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# REPRODUCTIVE RESPONSE AT ILE DE FRANCE EWES AFTER 5 DAYS PROGESTAGEN TREATMENT PLUS OR WITHOUT PREGNANT MARE SERUM GONADOTROPIN (PMSG)

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***Abstract***

*The aim of the present study was to study the reproductive response at Ile de France ewes after 5 days progestagen treatment plus or without pregnant mare serum gonadotropin (PMSG). The experiment was carrired out with 26 multiparous ewes. Three experimental groups were formed depending on whether a PMSG was used or not, as well as the time of treatment with a synthetic analogue of PGF 2α (before or after the removal of sponges). Group 1 (PGF /Pg/PMSG) (n=8) - at the time of the placement of the sponges, the ewes were treated with a synthetic analogue of PGF 2α. At the time of the sponge removal, 300 UI PMSG was put i.m. Group 2 (PGF /Pg) (n=9) - at the time of the placement of the sponges, the ewes were treated with a synthetic analogue of PGF 2α. Group 3 – (Pg/ PGF) (n=9) - the ewes were treated with a synthetic analogue of PGF 2α at the time of the sponge removal. The protocol, that consisted PMSG, successfully synchronized estrus (100%) with high levels of fertility (87.5%) and fecundity (171.4%). Protocols, which were without PMSG, were not suitable for ES, because of unsatisfactory results, that were obtained.*

***Key words:*** *estrus, ewes, PMSG, progestagens, synchronization.*

## INTRODUCTION

The synchronization of fertilities and births of ewes are main elements of the reproductive management in sheep breeding. Synchronization of estrus allows control and shortening of lambing and kidding, with synchronization of weaning and uniform batching of animals to slaughter; it also allows more efficient use of labor and animal facilities (Abecia et al., 2011). In Europe, the most common hormonal method for estrus synchronization (ES) of small ruminants is with intravaginal sponges, impregnated with progestagen (flurogestone acetate FGA or medroxyprogesterone acetate MGA) (Danko, 2003; Menchaca and Rubianes, 2004; Abecia et al., 2011). In the traditional protocols for synchronization of oestrus with intravaginal sponges, they stay in the vagina for 12 to 14 days (so-called long term treatments), followed by a PMSG (eCG) injection during the removal of sponges and after about 48 hours an oestrus occurs and the sheep can be inseminated (Abecia et al., 2011). The long-term progesterone treatments efficiently synchronize estrus, but with variable fertility (Menchaca and Rubianes, 2004) and also related to the development of vaginitis and problems with lack of sponge retention (Suarez et al., 2006; Martins et al., 2009), which are not consistent with what is desired from an animal welfare and health perspective (Maritnez-Ros et al., 2018).

According to the new concepts of follicular growth (the wave model, and that each follicular wave appears every 5-7 days), were developed various alternative, short-term progestagen treatments in sheep and goats, consisting of the induction of 5-7 days progesterone background followed by the injection of PMSG (Menchaca and Rubianes, 2004). Short progestagen treatments are effective both during the non-breeding season (Ungerfeld and Rubianes, 1999; Ataman et al., 2006; Martinez-Ros et al., 2018[1]; Maritnez-Ros et al, 2019[1]) and during the breeding season (Viňoles et al., 2001; Ataman et al., 2006; Ustuner et al., 2007; Karaca et al., 2009; Metodiev and Raicheva, 2011; Cox et al., 2012; Metodiev et al., 2014; Maritnez-Ros et al., 2019[1], Maritnez-Ros et al., 2018[2]; Metodiev, 2019; Maritnez-Ros et al, 2019[2]; Maritnez-Ros et al, 2019[3]; Maritnez-Ros and Gonzalez-Bulnes, 2019). Short-term protocols are more and more frequently used for sheep artificial

insemination under field conditions, but even with the advantages with use of this protocol there is still less use of this progestogen treatment regimen than that of the classical long-term treatments (Maritnez-Ros et al., 2018[1]).

In fact, all protocols for ES that used progestagens ends with PMSG treatment. Currently, there is a highly active movement in European countries against the use of eCG and it is necessary to look for alternative protocols for the induction and ovulation which would not include eCG (Maritnez-Ros et al., 2019[1]).

The aim of the present study was to study the reproductive response at Ile-de-France ewes after 5 days progestagen treatment plus or without PMSG.

## MATERIALS AND METHODS

The experiment was carrired out with 26 ewes of the Ile-de-France breed, raised in the Experimental farm of the IAS - Kostinbrod during May, 2019. Before and after the experiment, the animals were raised outdoors, grazed on pasture with supplement of 300 g. of sheep concentrate mix (12% crude protein, produced in a forage factory) per ewe per day. During the time when the ewes were with sponges, they were raised indoors, fed with meadow hay ad libitum with supplement of 300 g. of sheep concentrate mix (12% crude protein) per ewe per day. All experimental ewes were multiparous, aged 2-4 years, live weight (70-75 kg), body condition (BCS= 3.0-3.5), clinically healthy and with normal deliveries in the previous lambing. The intravaginal sponges (30 mg FGA Synchropart®, Ceva Sante Animale, France) were put on day 15 after the begining of controlled matings to all experimental animals for 5 days. Control matings (or mating campaign) means that every day at ewes were introduced teasers (1 teasers to 50 ewes) and ewes in heat were inseminated naturally with rams according the breeding plan. Usually mating campaigns with all flocks in Experimental farm of the IAS - Kostinbrod lasts 40-45 days. For those 15 days, 23 from 110 multiparous ewes came in estrus. The three experimental groups were formed with ewes depending on whether a PMSG was used or not, as well as the time of treatment with a synthetic analogue of PGF 2α (before or after the removal of sponges). The ewes in the experimental groups did not come in estrus up to this moment.

Group 1 (PGF /Pg/PMSG) (n=8) - at the time of the placement of the sponges, the ewes were treated with a synthetic analogue of PGF 2α - 1.0 ml Alfabedyl (Ceva Sante Animale, France, contains Alfaprostol 2 mg/ml). At the time of the sponge removal, 300 UI PMSG (Folligon®, MSD Animal Health) was applied.

Group 2 (PGF /Pg) (n=9) - at the time of the placement of the sponges, the ewes were treated with 1.0 ml Alfabedyl. At the time of the sponge removal, no treatment.

Group 3 – (Pg/ PGF (n=9) - at the time of the placement of the sponges, the ewes were not treated with a synthetic analogue of PGF 2α. At the time of the sponge removal, ewes were treated with 1.0 ml Alfabedyl.

The ultrasound screenings of the ovaries were done on next days: the day of the placement of the sponges (Day 1), the day of the removal of the sponges (Day 5) and 47 hours after sponge removal (Day 7). The equipment included a digital portable ultrasound system ALOKA ProSound 2 (Aloka Co., Ltd.) supplied with electronic linear transducer UST 5820, with frequency of 7.5 MHz. The total number and mean diameters of ovulatory sized follicles (diameters ≥ 5.00 mm) were counted for every ewe. Also presence or not of corpus luteum was observed. If there was of corpus luteum, the number of it was counted.

At 48 h after the sponge removal, the ewes were tested for presence of a heat with an estrous detector (Draminski Ltd). All ewes that had electrical resistance ≤ 350 units were considered to be in estrus (according to users' manual and our field observations). Ewes with boundary values for estrus (360-440 units) were also recorded. At 49 h after sponge removal ewes in heat according estrus detector and those with bound values were put to individual mating. At 56 h after sponge removal, the ewes that were not mated at 49 hour were tested again for presence of a heat with an estrous detector (Draminski Ltd) and the same procedure, like 48 hours, was done. Those in heat and with bound values were put to mating on 57 hour. For every mating only

one service was done. Six clinical healthy rams were used. Every ram did maximum 4 services per day (2 at 49 h and 2 at 57 hours).

**Statistical analysis**

The data for follicles and corpora lutea was presented in number means ± SEM. The mean diameters of follicles were compared by one way - ANOVA, Data analysis, Excel, Microsoft Office). All ovarian data was presented in number, means ± SEM.

The following reproductive parameters were studied:

- Estrus synchronization rate (ESR) – all ewes in estrus on Day 2 after sponge removal (detected by to estrus detector and by ram mating) - ewes in a heat/ all ewes x 100.
- Fertility - lambed ewes/ inseminated ewes x 100.
- Fecundity - the number of born lambs (included all born lambs - live or dead) / lambed ewes x 100.

Fertility and fecundity were calculated after lambing. All results were presented in percentage. 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/).

## RESULTS AND DISCUSSIONS

During the treatment, ewes from the experimental groups had similar number of ovulatory follicles (Table 1.) At Day 7 (47 hours after sponge removal) ewes from Group1 had the biggest number of ovulatory sized follicles – 21, whereas the Group 2 had the smalest – 16, but with the biggest mean size – 6.41 mm. The mean sizes follicles were not significantly different at all studied days. At the day of placement of sponges (Day 1) all ewes from Group 1 had corpora lutea, whereas 1 from Group 2 and 2 from Group 3 didn't (Table 1).

Table 1. Descriptive values for the follicles and corpora lutea

| | Day 1 | | | | Day 5 | | | | Day 7 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Total number of follicles | Mean diameter of follicles | Number of ewes with corpora lutea | Mean number of corpora lutea per ewe | Total number of follicles | Mean diameter of follicles | Number of ewes with corpora lutea | Mean number of corpora lutea per ewe | Total number of follicles | Mean diameter of follicles | Number of ewes with corpora lutea |
| First (n=8) | 14 | 6.17±0.20 | 8 | 1.87 | 20 | 5.88±0.15 | 0 | | 21 | 6.35±0.17 | 0 |
| Second (n=9) | 10 | 5.8±0.17 | 8 | 1.87 | 18 | 5.67±0.13 | 0 | | 16 | 6.41±0.13 | 0 |
| Third (n=9) | 14 | 5.8±0.12 | 7 | 1.71 | 17 | 5.59±0.11 | 8 | 1.62 | 18 | 6.17±0.15 | 0 |

At the day of sponge removal there weren't ewes from Group 1 and Group 2 with corpora lutea, whereas only 1 ewe from Group 3 did not have coprus luteum. After 47 hours, all ewes from Group 3 didn't have corpora lutea.

The ESR, according to estrus detector, was similar at Group 1 and Group 2 - 7 ewes, whereas the results for Group 3 were worse - 5 ewes. The ESR, according to ram service, was highest at Group 1 - 100% and its value was significantly different from values of Group 2 and Group 3 (Table 2).

Fertility for Group 1 was 87.50% and its values was significantly different from values of Group 3 - 0% (neither of three ewes in estrus was pregnant after ram service) (Table 3). Fertlity of Group 2 was 66.67 % but with low fecundity - 1 lamb from 1 ewe. Fecundity for Group 1 was 171.4%.

Table 2. Estrus synchronization rate (ESR) of the three experimental groups according to estrus detector and ram service

| Group | Total number of ewes in estrus, detected by ED | Total number of ewes, that were serviced by ram | ESR according to estrus detector, % | ESR according to ram service, % |
|---|---|---|---|---|
| First (n=8) | 7 | 8 | 87.5 | 100.0* |
| Second (n=9) | 7 | 3 | 77.77 | 33.33 |
| Third (n=9) | 5 | 3 | 55.55 | 33.33 |

**Note.** * Significant differences at P< 0,01 between Group I and Group II, Group I and Group III

Table 3. Fertility and Fecundity of the three experimental groups

| Group | Fertility, % | Fecundity, % |
|---|---|---|
| First (n=8) | 87.50* | 171.4 |
| Second (n=3) | 66.67 | 100 |
| Third (n=3) | 0 | |

**Note.** * Significant differences at P< 0,001 between Group I and Group III

## DISCUSSIONS

The aim of the present study was to study the reproductive response at Ile de France ewes after 5 days progestagen treatment plus or without PMSG. All of our experiments so far with short progestogen treatments were at the beginning of the breeding campaign. Over the last few years (2016, 2017, 2018, personal observations) the breeding campaign with the sheep from the flock have been stretched over time. Therefore, we chose to postpone the experiment two weeks after the onset of the breeding campaign to accelerate the campaign and also to see whether cyclic activity has started in sheep that have not exhibited so far estrus.

The presence of corpora lutea in 23 of all 26 sheep proved that sheep started to cycle (Table 1). The treatment of the first and second groups with a synthetic analogue of PGF 2α proved effective luteolysis. In the third group, which did not receive a synthetic analogue of PGF 2α, demonstrated that a 5-day treatment with synthetic progestogen was insufficient to regress the corpus luteum. This finding was in accordance with conclusion of Menchaca and Rubianes (2004), that in order to obtain good results in estrus induction after short treatment during the estrus season, it is necessary to provide regression of the corpus luteum. If luteolysis is induced at the beginning of short treatments, all females will maintain similar and adequate serum levels of exogenous progesterone during treatment. In our previous experiment (Metodiev and Raicheva, 2011) we evaluated the effect of 6 day progestagen treatments plus PMSG prior ram introduction on the estrus synchronization and the fertility of Ile de France ewes in the beginning of breding campaign we had two experimental groups – Group with Alfabedyl treatment and Group without Alfabedyl. Both groups had 91.66 ES, but with different fertility - 63.64% vs. 45.45%. On the basis of present and previous studies, we can conclude that treatment with a synthetic analogue of PGF 2α is obligatory at sponge placement, when scheme with short-term progestagen treatment are used.

In all three ultrasound observations, the state of ovaries in terms of the number and average size of the ovulatory follicles were the same. During the treatment, ewes from the experimental groups had similar number of ovulatory follicles and mean sizes follicles were not significantly different at all studied days (Table 1). These observations confirmed the conclusions of Menchaca and Rubianes (2004) that short-term progestagen treatments were adequate in the duration of a follicle wave.

The present experiment was the first, in which we used two different methods to detect estrus synchronization - estrus detector and rams. In our previous experiments we used only one of them. But in all previous experiments we used PMSG after sponge removal. The ESR according to the estrus detector was similar at Group 1 and Group 2 - 7 ewes, whereas the results for Group 3 were worse - 5 ewes. ESR according to ram service was highest at Group 1 – 100% and its value was significantly different from values of Group 2 and Group 3. The ESR in Group 1 was consistent with the results obtained in our previous experiments and also to these, reported by other authors – 80.0 and 100% until 144 hours after sponge removal (Ungerfeld and Rubianes, 1999; Viñoleset al., 2001; Aköz et al., 2006; Ustuner et al., 2007; Karaca et al., 2009; Martemucci and D'Alessandro, 2011; Metodiev and Raicheva, 2011; Metodiev and Raicheva, 2014; Metodiev et al., 2018, Martinez-Ros et al., 2018[1]; Martinez-Ros et al., 2019[3]; Martinez-Ros and Gonzalez-Bulnes, 2019)

The results for Groups 2 and 3 are more puzzling. Judging by the results of the estrus detector, those of Group 2 were similar to those of Group 1, while those of Group 3 were slightly lower in value. It was interesting why in sheep with ovulatory follicles and electrical resistance of the vagina responding to animals

with oestrus, these animals were not allowed to be covered. We suppose, this was due to the lack of sufficiently strong stimuli for long-term secretion of GnRH from the hypothalamus. Caraty et al. (2002) found that GnRH is involved in the control of receptivity in a ruminant species and suggested that in the cycling ewe the sustained preovulatory GnRH plays a physiological role in extending the duration of estrus.

We could conclude that if schemes without PMSG are used, stimuli of axis GnRH/LH should be done, for example external GnRH or new products, like the kisspeptin-10 analog C6. Martinez-Ros and Gonzalez-Bulnes (2019) concluded, that 5 days progestagen protocols with one dose GnRH, put on 56 hour after sponge removal, or two dose GnRH (put on sponge placement and second put on 56 hour after sponge removal) offer similar yields to eCG 5-days protocols. Decourt et al. (2019) examined the effect of the kisspeptin-10 analog C6 (palm-γ-Glutamyl-Tyr-Asn-Trp-Asn-Ser-GlyC[Tz]Leu-Arg (Me)-Tyr-NH2) as alternative of PMSG and the obtained results were promising.

Fertility for Group 1 was 87.50% and its values was significantly different from values of Group 3-0% (neither of three heat ewes was pregnant after ram service). Fertlity of Group 2 was 66.67% but with low fecundity - 1 lamb from 1 ewe. Fecundity for Group 1 was 171.4%. High fertility in Group 1 was in conformity with the suggestion of Menchaca and Rubianes (2004) that high short-term progestagen treatments follow PPSG treatment may better control follicular dynamics and improve fertility in small ruminants than long-term programs. In general, the results of fertility for Group 1 and Group 2 were in correspondence with the results to other authors, obtained after short terms progestagen treatment, natural mating and multiparous ewes (Ungerfeld and Rubianes, 1999 - 66.7%, Viñoles et al., 2001 - 87.0%; Karaca et al., 2009 - 71.6%, Martemucci and D'Alessandro, 2011 - 80%, Martinez-Ros and Gonzalez-Bulnes, 2019 - 76.5%, Martinez-Ros et al., 2019[1] - 80-90% (in breeding season), Martinez-Ros et al., 2019[3] - 60-80%).

Low values of fecundity (100%) of Group 2, compared to fecundity of Group 1 (171.4%), as well as zero fertility of Group 3 (Table 3), demonstrated that treatment without PMSG was unreliable for usage.

## CONCLUSIONS

The protocol that consisted 5-day progestagen treatment plus synthetic analogue of PGF 2α put at the time of the placement of the sponges and PMSG at the time of the sponge removal successfully synchronized estrus with high levels of fertility and fecundity. Protocols, that consisted 5-day progestagen treatment plus synthetic analogue of PGF 2α put at the time of the placement of the sponges or removal of the sponges without PMSG are not suitable for ES, because of unsatisfactory results, that were obtained.

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# TECHNOLOGIES OF ANIMAL HUSBANDRY