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PROGESTERONE PROFILES DURING ESTROUS SYNCHRONIZATION IN YOUNG OSSIMI EWES (With One Table and 4 Figures)

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مستوى هرمون البروجيستيرون أثناء توافق دورة الشبق
في النعاج الأوسيمي الصغيرة

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وضعت خطة لتوافق دورة الشبق في مجموعة من النعاج الأوسيمي (عددها=15) و تلي ذلك تلقحها اصطناعيا بعد وقت محدد . وكانت طريقة المعالجة كالتالي: تم حقن تلك النعاج مرتين بواسطة البروستاجلاندين $F_{2\alpha}$ بينهما تسعة أيام و جمعت عينة دم من جميع النعاج في اليوم الأول لحقن الجرعه الأولى و ثلاثة أيام بعدها وكذلك في اليوم الأول لحقن الجرعة الثانية وثلاثة أيام بعدها . تم تلقح جميع النعاج في اليوم الثاني و الثالث بعد حقن الجرعة الثانية . كانت إستجابة النعاج لحقن البروستاجلاندين $F_{2\alpha}$ مختلفة حيث استجابت 6 نعاج للجرعة الأولى و الثانية واستجابت ثلاثة نعاج للجرعة الأولى دون الثانية وكذلك استجابت ثلاثة أخرى للجرعة الثانية دون الأولى كما لم تستجب ثلاثة نعاج لكلا الجرعتين . لم تستجب بعض النعاج لحقن البروستاجلاندين $F_{2\alpha}$ بالرغم من وجود مستويات عالية من البروجيستيرون فيها كما انه لم يحدث إخصاب في جميع الأغنام ذات البروجيستيرون مرتفع المستوى أثناء التلقيح . وقد تم الإخصاب في ثلاثة نعاج فقط من أصل تسعة تم تلقحهم لوجود البروجيستيرون بمستوى منخفض فيهم . ومن الدراسة نجد انه بالرغم من إمكانية استعمال البروستاجلاندين في توافق دورة الشبق في الأغنام فإن بعض النعاج لم تستجب له بكفاءة .

SUMMARY

A protocol of estrus synchronization followed by fixed-time insemination was applied in a group of 15 maiden Ossimi ewes. The treatment was accomplished by using two injections of prostaglandin $F_{2\alpha}$ 9 days apart. Jugular venous blood was collected from all ewes at the time of the first $PGF_{2\alpha}$ -injection, 3 days after the first $PGF_{2\alpha}$ -injection, at the time of the second $PGF_{2\alpha}$ -injection and 3 days after the

second PGF₂α-injection. All ewes were inseminated on the second and third days of the second PGF₂α-injection. The treated ewes reacted differently to the prostaglandin as follows: 6 ewes responded to the first and the second PGF₂α-injection, 3 ewes responded to the first but not to the second injection, 3 ewes responded to the second but not the first injection, and 3 ewes did not respond either to the first or the second injection. In some ewes, although the progesterone (P4) level was high at the time of prostaglandin-application, they did not respond to the treatment. None of the ewes, that revealed high P4 concentration at the time of insemination, conceived. Out of the 9 ewes, which showed low P4-level at the time of insemination, only three of them (30%) conceived. It could be concluded that, although prostaglandin can be used to synchronize estrus in young Ossimi ewes, some cases did not respond efficiently to this regime.

Key words: Ewes, Estrous synchronization, PGF₂α, Progesterone

INTRODUCTION

Attempts to control the occurrence of estrous and ovulation in sheep are usually based either on trying to simulate the activity of the cyclic corpus luteum by using progesterone or progestagen preparations or by inducing luteolysis of the cyclic corpus luteum (Boland *et al.*, 1978; Acritopoulou-Fourcroy *et al.*, 1982; Godfrey *et al.*, 1997). The available literature on the use of prostaglandin in cyclic sheep is much less than that for cattle. Part of this is due to the fact that PGF₂α is not relevant to control sheep reproduction during the anestrous period. However, in Egypt the subtropical Rahmani and Ossimi breeds of ewes did not have a distinct period of acyclicity as in the seasonal temperate breeds (Aboul-Naga *et al.* 1987, 1991).

PGF₂α are unsaturated fatty acids that are synthesized in several tissues in the body, especially the uterus (Horton and Poyser, 1976). PGF₂α and its analogues are potent luteolytic agents in cycling ewes when given in a single intramuscular injection (Hughes *et al.* 1976).

Variation in fertility has been reported after the use of PGF₂α or its analogues for synchronizing of estrus in ewes. Although some reports showed little evidence of any adverse effect on fertility after the use of PGF₂α in estrus synchronization (Fairnie *et al.*, 1977; Lightfoot *et al.*, 1976; Godfrey *et al.*, 1997), other results were not always reassuring (Boland *et al.* 1978).

The aim of this study was to analysis the progesterone profiles in young Ossimi ewes after application of double PGF₂α injections and to associate these profiles with the subsequent conception rate.

MATERIALS and METHODS

Fifteen maiden Ossimi ewes between 12 and 16 months old and with 50.13 ± 9.4 kg mean body weight were used in the present study. They were maintained on concrete floor in a semi-opened pen of 5 x 5 meter in the agriculture farm of Assiut University. Natural lighting was provided throughout the experimental period. Within the pen, ewes were given a concentrate feed mixture consisting of 77% ground yellow corn, 20% uncorticated cotton seed meal, 2% lime stone and 1% sodium chloride. A mineral mixture was also added at a rate of 3 kg/ton of the concentrate mixture. These ewes had apparently normal genital tract, as indicated by ultrasonographic examination, and they did not show any abnormal vaginal discharge during the mating period. Ultrasound examination was done using 100 LC scanner attached with 6/8 changeable probe (Pie Medical, Holand).

Prostaglandin F₂α analogue was used for estrous synchronization in all ewes during the breeding season. Treatment was accomplished by using a double injection regimen consisting of two injections each of 2 ml PGF₂α (Iliren, 0.15 mg tiaprost/ml, intervet). The first injection was given i.m., the second injection was also given i.m. nine days after the first injection. Forty two hours after each injection and for 5 successive days, estrus was detected by using two rams. Each ram was exposed to ewes for 20-minute daily on the morning to detect estrus. A ewe was considered to have responded to PGF₂α if she was in estrus between 1-3 days after treatment and showed a marked decrease in the serum progesterone (P4) concentration 3 days after treatment (< 1 ng/ml).

Jugular venous blood was collected from each ewe at the following times:

- a. At the time of the first PGF₂α-injection
- b. 3 days after the first PGF₂α-injection (expected day of estrus)
- c. At the time of the second PGF₂α-injection
- d. 3 days after the second PGF₂α-injection (expected day of estrus).

Blood serum was harvested after centrifugation at 3000 rpm for 20 minutes and was stored at - 20 °C until assayed. The P4 concentration was determined in each sample by using an ELISA technique reported by Biosource, 1995. The coefficient of variance of

intra- and interassay were 13.5 and 14% for the P4 assay, respectively.

All ewes, whether they showed estrus symptoms or not, were inseminated with a fresh diluted (egg yolk citrate diluent) semen obtained from a fertile ram. The inseminating dose was 0.5 ml containing about 100 million alive and progressively motile sperm. The insemination was done on the second and the third days from the second PGF₂α-injection. All inseminated ewes were examined ultrasonographically 35 days after insemination to determine pregnancy.

Changes in the P4 concentrations in relation to the application of PGF₂α were compared by the ANOVA-test using SPSS-program version 10. The difference was considered significant at a probability of $p < 0.05$.

RESULTS

Changes in P4 concentrations after double injections of PGF₂α injection is shown in (Table 1). At the time of the 1st PGF₂α-injection 11/15 ewes had P4 levels higher than 1 ng/ml serum, while the other 4 ewes (Ewes 113, 120, 124, 106) showed P4 levels lower than 1 ng/ml. Three days after PGF₂α-injection, the P4 level decreased to levels lower than 1 ng/ml in 9/11 ewes, while it remained high in 2 ewes (Ewes 114, 131). The interval from the first PGF₂α-application to the first appearance of heat signs was one day in 3 ewes. Then, the number increased to 9 ewes on the second and third days of PGF₂α-injection. At the time of the 2nd PGF₂α-injection, all the ewes showed P4 level higher than 1 ng/ml. However, only 9/15 ewes responded to the 2nd PGF₂α-injection by decreasing the P4 level lower than 1 ng/ml. The interval from the second PGF₂α-injection to the appearance of heat of the 9 reacted ewes was two days.

According to the reaction to the first and second PGF₂α-injections the 15 ewes could be classified into:

- Group 1:** ewes responded to the first and second PGF₂α-injection (n=6, Ewes 108, 109, 112, 118, 130, 132, Fig 1),
- Group 2:** ewes responded to the first but not the second PGF₂α-injection (n=3, Ewes 110, 115, 140, Fig 2),
- Group 3:** ewes responded to the second but not the first PGF₂α-injection (n=3, Ewes 113, 120, 124, Fig 3),
- Group 4:** ewes did not respond either to the first or the second PGF₂α-injection (n=3, Ewes 106, 114, 131, Fig 4).

None of the ewes, that revealed high P4 concentration at the time of insemination, conceived. Out of the 9 ewes, which were in estrus and

showed low P4-level at the time of insemination, only three of them (30%) conceived. The first two ewes were among those, responded to the first and second PGF₂α treatment (Ewes 118, 132), while the third was among those, responded to the second PGF₂α treatment (Ewes 124).

Table 1: Progesterone levels after prostaglandin F₂α analogue injection in young Ossimi ewes

Ewe Number	Progesterone level (ng/ml) at the time of:				conception
	1 st PGF ₂ α	3 days after 1 st PGF ₂ α	2 nd PGF ₂ α	3 days after 2 nd PGF ₂ α	
Ewe 108	3	0.7	5	0.9	-
Ewe 109	4	0.9	7.3	0.7	-
Ewe 112	6	0.3	8	0.3	-
Ewe 118	2.6	0.7	5	0.2	+
Ewe 130	4.2	0.7	2.3	0.3	-
Ewe 132	8	0.8	5.9	0.2	+
Ewe 110	3.6	0.8	3.6	3.6	-
Ewe 115	3.6	0.2	5.4	2.6	-
Ewe 140	12	0.4	12	8	-
Ewe 113	0.5	0.2	7.4	0.4	-
Ewe 120	0.7	3.6	6	0.9	-
Ewe 124	0.8	0.7	10	0.2	+
Ewe 106	0.6	4.1	8.5	5.2	-
Ewe 114	10	12	20	8.4	-
Ewe 131	6.1	5.4	12	16	-
Mean	4.38 ^a	2.10 ^{bc}	7.89 ^b	3.19 ^c	
± SD	± 3.4	± 3.1	± 4.2	± 4.4	
(range)	(0.5-12)	(0.2-12)	(2.3-20)	(0.2-16)	

^{abc} Values with different superscript letters differ significantly (p<0.05).

Interval between 1st and 2nd prostaglandin F₂α injection is 9 days.

DISCUSSION

The data presented here are based on the use of PGF₂α analogue to synchronize the estrus in young Ossimi ewes. The treated ewes reacted differently to the double injections of prostaglandin F₂α. While some ewes responded to the first and second injection, others ewes reacted only to the first or the second treatment, and others did not react to the first or second injection. At the time of injection of the first dose of PGF₂α, two third of the ewes showed high P4 level (luteal phase) while one third revealed a low concentration (follicular phase). This distribution of ewes in the estrus cycle is normal and an acceptable one. Normally, the ewes show cycles of 16-17 days, in general most cycles range from 14 to 18 days (Asdell, 1964; Hafez, 1952; Aboul-Naga *et al.*,

1987). Progesterone concentration in the blood reflects the activity of corpus luteum in ewes (Bostedt *et al.* 1981). The P4 concentration in the circulation is lowest during estrus but begins to rise immediately after formation of the corpus luteum and reaches the maximum level later in the cycle. The sheep corpus luteum attains full secretory activity by about the sixth to eighth day of the cycle and continues secreting P4 at a fairly constant level until about day 15. The maximum levels being reached at about day 8 and then begins to fall a day or two before the next cycle (Cunningham *et al.* 1975).

After the first PGF₂α-administration, peripheral blood P4 concentration sharply decreased within 3 days of injection in 9 ewes. Irrespective to the day of estrous cycles a single i.m. injection of prostaglandin resulted in estrus within 24-50h in 80-86.6 % of the ewes (Hughes *et al.*, 1976; Kunchev and Doichev, 1979; Light *et al.*, 1994). Ewes with low P4 level at the time of first PGF₂α-injection (n=4) did not respond to the PGF₂α- administration. To be effective, the prostaglandin F₂α should be injected in ewes with an active corpus luteum. The corpus luteum of the ewe is responsive to an analogue of PGF₂α only between days 5 and 14 of the estrous cycle (Chamley *et al.*, 1972; Acritopoulou-Fourcroy and Haresign, 1980). Thus it appears that these animals did not have corpus luteum, growing or regressing corpus luteum or that lack receptors to prostaglandin

In two ewes although the P4 was high at the time of the first PGF₂α-injection, both ewes failed to respond to the PGF₂α- application. The same two ewes failed also to respond to the second PGF₂α-injection. This condition opens a question about the sensitivity of corpus luteum of ewes to the exogenous application of PGF₂α or its analogues. A nearly similar phenomenon were observed in a group of ewes with persistence of corpus luteum (Hooper and Thorburn, 1987). They found that, although a PGF₂α analogue was infused into the uterine vein of two ewes with persistent corpora lutea, it failed to induce luteolysis. Also, there has been some evidence that the estrous response may be influenced by PGF₂α dose level (Hackett and Robertson, 1980). In South Africa, lower doses of the analogue were often insufficient to induce complete luteolysis, as indicated by an initial decline in P4 level followed by a gradual rise in the steroid., suggesting some recovery of luteal function (Greyling and Van der Westhuysten, 1979). This ability of the corpus luteum to recover after PGF₂α had been previously recorded (Thorburn and Nichol, 1971).

At the time of the second PGF₂α-administration, all the fifteen ewes showed a high level of blood P4. In fact, this is an ideal result, as the goal of the first PGF₂α-injection was to bring all the treated ewes after 9 days with a functional corpus luteum. However, after the second PGF₂α-injection only 9/15 ewes responded to this application, while 6 ewes failed to react. Two of these six ewes did not respond either to the first or the second dose. Failure of the corpus luteum of some investigated ewes to respond to the exogenous PGF₂α analogue application may be explained as such ewes needed a higher dose of PGF₂α than normal required, or the corpus luteum recovered again after an initial state of regression, or such corpus luteum is a persistent one, which is unsusceptible to the prostaglandin. Unfortunately, this phenomenon needs further and more extensive study to clarify it.

In the present study, the incidence of estrous expression after two doses of PGF₂α-injection is a lower one (60%). In the literature this incidence is much variable. After two doses of PGF₂α-injection with 7-10 days interval, the incidence of estrus expression was 72-82% within 30-54h (Godfrey *et al.* 1999, 2001). Age and breed of ewes may be a cause for this difference.

After the fixed time insemination on the second and third days of the second PGF₂α-administration, non of the ewes with high P4 level at the time of insemination conceived. This means that, it is not necessary to inseminate ewes that could not exhibit the external signs of estrus. A total of 3 out of 9 ewes conceived on the induced estrus, which is a lower one. In fact, as with progestagens, the reduced fertility at the estrus immediately following treatment with PGF₂α has also been a problem (Quinlivan, 1980). There were indications that PGF₂α treatment could have a very rapid and dramatic effect on steroid synthesis in the lutein cell whereas normal luteolysis would seem to involve more gradual regression of the gland (Stacey *et al.* 1976). This may have some relevance in explaining the low fertility of sheep after this form of treatment. Also, experience with artificial insemination in ewes might be an additional cause for the low conception rate obtained in this study. Trans-cervical insemination 48h after the second dose of PGF₂α resulted in a conception rate 8.7% in the first trial and 52.9% in the second trial (Godfrey *et al.* 1999).

According to the results of the present study it could be observed that while some young ewes responded ideally others did not react to this regime of estrus synchronization. This variation might be partially

the cause of the low fertility associating this program.

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Fig 1. Progesterone level in ewes, which responded to the first and the second PGF₂α-injection (Group1, n=6)

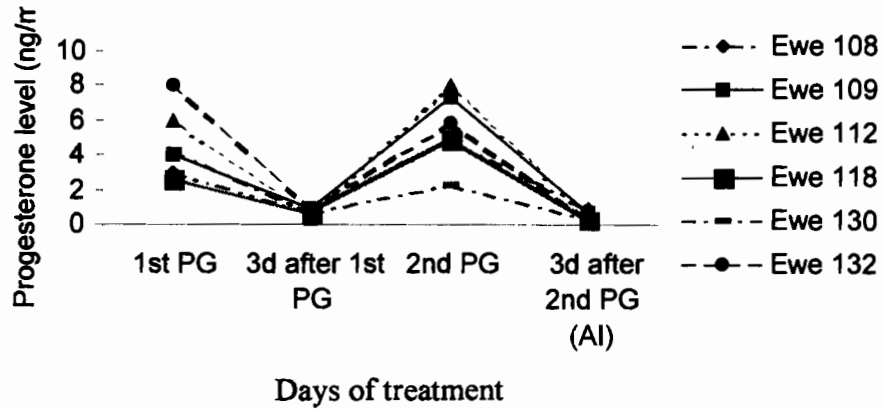
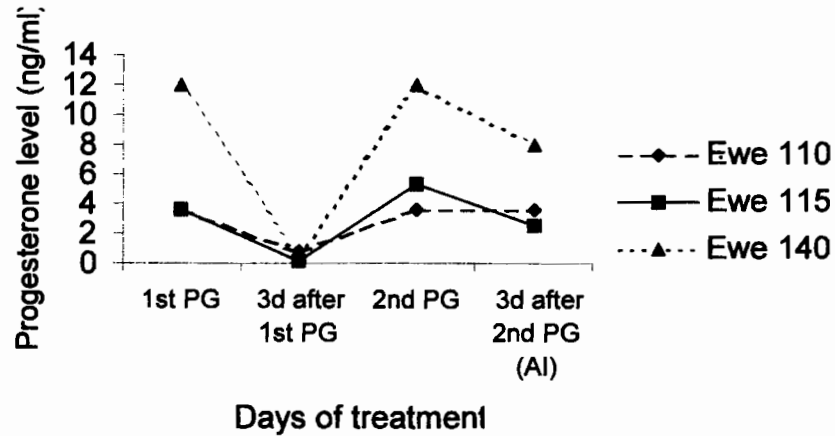


Fig 2. Progesterone level in ewes, which responded to the first but not the second PGF₂α-injection (Group2, n=3)



PG: PGF₂α-injection
AI: Artificial Insemination

Fig 3. Progesterone level in ewes, which responded to the second but not the first PGF₂α-injection (Group3, n=3)

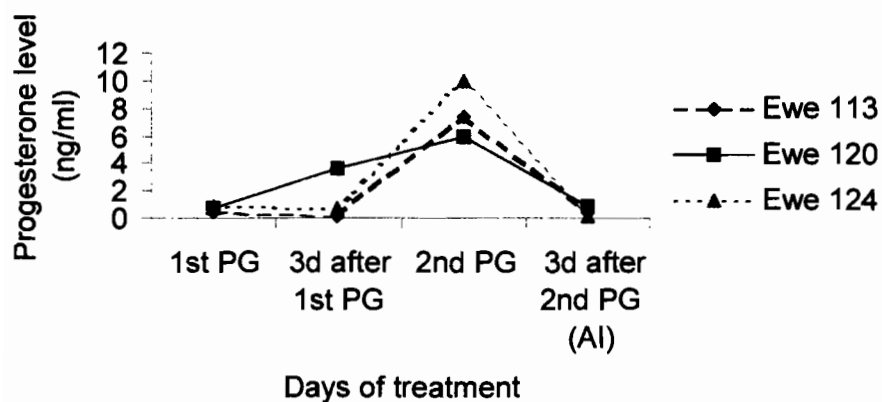
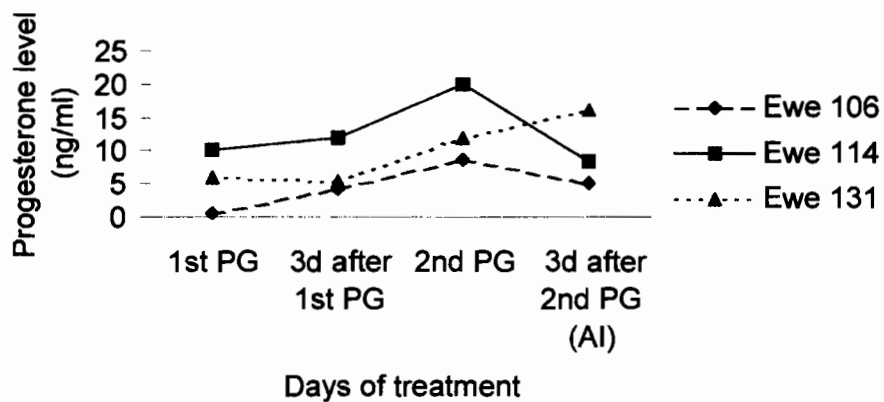


Fig 4. Progesterone level in ewes, which not respond either to the first or the second PGF₂α-injection (Group4, n=3)



PG: PGF₂α-injection
AI: Artificial Insemination