Assiut Vet. Med. J. Vol. 36 No. 72, January 1997

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SYNCHRONIZATION OF OESTRUS AND FERTILITY IN SHEEP WITH TWO INJECTIONS OF FERTIRELIN ACETATE (GnRH ANALOGUE) AND PROSTAGLANDIN $F_2\alpha$.

(With 2 Tables and One Figure)

BY

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(Received at 17/12/1996)

تواقت (تزامن) الشبق والخصوبة فى الأغنام بحقن كل من شبيه الهرمون الحاث للغدة المنسليه والبروستاجلاندين ف ٢∝

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أجريت هذه الدراسه بهدف معرفه عم اذا كان الحقن بهرمون البروستاجلاندين (١٥ ملى جرام) بعد الحقن بخمسه ايام بشبيه الهرمون الحاث للغدة المنسلية (٥٠ ميكروجرام) يعطى مستوى مقبول من تواقت الشبق والخصوبة فى الأغنام بالمقارنة بالحقن بالبروستاجلاندين فقط. أستخدمت فى هذه الدراسة عدد ٤٥ نعجة بالغة منتظمة الدورة . والتى قسمت الى أربعة مجاميع علاجية : حقنت نعاج المجموعة (أ) ب ٥ر١ ملى من محلول كلوريد الصوديوم (٩ ر٠ ٪) و حقنت نعاج المجموعة (ب) بحقنه واحده من البروستاجلاندين (١٥ ملى جرام) و حقنت نعاج المجموعة (ج) بحقنتين من البروستاجلاندين بينهما ١١ يوم و حقنت نعاج المجموعة (د) بحقنه من البروستاجلاندين (١٥ ملى جرام) بعد خمسه ايام من الحقن بشبيه الهرمون الحاث للغدة المنسلية (٥٠ ميكروجرام) . كانت نسبه حدوث الشبق فى النعاج بعد خمسه ايام من الانتهاء من الحقن فى المجاميع المختلفة (أ – د) هى ٥ر ٤٥ ٪ ، ٧ر٧٢ ٪ ، ٩ر٩٠٪ ، ٧ر٩١٪ على التوالي . ولوحظ ايضا أن نسبه ضبط إحداث الشبق خلال ثلاث ايام من الانتهاء من الحقن كان عالى ($P< 0.05$) فى النعاج التى حقنت بحقنتين من البروستاجلاندين (مجموعه ج) أو فى النعاج التى حقنت بالبروستاجلاندين بعد خمسه ايام من الحقن بشبيه الهرمون الحاث للغده المنسلية (مجموعه د) عن النعاج التى حقنت بحقنه واحده من

البروستاجلاندين (مجموعه ب) ٠ وجد ايضا أن نسبه الحمل فى المجموعة د (٣ر٨٣٪) كانت أعلى من المجموعة أ الضابطة (٣ر٢٧٪) وكذلك المجموعة ب (٥ر٤٥ ٪) التى حقنت بحقنه واحده من البروستاجلاندين ٠ وكذلك وجد أنه لا يوجد اختلافات معنوية فى نسبه حدوث الشبق والمقاييس الإنتاجية للنعاج بين المجموعتين ج و د. دلت هذه النتائج أنه يمكن الحصول على مستوى مقبول من توافت الشبق والخصوبة وكذلك ضبط حدوث الشبق بعد ثلاث أيام من نهاية الحقن باستخدام الجمع بين الحقن بالبروستاجلاندين بعد خمسه أيام من الحقن بشبيه الهرمون الحاث للغده الصنسلية فى الأغنام.

SUMMARY

A study was conducted to determine whether a single intramuscular injection of 50μg of fertirelin acetate (GnRH analogue) followed 5 day later by 15 mg of prostaglandin $F_2\alpha$ would give an acceptable level of synchronization of oestrus and fertility in comparison with prostaglandin $F_2\alpha$. A total of 45 mature cyclic crossbred ewes (Chios x Ossimi) were involved in the study and randomly allocated to one of four treatment groups [ewes received 1.5 ml saline (group A) or single injection of 15 mg $PGF_2\alpha$ (group B) or double injections of 15 mg of $PGF_2\alpha$ (group C) or GnRH-$PGF_2\alpha$ (group D)]. Oestrus occurred within 5 days from end of treatment in 45.5%, 72.7%, 90.9% and 91.7% of ewes in groups A to D. Precision of oestrus within 3 days period from end of treatment was higher ($P< 0.05$) in ewes treated with double injections of $PGF_2\alpha$ or GnRH followed 5 days later by $PGF_2\alpha$ than ewes treated with single injection of $PGF_2\alpha$. Overall pregnancy rate after treatment with GnRH followed 5 days later by $PGF_2\alpha$ (group D) was significantly ($P< 0.05$) greater 83.3 % than for control ewes 27.3% (group A) and treated ewes with single injection of $PGF_2\alpha$ 45.5% (group B). Moreover, there was no difference in response and reproductive parameters between groups treated with double injections of $PGF_2\alpha$ or GnRH followed 5 days later by $PGF_2\alpha$. These results indicated that acceptable levels of oestrus synchronization and fertility can be obtained with a combined injections of GnRH followed 5 day later by $PGF_2\alpha$ and the treatment can increase the precision of oestrus after $PGF_2\alpha$ within 3 days from end of treatment.

Key Words: *Sheep-Oestrus-GnRH-Synchronization.*

INTRODUCTION

Oestrus Synchronization is an important technique used in the management of domestic livestock and is an active area of research . Synchronization of oestrus has been used in sheep industry to improve production efficiency and to facilitate the use of artificial insemination and embryo transfer *(Brice et al., 1989 and Beck et al., 1993).*

It is generally accepted that prostaglandin $F_2\alpha$ and its analogues are the most effective luteolytic agent for synchronizing oestrus. Following $PGF_2\alpha$ or its analogue treatment, luteolysis and the consequent fall in plasma progesterone concentrations can be demonstrated to occur uniformly and rapidly but greater control over the phase of follicular maturation is desirable *(Macmillan and Thatcher, 1991)*. Many reports dealt with the benefits of using of either progestagen pessary, left in situ for 12 to 14 days *(Henderson et al., 1984 and Beck et al., 1987);* or prostaglandin $F_2\alpha$ given as a two injections separated by an interval of 9 or 11 days *(Henderson et al., 1984 and Beck et al., 1987; 1993)*, but it is considered a relatively long time period. Although, *Beck, et al. (1987; 1993)* demonstrated the effect of single injection of $PGF_2\alpha$, but if this treatment given randomly to cycling animals, only luteal phase ewes respond *(Acritopoulou and Haresign, 1980)*. Although, numerous studies have been conducted using the combined treatment with GnRH agonist followed by $PGF_2\alpha$ to improve the synchronization of oestrus and fertility in bovine species *(Macmillan et al., 1985; Guilbault et al., 1991 and Twagiramungu et al., 1992)* but, very little information is available for ovine species *(Beck et al., 1996).*

The objectives of the present study were to determine whether a combined treatment with fertirelin acetate (GnRH agonist) followed by $PGF_2\alpha$ at a 5 day interval give an acceptable level of synchronization of oestrus and fertility in comparison with prostaglandin $F_2\alpha$ and to assess the effect of pretreatment with GnRH on the precision of oestrus followed by $PGF_2\alpha$ injection.

MATERIAL and METHODS

Management and treatment

This study was conducted at the beginning of May 1996 (breeding season) at the Animal Production Research Station, Malawi, El-Minia governorate. The station belongs to Animal Production Research Institute,

Egypt. A total of 45 crossbred ewes (Chios X Ossimi), 3 -5 years old, with an average live weight 40 - 45 kg, had a successive lambing during the preceding season, were used in this study. Each ewe was submitted to a general physical examination and vaginal examination. In addition, the ewes were dewormed regularly and were flushed for 3 weeks prior to start of the study. All ewes were housed with teaser rams, under conditions of natural day-length and temperature. All ewes were grazing on Egyptian clover (T. alexandrinum) from December to May. Thereafter, they were fed on crop residues available in summer season and green forage (Darawa). In addition, the ewes were supplemented with concentrate mixture about 0.5 Kg/head and increased to 1 Kg / head at late pregnancy and lactation. All animals must have showed at least two normal estrous cycles before they were used.

The ewes were randomly assigned to one of four treatment groups. Group A, (n = 11) the ewes received a single dose of 1.5 ml i.m. injection of saline (0.9 % NaCl solution, placebo) and served as a control. Group B (n = 11) the ewes received a single i.m. injection of 15 mg $PGF_2\alpha$ (3 ml Lutalyse, Upjohn, Belgium). Group C (n = 11), the ewes received two injections of $PGF_2\alpha$ (3 ml Lutalyse, Upjohn, Belgium). Group D (n = 12) the ewes received a single i.m. injection of 50 µg fertirelin acetate GnRH analogue (1ml Conceral, Takeda, Co., Japan) followed 5 days later by 15 mg $PGF_2\alpha$ (3 ml Lutalyse, Upjohn, Belgium). All four treatments were timed to end together. The animals for each treatment were kept in a separate group with teaser ram of good libido. The ewes were observed for estrus twice daily for the following 5 days after the end of treatment. All ewes were hand mated on two occasions during the first 12 hours of estrus using fertile rams (1 ram / 7 ewes). All rams used as a teaser or for mating were flushed 3 weeks before the start of study.

Blood samples (10 ml) for serum progesterone determination were collected by jugular venipuncture and centrifuged at 3000 g for 10 min. then the serum was removed and stored at - 20°C until assayed. The samples were collected from GnRH-$PGF_2\alpha$ treated group as follow: just before GnRH injection to assess the luteal activity and at the 3rd day and 5th day after GnRH injection to assess the effect of GnRH on luteal activity. Moreover, blood samples were collected from all $PGF_2\alpha$ treated ewes after end of treatment on 3rd day and 5th day to assess the response to each treatment and after 20 days from last mating (progesterone level >1 ng/ml; *Shemesh et al, 1979*) for pregnancy diagnosis. Serum progesterone concentrations were determined by RIA method (Coated-A-count progesterone, Diagnostic

Products Co. Los Angeles, U.S.A.). All samples were analyzed in duplicate in the same assay.

Statistical analysis

The synchronization rate was defined as the percentage of ewes showed oestrus. The pregnancy rate was defined as the percentage of ewes pregnant per total number of treated ewes. Precision of estrus synchronization response was defined as the percentage of ewes which showed oestrus within fixed time intervals (3 or 5 days from end of treatment). The data were analyzed statistically with SAS program *(SAS, 1988)* as a completely randomized design. Differences in synchronization and pregnancy rates, precision of oestrus and progesterone level due to treatments were analyzed by Chi-square test *(Snedecor and Cochron, 1969)*. and T. test, respectively.

RESULTS

The effects of various treatment regimes on oestrus synchronization and fertility parameters in sheep are summarized in Table 1. Overall, the synchronization rate within 5 days after treatment was higher ($P< 0.05$) in ewes treated by double injections of $PGF_2\alpha$ (group C) and GnRH-$PGF_2\alpha$ (group D) than for control ewes (group A). However, the synchronization rate in ewes treated by single dose of $PGF_2\alpha$ (group B) tended to increase to levels higher than of control ewes (group A), but the difference was not statistically significant.

The percentage and distribution of occurrence of synchronized oestrus for ewes in each treatment group are presented in Table 1, and Fig.1. Of the ewes treated by either double injections of $PGF_2\alpha$ (group C) or GnRH followed by $PGF_2\alpha$ (group D), 7 (70%) and 9 (81.8%) ewes, respectively came into oestrus within 3 days after end of treatment, respectively. Though combined treatment of GnRH by $PGF_2\alpha$ tended to be more effective in precision of oestrus within 3 days from end of treatment than for treated by double injections of $PGF_2\alpha$, the difference was not statistically significant. However, the single injection of $PGF_2\alpha$ treatment induced synchronized oestrus in 3 ewes (37.5%) in group B. The differences between groups C, D and B were statistically significant ($P< 0.05$).

Reproductive parameters following different treatment regimes are shown in Table 1. The overall pregnancy rate for ewes either treated by either double injections of $PGF_2\alpha$ or GnRH followed 5 days later by $PGF_2\alpha$ were 72.7% and 83.3%, respectively. These values were significantly greater

($P< 0.05$) than for saline treated ewes. Moreover, the pregnancy rate for ewes treated with GnRH-$PGF_2\alpha$ (83.3%) was significantly ($P< 0.05$) greater than for ewes treated with single injection of $PGF_2\alpha$ (45.5%).

Serum progesterone levels (mean ± S.E) for ewes before and after treatment with either $PGF_2\alpha$ (single or double injections) or GnRH followed 5 days later by $PGF_2\alpha$ are summarized in table 2. Progesterone levels on day 0 just before treatment were similar between the three treatment groups. From day 0 to day 5 after GnRH injection, the progesterone levels increased from 2.52 ± 0.27 ng/ml at day 0 to 3.60 ± 0.22 ng/ml at day 3 and 3.87 ± 0.11 ng/ml at day 5 and the differences were statistically highly significant ($P< 0.01$). After administration of $PGF_2\alpha$, no difference in progesterone level (basal level at oestrus, in ewes respond) was observed between treated groups within 3 to 5 days from end of treatment.

DISCUSSION

Controlled breeding, involving the manipulation of normal estrous cycle, is usually designed to achieve a degree of ovulation control sufficient to allow the insemination of high proportion of treated animals over a short period. Programming follicular development by injection of GnRH agonist *(Macmillan and Thatcher, 1991)* provided a basis for development of an oestrus synchronization system in which both follicular development and corpus luteum regression are controlled *(Thatcher et al, 1989)* in the bovine.

Recent study has been only conducted in England *(Beck et al., 1996)* into the possibility of using sequential treatment with GnRH agonist and $PGF_2\alpha$ for oestrus synchronization in sheep. The results of our study agree with those of Beck et al. (1993; 1996) who also found that 91% and 100% ewes either treated with GnRH agonist or progestagen followed 5 days later by $PGF_2\alpha$ were mated by fertile rams compared with 94 % ewes treated with two injections of $PGF_2\alpha$ given 11 day apart. Furthermore, they reported that there was no effect of treatment on the reproductive performance of treated ewes. The reason for the combined treatment being so effective is that GnRH agonist pretreatment seems to prolong corpus luteum lifespan and or partially protect corpus luteum against spontaneous regression or induce depletion of antral follicles through luteinization of these growing follicles. Since the presence of follicles sensitive to LH *(Driancourt et al., 1990)* and growth of the follicles to diameters of 4-6 mm *(Webb and Gauld, 1985)* have been documented during the luteal phase of the estrous cycle in ewes. Thus, this

depletion and or luteinization of antral follicles (≥ 2 mm) reduced follicular production of estradiol *(Tsonis et al., 1983)* which in turn prevent increase in the concentration of endometrial oxytocin receptors and thereby prevent stimulation of $PGF_2\alpha$ production by luteal oxytocin. Recent study by *Beard and Lamming, (1994)* demonstrated that the estradiol concentration affects the timing, the magnitude and the pattern of the $PGF_2\alpha$ response to oxytocin in ewes. It is therefore, no ewes showed oestrus beheviour through 5 days before $PGF_2\alpha$ injection in our study. GnRH agonist (fertirelin acetate) acts via pituitary release of gonadotrophins and dose not have a direct effect on the ovaries of ewes since specific GnRH receptors have not been detected in the ovine ovaries *(Brown and Reeves, 1983)*. Moreover, in both primates and domestic ruminants luteinizing hormone (LH) is essential for secretion of normal amounts of progesterone at all stages of the luteal phase *(Baird, 1992)*.

In addition, these results demonstrated that pretreatment with fertirelin acetate (GnRH agonist) had a tendency to improve the precision of oestrus after $PGF_2\alpha$ injection. This tendency was further confirmed by observation that the proportion of ewes in oestrus after 1st day to 3 rd day was higher in GnRH- $PGF_2\alpha$ than in $PGF_2\alpha$ treated groups. This is in agreement with a part of recent report by *Beck et al ., (1996)* who indicated that 91% of pretreated ewes with GnRH were in oestrus within 3 days after $PGF_2\alpha$ injection. *Hunter et al., (1986)* found that the injection of progesterone was associated with a transient reduction in pulsatile LH secretion. In our study, pretreatment with GnRH increased progesterone concentration within 5 days before $PGF_2\alpha$. Similar observation reported by *Twagiramungu et al. (1992)* who indicated that injection of GnRH agonist (buserelin) induced increase in progesterone level in cycling cows. It is Known that pulsatile LH secretion is involved in the maturation of the preovulatory follicles *(Basiouni, et al., 1996)*. In addition, progesterone may exert its beneficial effect on subsequent luteal function by suppressing and therefore effectively synchronizing the early stages of follicular development *(Keisler and Keisler, 1989)*. This would insure that all potential ovulatory follicles were then at an appropriate stage of development to respond adequately to GnRH-induced follicular phase and develop into normal corpora lutea after ovulation *(Basiouni et al ., 1996)*. This coincides with a part of our data that demonstrates higher pregnancy rate for pretreated GnRH group than $PGF_2\alpha$ treated regimes alone. Therefore, it is possible that 5 days after GnRH injection resulted in a greater homogenecity of

ovarian follicles inventories and sensitive luteal structures to $PGF_2\alpha$ among ewes at time of $PGF_2\alpha$ induced luteolysis such that the precision of oestrus was improved. Oestrus response and reproductive performance of ewes in this study tended to be less than that obtained by *Beck et al., (1993; 1996)* and this may be attributed due to breed differences. It has been proposed that there is a variation between breeds in the sensitivity of hypothalamus / pituitary glands to the negative feed back effect of gonadal hormones *(Bindon and Piper, 1984).*

In this study, oestrus response and fertility parameters to a single injection of $PGF_2\alpha$ were slightly less than to the double regime. Other investigators found similar observations following single injection of PGF analogue *(Acritopoulou and Haresign, 1980; Henderson et al., 1984; Beck et al., 1987).* Moreover, the results of the luteolytic action of $PGF_2\alpha$ imposed on day 5 after GnRH injection on progesterone level in this study corroborate with those of *Douglas and Ginther (1973) and Ott et al. (1980)* where progesterone level fall to a level less than 0.5 ng/ml at oestrus.

Thus it can be concluded that an acceptable levels of oestrus synchronization and fertility can be obtained with a combined GnRH injection followed by 5 days later by $PGF_2\alpha$ comparable with other methods and increase the precision of oestrus within 3 days after $PGF_2\alpha$ injection.

ACKNOWLEDGMENT

The authors wish to thank Prof. Dr. T. Nakao, head of Dept. Obst. & Gyn., Rakuno Gakuen University, Hokkaido and Takeda Chemical Industries, Japan for donating the fertirelin acetate used in this study.

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Table 1: Estrus synchronization and fertility in ewes after treatment with saline or prostaglandin $F_2\alpha$(single or double injections) or GnRH analogue followed 5 days later by $PGF_2\alpha$.

	Treatment groups			
	Saline	Prostaglandin $F_2\alpha$		GnRH-$PGF_2\alpha$
		Single	Double	
	(A)	(B)	(C)	(D)
Ewes treated (No.)	11	11	11	12
Total ewes respond (%)	5(45.5)[a]	8(72.7)[ab]	10(90.9)[b]	11(91.7)[b]
Ewes respond ≤ 3 days of treatment (%)	2(40.0)	3(37.5)[a]	7(70.0)[b]	9(81.8)[b]
Ewes respond > 3-5 days of treatment (%)	3(60.0)	5(62.5)	3(30.0)	2(18.2)
Pregnancy rate (%)	3 (27.3)[a]	5(45.5)[b,c]	8(72.7)[b]	10(83.3)[b,d]

[a,b,c,d]: Values in the same rows with different superscripts are different ($P < 0.05$).

Table 2: Progesterone concentrations (ng/ml) in responded ewes either treated with $PGF_2\alpha$ (single or double injections) or GnRH analogue followed 5 days later by $PGF_2\alpha$.

Treatment groups	No. of ewes respond[1]	Days after treatment with				
		day 0[2]	GnRH analogue		$PGF_2\alpha$	
			3rd day	5th day	3rd day	5th day
Single $PGF_2\alpha$ (group B)	8	2.07± 0.20[a]	——	——	0.47± 0.08 (3)	0.36± 0.04 (5)
Double $PGF_2\alpha$ (group C)[3]	10	2.39±0.78	——	——	0.33± 0.06 (7)	0.29±0.09 (3)
GnRH- $PGF_2\alpha$ (group D)	11	2.51± 0.27[a]	3.60± 0.22[b]	3.87± 0.11[b]	0.34±0.05 (9)	0.53±0.03 (2)

[a,b]: Values in the same rows with different superscripts are different ($P < 0.01$).
[1] Ewes in oestrus within 3- 5 days from $PGF_2\alpha$ injection. [a]: mean (± S.E.).
[2] Day 0: Just before injection of $PGF_2\alpha$ or GnRH.
[3] P_4 levels after 2nd injection of $PGF_2\alpha$.
( ) Number of ewes (oestrus) at different intervals from $PGF_2\alpha$ injection.

Fig.1: Distribution of estrus between days 1 and 5 in ewes after treated either with prostaglandin F (single or double injections) or two injections each of fertirelin acetate (GnRH analogue) and prostaglandin F