the dosage of GnRH used in timed artificial insemination (TAI) protocol (OvSynch) could be reduced from 100 to 50 µg, thereby decreasing the cost of TAI protocol without impacting fertility. Similar results have been reported in beef cattle by Sasongko *et al.* (2000) and in lactating dairy cows by El-Shama'a (2003), however Funk and Anderson (2000) found that decreasing the GnRH dosage reduced conception rates in beef cattle. Another potential method to decrease the cost of this program is to use estrus detection in lieu of the second GnRH injection (Jordan *et al.*, 2002). Conception rates after $PGF_{2\alpha}$ treatment are higher for cows inseminated at observed estrus than that inseminated at fixed time in some studies (Lucy *et al.*, 1986; Stevenson *et al.*, 1987; Archbald *et al.*, 1992; Stevenson *et al.*, 1996, 1999), but not all (Pursley *et al.*, 1997_b; Cartmill *et al.*, 2001). When AI occurs only upon observed estrus, the 100% AI submission rate, which is the advantage of programmed breeding system with fixed TAI is lost. But, by combining visual estrus detection with fixed time AI, the advantage in conception rate and submission rate could be captured in one protocol. The aim of this study was to compare 1) the effectiveness of two protocols in which a decreased dose of GnRH (50 µg) was used for synchronization of estrus and ovulation, one based on visual estrus detection combined with TAI and one with solely TAI with that of standard, 1 or 2 injections of $PGF_{2\alpha}$ in subestrus lactating dairy cows; 2) the efficacy of these protocols on increasing the reproductive performance.

MATERIALS AND METHODS

Thirty three primiparous and multiparous lactating Friesian cows 60 to 90 d postpartum. The cows were carefully selected to be subestrus, as defined by the absence of detectable estrus from calving and by the presence of a CL detected by palpation per rectum. Cows diagnosed with abnormal uterine involution or ovarian cysts were also used. Treatment of cows were carried out at Sakha Experimental Station, Ministry of Agriculture during the period from Oct.2003 to April 2004. All cows were free from clinical signs of mastitis and fed complete diets based on milk production according to the standard allowances recommended by NRC (1989). Cows were machine milked twice daily.

Hormone protocols

Cows were randomly allotted to three similar groups (11 cows each), by parity and calving date. Group1(OvSynch) cows received intramuscularly (i.m) an initial injection (Day 0) of 50 μg GnRH (Fertagyl; Intervet International B.V., Boxmeer, Holland); on Day 7, the cows received 500 μg of $PGF_{2\alpha}$ (Cloprostenol, Estrumate, Essex Animal Health Friesoythe, Germany); and on Day 9, a second injection of 50 μg GnRH was administrated. Cows underwent a timed AI within 8 to 20 h after the second GnRH injection (Fig.1).

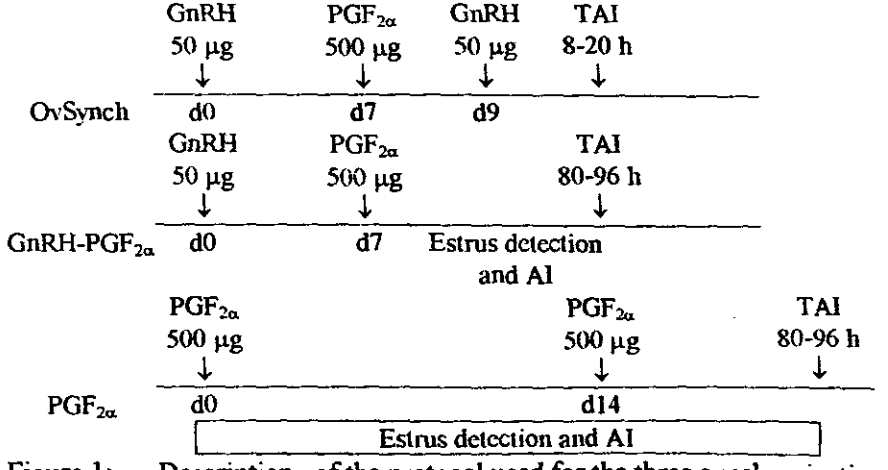

Figure 1: Description of the protocol used for the three synchronization protocols, OvSynch, GnRH- $PGF_{2\alpha}$ and $PGF_{2\alpha}$, identifying timing of injection.

Group 2 (GnRH- $PGF_{2\alpha}$) cows were injected by 50 µg GnRH on d 0 and 500 µg of $PGF_{2\alpha}$ on d 7 and then cows were visually observed for estrus 4 times daily and artificially inseminated with frosen semen (AI) 12 h after exhibiting behavioral estrus during d 7 to d 10. For the cows not exhibiting estrus within 3-4 days after $PGF_{2\alpha}$ injection, AI was performed at 80-96 h after $PGF_{2\alpha}$ injection (Fig.1). Estrus was defined as standing to be mounted by another cow and (or) visual signs of mucus.

Group 3 ($PGF_{2\alpha}$) cows were treated with 500 µg of $PGF_{2\alpha}$ on d 0 and were inseminated 12 h after observed estrus. If estrus not observed, the cows received a second injection of 500 µg $PGF_{2\alpha}$ on d 14 and AI was done 12 h after observed estrus or at 80-96 h after the second injection of $PGF_{2\alpha}$ (Fig.1) for cows not exhibiting estrus. For Group 2 and 3, all cows were observed for estrus 4 times/day.

Pregnancy diagnosis was determined approximately 35 and 65 d post AI by transrectal palpation. Cows that returned to estrus following the first AI did not receive subsequent hormonal treatment.

Cost analysis:

Semen and labor costs were similar for the three treatment groups and were not included in the cost analysis, which were calculated based on treatment differences in hormone costs alone(i.e., GnRH and $PGF_{2\alpha}$). For each group, the per pregnancy costs were calculated as the total cost of hormones used for synchronization divided by the number of cows diagnosed pregnant at 65 d post AI.

Statistical analysis:

The obtained data were statistically analyzed using General Linear Models Procedures Adapted by SPSS (1997) for User's Guide.

RESULTS AND DISCUSSION

Rate of estrus synchronization, pregnancy rates and other measures of reproductive performance of cows in each of the three treatment groups are summarized in Table 1. Although only 81.8 and

63.6% of the cows in the GnRH- $PGF_{2\alpha}$ and $PGF_{2\alpha}$ were detected in estrus, all undetected cows were inseminated at 80-96 h after $PGF_{2\alpha}$ injection. Therefore, submission rates for AI were 100% for all cows within treatments. Of those cows detected in estrus, the mean intervals to estrus after $PGF_{2\alpha}$ injection tends ($P< 0.1$) to be less for cows in the GnRH- $PGF_{2\alpha}$ group than that of cows in the $PGF_{2\alpha}$ group. This finding was similar to that of Stevenson *et al.* (1999). Estrus detection can be eliminated by the administration of the second GnRH injection in conjunction with timed AI in beef (Twagiramungu *et al.*, $1992_{a,b}$) and dairy (Pursley *et al.*, 1995 and 1997_a) cattle without altering pregnancy rates compared with AI 12 h after the onset of detected estrus. In the present study 9.1% of cows treated with OvSynch protocol was observed in estrus before AI as compared with their respective in the treatments. This GnRH injection other might caused estrogen secretion (Kobayashi, 1995), and generally limited further mounting and standing activity by most cows. These results are in consistent with that of Mialot *et al.* (1999) who reported that 11 to 14% of OvSynch treatment cows were observed in estrus on a day other than d 10. The rate of visual estrus detection after the GnRH- $PGF_{2\alpha}$ treatment was significantly ($p<0.05$) higher than that obtained after the two injections of $PGF_{2\alpha}$.

Table 1: Estrus, conception and pregnancy outcomes of lactating dairy cows in postpartum subestrus treated with OvSynch, GnRH-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ protocols.

Item	Treatment group					
	OvSynch		GnRH-$PGF_{2\alpha}$		$PGF_{2\alpha}$	
	No.	%	No.	%	No.	%
No. Cows	11		11		11	
Estrus detection rate	1	9.1	9	81.8	7	63.6
$PGF_{2\alpha}$ to estrus (hrs)	-	-	68		77	
AI submission rate	11	100	11	100	11	100
Cows excluded	-	-	2	18.2	1	9.1
Conception rate at 35d	7	63.6^a	7	77.8^b	5	50.0^c
Pregnancy rate at 65d	6	54.6^a	6	66.7^b	4	40.0^c
Pregnancy losses	1	14.3	1	14.3	1	20.0

^{a,b,c}Values within each row carrying different superscripts differ ($P<0.05$.)

These results were comparable to that of Stevenson *et al.* (1999) and slightly higher than that previously reported by Lemaster *et al.*(2001) in beef cows and Stevenson *et al.* (1996) in lactating dairy cows using the same protocol in which a manufacturer's recommendation dose (100 µg) GnRH was used. Thereby, this finding showed that the improved detection rate in the current study after the combined GnRH- $PGF_{2\alpha}$ treatment and the earlier occurrence of estrus in cows show a clear advantage for this treatment and further reinforces that the GnRH- $PGF_{2\alpha}$ treatment synchronize follicular development and corpus luteum regression.

Two cows from group 2 (GnRH- $PGF_{2\alpha}$) and one cow from group 3 ($PGF_{2\alpha}$) were excluded from the calculation of conception, pregnancy and pregnancy losses because they were sold before pregnancy diagnosis (Table 1). In this study, although the conception rates for cows treated with OvSynch were significantly ($P<0.05$) higher (63.6%) than that for cows treated with $PGF_{2\alpha}$ (50%), the cows treated with GnRH- $PGF_{2\alpha}$ presented the highest conception rate (77.78%; $P<0.05$) compared with the other treatments. These results were lower than that previously reported by El-Shama'a (2003) for cows treated with OvSynch (100%) in which 50 µg GnRH was used, and comparable to that reported by Stevenson *et al.* (2000) and DeJarnette *et al.* (2001) in beef cows. However, our results were markedly higher than that previously reported by Pursley *et al.* ($1997_{a,b}$); Fricke *et al.* (1998) using OvSynch; Stevenson *et al.* (1999) using GnRH- $PGF_{2\alpha}$ and OvSynch protocols in lactating dairy cows and Momcilovic *et al.* (1998); Jobst *et al.* (2000) using a similar synchronization protocols.

The pregnancy rates diagnosed at d 65 post AI for cows in OvSynch, GnRH- $PGF_{2\alpha}$ and $PGF_{2\alpha}$ treatment groups were 54.6, 66.7, and 40%, respectively. These results demonstrated that pregnancy rates were greater ($P<0.05$) when GnRH was administered 7 d before $PGF_{2\alpha}$ injection than cows received no GnRH, however, the pregnancy rate for cows treated with GnRH- $PGF_{2\alpha}$ was the best ($P<0.05$). These results are consistent with similar studies indicating that GnRH pretreatment tends to improve reproductive performance following GnRH- $PGF_{2\alpha}$ based on estrus synchronization protocols(Twagiramungu *et al.*, 1992_a; Stevenson *et*

al., 2000; DeJarnette *et al.*, 2001). In $PGF_{2\alpha}$ treatment, lactating cows, expressed poor rate of estrus after two injections of $PGF_{2\alpha}$ and thus limited the success of such treatments (Lucy *et al.*, 1986; Stevenson, 1987; Stevenson *et al.*, 1996). This may be due to the variations in the stage of estrus cycle at the time of $PGF_{2\alpha}$ administration which affect the percentage of cows that expressed estrus in response to $PGF_{2\alpha}$ and pregnancy rate (Xu *et al.*, 1997). This may partially explain the lower rates of estrus detection, conception and pregnancy obtained for cows treated with $PGF_{2\alpha}$ in this study. Collectively, these results suggested that induction and regression of luteal structures with GnRH - $PGF_{2\alpha}$ at 7 d intervals mediates the physiological processes responsible for the short lived corpus luteum and thereby, attenuates their occurrence and negative impact on fertility.

Pregnancy losses contribute to the failure of reproductive efficiency in lactating dairy cows because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy losses (Fricke *et al.*, 1998). In dairy cows, only 48% of embryos were classified as a normal on d 7 post AI (Weibold, 1998). Thus, substantial pregnancy losses probably occurred before ultrasound examination calculated at d 28 or transrectal palpation at d 35 post insemination. In the current study, pregnancy losses from d 35 to d 65 post AI was lower for cows treated with OvSynch and GnRH- $PGF_{2\alpha}$ (14.3% or 0.48% per day) than that of cows treated with $PGF_{2\alpha}$ (20% or 0.66% per day). This rate of pregnancy losses was similar to the finding of Smith and Stevenson (1995; 19%), Vasconceles *et al.* (1997; 16.8%), Pursley *et al.* (1998; 20%), and Fricke *et al.* (1998; 13.3%) and was considerably lower for other published report (El-Shama'a, 2003; 30.7%) during comparable stage of pregnancy in lactating dairy cows. But, it was slightly higher that reported by Thurmond and Picanso (1993; 10.7%) and Thurmond *et al.* (1990; 10.6%) after routine pregnancy diagnosis by rectal palpation. The specific physiological mechanisms responsible for pregnancy losses in lactating dairy cows are unknown, but may include lactational stress associated with an increase of milk production (Nebel and McGilliand, 1993), negative energy balance (Butler and Smith, 1989), toxic effect of urea and

nitrogen (Butler *et al.*, 1995), the age of oocyte at fertilization and the time at which AI was performed (Pursley *et al.*, 1998); also, oviductal and uterine environments exposed to abnormal hormonal pattern may not be suitable for subsequent embryonic development and survival (Moreira *et al.*, 2001).

The estimate cost per pregnancy was based on 6, 6, and 4 pregnancies from 11, 9, and 10 cows for cows in OvSynch, GnRH-$PGF_{2\alpha}$ and $PGF_{2\alpha}$, respectively (Table 2).

Table 2: Cost analysis of synchronized postpartum subestrus lactating dairy cows treated with OvSynch, GnRH-$PGF_{2\alpha}$ or $PGF_{2\alpha}$ protocols.

Item	Treatment groups		
	OvSynch	GnRH-$PGF_{2\alpha}$	$PGF_{2\alpha}$
No. of cows	11	9 (11-2)	10 (11-1)
No. pregnancies[a]	6	6	4
Cost of GnRH[b]			
1-LE/ cow	7.0	3.5	
2- LE/ pregnancy[c]	12.83[A]	5.25[B]	
Cost of $PGF_{2\alpha}$ [b]			
1-LE/ cow	12	12	15.6
2- LE/ pregnancy[c]	22[A]	18[A]	39.0[B]
Total cost of hormones			
1-LE/ cow	19.0	15.5	15.6
2- LE/ pregnancy[c]	34.83[A]	23.25[B]	39.0[A]

[a]For each treatment group, number of cows pregnant at d 65 post AI,
[b]Mean hormone costs was LE 3.5 per 50 µg GnRH and LE 12 per 500 µg $PGF_{2\alpha}$,
[c]Per pregnancy costs for each treatment group were calculated as the total cost of hormones by the number of cows diagnosed pregnant at d 65 post AI.
[A,B]Values within each row carrying different superscripts differ ($P < 0.05$).

Cost per cow and per pregnancy for GnRH were LE 3.5 and LE 7.58 less for cows in GnRH-$PGF_{2\alpha}$ versus OvSynch, respectively. Whereas, PGF_{α} cost per cow was identical for both OvSynch and GnRH-$PGF_{2\alpha}$ protocols. Pregnancy costs for PGF_2 were LE 4.0 and LE 21.0 less for cows in GnRH-$PGF_{2\alpha}$ compared with OvSynch and $PGF_{2\alpha}$, respectively. Total cost of hormones per cow required for synchronization of estrus and ovulation was LE

19.0, LE 15.5 and LE 15.6 for cows in the OvSynch, GnRH-$PGF_{2\alpha}$ and $PGF_{2\alpha}$, protocols, respectively. Whereas, total cost of hormones per pregnancy was LE 34.83, LE 23.25 and LE 39.0 for the three treatment groups, respectively ($P<0.05$). The 50 µg GnRH used in OvSynch and GnRH-$PGF_{2\alpha}$ treatment groups appeared to be effective to induce a higher response compared with using double doses of $PGF_{2\alpha}$. These results confirmed the other studies which reported that 50 µg GnRH may effectively ovulate normally growing, non cystic follicles during synchronization of ovulation (Fricke *et al.*, 1998 and El-Shama'a, 2003) and improved conception rate and reduced pregnancy losses.

Applying the cost saving associated reducing the dose of GnRH required for OvSynch and GnRH-$PGF_{2\alpha}$, by 50%, the estimated cost per pregnancy could be decreased by LE 4.17 and LE 15.75 for cows in both treatment groups compared with $PGF_{2\alpha}$ treatment groups. Thus, decreasing the dose of GnRH in GnRH-$PGF_{2\alpha}$ protocol significantly ($P<0.05$) reduced synchronization cost per pregnancy and reduced pregnancy losses and may be an effective method for establishing pregnancy in subestrus postpartum dairy cows. In conclusion, the treatment of subestrus lactating dairy cows can be achieved as effectively in GnRH-$PGF_{2\alpha}$ protocol using a decreasing dose of GnRH when estrus detection is good or with OvSynch protocol when estrus detection is poor. But the reproductive performance was always better for cows given GnRH-$PGF_{2\alpha}$ compared with that given other protocols.

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تقييم استخدام بروتوكولات مختلفة لإحداث الشياع والتبويض فى أبقار اللبن الحلابة الخاملة جنسيا بعد الولادة

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الملخص العربى

استخدمت فى هذه الدراسة (٣٣) بقرة فريزيان خاملة جنسيا فى فترة ما بعد الولادة لمقارنة بروتوكولين لإحداث الشياع والتبويض مع تلك الذى يستخدم فيه حقنة أو حقنتين من البروستاجلاندين. قسمت هذه الأبقار عشوائيا إلى ثلاث مجموعات كالأتى: المجموعة الأولى تم حقنها عضليا بالهرمون المنبه لتحرير الهرمونات الجوتادوتروفيه GnRH (٥٠ ميكروجرام) عند اليوم صفر ثم حقنت بـ ٥٠٠ ميكروجرام بروستاجلاندين عند اليوم السابع وفى اليوم التاسع تم حقنها بجرعة ثانية من الـ GnRH قدرها ٥٠ ميكروجرام بعدها لقحت الأبقار عند ٨-٢٠ساعة تلقيحا صناعيا. أما المجموعة الثانية فتم معاملتها بنفس الطريقة عدا أن الجرعة الثانية من الـ GnRH قد ألغيت حيث استعيض عنها بمتابعة علامات الشياع وتلقيح الأبقار بعد ١٢ ساعة من بدأ ظهور علامات الشياع عليها ، بينما الأبقار التى لم تظهر عليها الشياع فتلقح عند ٨٠-٩٦ ساعة. تم حقن أبقار المجموعة الثالثة بـ ٥٠٠ ميكروجرام من البروستاجلاندين فى العضل بعدها لقحت الأبقار بعد ١٢ ساعة من بدأ ظهور علامات الشياع عليها بينما أعطت الأبقار التى لم يظهر عليها علامات الشياع جرعة ثانية من البروستاجلاندين (٥٠٠ ميكروجرام) عند اليوم ١٤ ثم يلاحظ بعدها الشياع فى الأبقار حيث تلقح

الأبقار بعد ١٢ ساعة من بدأ ظهور علامات الشياع عليها ، بينما تلقح تلك التى لم يظهر عليها شياع عند ٨٠-٩٦ ساعة من الحقنة الثانية للبروستاجلاندين.

أظهرت النتائج أن نسبة الحمل عند اليوم ٣٥ بعد التلقيح بالنسبه لأبقار المجموعة الثانية (٧٧,٨%) أعلى من تلك التى للمجموعة الأولى (٦٣,٦%) والثالثة (٥٠%) على الشبية من ذلك كانت نسبة الحمل عند اليوم ٦٥ بعد التلقيح لأبقار المجموعة الثانية أيضا أعلى (٦٦,٧%) من تلك التى لأبقار المجموعة الأولى (٥٤,٦%) والثالثة (٤٠%) بينما لم يكن هناك أى اختلاف بين المجاميع بالنسبه لمعدل فقد الحمل من اليوم ٣٥ إلى اليوم ٦٥ (١٤,٢٩ ، ١٤,٢٩ ، ٢٠%) للمجاميع الثلاث على التوالى. كان مقدار التوفير فى تكاليف الهرمون المستخدم لإحداث الشياع والتبويض أفضل فى المجموعة الثانية عن المجموعة الأولى والثالثة بمقدار ٤,١٧ جنيها ، ١٥,٧٥ جنيها لكل حمل على التوالى.

الخلاصة: نستنتج أن استخدام نظام الهرمون المنبه لتحرير الهرمونات الجونادو تروفية بجرعته المنخفضة + هرمون البروستاجلاندين سواء وحدة أو بجرعا بحقنه من الـ GnRH يعتبر طريقة فعالة ومؤثرة فى إحداث الشياع المخصب وتحقيق الحمل فى أبقار الفريزيان الحلابة الخاملة جنسيا بعد الولادة. فضلا عن ذلك ، أفضل كفاءة وأداء تناسلى تم ملاحظته بالنسبه للأبقار المعاملة بالهرمون المنبه لتحرير الهرمونات الجوتادوترفية + البروستاجلاندين فقط.