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Induced breeding in anestrous milking ewes by using twice-repeated PMS combined with estradiol-17 β

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Summary. A total of 114 anestrous milking Karagouniko ewes were divided randomly into 3 equal groups at 2 months postpartum. Ewes in group I were treated with 375 mg progesterone implants for 14 days and two 1 000 IU injections of PMS given at an interval of 16 days. Immediately before mating each ewe, 30 μg estradiol- 17 β was injected only at the estrus exhibited after the first PMS injection. Ewes in group II received the same treatment without estradiol-17 β. Ewes in group III were untreated and served as controls. The percentage of estrus found after first PMS injection, second PMS injection and after both PMS injections was 100, 44.5 and 100, respectively, for group I, 89.5, 55.0 and 100, respectively, for group II, and 0, 2.6 and 2.6, respectively, for group III. The lambing percentage after the first, second and both PMS injections was 23.6, 58.8 and 50 respectively, for group I, 56.0, 47.6 and 76.3, respectively, for group II, and 0, 2.6 and 2.6, respectively, for group III. Prolificacy for group I was 167.7, for group II it was 163.7, and for group III, 143.8. It was found that two injections of 1 000 IU PMS at an interval of 16 days after progesterone treatment resulted in 76.3 p. 100 lambing. However, when 30 μg estradiol-17 β was injected immediately before mating, the conception rate dropped to 50.0 p. 100.

Introduction.

Many papers have been published on inducing fertile estrus during all types of anestrous periods (postpartum, lactation and/or seasonal anestrus). It was concluded that breeding depended on the season (Hulet and Foote, 1967 ; Thimonier and Mauleon, 1969 ; Kostov, 1974 ; Cognie, Hernandez-Barreto and Saumande, 1975), the breed of ewe (Cognie, Hernandez-Barreto and Saumande, 1975 ; Hulet, 1976), ewe nutritional state (Coop, 1966 ; Hulet, Foote and Price, 1967 ; Hunter and Van Aarde, 1973 ; Tilev, 1974), udder functioning condition (Rahman and Kitts, 1967 ; Cognie, Cornu and Mauleon, 1974 ; Kostov, 1974 ; Cognie, Hernandez-Barreto and Saumande, 1975), type and form of hormonal application (Thimonier *et al.*, 1968 ; Bankov, Tilev and Lazarov, 1974 ; Eleftheriou *et al.*, 1974*a* ; Kostov, 1974 ; Margaritis, Samouelidis and Semadopoulos, 1974 ; Otel, 1974 ; Pekrov, Sokarovski and Tokovski, 1974 ; Poilas *et al.*, 1974 ; Tsamis *et al.*, 1974*b* ; Hulet, 1976), interval between parturition and mating (Hunter, 1968 ; Petkov, Sokarovski and Tokovski, 1974 ; Cognie, Hernan-

dez-Barreto and Saumande, 1975), number of lambs suckled per ewe, level of lactation (Cognie, Cornu and Mauleon, 1974 ; Cognie, Hernandez-Barreto and Saumande, 1975), mating by natural or artificial insemination (Eleftheriou, Margaritis and Samadopoulos, 1974 ; Gordon, 1975), number of lambs born per ewe (Cognie, Cornu and Mauleon, 1974 ; Cognie, Hernandez-Barreto and Saumande, 1975).

The present research was undertaken in view of establishing an hormonal treatment for successfully breeding lactating anestrous ewes using twice-repeated PMS combined with estradiol-17 β for improving conception rate (Hawk, 1974 ; Hawkand Cooper, 1975).

Material and methods.

One hundred and fourteen lactating Karagouniko ewes, a native milking breed, with weaned lambs were used. This breed shows seasonal estrous cycle activity during early autumn and an anestrous period during spring and summer. The experimental ewes lambed during December and January and were treated hormonally 2 months later on March 1. They were subjected to standard management practice as to housing and nutrition, and were milked twice daily. The ewes were randomly assigned to 3 experimental groups of 38 animals each. Three ewes in each group were laparotomized 3 days before starting the hormonal treatment to determine if the ovaries were in an anestrous state. Silicone rubber implants containing 375 mg progesterone were placed in all ewes of group I ; they were withdrawn 14 days later. Immediately after implant withdrawal, 1 000 IU PMS were injected intramuscularly into each ewe and 3 painted vasectomized rams were put with the flock. Each ewe from the group showing estrus was injected intramuscularly with 30 μg estradiol-17 β in corn oil and led to the ram for hand mating. These mated females were then placed in a nearby pen with rams of proven fertility for free mating. A total of 5 rams was used for each experimental group. A second PMS treatment of 1 000 IU was applied on all

TABLE 1

Experimental design for hormone-induced breeding in anoestrous milking ewes

No. of group	Hormonal treatment					
	Type of hormone	Route of administration	Duration of treatment	Days of treatment	Amount administ.	No. o ewes
I	Progesterone (1)	implant	14 days	1st-14th	375 mg	38
	PMS (2)	i. m.	twice	14th and 32nd	1 000 IU	
	Estradiol-17 β (3)	i. m.	once	day of mating	30 μg	
II	Progesterone	implant	14 days	1st-14th	375 mg	38
	PMS	i. m.	twice	14th and 32nd	1 000 IUv	
III	Controls (4)	—	—	—	—	38

(1) Silicone rubber implants. Trade name « Sil-Estrus », Abbot Co., Greece. (2) Pregnant mare serum. Trade name « Gestyl », N. V. Organon-Niadas, Greece. (3) Estradiol-17β was injected immediately before mating. (4) No treatment.

the ewes of the group, and the ones showing estrus were mated to fertile rams without receiving estradiol-17 β. The ewes in group II were subjected to the same hormonal treatment, but the administration of 30 μg estradiol-17 β immediately before mating was omitted. The ewes in group III were not treated and served as controls. Procedures for checking estrus and mating were similar in all groups. The experimental design is illustrated in table 1. The results were statistically analyzed by using simple Chi-square comparisons (Ostle, 1969).

Results and discussion.

The ovaries of the 3 laparotomized ewes from each experimental group were in a stage of deep anestrus (ovaries were white and small with no follicular or luteal formations on the surface). The lack of cycling ewes in the controls also provided indirect evidence of the anestrous stage of the experimental ewes. The induction of estrus was successfully ($P < 0.1$) achieved by hormones, as shown in table 2. The percentage of estrus after the first injection of 1 000 IU PMS rose to 100 in ewes of group I and to 89.5 in ewes of group II. None of the controls exhibited estrus at the same time. After the second injection of 1 000 IU PMS, 44.5 p. 100 of ewes of group I and 55.0 p. 100 of ewes of group II exhibited estrus, while at the same time the percentage of estrus found in control group ewes was only 2.6 ($P < 0,01$). Finally, all the ewes (100 p. 100) of the hormonally treated groups I and II exhibited estrus, while only one ewe (2.6 p. 100) of the controls cycled ($P < 0.01$). The proportion of lambed ewes treated was also satisfactory, as shown in table 2. Fifty percent of the ewes in group I with estradiol-17 β and 76.3 p. 100 in group II without estradiol-17 β lambed versus 2.6 p. 100 ($P < 0.01$) of the control ewes. These results are comparable to those reported by Petcu *et al.* (1974), Eleftheriou *et al.* (1974*a*) and Bankov, Tilev and Lazarov (1974). The results of the present experiment showed that the second injection of 1 000 PMS brought back to estrus 44.5 p. 100 of the cycled ewes from the first PMS injection in both treated groups and induced 10.5 p. 100 first estrus in ewes of group II (table 2). This favorable effect of the second injection of PMS agrees with earlier work by Gordon (1963), Wagner (1964) and Hulet and Foote (1967). The second PMS injection also improved the lambing percentage from 23.6 to 50 in group I ewes and from 56.0 to 76.3 in group II animals (table 2). Comparable improvement in lambing percentage of anestrous ewes, treated with 2 injections of PMS was reported recently by Kostov (1974), Tsamis *et al.* (1974*a*) and Bankov, Tilev and Lazarov (1974).

The administration of estradiol-17 β at mating aimed at improving sperm transport since it is known that progestagens and progesterone, mostly used for regulation of ovulation and priming of the reproductive tract in ewes, inhibit sperm transit into or through the genital tract thus causing failure in ovum fertilization (Quinlivan and Robinson, 1967, 1969 ; Hawk and Conley, 1971, 1972). Hawk (1974) reported that estradiol-17 β increases the number of spermatozoa recovered from the oviducts by facilitating sperm transport through the genital tract. Hawk and Cooper (1975) further found that 30 μg estradiol-17 β, injected at the time of mating, significantly increased by more than tenfold the number of sperm cells recovered from the uterus and oviducts. Contrary to the expected beneficial effect, the results of the present

TABLE 2

Oestrus exhibition (p. 100) *and lambing percentage of milking ewes, treated hormonally and mated during estrous period*

No. of group	Hormonal treatment	No. of ewes	After 1st PMS injection		After 2nd PMS injection				After 1st and 2nd PMS injection		Prolificacy
			Oestrus exhibition and mating	Lambing (p. 100)	Oestrus exhibition First œstrus	Oestrus exhibition Return	Total œstrus exhibition and mating	Lambing (p. 100)	Oestrus exhibition and mating	Lambing (p. 100)	
I	Progesterone 375 mg (1) PMS 1 000 IU (2) Estradiol-17 β 30 μg (3)	38	100.0[a]	23.6[ac]	0	44.5	44.5[a]	58.8	100.0[a]	50.0[ae]	167.7
II	Progesterone 375 mg (1) PMS 1 000 IU (2)	38	89.5[a]	56.0[ad]	10.5	44.5	55.0[a]	47.6	100.0[a]	76.3[af]	163.7
III	Controls (4)	38	0.0[b]	0.0[b]	2.6	0.0	2.6[b]	100.0	2.6[b]	2.6[b]	143.8

(1) Silicone rubber implants for 14 days, subcutaneously. (2) Pregnant mare serum, injected twice i. m. at day 14th (day implant withdrawal) and 32nd. (3) Injected i. m. immediately before mating each ewe, after the first PMS injection only. (4) No treatment.

$P < 0.01$ for comparison a to b and c to d (same column). $P < 0.05$ for comparison e to f (same column).

experiment disclosed an adverse effect of estradiol-17 β on the conception rate (table 2). Thus, estradiol-17 β resulted in a significantly ($P < 0.01$) lower conception rate in group I (23.6 p. 100) in comparison to group II (56.0 p. 100). A similar significant ($P < 0.05$) adverse effect was shown on the total conception rate between groups I and II (50.0 *versus* 76.3 p. 100 respectively). However, the adverse effect of estradiol-17 β on conception rate of ewes in group I was not shown at estrus mating after the second PMS injection when the administration of estradiol-17 β was discontinued (47.6 p. 100 *versus* 58.8 p. 100 respectively). An adverse effect of estradiol was already observed by Pelletier and Thimonier (1973) who noticed a tendency ($0,05 < P < 0.1$) towards a reduced ovulation rate when 50 μg estradiol benzoate was given 24 hrs after sponge withdrawal.

From the results of the present experiment, it seems that breeding milking ewes 2 months postpartum during the spring anestrous period, is satisfactorily feasible using progesterone implants with twice-repeated injections of 1 000 IU PMS.

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Résumé. La combinaison du traitement progestérone-PMS avec de l'estradiol-17 β a été essayée pour rétablir la fertilité des brebis laitières Karagouniko pendant la période d'anoestrus, dans les deux mois suivant l'agnelage.

Un total de 114 brebis a été divisé au hasard en 3 groupes de nombre égal. Les brebis du groupe I ont été traitées avec des implants de progestérone (375 mg) pendant 14 jours et deux injections de 1 000 UI de PMSG chacune ont été administrées à un intervalle de 16 jours. Juste avant la saillie de chaque brebis, 30 μg d'estradiol-17 β furent injectés, seulement aux brebis qui avaient manifesté des chaleurs après la 1re injection de PMS. Les brebis du groupe II ont reçu le même traitement sans estradiol-17 β. Les brebis du groupe III ont servi de témoins.

Les pourcentages de brebis en estrus après la 1re injection de PMS, la 2e injection de PMS et deux injections, ont été respectivement : pour le groupe I : 100, 44,5 et 100 ; pour le groupe II : 89,5, 55,0 et 100 ; pour le groupe III : 0, 2,6 et 2,6. Les pourcentages d'agnelage ont été : pour le groupe I : 23,6, 58,8 et 50 ; pour le groupe II : 56,0, 47,6 et 76,3 et pour le groupe III : 0, 2,6 et 2,6. Le nombre d'agneaux nés pour 100 brebis qui ont mis bas a été en moyenne : pour le groupe I, 167,7 ; pour le groupe II : 163,7 et pour le groupe III : 143,8.

Deux injections de 1 000 UI de PMS à un intervalle de 16 jours après un traitement à la progestérone permettent 76,3 p. 100 d'agnelage ; 30 μg d'estradiol-17 β injectés immédiatement avant la saillie abaissent le taux d'agnelage à 50,0 p. 100.

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