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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 البروجيسترون أثناء توافق دورة الشبق في النعاج الأوسيمي الصغيرة

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وضعت خطة لتوافق دورة الشبق في مجموعة من النعاج الأوسيمي (عددها=١٥) و تلي ذلك تلقيحها 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 تلقيح جميع النعاج في اليوم الثانى و الثالث بعد حقن الجرعة الثانية.كانت إستجابة النعاج لحقن البروستاجلاندين $F_2\alpha$ مختلفة حيث استجابت ٦ 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 البروجيسترون 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 تلقيحهم 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 $PGF_2\alpha$-injection. All ewes were inseminated on the second and third days of the second $PGF_2\alpha$-injection. The treated ewes reacted differently to the prostaglandin as follows: 6 ewes responded to the first and the second $PGF_2\alpha$-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,* $PGF_2\alpha$, 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 $PGF_2\alpha$ 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).

$PGF_2\alpha$ are unsaturated fatty acids that are synthesized in several tissues in the body, especially the uterus (Horton and Poyser, 1976). $PGF_2\alpha$ 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 $PGF_2\alpha$ 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 $PGF_2\alpha$ 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 $PGF_2\alpha$ 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 $F_2\alpha$ 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 $PGF_2\alpha$ (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 $PGF_2\alpha$ 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 $PGF_2\alpha$-injection
b. 3 days after the first $PGF_2\alpha$-injection (expected day of estrus)
c. At the time of the second $PGF_2\alpha$-injection
d. 3 days after the second $PGF_2\alpha$-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 $PGF_2\alpha$- 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 $PGF_2\alpha$ 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 $PGF_2\alpha$ injection is shown in (Table 1). At the time of the 1st $PGF_2\alpha$-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 $PGF_2\alpha$-injection, the P4 level decreased to levels lower than 1 ng/ml in 9/11 ewes, while it remained hegh in 2 ewes (Ewes 114, 131). The interval from the first $PGF_2\alpha$-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 $PGF_2\alpha$-injection. At the time of the 2nd $PGF_2\alpha$-injection, all the ewes showed P4 level higher than 1 ng/ml. However, only 9/15 ewes responded to the 2nd $PGF_2\alpha$-injection by decreasing the P4 level lower than 1 ng/ml. The interval from the second $PGF_2\alpha$-injection to the appearance of heat of the 9 reacted ewes was two days.

According to the reaction to the first and second $PGF_2\alpha$-injections the 15 ewes could classified into:

Group 1: ewes responded to the first and second $PGF_2\alpha$-injection (n=6, Ewes 108, 109, 112, 118, 130, 132, Fig 1),

Group 2: ewes responded to the first but not the second $PGF_2\alpha$-injection (n=3, Ewes 110, 115, 140, Fig 2),

Group 3: ewes responded to the second but not the first $PGF_2\alpha$-injection (n=3, Ewes 113, 120, 124, Fig 3),

Group 4: ewes did not respond either to the first or the second $PGF_2\alpha$-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 $PGF_2\alpha$ treatment (Ewes 118, 132), while the third was among those, responded to the second $PGF_2\alpha$ treatment (Ewes 124).

Table 1: Progesterone levels after prostaglandin $F_2\alpha$ analogue injection in young Ossimi ewes

Ewe Number	Progesterone level (ng/ml) at the time of: 1st $PGF_2\alpha$	3 days after 1st $PGF_2\alpha$	2nd $PGF_2\alpha$	3 days after 2nd $PGF_2\alpha$	conception
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^{az}	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 $F_2\alpha$ injection is 9 days.

DISCUSSION

The data presented here are based on the use of $PGF_2\alpha$ analogue to synchronize the estrus in young Ossimi ewes. The treated ewes reacted differently to the double injections of prostaglandin $F_2\alpha$. 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 $PGF_2\alpha$, 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 $PGF_2\alpha$-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 $PGF_2\alpha$-injection (n=4) did not respond to the $PGF_2\alpha$- administration. To be effective, the prostaglandin $F_2\alpha$ should be injected in ewes with an active corpus luteum. The corpus luteum of the ewe is responsive to an analogue of $PGF_2\alpha$ 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 $PGF_2\alpha$-injection, both ewes failed to respond to the $PGF_2\alpha$- application. The same two ewes failed also to respond to the second $PGF_2\alpha$-injection. This condition opens a question about the sensitivity of corpus luteum of ewes to the exogenous application of $PGF_2\alpha$ 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 $PGF_2\alpha$ 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 $PGF_2\alpha$ 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 $PGF_2\alpha$ had been previously recorded (Thorburn and Nichol, 1971).

At the time of the second $PGF_2\alpha$-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 $PGF_2\alpha$-injection was to bring all the treated ewes after 9 days with a functional corpus luteum. However, after the second $PGF_2\alpha$-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 $PGF_2\alpha$ analogue application may be explained ass such ewes needed a higher dose of $PGF_2\alpha$ 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 $PGF_2\alpha$-injection is a lower one (60%). In the literature this incidence is much variable. After two doses of $PGF_2\alpha$-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 $PGF_2\alpha$-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 $PGF_2\alpha$ has also been a problem (Quinlivan, 1980). There were indications that $PGF_2\alpha$ 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 $PGF_2\alpha$ 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.

REFERENCES

Aboul-Naga, A.M.; Aboul-Ela, M; El-Nakhla, S.M. and Mehrez, A.Z. (1987): Oestrous and ovarian activity of subtropical fat-tailed Rahmani sheep and their response to light treatment. J Agr Sci Cambridge 108: 617-621.

Aboul-Naga, A.M.; Mansour, H.; Aboul-Ela, M.B. and Almahdy, H. (1991): Breeding activity of two subtropical sheep breeds under accelerated lambing system. Small Rum Res 4, 277-283.

Acritopoulou- Fourcroy, S. and Haresign, W (1980): Response of ewes to a single injection of an analogue of $PGF_2\alpha$ given at different stages of the estrous cycle. J Reprod Fertil 58, 219-221.

Asdell, S.A. (1964): Patterns of mammalian Reproduction. Cornell University Press, Itheca, New York.

Boland, M.P.; Gordon, I. and Kelleher, D.L. (1978): The effect of treatment by prostaglandin analogue (ICI-80996) or progestagen (SC-9880) on ovulation and fertilization in cyclic ewes. J Agr Sci Cambridge 91, 727-730.

Bostedt, H.; Stolla, R. and Klenner, A. (1981): Progesteronkonzentartionen im Blutplasma laktierender und nicht laktierender Schafe in der ersten Wochen nach der Geburt. Zschr Tierzucht Zuchtungsbiol 98, 11-20.

Chamley, W.A.; Buckmaster, J.M.; Cain, M.D.; Cerini, M.E.; Cunningham, I.A. and Goding, J.R. (1972): The effect of $PGF_2\alpha$ on progesterone, oestradiol and LH secretion in sheep with ovarian transplantation. J Endocrionology 55, 253-263.

Cunningham, N.F.; Symons, A.M. and Saba, N. (1975): Levels of progesterone, LH and FSH in the plasma of sheep during the estrous cycle. J Reprod Fertil 45, 177-180.

Fairnie, I.J.; Wales, R.G. and Gherardi, P.B. (1977): Time of ovulation, fertilization rate and blastocyst formation in ewes following treatment with prostaglandin analogue (ICI 80996). Theriogenology 8, 183 (abst).

Godfrey, R.W.; Gray, M.L. and Collins, J.R. (1997): A comparison of two methods of oestrous synchronization of hair sheep in the tropics. Anim Reprod Sci 47, 99-106.

Godfrey, R.W.; Collins, J.R.; Hensley, E.L. and Wheaton, J.E. (1999): Estrus synchronization and artificial insemination of hair sheep ewes in the tropics. Theriogenology 51, 985-997.

Godfrey, R.W.; Collins, J.R. and Hensley, E.L. (2001): Behavioral and endocrine responses of hair sheep ewes exposed to different mating stimuli around estrus. Theriogenology 55, 877-884.

Greyling, J.P.C. and Van der Westhuysten, J.M. (1979): The synchronization of oestrus in sheep. II. Dose effect of prostaglandin in the double injection regime. South Africa J Anim Sci 9, 193-195.

Hackett, A.J. and Robertson, H.A. (1980): Effect of dose and time of injection of prostaglandin $F_2\alpha$ in cycling ewes. Theriogenology 13, 347-351.

Hafez, E.S.E. (1952): Studies on the breeding season and reproduction of ewe. J Agr Sci Cambridge 42, 189-265.

Hooper, S.B. and Thorburn, G.D. (1987): Prostaglandin $F_2\alpha$ and oxytocin release during persistence of the corpus luteum in sheep. Acta Endocrinol. (Copenh) 115, 469-477.

Horton, E.W. and Poyser, N.L. (1976): Uterine luteolytic hormone: A physiological role for prostaglandin $F_2\alpha$. Physiological review 56, 595-651.

Hughes, F.; Lucas, J.M. and Notman, A.B. (1976): The synchronization of oestrus and subsequent fertility in ewes following treatment with a synthetic prostaglandin analogue (ONO 453). Prostaglandins 11, 1033-1039.

Kunchev, L. and Doichev, S. (1979): Estrus synchronization in sheep with ICI 80 99. Vet Med Nauki 16, 12-16.

Light, J.E., Silvia, W.J. and Reid, R.C. (1994): Luteolytic effect of prostaglandin $F_2\alpha$ and two metabolites in ewes. J Anim Sci 72, 2718-2721.

Lightfoot, R.J.; Croker, K.P. and Marshall, R. (1976): Use of prostaglandin analogue (ICI 80996) for the synchronization of estrus and lambing in Merino ewes. Proc Inter Sheep Breeding Cong (Muresk), pp. 449-454.

Quinlivan, T.D. (1980): Estrous synchronization and control of estrous cycle in sheep. In: Current Therapy in Theriogenology, Morrow, D.A. (ed). W.B. Saunders Company, pp. 950-954.

Stacey, B.D.; Germmell, R.T. and Thorburn, G.D. (1976): Morphology of the corpus luteum in the sheep during regression induced by prostaglandin $F_2\alpha$. Biol Reprod 14, 280-291.

Thorburn, G.D. and Nichol, D.H. (1971): Regression of ovine corpus luteum after infusion of $PGF_2\alpha$ into ovarian artery and vein. J Endocrinology 51, 751-752.

Fig 1. Progesterone level in ewes, which responded to the first and the second $PGF_2\alpha$-injection (Group1, n=6)

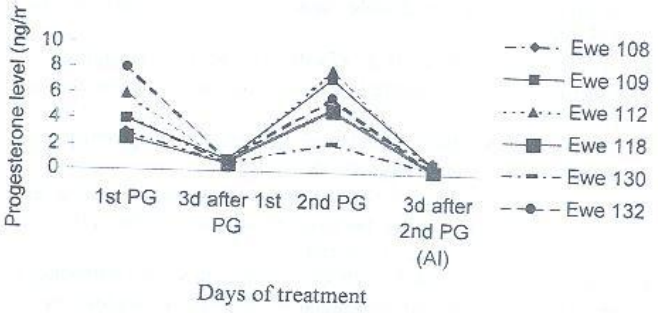
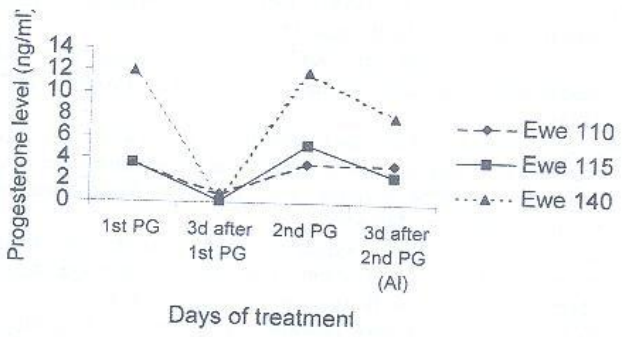


Fig 2. Progesterone level in ewes, which responded to the first but not the second $PGF_2\alpha$-injection (Group2, n=3)



PG: $PGF_2\alpha$-injection
AI: Artificial Insemination

Fig 3. Progesterone level in ewes, which responded to the second but not the first $PGF_2\alpha$-injection (Group3, n=3)

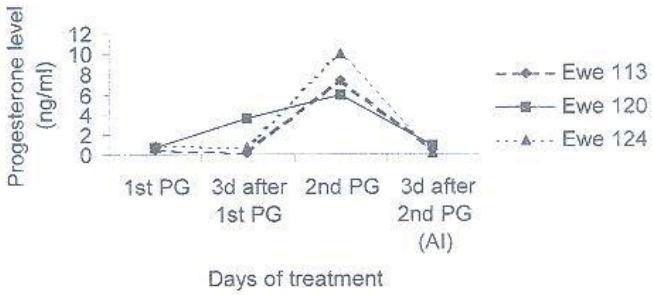
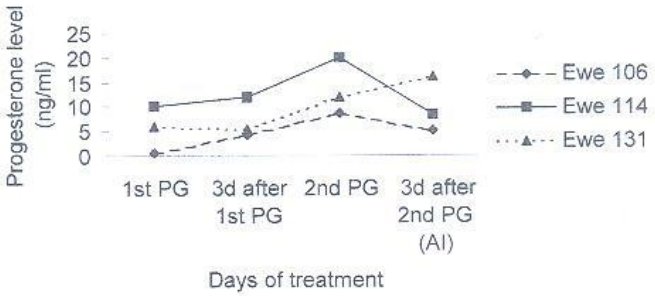


Fig 4. Progesterone level in ewes, which not respond either to the first or the second $PGF_2\alpha$-injection (Group4, n=3)



PG: $PGF_2\alpha$-injection
AI: Artificial Insemination