OESTRUS SYNCHRONIZATION AND FERTILITY IN ROMANOV CROSSBRED EWES USING GnRH AND $PGF_{2\alpha}$

By

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ABSTRACT

Sixty Romanov crossbred ewes aged 2.5-3 years and weighing 40-50 kg, were used to compare three method of estrus synchronization. The ewes were divided randomly into three similar groups. The first group (PFP) received intravenous two injections each of 125 µg $PGF_{2\alpha}$ (cloprostenol-estrumate) 11 d apart and a single intravenous injection of 20 µg GnRH (Fertagyl) 5 d prior to the second $PGF_{2\alpha}$ injection. The second group (PF) was given intravenous 20 µg Fertagyl followed 5 days later by intravenous injection of 125 µg cloprostenol. The third group (PP) was intravenous injected with 250 µg cloprostenol, 11 d apart, and served as control. All ewes were naturally inseminated (NI) on observed standing estrus or on fixed time 72-80 hrs after the 2nd $PGF_{2\alpha}$ administration if ewes that failed to display standing estrus. Pregnancy was diagnosed 50 d post NI using ultrasonic technique. The percentage of exhibition estrus was 55.0; 36.8 and 16.7% for the PFP; FP and PP treatments, respectively. The synchronized pregnancy rate by ultrasonic technique was 54.6; 71.4 and 100% for ewes displayed standing estrus in different three treated groups (PFP; FP and PP, respectively), while it was 44.4, 25.0 and 0.0% for ewes inseminated at fixed time in the corresponding three groups. The lambing rate was 45.0; 52.6 and 16.7% and the litter size was 1.89, 1.3 and 1.0 in PFP; FP and PP treatments, respectively.

Estimated saving in hormone costs using lower doses of $PGF_{2\alpha}$ and GnRH (Fertagyl) for synchronization of estrus and fertility in FP treatment were LE 3.25 and LE 8.35 per ewe and LE 8.66 and LE 69.16 per lambing less than PFP and PP treatment respectively. It was concluded that Fertagyl and cloprostenol, given 5 days apart, will produce intermediate levels of estrus

synchronization, fertility, less laborious and has short duration comparable with a double dose of $PGF_{2\alpha}$ with or without GnRH.

INTRODUCTION

Maintenance of the reproductive efficiency of sheep is an ongoing challenge for sheep producers. Therefore, estrus synchronization is used in the sheep industry to improve production efficiency and to facilitate the use of artificial insemination and embryo transfer techniques (Beck *et al.*, 1996). The method most commonly used is the progestagen impregnated intravaginal sponge, left *in situ* for 12 to 14 days (Gordon, 1983). A less commonly used but equally effective method is two injections of $PGF_{2\alpha}$ separated by an interval of 9 to 11 days (Dahlen *et al.*, 2003). Although both these methods give high level of synchronization and fertility but they need a relatively long period. Therefore it would be advantageous to develop a method that requires less time and labour. Beck *et al.*, (1996) demonstrated that an injection of 4 μg GnRH analogue (buserelin) 5 days prior to the $PGF_{2\alpha}$ treatment, will result in an acceptable level of synchronization and fertility in Welsh Halbred ewes comparable with a standard double dose $PGF_{2\alpha}$ regime. Anestrus in ewes is characterized by the absence of estrus and ovulation due to decreased LH pulse frequency in response to increased hypothalamic sensitivity to the negative feedback effect of estradiol (Legan and Karsch, 1980 and Goodman *et al.*, 1982). An increase in LH pulsatility by GnRH given during the luteal phase. can result in either ovulation or atresia of the dominant follicle (Webb *et al.*, 1992; Rubianes *et al.*, 1997 and Tasende *et al.*, 2002) and when $PGF_{2\alpha}$ is given a few days later a new wave of follicles is synchronously developing (Wolfenson *et al.*, 1994 and DeJarnette *et al.*, 2001). The objective of this study was to determine the effect of GnRH when used in conjunction with one or two injections of $PGF_{2\alpha}$ on estrus synchronization and fertility in anestrus Romanov crossbred ewes comparable with control group received two injections of $PGF_{2\alpha}$ without GnRH.

MATERIAL AND METHODS

A total of sixty mature, non pregnant crossbred Romanov ewes, 2.5-3 years of age with on the average weighed 40 to 50 kg.

were used for the current work. Animals were belonging to the Mehallet Mousa Station, Animal Production Research Institute, Ministry of Agriculture during the anestrus period (May 31 to early summer, 2003). Ewes were housed in semi open pens under conditions of natural day length and were fed on (0.25 kg) concentrate mixture, rice straw (0.6 kg) and berseem hay according to the standard allowances recommended by the Ministry of Agriculture. Water and Minerals blocs were always available.

Ewes were allocated randomly into three equal treatment groups (20 ewes/each, Figure 1). GrouPPFP received two intravenous injections each of 125 µg cloprostenol, $PGF_{2\alpha}$ (Estrumate, Coopers Tiereazneimittel GmbH, Burghwedel, Germany) given 11 days apart and a single intravenous injection of 20 µg GnRH (Fertagyl; Intervet, Millsboro, DE) on days 5 prior to the second injection of PG as follows: Day 0, PG; Day 6, GnRH; Day 11, PG. Group FP. Group FP: Was treated with a single intravenous injection of 20 µg Fertagyl (GnRH) followed 5 days later by intravenous injection of 125 µg cloprostenol. Group PP: (considered as control) received two intravenous injections each of 250 µg cloprostenol ($PGF_{2\alpha}$), 11 days apart.

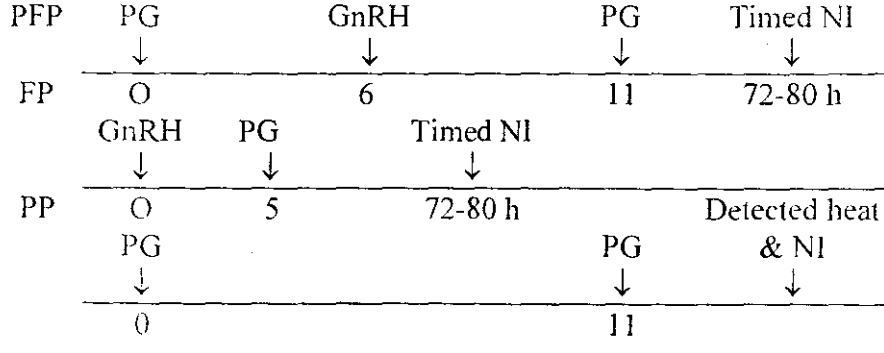

Fig. (1): Timeline for administration of hormones to experimental groups

All ewes were detected for the onset of estrus three times daily and ewes which were seen to be receptive and stood for mounting by the ram were considered to be in estrus. All ewes were naturally inseminated (NI) on standing estrus or on fixed time 72-80

hrs after PG administration to ewes that failed to display standing estrus. Ewes were examined for pregnancy diagnosis at day 50 post breeding using ultrasonic technique (Bedienuny Sanleitung-Germany). Lambing rates and litter size (number of lambs born/ ewe) were recorded. Individual blood samples were taken via Jugular vein puncture just before hormonal injection, centrifuged at 3000 rpm for 15 minutes and stored at -20°C for later progesterone (P_4) assay using RIA technique. Analysis of variance was done according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The plasma progesterone concentrations of the 3 treated groups are shown in Table (1). There was a marked increase in the concentration of progesterone after 6 days of giving the 1st dose of PG in PFP ewes (2.39 ± 0.12 ng/ml) compared with those treated by FP (0.54 ± 0.03 ng/ml). It was reported that during anestrus, ovulation can be induced with LH or GnRH (McLeod and Haresign, 1984 and Legan *et al.*, 1985), but these treatments always result in a high proportion of ewes with premature luteal regression. This can be avoided by previous priming with luteal-phase concentrations of progesterone by using a combination of PG, GnRH and PG treatment (PFP). This result explain progesterone priming in anestrus ewes since the reduction of uterine sensitivity to estradiol in early diestrus allows normal luteal function and it may affects the ovarian dynamics, as being observed in cows (Sirois and Fortune., 1990 and Bergfelt *et al.*, 1991) and in sheep (Leyva *et al.*, 1998). The data for one ewe from FP and two ewes from PP, that died during the trial were excluded from the results.

Table (1): Plasma progesterone concentration (P_4) ng/ml of anestrus Romanov crossbred ewes treated with either $PGF_2\alpha$ and GnRH $PGF_2\alpha$ alone.

Treatments	P_4 concentration (ng/ml) on day of		
	PG injection	GnRH injection	PG injection
PFP (0-6-11)	0.08 ± 0.0	2.39 ± 0.12	1.29 ± 0.09
FP (0-5)	-	0.54 ± 0.03	0.83 ± 0.02
PP (0-11)	1.87 ± 0.14	-	1.95 ± 0.055

A percentage of ewes exhibited synchronized estrus after treatment was 55% (11/20) PFP and 36.8% (7/19) in FP being significantly higher than 16.7% (3/18) in PP, Table (2). These results were confirmed by previous studies, which suggested that analogous of GnRH, administered 1 week prior to $PGF_{2\alpha}$ improved the rate and precision of synchronization of subsequent estrus (LeBlanc *et al.*, 1998 and Jobst *et al.*, 2000), it also increased the size of ovulatory follicle and raised plasma estrogen concentration at estrus (Wolfenson *et al.*, 1994). The mode of action in response to the application of GnRH is to reset the follicular wave cycle, leading to selection of a dominant follicle 1 to 2 d after GnRH treatment (Twagiramungu *et al.*, 1995). In the present study, the percentage of standing heat in PFP treated ewes was higher than that treated with FP but the differences in this respect were not significant. This finding disagrees with Beck *et al.* (1996) who obtained 88.8% synchronization using a combination of buserelin (GnRH) and $PGF_{2\alpha}$ in sheep during the breeding season. The effects of GnRH on the corpus luteum (CL) present at the time of treatment are equivocal (Macmillan and Thatcher, 1991). The variability in the response may be related to the fate of large follicles and the subsequent formation of accessory CL following luteinization. In PFP treatment 6 (54.6%) out of 11 ewes inseminated at observed standing estrus and 4 (44.4%) out of 9 ewes inseminated at fixed time were diagnosed pregnant after 50 d post breeding (Table 2). The corresponding values for FP treatment were 71.4% and 25% respectively. The total ewes diagnosed pregnant in the previous two groups were 10 (50%) and 8 (42.1%), respectively, but the difference between the two groups was not significant. The percentage of ewes diagnosed pregnant in PP control group (16.7%) was significantly ($P < 0.05$) lower than that of the other two groups. The present findings were agreement with that reported by Stevenson *et al.* (1996) but it was lower than that reported by Beck *et al.* (1996). The high pregnant rate following the combined treatments suggests that the Fertagyl treatment ensured 42.1 to 50% of the ewes were responsive to $PGF_{2\alpha}$ 5 days later. This was probably due to a Fertagyl induced LH surge causing ovulation or

luteinization of ovarian follicles or alternatively the prolongation of luteal function (Beck *et al.*, 1996). Lambing rate, in PFP (45%) and FP (52.6%) treatments, was significantly ($P < 0.05$) higher than that in control (PP) treatment (16.7%), Table (2). The present results was lower than that obtained by Gordon (1983), Beck *et al.* (1987), Beck *et al.* (1996) and El-Shamáa *et al.* (2003).

Table (2): Estrus response, synchronized pregnancy rate diagnosed on d 50 by ultrasonic technique and lambing rates in Romanov crossbred after estrus using GnRH and $PGF_{2\alpha}$ treatments.

Treatments	No. of treated ewes	Synchromized estrus		Failed to show standing estrus		Pregnancy rate on d 50 after NI at				Total		Lambed ewes	
						Standing estrus		Fixed time NI					
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
PFP	20	11	55.0 b	9	45.0 a	6	54.6 a	4	44.4	10	50 b	9	45.0 b
FP	19	7	36.8 b	12	63.2 a	5	71.4 a	3	25.0	8	42.1 b	10	52.6 b
PP	18	3	16.7 a	15	83.3 b	3	100.0 b	0.0	0.0	3	16.7 a	3	16.7 a
Total	57	21	36.8 A	36	63.2 B	14	66.7 B	7	19.4 A	21	36.8	22	38.6

a. b: Percentages in column with different superscripts significantly differ ($P < 0.05$)
A. B: Percentages in row with different superscripts significantly differ ($P < 0.05$).

Fertagyl when used in conjunction with either two or one injections of $PGF_{2\alpha}$ (PFP and PF) leading to increase the incidence of twining (Table, 3) compared with the control group (PP) received two injections of $PGF_{2\alpha}$ without GnRH. Mean of litter size per ewe was higher ($P < 0.05$) in PFP than in FP and PP treatments (1.89 vs 1.3 & 1.0, respectively, Table 3). The present results were lie with the range obtained by Laliotis *et al.* (1998).

Table (3): Number of ewes lambing to synchronized oestrus and litter size distribution after different treatments.

Treatments	No. of ewes lambed	No. of lambs						Total of lambs	Litter size ewe
		Single		Twins		Triplets			
		No.	%	No.	%	No.	%		
PFP	9	3	33.3	4	44.4	2	22.2	17	1.89 c
FP	10	7	70.0	3	30.0	0.0	0.0	13	1.30 b
PP	3	3	100.0	0.0	0.0	0.0	0.0	3	1.00 a
	22	13	59	7	31.8	2	9.1	33	1.50

Cost analysis of estrus synchronization were based on 9, 10 and 3 lambed ewes from 20, 19 and 18 ewes treated with PFP; FP and PP treatments, respectively, (Table 4). Total cost of hormones per ewe required for estrus synchronization was LE 7.9; 4.65 and LE 13 for ewes treated with PFP; FP and PP, respectively. However, the total cost of hormones per lambing was LE 17.5, 8.84 and 78 for ewes in PFP; FP and PP treatments, respectively. Compared with other methods of synchronization, the administration of Fertagyl 5 d before one injection of $PGF_{2\alpha}$ (FP treatment) has, from a practical point of view, a number of advantages. It has a short duration, thus allowing an overall reduction in the time required for synchronization procedures and this might be useful for shortening artificial insemination and embryo transfer programmes (Beck *et al.*, 1996). In addition, the total cost of hormones per ewe was LE 3.25 and LE 8.35 less for ewes in FP treatments versus the PFP and PP treatments, respectively, while the total cost of hormones per lambing was LE 8.66 and LE 69.16 less for ewes in FP treatments versus the PFP and PP treatments, respectively.

Table (4): Cost analysis of synchronization of estrus in anestrus Romanov crossbred ewes after administration of GnRH and $PGF_{2\alpha}$.

Treatments	PFP	FP	PP
No. of ewes	20	19 (20-1)	18 (20-2)
No. of lambed ewes	9	10	3
Cost of $PGF_{2\alpha}$[A]			
1- LE/ewe	6.5	3.25	13
2- LE/lambing[B]	14.4 b	6.18 a	78 c
Cost of GnRH[A]			
1- LE/ewe	1.4	1.4	-
2- LE/lambing[B]	3.1	2.66	-
Total cost of hormones			
1- LE/ewe	7.9	4.65	13
2- LE/lambing[B]	17.5 b	8.84 a	78 c

A. Mean hormone costs was LE per 13 per 500 µg $PGF_{2\alpha}$ and LE 7 per 100 µg GnRH

B. Per pregnancy costs for each treatment were calculated as the total cost of hormones divided by the number of ewes lambed

a,b,c Values in row with different superscripts significantly differ ($P < 0.05$).

In conclusion, the results of this study suggest that Fertagyl and one injection of $PGF_{2\alpha}$, given 5 days apart. is effective treatment and is less laborious as an 11 days double dose $PGF_{2\alpha}$ regime with or without GnRH for synchronization estrus during the out breeding season.

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تنظيم الشياع والخصوبة فى نعاج الرومانوف الخليط باستخدام الهرمون المحرر للجرنادوترفنيات والبروستاجلاندين $F_{2\alpha}$

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الملخص العربى

استخدم فى هذه الدراسة ستون نعجة من الرومانوف الخليط عمرها من ٢,٥-٣ سنة وتزن من ٤٠-٥٠ كجم. قسمت النعاج عشوائيا الى ثلاثة مجاميع متماثلة. المجموع الاولى تم حقنها وريديا بجرعتين من البروستاجلاندين تحتوى كل منها على ١٢٥ ميكروجرام كلوبروستينول بفاصل ١١ يوم كما حقنت بـ ٢٠ ميكروجرام GnRH (فيرتاجيل) قبل الحقنة الثانية للبروستاجلاندين بخمسة أيام اما المجموعة الثانية فتم حقنها بـ ٢٠ ميكروجرام GnRH (عند اليوم صفر) ثم بعد خمسة أيام حقنت بـ ١٢٥ ميكروجرام كلوبروستينول ، أما المجموعة الثالثة فحقنت بالوريد بـ جرعتين من البروستاجلاندين كل منها ٢٥٠ ميكروجرام كلوبروستينول بفاصل ١١ يوم واستخدمت كمجموعة ضابطه (كنترول). تم تلقيح جميع النعاج طبيعيا بالنسبة للنعاج التى اظهرت شياع وكذلك بالنسبة للتى لم تظهر شياع عند وقت محدد للتلقيح ٧٢-٨٠ ساعة بعد الحقنة الاخيرة من البروستاجلاندين. وتم تشخيص الحمل للنعاج بعد ٥٠ يوم من تاريخ التلقيح باستخدام الموجات فوق الصوتية. كانت نسبة النعاج التى اظهرت شياعا هى ٥٥% ، ٣٦,٨% و ١٦,٧% لكل من المجموعة الاولى والثانية والثالثة على التوالى. وكان معدل الحمل الناتج عن تزامن الشياع عند اليوم ٥٠ بعد التلقيح باستخدام الموجات فوق الصوتية هى ٥٤,٦% ، ٧١,٤% و ١٠٠% بالنسبة للنعاج التى اظهرت شياع فى المجاميع الثلاثة المعاملة على التوالى. بينما كانت ٤٤,٤% ، ٢٥% ، صفر% بالنسبة للنعاج التى لم تظهر عليها شياع ولقحت عند وقت محدد ٧٢-٨٠ ساعة من الحقنة الاخيره من البروستاجلاندين فى المجموعات الثلاثة المتعاقبة على التوالى. ايضا كان معدل الولادات ٤٥% ، ٥٢,٦% و ١٦,٧% ومتوسط معدل الحملان لكل نعجه ١,٨٩ ، ١,٣ و ١ فى المجاميع

المعامله على الترتيب. وبحساب تكاليف المعامله الهرمونية لتنظيم الشياع والخصوبة فى المعامله الثانيه كانت اقل بمقدار ٣,٢٥ جنيه و ٨,٣٥ جنيها لكل نعجه وأقل بمقدار ٨,٦٦ جنيها و ٦٩,١٦ جنيها لكل ولاده عند مقارنتها بتكاليف المجموعة الاولى والثالثة على الترتيب ومن هذا نستنتج ان المعامله بالفيرتاجيل (GnCH) والكلوبروستينول المعطى بفاصل خمسة أيام بينهما اعطت مستويات من تنظيم الشياع والخصوبة وبأقل تكاليف معنوية وإحتياجها الى فتره زمنيه قصيره لتطبيقها بالمقارنه لطريقه حقن جرعتين من البروستاجلاندين مع او بدون الحقن بالفيرتاجيل.