Including Natural and Synthetic PGF2α in a 11-day FGA-Based Estrus Synchronization Protocol in Sheep: an Efficacy Comparison of Dinoprost and Cloprostenol

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Abstract
There are a variety of hormonal protocols and products on the market, but it is still unclear how they will work on specific field conditions. The efficacy of using different forms of prostaglandin F2α analogues such as dinoprost and cloprostenol in a FGA-based estrus synchronization protocols were compared in sheep. For this purpose, on the basis of a completely randomized design, 60 ewes (Tsigai breed, Rusty variety; 2–4-years old, mean body score of 2.5 ± 0.5) were divided into two estrus synchronization treatment groups, which included: fluorogestone acetate (FGA) sponges for 11 days, with the administration of an intramuscular injection of 5 mg of dinoprost on the 9-day, followed by 300 IU PMSG at the time of sponge withdrawal (FGA-D-PMSG group, n=30), and for the other group, the same synchronization protocol was followed, with the difference that instead of dinoprost, ewes received 75 µg of cloprostenol (FGA-C-PMSG group, n=30). The estrus response rate percentage (%ERR) ranged between 76.66% (FGA-C-PMSG group) and 93.33% (FGA-D-PMSG group). In this study, it is shown that the type of PGF2α (natural or synthetic) can influence the results of a 11-day FGA-PGF2α-PMSG synchronization protocol, in terms of occurrence of estrus behaviour. Additionally, hormonal treatments cost for each protocol was calculated in order to determine the most cost-effective method and whether it can be implemented in small and large-scale sheep farming.

Keywords: FGA; PMSG; dinoprost; cloprostenol; synchronization; ERR; NRR; sheep

1. Introduction

Estrus manipulation is an efficient method in order to reduce the increasing cost of labor, by concentrating work in a well-defined period. It is also a method of improving the quality and quantity of deliverable lamb. This can increase the income of sheep farms, because the farmer can plan and meet his requirements regarding delivery terms and the quantity/quality of deliverable lamb. The starting point of all ARTs protocols (assisted reproduction technologies) is represented by synchronization of estrus and ovulation (SEO), because, since sheep are seasonal breeders, SEO is a crucial component of artificial insemination (AI), multiple ovulation and embryo transfer (MOET) and laparoscopic ovum pick up (LOPU) [1].

In order to synchronize estrus in sheep, progesterone treatments are increasingly used, both in large and small farms. According to reports, progesterone treatments are successfully used in order to synchronize estrus in sheep during both breeding [2-5] and non-breeding seasons [3,6-9]. Progesterone (P4) treatments are typically administered as intravaginal sponges, injectables, or controlled internal drug releasing (CIDR) devices. Commercially available intravaginal sponges are generally impregnated with different

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concentrations of fluorogestone acetate (FGA) or medroxyprogesterone acetate (MPA). P4 treatments are used both for short-term protocols (5-7 days of exposure to progesterone) and for long-term protocols (12-14 days), in combination with gonadotropins [10]. However, prolonged use of intravaginal sponges is frequently linked to vaginitis [11].

Both in breeding and non-breeding seasons, in order to improve estrus and ovulation rates, in addition to intravaginal insertion of P4-impregnated sponges, pregnant mare serum gonadotropin (PMSG) is injected intramuscularly and estrus begins in 24 to 48 hours [12].

In recent years, there has been an increased interest in testing different estrus synchronization protocols, which, in addition to P4 treatment combined with gonadotropins, also contain a luteolytic agent, mainly PGF2α and its analogues [13,14].

In ruminants and other domestic species, PGF2α (dinoprost tromethamine) and its analogues (e.g. cloprostenol) are powerful luteolytic agents that have been used in estrus synchronization programs either alone or in combined with progestogens [15].

PGF2α is produced by endometrial glands in response to oxytocin to induce luteolysis at the end of the luteal phase when there is no pregnancy. This process starts in ewes and does on days 11 to 12 and around day 13 following estrus, respectively [16]. Reproduction management is strongly influenced by the ability to manipulate the luteolytic activity of the ovary through pharmaceutical methods [17]. Intramuscular injection of a suitable dose of natural PGF2α, or its analogues, is an effective method of inducing luteolysis [18].

PGF2α (dinoprost) has a short half-life, showing accelerated metabolism in the organism, which reduces its effectiveness [19]. Cloprostenol is obtained by chemical synthesis, and is represented by two isomers, in a dextrorotatory and levorotatory form (D- and L-cloprostenol), but also a mixture of both, DL-cloprostenol, whose effect is 10 times lower than that of D-cloprostenol [20]. However, only the D-isomer of cloprostenol exhibits luteolytic activity [20]. The most frequently used hormones for estrous synchronization are presented in Figure 1.

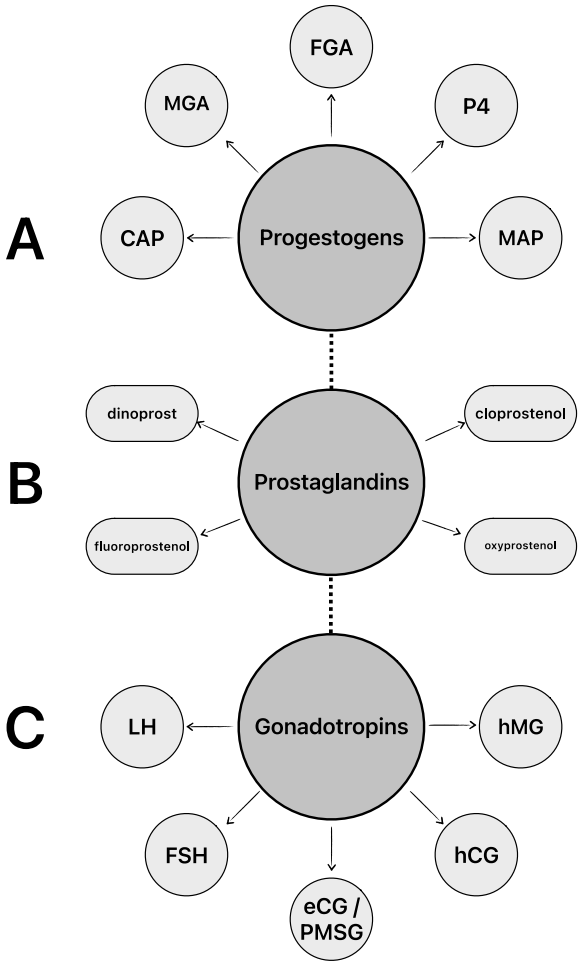

Figure 1. Hormones used for estrous synchronization. **(A)** Progestagens: chlormadinone acetate (CAP); melengestrol acetate (MGA); fluorogestone acetate (FGA); progesterone (P4); medroxyprogesterone acetate (MPA); **(B)** Prostaglandins: dinoprost; cloprostenol; fluoroprostenol; oxyprostenol; **(C)** Gonadotropins: follicle-stimulating hormone (FSH); equine chorionic gonadotropin (eCG) / pregnant mare serum gonadotropin (PMSG); human chorionic gonadotropin (hCG); human menopausal gonadotropin (hMG).

In the last decade, many studies have tested the effectiveness of dinoprost [21-24] and cloprostenol [25-30] used in protocols that involves one or two intramuscular injections at different time intervals. There has also been increased interest in determining which of these two is more effective [17,25,31]. However, none of these studies compared the luteolytic efficiency of these prostaglandins when used in combination with P4 (or analogues) and gonadotropins.

Considering all this, the purpose of this study was to test a mid-term protocol (11 days) in order to avoid the unwanted effects of long-term protocols (14 days), like vaginitis, but still not to decrease the positive effects of longer exposure to progesterone. Also, we wanted to compare the effectiveness of dinoprost and cloprostenol, which were used as luteolytic agents.

2. Materials and methods

This study was carried out in the breeding season (September), at Agricultural Research and Development Station Turda. Healthy multiparous ewes (n=60; Tsigai breed, Rusty variety; 2–4-years old, mean body score of 2.5 ± 0.5), were divided into 2 groups (n=30) randomly.

On day 0, all ewes received one intravaginal FGA-impregnated sponge (20 mg fluorogestone acetate, FGA, Chronogest®; MSD Animal Health, Madrid, Spain) for 11 days.

Two days before removing sponges (day 9), ewes received a prostaglandin-intramuscular injection (IM). Thus, the first group (FGA-D-PMSG, n=30) received 5 mg dinoprost (Dinolytic, IM; Pfizer AG, Zurich, Switzerland), and the second group (FGA-C-PMSG, n=30) received 75 µg D-cloprostenol (Genestran, IM; Vetcare Ltd., Finland).

At the time of sponge removal (day 11) animals from both groups received a dose of 300 IU PMSG (Sergon, IM; Bioveta, Czech Republic). Also, loss of sponge and vaginitis were recorded. Vaginitis was recorded when purulent discharge was presented at the time of sponge removal.

Ewes in estrus were detected using aproned rams, once a day. Females were considered in estrus when exhibited standing reflex. Based on these recordings, estrus response rate percentage (%ERR) was calculated. Ewes were mated once, by a selected ram. The detection of ewes in estrus with aproned rams continued for 35 days (daily) after mating, thus we were able to calculate the non-return rate percentage (%NRR).

All the treatments performed in this study are presented in Figure 2.

Data was statistically analyzed using GraphPad Prism (Version 9.3.1). An exact Fisher's test was performed to compare the results of the two protocols in terms of %ERR and %NRR.

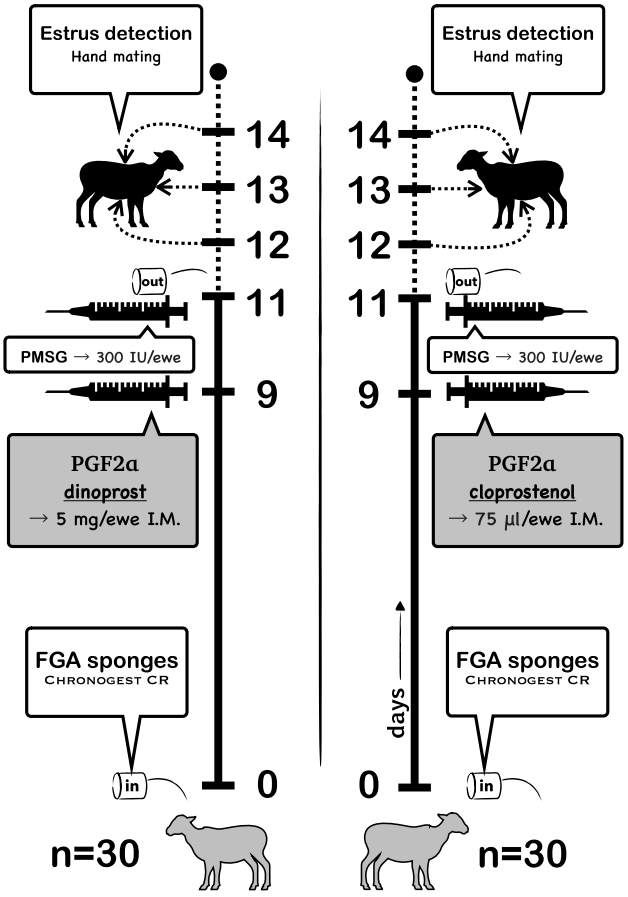

Figure 2. Protocols used in this study for estrus synchronization and the time frame of treatments: FGA-D-PMSG group (left) and FGA-C-PMSG (right).

Additionally, hormonal treatments cost for each group was calculated in order to determine the most cost-effective method and whether it can be implemented in small and large-scale sheep farming.

3. Results and discussion

Considering that the main indicator of a successful estrus synchronization protocol is represented by the estrus response rate percentage (%ERR), ewes that showed behavioral signs of estrus (standing reflex) within 72 h after the withdrawal of the sponge were recorded. Since estrus detection was done once a day with aproned rams, the 72-hour time after removing the sponges was divided into 3 time periods, as can be seen in Table 1.

In the first 24 h, no ewe showed estrus behavior, most females showing estrus in the 24-48 h interval (both groups).

Table 1. Estrus response rate (%ERR) in different time periods

Group	0-24 h	24-48 h	48-72 h
FGA-D-PMSG	0% (0/28)	78.57% (22/28)	21.43% (6/28)
FGA-C-PMSG	0% (0/23)	69.57% (16/23)	30.43% (7/23)

Regarding the onset of estrus, no significant differences (p>0.05) were identified between the two groups. However, the ewes from the FGA-D-PMSG group showed an earlier onset of estrus than the FGA-C-PMSG group (78.57% vs. 69.57%).

Estrus performance of ewes following the synchronization protocols is presented in Table 2. None of ewes lost the sponge and only one case of vaginitis was recorded (FGA-C-PMSG). Twenty-eight ewes from FGA-D-PMSG group were detected in estrus and in FGA-C-PMSG group 23 ewes showing estrus sings were identified.

Table 2. Estrus performance of ewes following the synchronization protocols

Parameter	FGA-D-PMSG	FGA-C-PMSG
Sponge loss	0% (0/30)	0% (0/30)
Vaginitis rate[1]	0% (0/30)	3,33% (1/30)
ERR[2]	93.33% (28/30)	76.66% (23/30)
NRR[3]	85.72% (24/28)	82.60% (19/23)

[1]Vaginitis rate (%) = ewes exhibiting vaginitis/number of ewes treated with intravaginal sponge × 100
[2]ERR (%; estrus response rate) = ewes exhibiting estrus/number of ewes treated × 100
[3]NRR (%; non-return rate) = ewes not returning to estrus (35 days)/mated ewes × 100

Our results show that a mid-term protocol (11 days) can prevent the unwanted effects of long-term protocols regarding the loss of sponges, which in our case was 0/60. Also, we could record a small percentage of vaginitis (1/60), even if we did not use antibiotics [32] or probiotics [33]. Therefore, by using vaginitis-preventing antibiotics applied with intravaginal sponges, there may be a risk that their residues will be found in milk [34]. This can be a serious problem for dairy sheep farms. Likewise, using antibiotics or probiotics would have increased the protocols cost.

There were significant differences ($P < 0.05$) in terms of %ERR between the two groups (figure 3).

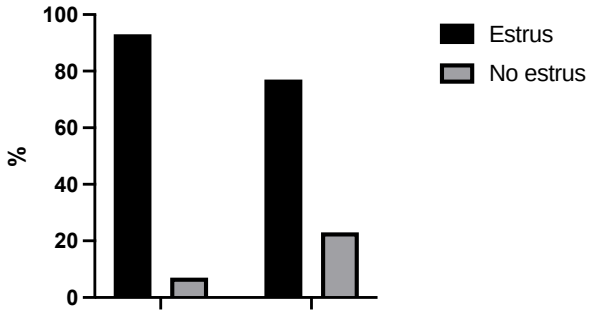

Figure 3. Estrus response rate (%ERR)after FGA-D-PMSG (left) and FGA-C-PMSG (right) synchronization

The average percentage of non-return rate (%NRR) was not significantly different (p>0.05) (figure 4).

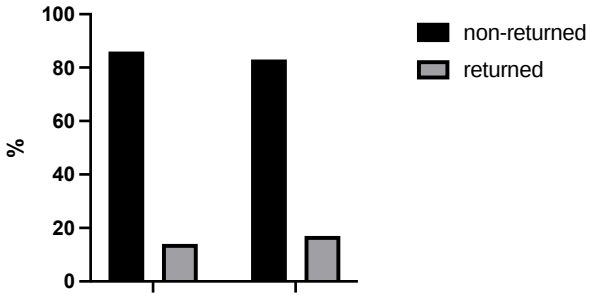

Figure 4. Non-return rate (%NRR) after FGA-D-PMSG (left) and FGA-C-PMSG (right) synchronization

Ewes from the FGA-D-PMSG group showed a higher estrus response rate (%ERR) than those from FGA-C-PMSG group (93.33% vs. 76.66%), which could be influenced by a superior luteolytic efficiency of dinoprost. Previous hypotheses regarding the lutelotic efficacy of these pharmaceutical products are controversial, with some showing that either dinoprost [31] or cloprostenol [17] are more effective in terms of %ERR. However, these studies compare the effectiveness of these hormonal products in a protocol that involves one or two intramuscular injections of PGF2α at a certain time interval, not in combination with P4 and PMSG. We wanted to determine if the type of PGF2α can influence the results of an FGA-based estrus synchronization protocol, which involved exposing ewes to an 11-

day P4 treatment, with a luteolytic effect injection (PGF2α) on day 9, and at the time of sponge removal a dose of PMSG. FGA-D-PMSG group showed better results than FGA-C-PMSG group regarding: vaginitis rate (0% vs. 3.33%), %ERR (93.33% vs. 76.66%) and %NRR (85.72% vs. 82.60%).

Dinoprost induces a faster luteolytic response than cloprostenol [35], which could determine a superior luteolytic effect when used in combination with P4 and PMSG. Thus, considering that there is no knowledge in this regard, in order to test this hypothesis, more studies are needed. Also, dinoprost determines much higher levels of nitric oxide (NO), increases blood flow area (BFA) and blood velocity of the corpus luteum (CL) in the first hours after treatment [25]. Thus, dinoprost could induce a faster and more luteolytic effect than cloprostenol.

Hormonal drugs cost for both groups was similar (Table 3), but considering the differences in performance, the protocol used in FGA-D-PMSG group seems to be more cost-effective.

Table 3. Hormonal drugs cost per ewe (Euro)

Group	FGA-D-PMSG	FGA-C-PMSG
FGA	2.29	2.29
PGF2α	0.87	0.90
PMSG	2.53	2.53
Total	**5.69**	**5.72**

Considering all the advantages that estrus synchronization brings to the farm's income, the cost of approximately 5.7 Euro/ewe may be economically justified.

4. Conclusions

Considering the results regarding sponge loss, vaginitis rate, estrus response rate percentage (%ERR) and non-return to estrus rate percentage (%NRR), both protocols tested in this study determined satisfactory percentages. However, the protocol used for the FGA-D-PMSG group was superior to the FGA-C-PMSG, which makes it more cost-effective. Hence, these protocols are suitable for use in small and large-scale sheep farming.

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