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# THE ESTRUS INDUCTION AND SYNCHRONIZATION PROTOCOL THROUGH THE MALE EFFECT AND PROSTAGLANDINS IN OUT OF THE BREEDING SEASON AT CARPATHIAN GOAT

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***Abstract***

***The objective of this study was to validate the prostaglandin-based protocols using the male effect for inducing and synchronising ovulations out of the breeding season along with photoperiodic treatment. The studies were conducted at 2 farms of private breeders of goats from Romania on 25 Carpathian goats at each farm. The experiment started with the male effect applied at goats (Day 0 – on 18 July 2013) and the injection of PG (Reglandin, cloprostenol 0.75µg/goat) realised in day 17. After 70 hours from PG injection, the goats were artificial inseminated with refrigerated semen. After goat's insemination (day 20), the bucks were removed from lot of goat treated with prostaglandin. The control lot, 25 Carpathian goats at each farm, was treated with FGA sponges (Chronogest, Intervet, France, 20 mg FGA) associated with PMSG 400 UI injection (Folligon 1000 UI, MDS, Holland) and Reglandin (0.75µg cloprostenol /goat).For the experimental lot of goats treated with prostaglandin and male effect, occurrence of oestrus was 92% (23/25) on farm 1 and 80% (20/25) on farm 2 and fertility was 68% (17/25) for each farm. Fertility after artificial insemination of goats not presented statistical differences between experimental lots of goats treated with prostaglandins and male effect comparing with hormonal treatment applied on goats (68% vs. 70%, p≥0.05).***

Keywords: male effect, prostaglandin, out of breeding season, artificial insemination.

## 1. INTRODUCTION

Goat breeding in Romania has been registering a growing numeric evolution, firstly because of the favorable breeding conditions existing in Romania and secondly because the consumers have gradually acknowledged the quality of the meat and milk of these two species. The social demand for the goat milk and meat is more and more oriented towards the consumption of ecologically obtained products, without the intervention of chemicals or hormones in the breeding technologies.

Romanian sheep and goats exhibit seasonal changes in reproductive activity, accompanied by variations in the availability of products over the year (L. Moise [12]). In order for the market to grow and satisfy its growing demand, solutions need to be found to enable goat's milk to be produced all year round. The main way to influence milk production is represented by modulating hormonal or non-hormonal reproduction means (L. Moise [13]).

Artificial control of reproduction provides some advantages for goat keepers. Control of reproduction is necessary both to eliminate undesirable consequences of reproduction like: inbreeding, kidding at long duration and contribute to the making of more productive genotypes in better environmental conditions (Corteel, [5]).

Reproduction of goats outside the natural breeding season can be achieved by several strategies: hormone treatments, day-length (referred to as the 'photoperiod') manipulation and the 'male effect' (a natural way based on social interactions male-female). Procedures based on the use of hormones have become widespread in the community of breeders over Europe, especially as hormone treatments allow reproduction by artificial insemination (AI). Artificial insemination is the privileged way to accelerate genetic progress of goat breeds subjected to selection programs, resulting in the rise of sanitary conditions, quality and quantity of the milk produced (B. Leboeuf [7]). The treatments used so far are based on the intravaginal administration of progestagenes for 11 days in goats and for 13 days in sheep, followed by the injection with PMSG (Folligon) and prostaglandin F2α (PGF2α) or its analogues, two days before the removal of the intravaginal sponges. There are several disadvantages associated to the administration of hormones. Firstly, since PMSG (pregnant mare serum gonadotropin) is an animal hormone, there are concerns regarding its use interspecifically. However, the main problem is connected to the occurrence of anti-PMSG antibodies in the case of repeated use and the consequence is the reduction of fertility. Moreover, the use of progestagenes is strictly limited in the European Union through the regulation RD 2178/2004 regarding the Maximum Residue Limits, which stipulates that any residues in milk or meat are strictly forbidden in order to reduce the possibility of affecting human health.

The objective of this study was to validate the prostaglandin-based protocols using the male effect (a natural way based on social interactions male-female) and the day-length manipulation (light treatments based on changing the length of the photoperiod during a

scheduled period of time) for inducing and synchronising ovulations out of the breeding season.

## 2. MATERIAL AND METHOD

### 2.1. Location and animals

Experiment started at July 2013, succeeding the photoperiodical treatment. The experiments were developed on 100 Carpathian breeding goats from two farms, aging 2-5 years, 45-55 kg weight. For each farm, the goats were dived into two lots, one for experimental protocol (with prostaglandin) and the second for control (with progestagen):

a. (PG1) Goats (n=25) treated with prostaglandin (Reglandin, Zoovet Impex S.R.L, Romania with active substance -cloprostenol 0.75µg/goat) and male effect (n=5); male/female ratio1:10.

b. (HT) Goats (n=25) treated with FGA sponges (Chronogest, Intervet, France, 20 mg FGA) associated with PMSG 400 UI injection (Folligon 1000 UI, MDS, Holland) and Reglandin (0.75µg cloprostenol /goat).

Also for the two lots of goats were examinated pseudopregnancy statutes of goats before 19 days of experiment beginning.

### 2. Protocols applied

The goats and the bucks were maintained in photoperiod conditions with 90 LD and 60 SD (using melatonin implants).

### 2.1. Prostaglandin associate with male effect treatment (PG)

For male effect the goats were isolated from males before 60 days to starting the experiment.

Start of experiment was represented by the male effect applied at goats (Day 0 – on 18 July 2013) and the injection of PG (Reglandin, cloprostenol 0.75µg/goat) realised in day 17. The optimal ratio is 1 buck per 10 does. The males were introduced in day 0 with aprons. Daily rotation of the bucks must be used to maintain optimal stimulation of the females. The contact between males and females should be effective and unrestricted. Bucks should be placed with females 24 h a day continuously until AI.

After 70 hours from PG injection, the goats were artificial inseminated with refrigerated sperm. After goat's insemination (day 20), the bucks were removed from lot of goat treated with prostaglandin.

After 21 days from insemination of goats, the bucks were reintroduced in this lot of goats, for natural mating of goats which were returning in oestrus.

### 2.2. FGA sponges associated with PMSG treatment

For all goats from this lot were applied the FGA sponges (Day 0) and in day 9 were realized the injections with Folligon (PMSG 400 UI) and Reglandin (cloprostenol 0.75µg/goat). After 43 hours from removal of sponge, all goats were artificial inseminated with refrigerated sperm. For each farm were inseminated all the goats.

Artificial insemination at goats was done with refrigerated sperm with progressive motility and viability more than 75%. Quality of sperm was determinate by flowcytometric and microscopic methods. Refrigerated sperm was maintained at 4℃ and was used after 2-3 hours from collection.

**Figure 1. Artificial insemination of goats**

After 45 days from insemination of goats, all goats were examined by ultrasonographies for pregnancy diagnosis.

For all goats were recorded the next parameters: percent of oestrus detection, percent of returning of goats in oestrus, fertility, prolificacy.

## 3. RESULTS AND DISSCUSION

The study responds to the current tendencies in the management of breeding goats by applying non-hormonal techniques of controlled reproduction by the use of the "male" effect and treatment with prostaglandins or their analogues. The result of their association is the induction of estrus and fertile ovulation in seasonal anoestrus.

Goats are polyoestric animals with a 20-21 day oestrus interval during the breeding season that is dependent on photoperiod. Oestrus cycle of goats contains two different phases, the follicular (2-4 days) and luteal phases (16-17 days). Mating occurs only if the animal is in oestrus (Zamfirescu, [18]).

Up to now, several protocols have been described for the synchronization of ovulation based on the make effect and also various hormonal treatments with progesterone, progestagenes or PGF2α, where artificial insemination was done without the previous detection of estrus. The PGF2α prostaglandin and its analogues are not restricted pharmacologically and their use is not limited from the standpoint of residual metabolites. (Nuti, [6]). Estrus and ovulation can be induced by the administration of a unique dose of PGF2α in the luteal phase induced by the male effect. The administration of PGF2α at the beginning of the luteal phase induces the shortening of the interval to the occurrence of ovulation and a better quality of the induced corpus luteum (Mellado M, [11]).

Reproduction out of breeding season can be achieved using strategies based on changing the length of the photoperiod (B. Malpaux [8]).

Introduction of a male to a group of previously isolated anovulatory goats can induce oestrus and ovarian activity. The induced ovulation occurs within 4 days following exposure (G. Baril [1], [2]). Such induced corpora lutea ofen have a short lifespan and exhibit early regression after transitory progesterone release (P. Chemineau [3]). Early regression could be attributed to the lack of majority of preovulatory follicles (GB Martin [9]). Priming with a single injection of 20 mg of progesterone or with a progestagen for several days, eliminate the abnormal ovarian cycles (Y. Cognie [4]). Various studies have highlighted the role of uterine PGF2α in the early regression of induced corpora lutea (N. Lassoued [15]).

Our previous research showed that the breeding season on Carpathian goats was observed between late Septembers to November, when the goats presents the cyclic variations of progesterone concentrations. On middle-late Spring the progesterone concentrations down to basal level, which characterized the anoestrus period. (E. Sogorescu, [17]). Female goats become anovulatory out of the natural breeding season

Also, the Carpathian bucks shows variations in their sexual activity. They present a decrease of their sexual activity from February to April, and the reproductive season starts in September and finish in December

Sexually active males are very efficient to induce highly synchronized ovulations in anovulatory but not in cycling females.

Photoperiodic treatments are thus recommended to be applied in order to improve the male effect. Previous experiments showed that the treatment of both males and females is necessary to improve the ovulatory response and avoid delayed responses after male exposure.

In farm 1 the ooccurrence of oestrus at goats was 92% (23/25) for the experimental lot of goats treated with prostaglandin and male effect, 80% (20/25) lot of goats treated with FGA sponge and Folligon. Returning of goats in oestrus was 20% (5/25) for the goats after prostaglandin and male effect treatment, 8% (2/25) for treatment of goats with FGA sponge.

In farm 2 the occurrence of oestrus at goats was 80% (20/25) for the experimental lot of goats treated with prostaglandin and male effect, 92% (23/25) lot of goats treated with FGA sponge and . Returning of goats in oestrus was 8% (2/25) for the goats after prostaglandin and male effect treatment, 0% (0/25) for treatment of goats with FGA sponge.

For both farms, our study shows that ooccurrence of oestrus (DE) for both treatments applied on goats was higher than 85% and it has the same values for each treatment (86±6%, table 1). Percent of returning in oestrus of goats (RE) was 14% for PG protocol and only 4% for HT, presenting statistical differences between experimental lots of goats ($p<0.05$). Fertility after artificial insemination of goats not presented statistical differences between experimental lots of goats treated with prostaglandins and male effect comparing with hormonal treatment applied on goats (68% vs. 70%, $p\geq0.05$).

Prolificacy of goats treated with prostaglandins and male effect was 130% comparing with 135% for the lot of goats treated with Folligon injection and Chronogest.

In table 1 were shown all the reproductive indicators for PG and HT treatments in both farms.

**Table 1**: Reproduction parameters on goats (means ±S.E.M)

| **Parameters** | **(%) PG** | **(%) HT** |
|---|---|---|
| Occurrence of oestrus (DE) | 86±6 | 86±6 |
| Bucks' re-introduction (natural mating of goats returning in oestrus) (RE) | $14\pm6^{a}$ | $4\pm4^{b}$ |
| Fertility after artificial insemination (FIA) | 68±1.3 | 70±1 |
| Prolificacy of goats (PR) | 130±1.20 | 135±2.6 |

The introduction of males to anovulatory females can induce ovulatory activity during seasonal anoestrus (J.T. Pierson [16]). Effective during the breeding season, PG use (in combination with the male effect) offer a flexible, economical method for syncronization to shorten the breeding season in a natural mating situation (M. Medan [10]). Our study shows that prostaglandin treatments associate with photoperiodic treatments and male effect are efficient for synchronisation of oestrus and ovulation on non-cycling goats in 80-85% animals.

Pregnancy rates in goats achieved after the use of the single prostaglandin injection was 68% compared to a mean conception rate of 70% achieved hormonal treatment.

## 4. CONCLUSIONS

The study results contributes to the management of goat milk industry in Romania, given that raising goats in Romania recorded a numerical trend upward, primarily due to favorable growth conditions existing in our country and secondly because of the gradual recognition by consumer qualities that shows milk of this species.

Promotion and application of non-hormonal protocols support the new food behavior that promote the sane and safe aliments, without hormones or various other residues potentially carcinogenic, mutagenic or that may affect human health.

Even if PMSG administration could be recommended to breeders in order to increase the number of kids in the breeding season at goats, this treatment have limitations: a. PMSG have a long-acting biological activity, causing it to continually recruit antral follicles, which results a large number of unovulated follicles and b. appearance of anti-PMSG antibodies after long-term use of this declining the fertility of goats (Moise L, [14]).

During the out of breeding season, a prostaglandin treatment is sufficient to synchronize oestrus in more than 85% of treated goats and can easily replace the hormonal treatment.

Fertility and prolificacy did not differ between the experimental and control lots, concluding that single prostaglandin injection associated with the male effect is

a alternative to progestagen protocol for inducing and synchronising oestrus during anoestrus in AI programs.

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