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EFFICACY OF OVULATION SYNCHRONIZATION WITH GNRH AND PGF2α IN SUBFERTILE DAIRY COWS

(With 4 Tables and One Figure)

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كفاءة استخدام الهرمون المحرر للحاثه المنسلية وهرمون البروستاجلاندين في توافق الشبق في الأبقار الحلابة منخفضة الخصوبة

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هدف هذه الدراسة هو تقييم برنامجين في توافق الشبق في الحيوانات الحلابة منخفضة الخصوبة والتى تعانى من تكرار الشبق اوعدم الشيوع. أجري هذا البحث علي عدد ١٨ بقرة حلوب خلال الفترة من اليوم العشرون بعد المائة إلي اليوم الخمسين بعد المائتين في الفترة التي تلي الولادة. بدأ تطبيق البرنامج العلاجي علي هذه الحيوانات بغض النظر عن المرحلة من طور الدورة التي تمر بها خلال فترة العلاج. قسمت الحيوانات إلي ثلاث مجموعات علي حسب البرنامج الموضوع سلفاً في خطة البحث. المجموعة الأولي و تضم ٦ حيوانات تم حقنها بالهرمون المحرر للحاثه المنسلية في اليوم الأول (يوم بداية البرنامج) ثم بهرمون البروستاجلاندين في اليوم السابع ثم مرة أخري بالهرمون المحرر للحاثه المنسلية في اليوم التاسع. تم تلقيح الحيوانات في اليوم العاشر بواسطة طلوقة عالي الخصوبة بصرف النظر عن ظهور علامات الشبق علي الإناث المحقونة أم لا؟ المجموعة الثانية (ستة حيوانات) تلقت جرعة من الهرمون الحاث للغدة المنسلية في اليوم الأول ثم هرمون البروستاجلاندين في اليوم السابع ثم تم تلقيح هذه الحيوانات عند ظهور علامات الشبق عليها. المجموعة الثالثة فبقيت بدون أية معاملات (مجموعة ضابطة عددها ستة حيوانات) وتم تلقيح أفراد هذه المجموعة عند ظهور علامات الشبق عليها. أظهرت النتائج المتحصل عليها أن ٣ من ٦و ٦ من٦ و ٣ من ٦ من الأبقار أظهرت علامات الشبق بالنسبة للمجموعة ١, ٢, ٣ علي التوالي. تم تشخيص الحمل في كل الحيوانات بواسطة جهاز الموجات فوق الصوتية بعد مرور ٣٠ يوم من التلقيح. كانت نسبة الإخصاب ٦٦,٦% , ٥٠,٠ % ٣٣,٣, % في المجموعة ١, ٢, ٣ علي التوالي. استنتج من هذه الدراسة أن حقن الهرمون الحاث للغدة المنسلية ثم هرمون البروستاجلاندين ثم مرة أخري الهرمون الحاث للغدة المنسلية ثم تلقيح الحيوانات بصرف النظر عن ظهور علامات الشبق علي الإناث المحقونة مناسب لتحسين الكفاءة التناسلية في الأبقار الحلوب منخفضة الخصوبة.

SUMMARY

The objective of this study is to evaluate protocols for synchronizing ovulation in dairy cows suffering from some infertility problems. In this experiment, dairy cows with open period between 120 and 250 days at random stages of estrus cycle were assigned to one of the following treatments: Group I (n = 6) received GnRH agonist (buserelin in Day 1), PGF2α (cloprostenol in Day 7) and GnRH agonist (Day 9) then blindly mated within 24h after the second GnRH injection. In Group II (n = 6), one GnRH agonist was applied (Day 1) then PGF2α (Day 7) then was mated at observed estrus. While group III (n = 6) remained without treatments and mated at observed estrus. Three weeks before treatment, the ovarian changes were monitored twice weekly by trans-rectal, B-mode ultrasonography. After the beginning of the program, the examination was undertaken daily until mating. Pregnancy was determined by ultrasonography and rectal palpation 30 to 45 days after natural mating. In the treated groups, the percentage of animals detected in heat was 3/6 (50 %) and 6/6 (100 %) for group I and II, respectively. The conception rate reached 66.6 % (4/6) in group I and 50.0 % (3/6) in group II. The percentage of animals in control group (group III), which conceived after mating during the experiment was 33.3 % (2/6). Conception rate was higher in animals which were in the first half of the estrus cycle at the beginning of the programs. It was concluded that, in dairy subfertile cows, application of GnRH/PGF2α program was potentially effective to improve the conception rate.

Key words: Ovulation synchronization, GnRH, PGF2α, Subfertile Cows, Conceptin Rate.

INTRODUCTION

Reproductive efficiency has a major impact on the profitability of a dairy herd. In lactating dairy cows a 12 to 13 months calving interval is considered optimal under most management systems and production levels (Holmann *et al.*, 1984). Economic losses from longer calving intervals result from reduced milk produced per day of herd life, greater involuntary culling and birth of fewer replacement heifers (Pelissier, 1982 and Britt, 1985).

A herd average calving interval of 12 months requires that cows conceive by 85 days postpartum. Since all cows are acyclic for a variable interval postpartum and with a reported average conception rate 50% (Butler and Smith, 1989). The most common factors that affect the calving to the first estrus interval and eventual conception include energy and protein balance (Butler and Smith, 1989 and Osawa *et al*, 1996), ovarian cysts, other ovarian dysfunction (Thatcher *et al*., 1993, Garverick, 1997 and Opsomer *et al*., 1998).

In the last decade the characterization of bovine follicular dynamics by ultrasonography (Fortune *et al*., 1988, Pierson and Ginther, 1988 and Savio *et al*., 1988) provided the rational basis for pharmacological manipulation of the estrus cycle in order to synchronize ovulation and allow AI at a predetermined time without regard to estrus behavior.

The GnRH agonists have been shown to induce follicle luteinization or ovulation, followed by the emergence of a new follicular wave (Hanlon *et al*., 1996 and Thatcher *et al*., 1989). Administering GnRH agonist followed by PGF2α 7 days later is a synchronization system where the animals show a better homogeneity of follicular development at the moment of induced luteolysis (Thatcher *et al*., 1993, Twagiramungu *et al*., 1992a and b). If a second injection of GnRH agonist is administered, 36 to 48 h after PGF2α administration, the ovulation is synchronized (Burke *et al*., 1996, Pursley *et al*., 1997a and Twagiramungu *et al*., 1995). Timed AI 16 to 24 h after the second dose of GnRH results in pregnancy rates similar to those observed in beef (Twagiramungu *et al*., 1992 a & b and Twagiramungu *et al*., 1995) or dairy cows (Burke *et al*., 1996, Pursley *et al*., 1995, Schmitt *et al*., 1996 and Wiltbank *et al*., 1996) that were bred to a normal estrus.

The aim of the present work is to test, in field trails, the association of a GnRH agonist and PGF2α in order to confirm the efficiency of this protocol in subfertile cows under our local environment.

MATERIALS and METHODS

Animals:

The experiment was conducted on 18 holstein Friesian cows in a farm located in Bany Mor, Assiut province, Egypt, during autumn of 2003. The cows have a body condition score from 2.5 to 3.5 on a 0 to 5 scale (Edmondson *et al*., 1989), were maintained in milking parlour system throughout the experiment. During the period of examination, the animals were clinically healthy and free from any infectious or contagious diseases.

The animals included in this study were suffering from some infertility problems such as repeat breeder or anestrum and their open period was between 125 and 250 days. Those with severe endometritis were excluded.

Experimental design:

The studied animals were randomly divided into three groups: group I (n = 6) was treated intramuscularly blindly irrespective of the stages of the estrus cycle with a GnRH agonist (20 μg buserelin, Receptal®, i.m., Intervet International, B.V. Boxmeer, Holland on Day 1) followed by PGF2α on Day 7 (25 mg dinoprost teomethamin, Lutalyse®, Pharmacia N.V./S.A., Buurs, Belgium). On Day 9, the cows received a second injection of GnRH (20 μg buserelin) and within 24 h afterwards they were mated blindly without estrus detection. Group II animals (n = 6) were treated in the same way as Group I except that after Day 7-PGF2α, no further injections were applied. Instead, the animals were mated once they showed heat signs. Group III animals (n = 6) remained untreated (control) and were mated when they showed normal heat. The different animal groups were maintained in isolated paddocks away from the bull until they showed the estrus behavior (Group II and III) or the determined day for blind insemination (Group I). A highly fertile bull was used for mating of the studied animals.

Ultrasonographic examination:

Before beginning of the treatments, the genital tracts of all animals in the three groups were examined rectally and ultrasonographically (PIE MEDICAL 100 L. C. with 6/8 MHz linear transducer, Holland) twice weekly for three weeks. With the start of the experiment after the beginning of the treatments, the animals were examined daily. Follicular development, number of small, medium and large follicles, atresia and ovulation were considered. The dynamics of the corpora lutea after treatment were also recorded for each cow in a separate sheet for analysis. Sonograms were recorded and printed by Sony video graphic printer (UP-890 MD, Australia).

30 days after mating, pregnancy was diagnosed ultrasonically and confirmed rectally 15 days later. All rectal and ultrasonographic examinations were performed throughout the experiment by one and the same operator.

Statistical analysis:

Statistical analyses of the collected data were carried out according to procedures of completely random design, SAS (1995).

RESULTS

Clinical findings:

At the time of the first GnRH injection Day 1,3 from 6 of the group I,4 from 6 of the group II and 1 from 6 cows of the group III had a visible corpus luteum. Seven days later, the number of corpora lutea increased to 6, 5 and 2 cows respectively. Only 2 cows in group I, 1 cow in group II and 2 in group III had a dominant follicle (> 10 mm) while the remaining animals were not cyclic (Table 1).

Table 1: Number of animals with corpora lutea.

Day (Treatment)	Group I	Group II	Group III
1(1 ^ist^ GnRH)	3/6 (50%)	4/6 (66%)	1/6 (16.6)
7 (PGF2α)	6/6 (100%)	5/6 (83.3%)	2/6 (33%)
9 (2 ^nd^ GnRH)	2/6 (33%)	3/6 (50%)	2/6 (33%)
10 (Mating)	1/6 (16%)	No CL at the day of mating	2/6 (33%) then no CL at the day of mating

Follicular Development:

Average number of the small follicles (5-6 mm) was 1.6 ± 1.2 follicles in group I at the day of the first GnRH injection. Two days later, the number reached its maximum in the treated animals (9.2 ± 2.7 follicles), then decreased gradually reaching its minimum number at the day of the second GnRH injection (1.2 ± 1.1 follicles). There was slight increase in the follicle number (2.2 ± 1.3 follicles) at the day of mating (Table 2).

The medium sized follicles (ϕ = 7-9 mm) increased in number reaching the maximum at fourth day of the treatment (5.6 ± 2.4 follicles). At the second GnRH injection, the number was 0.9 ± 0.4 follicles and there were no medium sized follicles in most of the treated animals at the day of mating (Table 2).

Table 2: Changes in follicular size and number in group I (mean ± sd.).

Day	Treatment	Small sized follicle (5-6 mm)	Medium sized follicle (7-9 mm)
1	1 ist GnRH	1.6 ± 1.2^{a}	3.4 ± 2.5^{b}
2	--	3.2 ± 1.1^{a}	3.9 ± 1.1^{b}
3	--	9.2 ± 2.7^{b}	4.2 ± 1.4^{b}
4	--	8.2 ± 1.6^{b}	5.6 ± 2.4^{b}
5	--	6.2 ± 2.3^{b}	3.8 ± 1.8^{b}
6	--	4.0 ± 2.3^{ab}	1.4 ± 1.0^{a}
7	PGF2α	2.0 ± 1.8^{a}	0.9 ± 0.8^{a}
8	--	1.6 ± 1.5^{a}	1.3 ± 0.7^{a}
9	2 nd GnRH	1.2 ± 1.1^{a}	0.9 ± 0.4^{a}
10	Mating	2.2 ± 1.3^{a}	--

*values in means ± SD.
* Values with the same superscripts were nonsiginificantly different.
*Values with different superscripts were siginificantly different ($p < 0.05$).

At the day of the first GnRH injection, four cows from 6 had dominant follicles (ϕ ≥ 10 mm). Two cows ovulated within 24 h after the injection, then one cow showed ovulation after 48 h and the fourth cow ovulated after the third day of the injection (Table 3).

Table 3: Influence of the first GnRH injection on the development of the existing dominant follicle, group I (n = 6)

Parameter/ observations	Animals	
	N	%
Number of cows with dominant follicle (∅ ≥ 10 mm)	4/6	66.0
Reaction of the dominant follicle to Ovulation:		
Within 24 h after the injection	2/4	50.0
After 48 h	1/4	25.0
After 72 h	¼	25.0

There were three dominant follicles at the 7 th day of the program (day of PGF2α injection) on the ovaries of three cows, one of them had ovulation at the first GnRH injection. At the day of the second GnRH injection, each cow in group I had a dominant follicle which ovulated after the fixed mating in all cows (100% ovulation rate).

In group II, the course of the follicular development was similar to that in group I. The number of the small follicles was 2.3 ± 1.4 follicles at the day of the first GnRH injection. On the second day, the number reached its maximum in most of the treated animals (5.4 ± 2.6 follicles) then decreased gradually reaching 1.9 ± 0.7 follicles at the day of PGF2α injection (Table 4).

The medium sized follicles increased in number after two days of GnRH injection (3.2 ± 1.7 follicles) then decreased gradually to reach 1.7 ± 0.5 follicles at the day of PGF2α injection (Table 4).

Table 4: Changes in follicular size and number in group II (mean ± sd.).

Day	Treatment	Small sized follicle (5-6 mm)	Medium sized follicle (7-9 mm)
1	GnRH	2.3 ± 1.4^{a}	2.0 ± 1.4^{a}
2	--	5.4 ± 2.6^{b}	3.7 ± 1.6^{b}
3	--	5.1 ± 2.4^{b}	3.2 ± 1.7^{ab}
4	--	4.9 ± 1.5^{b}	2.5 ± 1.5^{a}
5	--	4.1 ± 1.2^{b}	2.9 ± 1.8^{a}
6	--	3.6 ± 0.7^{b}	1.9 ± 0.7^{a}
7	PGF2α	1.9 ± 0.7^{a}	1.7 ± 0.5^{a}
At the day of mating		1.1 ± 0.8^{a}	0.8 ± 0.4^{a}

*values in means ± SD.

* Values with the same superscripts were nonsiginificantly different.

*Values with different superscripts were siginificantly different ($p < 0.05$).

At the day of the GnRH injection, two cows from 6 had dominant follicles. They ovulated within 24 h after GnRH injection. After PGF2α, 3 cows showed ovulation till the time of the second GnRH for the group I. Two cows showed ovulation two days later and one cow had no ovulation.

In control group, only three cows were cyclic with follicular growth during the study period. The remaining cows were anestrus.

Percentage of animals showing estrus signs:

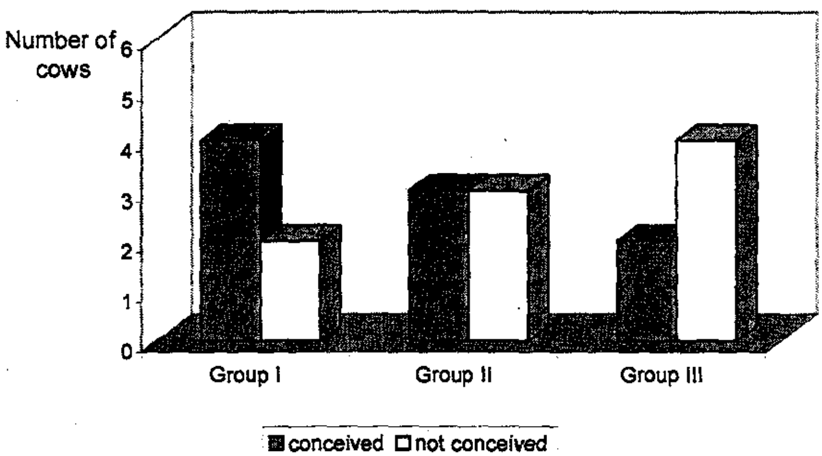
In group I, 2/6 cows (33.3%) had clear signs of heat on day of mating and only 1/6 cow (16.6%) showed weak signs. The remaining 3/6 cows (50%), were anestrus at the day of mating.

In group II, only 1/6 cows (16.6%) had signs of heat two days after PGF2α injection. On the third day, two cows (33.3%) showed signs of heat. On the fourth day, there was only one cow (16.6%) had signs of heat. The remaining cows (33.3%) were anestrus till the fifth day of injection.

Conception rate:

Pregnancy diagnosis of the studied animals using ultrasonography on Day 30 post mating revealed that 66.6% (4/6 cows, 50% (3/6 cows) and 33.3% (2/6 cows), of group I, II and III had conceived, respectively, (Fig.1).

Figure 1: Number of the conceived and not conceived cows in treated and control groups



DISCUSSION

At the time of the first GnRH injection, if a cow has a dominant and healthy follicle, this will ovulate in response to the GnRH-induced release of LH. Furthermore, the increase in FSH induced by the GnRH injection induces recruitment of a new pool of follicles in approximately 2 days (Day 7) and one of the follicles is selected to become the dominant follicle (Twagiramungu *et al.*, 1995 and Moreira *et al.*, 2000).

On day 12 of the cycle (7 days after the injection of GnRH), PGF2α is injected to regress both the original CL present at the day 5 of the cycle and the newly formed CL that was induced by the injection of

GnRH. The decrease in progesterone associated with regression of CL accelerates growth of the newly recruited follicle and a second injection of GnRH is made 2 days after the injection of PGF2α. The second injection of GnRH induces ovulation 24 to 32 hours later (Pursley *et al.*, 1995). The results of the present experiments concerning the use of Ovsynch protocol in the treatment of subestrous and subfertile dairy cows provide complimentary information to that reported by Pursley *et al.* (1995, 1997a and b).

In the present study, cows with some infertility problems were included and not those in the general population, the results are, therefore, not necessarily applicable to the general population of cows.

The effectiveness of the Ovsynch protocol may be attributed, in part, to the suggestion that the sequence of injections allows for better control of the developmental stage of the preovulatory follicle under the physiological conditions of dairy cows (Pursley *et al.*, 1995, Hussein, 2003).

The present results showed that the Ovsynch protocol, under field condition, did not allow for systematic synchronization of ovulation over an 8-h period on day 10, in contrast to previous findings of Pursley *et al.* (1995) and Hussein (2003). In this study, some of the treated cows began to cycle and showed follicular development after the first GnRH injection, However, the percent of animals returned to the cycle was somewhat low. Thus, the success of the program is dependent on whether lactating dairy cows are cycling or not as well as stage of the estrus cycle at the time the Ovsynch protocol is initiated. Clearly, if the cows within the herd are not cycling, pregnancy rate will be lowered.

From the obtained results, it was recorded that the small and medium sized follicles increased in the number and reached the maximum at the fourth day after the first GnRH injection. Similar results were obtained by Twagiramungu *et al.* (1995), Vasconcelos *et al.* (1999) and Hussein (2003). However, previous studies were carried on cyclic cows. The dominant follicles ovulated within 24 h to 72 h after the first GnRH injection (see Tab. 1). From the new follicular wave, one follicle will be selected to develop and may complete its growth. The mechanism of follicular selection is not clear. There are many suggestions to explain the selection and recruitment processes. Spicer and Echternkamp (1986) postulated that the selection of the follicles for dominancy is a result of increasing serum LH-pulses that leads to changes in the vascularization of ovarian tissues and intra follicular steroid concentration. Ginther *et al.* (1996) suggested that the drop in the

FSH-level at about two days from the appearance of a follicular wave is very important factor in the follicular selection. However, this study was not designed to investigate the mechanisms of the follicular selection.

Rather, because of the chronic low conception rate and infertility, this study was designed to evaluate a new technological approach to achieving pregnancy in lactating cows which suffer from some reproductive problems.

Treatment with buserelin induces the resumption of cyclic ovarian activity in postpartum anestrus cows, as determined by increased P4 concentrations, and fertility rate after buserelin-PGF2α-induced estrus is comparable to that of cyclic cows (Twagiramungu *et al.*, 1992a).

A higher percentage of cows in group I and group II exhibited estrus within 3 days after the second GnRH injection (group I) and 7 days after PGF2α injection. However, previous research (Archibald *et al.*, 1992) has shown that a higher percentage of cows were observed in estrus within 7 days after treatment with 2 luteolytic dosages of PGF2α 8 h apart compared with either 1 or 2 luteolytic dosages of PGF2α 24 h apart.

The conception rate of cows with Ovsynch was more successful (66.6 %) in group I than that in group II and III (50 % and 33.3 %). The highest pregnancy rate were observed in group I confirming the results of Pursley *et al.* (1995) Stevenson *et al.* (1996), Heuwiesser and Mansfeld (1999) and Sobiraj *et al.* (1999). The second dose of the GnRH injection in this program was necessary and resulted in a better conception rate than that without the second GnRH injection. In conclusion, the treatment of subfertile dairy cows can be achieved effectively with the Ovsynch protocol (GnRH + PGF2α + GnRH) with blind mating.

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