EFFECT OF PROSTAGLANDIN F-2α DOSAGE AND ROUTE OF ADMINISTRATION ON ESTRUS INDUCTION IN ROMANOV CROSSBRED EWES DURING THE END OF BREEDING SEASON

BY

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SUMMARY

Romanov crossbred ewes (n = 35) aged 22 months and weighing 40 kg live body weight were used to determine the influence of prostaglandin $F_2\alpha$ (Cloprostenol) dosage and route of administration on proportion of ewes showing estrus, interval to estrus, duration of estrus, conception rate and litter size. Ewes were randomly assigned to five groups (each of seven). Group I was intramuscular (IM) injected with 250 µg Cloprostenol ($PGF_{2\alpha}$). Groups 2, 3 and 4 were received 100 µg cloprostenol per ewe, administrated either deep cervical (DC), intravulvo-submucosa (IVSM) or intravenous (IV), respectively, while G_5 was served as control without any hormonal treatment. Ewes didn't show estrus after the first $PGF_{2\alpha}$ treatment, they were retreated with a second dose, 10 days apart, by the same route of PG administration. Ewes of groups 1, 2 and 4 were intramuscular injected with 450 IU pregnant mare serum at the 2nd PG treatment, while the 3rd group was intramuscular injected with 4 µg GnRH at the beginning of estrus. Ewes were observed three times daily for signs of behavioural estrus over 5 days. Percentage of ewes exhibiting estrus within 23 to 32.7 hrs following the application of prostaglandin via IVSM or IV was similar (100%) and higher than those IM injected (83.7%), DC (50%) or untreated ewes (28.6%). The time elapsed from treatment to estrus was significantly shorter ($P < 0.05$) in IVSM ewes (36.9 hrs) compared with 40.6 hrs in IV; 44.7 hr in DC and 47.7 hrs in IM group. The corresponding duration of the induced estrus was 23.1, 32.7, 41.3 and 34 hrs, respectively. All responded ewes were mated and observed for estrus 25 days post insemination. The lambing rate was higher in IVSM ewes than the other treated groups. The present study, indicated that 100 µg

cloprostenol administration via IVSM route should be an effective method for estrus synchronization and improved lambing rate in Romanov crossbred ewes.

INTRODUCTION

Estrous synchronization of sheep has been accomplished using several methods with varying degrees of success. Luteolytic agents such as prostaglandin $F_{2\alpha}$ or its synthetic analogs induce lysis of corpus luteum between the 4th and the 14th day of the estrous cycle (Deaver *et al.*, 1986).

Such agents are only effective in animals exhibiting ovarian cyclicity at the time of treatment (Wiltbank *et al.*., 1995 and Gordon, 1997). Doses of prostaglandin (Cloprostenol, Estrumate) ranging from 10 to 50 µg, administrated interamuscularly (IM) at d 10 of the estrus cycle have been used effectively for induction of estrus in ewes (Narayana, 1987). Intravulvo-submucosal (IVSM) administration in a small dose (10-40% of IM dose) is more economical for synchronization of estrus in ewes (Cardova *et al.*, 1990; Trivenidutt *et al.*, 1995 and Mohammed *et al.*, 2000) and in does (Mellado *et al.*, 1994 and Romano, 1998). The large variations in the estrus response observed after a synchronization treatment with prostaglandin and its analogue are the major factor limiting conception rate in sheep (Hackett and Robertson, 1980). These variations can be attributed to, the day of cycle when prostaglandin was given (Kastelic *et al.*, 1990); the wave of origin of the ovulatory follicle (Kastelic and Ginther, 1991), forms of hormonal therapy and route of injections (MacMillan and Peterson, 1993 and Mohammed *et al.*, 2000). Walker *et al.* (1989) demonstrated that administration of GnRH prior to insemination increased yield of fertilized ova from ewes, initiated a new wave of follicular development and improved the number of ovulations (Cognie, 1990).

The aim of this study was to evaluate the use of a low doses of estrumate (cloprostenol, $PGF_{2\alpha}$) at different sites of injections for estrus synchronization in Romanov crossbred ewes during the transition from breeding season to anestrus.

MATERIALS AND METHODS

Animals and management:

The present study was carried out on 35 Romanov crossbred ewes aged 22 months old and weighing 40 kg live body weight belonging to the Mehallet Mousa Experimental Station, Animal Production Research Institute, Ministry of Agriculture, during the period from mid-January to Mid-July, 2003.

Throughout the experiment, animals were housed in semi open pens under conditions of natural day length and were fed on 0.25 kg concentrate mixture (14% protein and 11% crude fiber) and Berseem according to the recommendations of the Ministry of Agriculture. Water and minerals blocks were always available.

Experimental design and procedure:

Ewes used in the present study didn't show signs of heat during the mating season in December (the end of the natural breeding season and the start of anestrus season). Ewes were randomly divided into five groups (each of seven) according to the dosage and route of $PGF_{2\alpha}$ (Estrumate, Coopers Tierazneimittel GmbH, Burghwedel, W. Germany, each ml contains 250 µg cloprostenol) administrated without knowledge of the stage of the estrus cycle in ewes as follows: group 1 (G_1) was received a single intramuscular (IM) injection of 250 µg cloprostenol. Groups 2, 3 and 4 received 100 µg cloprostenol (estrumate) per animal, via deep cervical (DC, ewes were lifted from hind limbs and by using small vaginal speculum and AI gun, the drugs were infused into the cervical canal deeply as possible and each ewe was raised at this position for about 5 min. after drugs administration, Mohammed *et al.*, 2000), intravulvosubmucosal (IVSM) and intravenous (IV), respectively. Ewes did not respond to the first injection, were injected with a second dose, 10 days apart, by the same route of $PGF_{2\alpha}$ administration. Ewes in G_1, G_2 and G_4 were IM injected with 450 IU PMSG (Folligon Intervet International B.V. Boxmeer, Holland) at the 2nd dose of PG injection. While the third group was IM injected with 4 µg GnRH (receptal-Hochst Veterinary GmbH, Germany) 36 hrs after the 2nd dose of $PGF_{2\alpha}$ injection. Group 5 was served as control without any hormonal treatment.

The onset of signs of heat was detected 3 times daily for 5 days after $PGF_{2\alpha}$ injection using a 3-yr old vasectomized ram. Ewes were considered in heat when they stood to be mounted by the male. Estrus duration was considered the time between the first and the last accepted mount. Ewes were naturally mated and observed for estrus 17-25 days after mating.

Pregnancy diagnosis was performed 60 days after insemination, using ultrasonography. Lambing rate and litter size (number of lambs born per ewe) were recorded.

The results were statistically analyzed using analysis of variance by SPSS (1997) for use's guide.

RESULTS AND DISCUSSION

Percentage of ewes in estrus after prostaglandin injections is presented in Table (1). Estrus response to the first injection and second injection was 44.4% and 73.3%, respectively ($P < 0.05$). Occurrence of estrus after PG injection in different routes, showed that all treated ewes (100%) of group 3 (IVSM) and group 4 (IV) expressed estrus signs and responded to synchronization treatment (Table, 1). The percentage of estrus exhibition in group 1 (IM) reached 83.7%, while the lowest percentage (50%) was observed in ewes treated with luteolytic drugs through DC infusion (group 2). Only 28.6% (2 from 7) of untreated ewes (control) exhibited estrus. The difference between these percentages was statistically significant ($P < 0.05$). Prostaglandins and its analogues have proven to be the most practical means for inducing luteolysis and synchronizing estrus (Wiltbank *et al.*, 1995). In this study the higher percentage of estrus exhibition was noticed in ewes that received PG via IVSM (G_3) and IV (G_4), since, the ability of PG to reach the uterine vein is faster and reaches to ovary directly via a counter current transfer mechanism in uterooverian pedicle (McCracken *et al.*, 1972) without passing through the pulmonary vascular bed, where it would be rapidly degraded to its inactive metabolites as in case of IM injections. Similarly, Cardova *et al.* (1990) suggested that effective estrus response to PG treatment through IVSM route may dependent on a unilateral pathway between the intravulvo-submucosa and ovary. Similar results were obtained by Khalifa (1999) in buffaloes and Mohammed *et al.* (2000) in Barki ewes, they reported that 100% and 96.6% estrous response, respectively.

was obtained after treating with $PGF_{2\alpha}$ via IVSM route. Results of present study support the concept that low doses of $PGF_{2\alpha}$ can be effective for estrus synchronization when administrated by the IVSM which is higher than that reported by Mellado *et al.* (1994) in goats.

Interval to onset of estrus was affected by both dose and route of $PGF_{2\alpha}$ administration (Table, 2).

Table (1): Effect of PG dosage and its route of injection on the incidence of heat in Romanov crossbred ewes.

Treatments	No. of treated ewes	No. of ewes responding to PG injection		No. of total responding ewes (%)
		after 1st dose (%)	after 2nd dose of PG (%)	
IM 250 µg PG (G_1)	7	5 (71.4)^{cd}	1 (50)^{b}	6 (83.7)^{cd}
DC of 100 µg PG (G_2)	6*	2 (33.3)^{b}	1 (25)^{a}	3 (50)^{b}
IVSM of 100 µg PG (G_3)	7	1 (14.3)^{a}	6 (100)^{c}	7 (100)^{d}
IV of 100 µg PG (G_4)	7	4 (57.1)^{bc}	3 (100)^{c}	7 (100)^{d}
Control (G_5)	7	-	-	2 (28.6)^{a}
Total	34	12 (44.4)^{A}	11 (73.3)^{B}	25 (73.5)

Percentages with different superscripts (a, b, c, d) in the same column differ significantly ($P < 0.05$)

Percentages with different superscripts (A & B) in the same row differ significantly ($P < 0.05$)

* One out of 7 ewes was detected pregnant before the beginning of treatment and excluded.

A higher proportion of ewes injected via IVSM route presented estrus earlier (36.9 $\pm$ 0.46 h) compared to ewes injected IM (47.7 $\pm$ 1.34 hr), followed by IV (40.6 $\pm$ 2.24 hr) and DC (44.7 $\pm$ 3.21 hrs).

These results suggest that, in ewes, $PGF_{2\alpha}$ injected via IVSM route reached the ovary faster, which caused earlier luteolysis and subsequent estrus. These results were confirmed by the previous finding of Mellado *et al.* (1994) in goats and Mohammed *et al.* (2000) in Barki ewes. In heifers, Alvarez *et al.* (1991) did not find differences in hours from treatment ($PGF_{2\alpha}$ given IM or IVSM) to estrus.

Table (2): Onset time to estrus (hrs) and duration of estrus (hrs) as affected by dose and route of PG administration.

Treatments	Onset time to estrus (hrs.)			Duration of estrus (hrs.)		
	Post 1st dose	Post 2nd dose	Total	Post 1st dose	Post 2nd dose	Total
G_1	47.4±1.9	49.0±0.0	47.7±1.3 ^{cd}	37.2±1.3	18.0±0.0	34.0±1.8^{b}
G_2	41.0±1.4	52.0±0.0	44.7±3.2^{c}	42.0±0.0	40.0±0.0	41.3±0.6^{c}
G_3	39.0±0.0	34.0±2.6	36.9±0.5^{a}	36.0±0.0	21.0±0.7	23.1±1.1^{a}
G_4	39.0±0.0	40.8±2.6	40.6±2.2^{b}	41.3±2.9	18.0±3.0	32.7±2.4^{b}
Total	41.6±0.8	43.8±1.2	42.5±1.4	39.1±0.9	24.3±2.7	32.8±1.2

Means with different superscripts (a, b, c, d) in the same column differ significantly ($P < 0.05$)

In accordance, Mutiga and Mukasa-Mugerwa (1992) found that the onset of estrus ranged from 43.5 ± 1.61 to 45.3 ± 1.43 hrs by $PGF_{2\alpha}$, while it was 35 to 67 hrs by different types of PG (Mohammed *et al.*, 2000). This interval depends on the phase of follicular development at the time of $PGF_{2\alpha}$ injection, animals that possess dominant follicles that are still growing will show estrus in 48 to 60 hrs, while animals with follicles at the plateau stage or regressing phase will take more than 3 days (Bo *et al.*, 1994 and Pinheiro *et al.*, 1998). Beal (1996) explained the variation in the timing of estrus to differences among animals in the rate of regression of the CL following $PGF_{2\alpha}$ treatment.

Concerning the length of estrus phase (duration of estrus), it averaged 23.1 ± 1.07; 32.7 ± 2.38; 34.0 ± 1.81 and 41.3 ± 0.58 after PG administrated via IVSM; IV; IM and DC route, respectively, Table (2). The differences between treatments were statistically ($P < 0.05$) significant. Variation between treatments with regard to duration of estrus might be due to the amount of oestrogen in the blood produced by induced luteolysis; estrogen level in blood is presumed to bring the animals into estrus and has a depressing effect on progesterone (Muna Ahmad *et al.*, 1998).

Non-return to estrus, pregnancy rates at 60 days post natural mating determined by ultrasonography, lambing rates and number of lambs born following different sites of PG administration are presented in Table (3). Non-return to estrus was highest (100%) in groups 1 (IM); 4 (IV) and 5 (control) followed by group 3 (IVSM, 71.4%) and group 2 (DC, 66.7%). The difference between these percentages was only significant ($P < 0.05$). Non-return to estrus

obtained in IVSM treatment was lower than that reported by Mohammed *et al.* (2000) in Barki ewes. The sign of non-return to estrus due to pregnancy is not physically different from anestrus at the end of the breeding season (like the present study). Therefore, pregnancy diagnosis based on non-return to estrus is not reliable in sheep and goats due to the seasonality in estrus behaviour (Sallam. 1999). Pregnancy diagnosis was confirmed using ultrasonography at 2 months of mating (Table 3). Irrespective to the route of PG injection, the conception rates were significantly ($P < 0.05$) lower in treated groups compared with that in the un-treated ewes. The present result was contrasted with that reported by Mohammed *et al.* (2000), while it was agreement with that finding by Mellado *et al.* (1994) working in Criollo goats compared natural $PGF_{2\alpha}$ by IVSM and IM routes without finding differences between routes or doses used.

Table (3): Effect of PG-dosage and its route of injection on the incidence of pregnancy rates in Romanov crossbred ewes.

Treatments		Conception rates by			No. of live lambs born per ewe	Predicted pregnant accuracy %
		Non-return to estrus (%)	60 d post insemination by sonography (%)	Lambing rate		
IM	(G_1)	6 (100)^{aB}	3 (50)^{a}	3 (50)^{a}	3	100 b
DC	(G_2)	2 (66.7)^{b}	1 (50))^{a}	1 (50)^{a}	1	100 b
IVSM	(G_3)	5 (71.4)^{bB}	2 (40)^{aA}	4 (80)^{bB}	4	-200 c
IV	(G_4)	7 (100)^{aB}	4 (57.1)^{a}	3 (42.9)^{a}	3	75 a
Control	(G_5)	2 (100)^{aB}	2 (100)^{Bb}	1 (50)^{aA}	2	50 a

Percentages with different superscripts (a, b, c, d) in the same column differ significantly ($P < 0.05$)

Percentages with different superscripts (A & B) in the same row differ significantly ($P < 0.05$)

Lambing rate, in the present study, was ranged between 42.9 and 80.0% (Table, 3). The present lambing rate in group 3 (IVSM, 80%) agreed with that reported by Mutiga and Mukasa-Mugerwa (1992, 80%) and it was slightly lower than that reported by Mohammed *et al.* (2000, 84.2%). Although the differences between the two percentages of conception rate at 2 months of gestation and at lambing were significant ($P < 0.05$) in group 3 (IVSM) and group

5 (control). The embryonic death was higher in control ewes than IVSM group treated with GnRH. Mohammed *et al.* (2000) stated that $PGF_{2\alpha}$ injection followed by GnRH (24 hrs) produced a high incidence of conception and the low early embryonic death. This may be due to the fact that ovine and bovine conspectuses secrete proteins, prostaglandin's and steroids which together with ovarian steroids modify uterine biochemistry and morphology which may lead to embryo mortality (Ashworth, 1992). There is evidence to suggest that luteal inadequacy is one of factors which lead to early embryonic death (Dowing, 1980). Controlling litter size in sheep is a critical step in improving productivity. The number of lambs born per treated ewe was 1.0 while it was 2 in untreated group (Table, 3).

The present results indicated that, small dose (40% of IM dose) of PG is effective for estrus synchronization in Romanov crossbred ewes when injected IV and IVSM. Administration of GnRH at the beginning of estrus in IVSM group results in an improved fertility of synchronized animals.

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تأثير جرعة البروستاجلاندين $F_{2\alpha}$ وطريقة اعطاءها على استحداث الشياع فى خلطان نعاج الرومانوف خلال نهاية موسم التربية

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الملخص العربى

خمسة وثلاثون نعجه من خلطان الرومانوف عمرهـــا ٢٢ شـــهر ووزن جسمها ٤٠ كجم استخدمت لتحديد تأثير جرعـــة البروســـتاجلاندين (كلوبروستينول) وطريقة اعطائها على اعداد النعاج التى يظـــهر عليـــها الشياع ، ووقت ظهور الشياع ، فترة الشياع ، معدل الخصوبـــة واعـــداد الحملان المولودة. وقد قسمت النعاج عشوائيا الى خمسة مجاميع (٧ فـــى كل منها).

المجموعـــة الاولـــى حقنـــت عضليـــا بـــ ٢٥٠ ميكروجـــرام كلوبروستينول. المجموعة الثانية والثالثة والرابعـــة عوملـــت بـــ ١٠٠ ميكروجرام كلوبروستينول لكل نعجه وتم اعطائها عن طريق نشرها داخل عنق الرحم ، حقنت فى الطبقة تحت المخاطية لشفرة فتحه الحيا وفى داخل الوريد على الترتيب بينما نعاج المجموعة الخامسة تركت بدون معاملـــة هرمونيه كمجموعة ضابطة.

النعاج التى لم يظهر عليها الشياع بعـــد المعاملـــة الاولـــى مـــن البروستاجلاندين عوملت مرة أخرى بجرعة ثانية بفاصل ١٠ ايام بنفـــس طريقة اعطائها. نعاج المجموعة الاولى والثانية والرابعة حقنت عضليا بـــ ٤٥٠ وحدة دوليه من مصل الفرسه الحامل عند وقت اعطـــاء المعاملـــة الثانية من البروستاجلاندين بينما المجموعـــة الثالثـــة حقنـــت عضليـــا ٤

ميكروجرام GnRH عند بداية ظهور الشياع. تم مراقبة الشياع ثلاث مرات يوميا ولمدة خمسة أيام. نسبة النعاج التى اظهرت الشياع خلال ٢٣-٣٢ ساعة عقب اعطاء البروستاجلاندين عن طريق الحقن فى الطبقة المخاطية لشفره فتحه الحيا وداخل الوريد كانت استجابتهما واحدة (١٠٠%) وكانت عالية بالمقارنة بالمحقونه عضليا (٨٣,٧%) والموضوع داخل عنق الرحم (٥٠%) او النعاج الضابطة ٢٨,٦%.

كان الوقت من المعاملة وحتى ظهور الشياع قصير معنويا فى النعاج المحقونه فى الطبقة المخاطية لشفره فتحة الحيا (٣٦,٩ ساعة) بالمقارنة بتلك المحقونه داخل الوريد (٤٠,٦ ساعة) وداخل عنق الرحم (٤٤,٧ ساعة) والمحقونه عضليا (٤٧,٧ ساعة) وكانت مدة الشياع المستحدث هى ٢٣,١ ، ٣٢,٧ ، ٤١,٦ ، ٣٤ ساعة على الترتيب.

كل النعاج التى ظهر بها شياع لقحت ولوحظ الشياع عليها لمدة ٢٥ يوما بعد تلقيحها كان معدل المواليد عالي فى المجموعة التى حقنت فى الطبقة المخاطيه لشفره فتحة الحيا بالمقارنة بباقى المجاميع.

واتضح من هذه الدراسة ان جرعة الـ ١٠٠ ميكروجرام من الكلويروستينول والمعطاه عن طرق الحقن فى الطبقة المخاطية لشفره فتحة الحيا يمكن ان تكون طريقة فعالة فى تنظيم الشياع وتحسين معدل الولادة فى خلطان نعاج الرومانوف.