

## **ESTRUS SYNCHRONIZATION OF EWES BY USING “RAM EFFECT” AND SINGLE TREATMENT WITH SYNTHETIC ANALOGUE OF PGF2A**

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### **Abstract**

METODIEV, N., 2015. Estrus synchronization of ewes by using “ram effect” and single treatment with synthetic analogue of PGF2 $\alpha$ . *Bulg. J. Agric. Sci.*, 21: 889–892

The aim of the present study was to induce synchronized estrus at ewes from Ile de France breed by using ram effect and single treatment with synthetic analog of PGF2 $\alpha$  in various days after ram introduction. After contacting of 7<sup>th</sup> days with rams, 35 ewes, that didn't show estrus, equal in age (from 2.5-to 3.5 years old), BCS (3.0-3.5), live weight of ewes (70-75 kg), clinically healthy and with normal last lambing, were assigned into 3 experimental groups: Group I – Treatment on day 7 (n = 12); Group II – Treatment on day 10 (n = 12); Group III – Control, without treatment (n = 11). On day 7<sup>th</sup>, 12 ewes randomly assigned (Group I) were treated with 125  $\mu$ g cloprostenol. The rest of ewes were observed and those with manifested estrus were inseminated, as they were assigned to the control group. On day 10<sup>th</sup> another 12 ewes were treated with 125  $\mu$ g cloprostenol and that was Group II. Ewes that remain untreated were assigned to control group, as they were observed for first manifested estrus. The ewes were inseminated naturally, by hand, as the first service was at first registered estrus and after that on 12 hour interval until the end of the estrus.

The obtained results showed that scheme that was used at Group II had better **effect of estrus synchronization (EES)** than this of Group I (100% vs. 66.66%), but the difference was not significant (\*P > 0.05). The best fertility had Group I – 85.7%, and the lowest - Group II – 70.0%. The fecundity had the lowest value at Group I - 100%, as there was significant differences (\*P < 0.05) between it and the values of Group II (157.14%) and Group III 166.66% (Table 1). The obtained results (100% ES, 70% pregnancy rate and 157.14 % fecundity) give us opportunity to recommend scheme, in which after 10 days of introducing the rams, ewes that didn't show estrus have to be treated with synthetic analogue of PGF2 $\alpha$ . The synchronization must be done after strict control of mating.

*Key words:* ewe, estrus, ram effect, prostaglandins

### **Introduction**

The synchronization of estrus of ewes is main element of the reproductive management in sheep-breeding. The methods of estrus synchronization can be classified as natural (non-hormonal) and pharmacological (hormonal).

One of the most popular non-pharmacological methods is the ram effect. This method is well established and studied since many years (Ungerfeld et al., 2004). According to Martin et al. (1986) there are differences of ovarian response at ewes after ram introduction which could lead to two surges of manifesting synchronized estrus induced by the ram effect- the first is between 17-20 days and the second is between 21-25 days after ram introduction.

Prostagalndin F2 $\alpha$  is the natural luteolytic agent that terminates the luteal phase (corpus luteum) of the estrus cycle (Hafez, 1993). The traditional schemes for estrus synchronization with PGF2 $\alpha$  or its analogues consist of two doses separated by 9 to 14 days (Bankov, 1989; Menchaca and Rubianes, 2004; Abecia et al., 2011) and were based on the refractoriness of young corpus luteum (till 5<sup>th</sup> day after ovulation) to lysis from PGF2 $\alpha$ . (Acritopoulou and Haresign, 1980). Rubianes et al. (2003) demonstrated that such refractoriness might be restricted to the first two days after ovulation.

The aim of the present study was to induce synchronized estrus at ewes from Ile de France breed by using ram effect and single treatment with synthetic analog of PGF2 $\alpha$  in various days after ram introduction.

## Material and Methods

### Arguments of the experiment

The aim of ram introduction is to form corpus luteum, if it is still missing. In our previous work (Metodiev et al., 2010; Metodiev and Raicheva, 2011; Metodiev et al., 2012) we concluded that Ile de France ewes from the flock of IAS-Kostinbrod normally cycled during the investigated season of the year (spring, April-May). So we designed the experiment to start in the beginning of mating season. We used “mating season”, not estrus season, because we didn't know the real bounds of anestrus and estrus for concrete flock and breed as whole for the conditions of Bulgaria. We proceed from the assumption that after 7 or more days of contact with rams, all of the ewe must have corpus luteum, according to the ram effect. The treatment with synthetic analog of PGF2 $\alpha$  will cause luteolysis and synchronized estrus till 3 day after treatment.

### Design of the experiment

The experiment was carried out during April, 2011. At the morning, during 7 days, the teasers were introduced to flock that was consisted of 210 ewes (in that number 26 nulliparous), aged 1.5 to 8.5 years. For those 7 days ewes with clinically manifested estrus was 55 (only multiparous) or these were 26.19% from whole flock. The ewes were inseminated according to the breeding plan.

On day 7<sup>th</sup> after starting the mating campaign, 35 ewes from the flock were assigned, equal in age (from 2.5-to 3.5 years old), BCS (3.0-3.5), live weight of ewes (70-75 kg), clinically healthy and with normal last lambing. Three experimental groups were done, according to the day of treatment.

On day 7<sup>th</sup>, 12 ewes randomly assigned (Group I) were treated with 0.5 ml Oestrofan  $\text{\textcircled{R}}$ , (BIOVETA, Czeck Republic, 125  $\mu\text{g}$  cloprostenol). The rest of ewes were observed and those with manifested estrus were inseminated, as they were assigned to the control group (Group III). On day 10<sup>th</sup> another 12 ewes were treated with 0.5 ml Oestrofan  $\text{\textcircled{R}}$ , (BIOVETA, Czeck Republic 125  $\mu\text{g}$  cloprostenol) and that was Group II. Ewes that remain untreated and these that were inseminated between Day 7<sup>th</sup> and Day 10<sup>th</sup> were assigned to control group (Group III), as they were observed for first manifested estrus.

The treatments for Group I and Group II were done in the evening, between 17 and 18 hour. The ewes were inseminated naturally, by hand, as the first service was at first registered estrus and after that on 12 hour interval until the end of the estrus. All the inseminations were according to the breeding plan, as the rams made up to 6 services per day (up to 3 in the morning and up to 3 in the evening). Before natural service, from all the rams, that were used, was collected semen by artificial vagina for estimation.

### Studied parameters

The following parameters were studied:

- Onset of estrus and effect of estrus synchronization (EES) - ewes in estrus were recorded twice a day by teasers, in interval of 12 h. The results were presented in hours. One ewe from Group II showed estrus after 12 h of treatment and was excluded from the analysis.
- Fertility is defined as the ratio of the number of ewes pregnant to the number of ewes, exposed to natural services at first estrus.
- Fecundity is defined as the number of born lambs from pregnant ewes (included all born lambs – live and dead). One ewe from control group was aborted, so we included the data about it only for the fertility, but not for the fecundity.

Note: Fertility and fecundity were calculated after lambing. One ewe from Group II died before the end of experiment, so its data was included only for the onset of estrus and EES.

### Statistical analysis

The significance of the differences between groups about the synchronization effect and fertility, were established by the Fisher's exact test (<http://graphpad.com/quickcalcs/contingency1/>). The significance of differences about fecundity was established by t –criteria of Student (t-Test: Two-Sample Assuming Unequal Variances, Data Analysis, Excel 2003, Microsoft).

## Results

The obtained results showed that scheme that was used at Group II had better EES than this of Group I (100% vs. 66.66%) (Table 1), but the difference was not significant (\*P > 0.05). The onset of estrus also differed between groups (Figure 1). The number of ewes with first signs of estrus from Group I was equal – 4 ewes at 36 h and 4 ewes at 48 h. In Group II there was a peak of ewes with first signs of estrus on 36 h – 8 for 11 ewes.

**Table 1**  
Effect of synchronization, fertility and fecundity of the studied groups

Groups	Effect of synchronization, %	Fertility, %	Fecundity, %
I	66.66	85.71	100.0
II	100	70.0	157.14*
III	-	81.82	166.66*

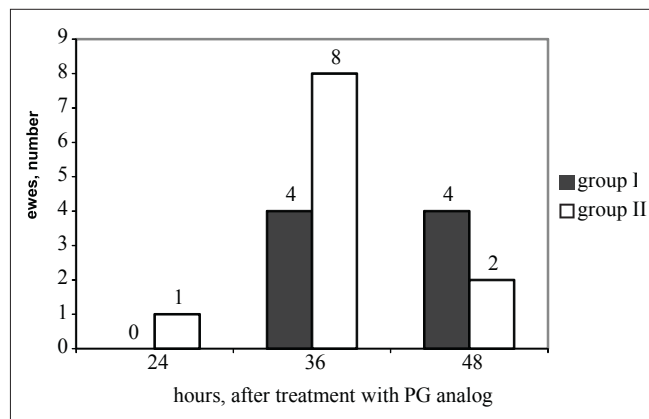


Fig. 1. Onset of estrus of Group I and Group II

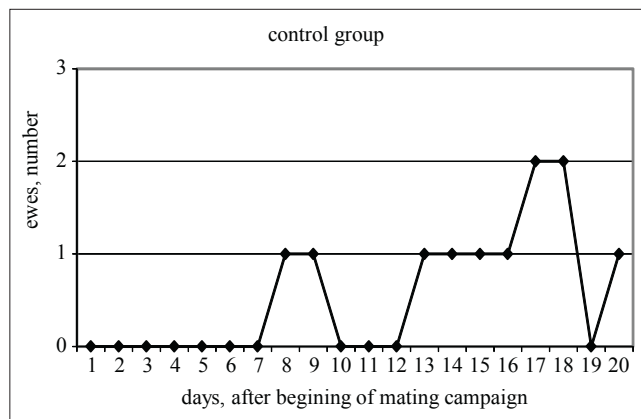


Fig. 3. Distribution of manifestation of first estrus of ewes in control group

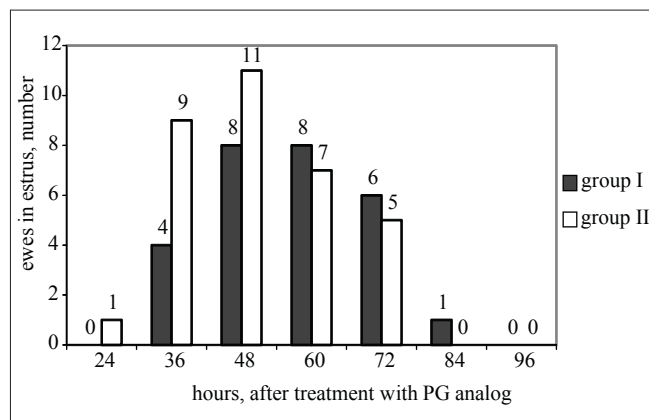


Fig. 2. Number of ewes in estrus by studied hours

At Figure 2 are presented number of ewes with estrus at every studied hour after treatment. For the Group I the biggest number of ewes in estrus was at 48 and 60 hours, but for the Group II this was 36 hour.

The distribution of ewes according to the onset of spontaneous estrus from the control group (Group III) was presented at Figure 3. Six from 11 ewes showed estrus between days 16 and 20 after starting the mating. The other 5 showed estrus between days 7 and 15.

The best fertility had Group I – 85.71%, and the lowest – Group II – 70.0% (Table 1). The ewes from Group III had mean value according to this trait – 81.22%. All the differences between groups were insignificant.

The fecundity had the lowest value at Group I - 100%, as there was significant differences ( $*P < 0.05$ ) between it and the values of Group II (157.14%) and Group III – 166.66% (Table 1).

## Discussion

The higher percent of estrus synchronization for Group II, compared to Group I, showed that all ewes had functional corpus lueum that was given in to lysis. Also, all the ewes form Group I and Group II, which were reacted to the treatment, were in estrus cycle. The irresponsiveness some ewes from Group I was probably due to passed short luteal cycle (according to the "ram effect") and forming of new corpus luteum, which was resistant to lysis.

The distribution of onset of estrus of the control group showed behavior, which was consequence of the ram effect - estrus between days 16 and 20 after starting the mating campaign. The other ewes became in estrous period before starting the breeding. The ram effect can be achieved without prior isolation of ewes from rams (Cusha et al., 1992). The ram effect is applicable in breeding season too, as the introduction of rams to cyclic ewes stimulates an increase in pulsatile LH secretion, independent of ewe genotype or stage of the estrous cycle (Hawken et al., 2007).

The high percent of fertility of both group, subjected to hormonal treatment, showed that the ovulations were of full value and inviolated transport of spermatozoa. It is well known, that pregnancy rate is generally lower in ewes, bred at synchronized estrus with PG or P4 compared with untreated ewes (review Fierro et al., 2013). Metodiev, 2013, in his PhD work found that after natural estrus was achieved the best results in fertility rate and fecundity, compared to induced estrus with double PG analogue treatment. The lower pregnancy rate after PG synchronizations was associated with decreased number of uterine contractions toward the oviduct or alternations of the vaginal mucus and hence the both reasons led to damage transport of spermatozoa and

their survival (review Fierro et al., 2013). It is important to note, that the inseminations were after natural mating, not artificial insemination and in that way more spermatozoa were capable to reach oocytes.

The significantly lower fecundity of Group I (100%, vs. 157.14% and 166.66%) gave us reason to suppose, that the final follicular growth was broken and lower follicles were ovulating. It is well known, that the treatment with PGF2 $\alpha$  and its analogues at the time of luteal phase of estrus cycle breaks normal follicle dynamic and normal luteogenesis (Barrett et al., 2002), probably influencing the endogenous rhythm of releasing FSH (Liu et al., 2006), breaks the function and final growth of ovulatory follicles (Abecia et al., 2011), reaching to differences in the time of ovulation (Barrett et al., 2002) as well as to lower levels of fertility (Barrett et al., 2002; Abecia et al., 2011) and according to us leading to variation of fecundity.

## Conclusion

The obtained results (100% ES, 70% pregnancy rate and 157.14% fecundity) give us opportunity to recommend scheme, in which after 10 days of introducing the rams, ewes that didn't show estrus have to be treated with synthetic analogue of PGF2 $\alpha$ . The synchronization must be done after strict control of mating. Also ultrasound screening ovaries for presence of corpus luteum must be done before PGF2 $\alpha$  treatment for better results.

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