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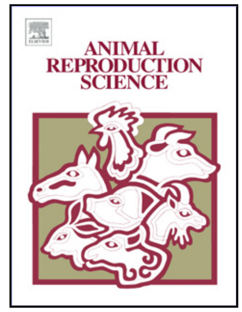

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Addition of eCG to a 14 d prostaglandin treatment regimen in sheep FTAI programs

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Highlights

- MAP+eCG showed higher pregnancy rate than the PG protocols.
- PG+eCG and MAP+eCG showed higher fecundity rates than PG alone.
- PGs are easy-to-apply technologies, with acceptable reproductive performance.

ABSTRACT

In the present study, there was evaluation of the alternative of adding eCG as part of a long-interval prostaglandin-$F_{2\alpha}$ (PG) treatment on the reproductive efficiency of Merino sheep during the breeding season. A total of 210 ewes and 182 ewe lambs were randomly assigned to three experimental groups to induce the timing of estrus among ewes in a: Long-interval PG, group being synchronized using two doses of PG 14 days apart; Long-interval PG+eCG group being synchronized using the same treatment regimen as Group PG with the addition of 200 IU eCG to the regimen, administered concomitantly with the second PG administration; and MAP+eCG group being synchronized with intravaginal progestin sponges for 14 days plus 200 IU eCG, administered at the time of sponge removal. The percentage pregnancy rate in ewes of the MAP+eCG group was greater than the ewes of the Long-interval PG and Long-interval PG+eCG groups (76.4% compared with 52.0% and 62.5%, respectively; $P<0.05$). The prolificacy rate was greater in the ewes of the Long-interval PG+eCG group compared with the other groups (114% compared with 100% and 103%, respectively; $P<0.05$). When considering the fecundity rate, ewes of the Long-interval PG+eCG and MAP+eCG groups had greater values than ewes of the Long-interval PG group (71.2% and 78.8% compared with 52.0%, respectively; $P<0.05$). The Long-interval PG+eCG is an alternative to the conventional progestin sponge plus eCG treatment regimen with there being a greater fecundity rate when this regimen is used compared with the Long-term PG and similar to MAP-eCG treatment regimens.

Keywords: Estrous synchronization; Prostagladin; Progestagens; Cervical fixed-timed artificial insemination; Ewe

1. Introduction

In the last decades, fixed-time artificial insemination (FTAI) has been developed as a technique for FTAI of a large number of ewes in a flock in the same timeframe after the last administration of a treatment regimen. With these approaches, therefore, there is no need for performing estrous detection. This technology minimizes animal handling, because detection of estrus and management of vasectomized males are not needed, reducing the time need for managing ewes and improving animal welfare (Olivera-Muzante et al., 2011; Vilariño et al., 2013; dos Santos-Neto et al., 2015). For achieving a desirable fertility rate after FTAI, it is necessary to apply methods of synchronization of estrus that concentrate a large number of females in estrus in a short period of time and with there being a great extent of synchrony between the onset of estrus and time when ovulation occurs (Ramos and Silva, 2018; Yu et al., 2019; Gonzalez-Bulnes et al., 2020).

Two of the most common methods of synchronization of estrus in sheep include the use of intravaginal progestin sponges in combination with equine chorionic gonadotropin (eCG) and the use of different prostaglandin treatment regimens (PG; Fierro et al. 2013; González-Bulnes et al. 2020). Wildeus (2011) summarized some of the results from research in the 1990s to establish optimal hormonal doses and timing of administration, to achieve a greater synchrony in time of estrus among ewes so as to facilitate the use of FTAI.

The use of PG in sheep reproduction has several practical advantages, including simple application (i.m. injection), reduced cost (70% of the cost per treated sheep) and less environmental contamination compared with the use of progestin intravaginal devices (Fierro et al., 2013). When there is a relatively longer interval between PG injections, there can be an increase in the duration of time in which there are longer periods of progesterone or progestin modulation of the hypothalamic-pituitary-ovarian axis during the follicular growth phase (Fierro et al., 2016). For that reason, reproductive performance has been improved when there are these relatively longer intervals between PG administrations compared with shorter PG intervals (Fierro et al., 2017). Treatments with PG, however, result in a slight reduction in pregnancy rates when there is FTAI with the use of these treatment regimens compared to treatment with the conventional intravaginal medroxyprogesterone acetate (MAP) sponge combined with eCG, due to a greater variation in time of expression of estrous behavior after imposing estrous synchronization treatment regimens (Gibbons et al., 2019). There, however, are differing thoughts on this topic (Olivera-Muzante et al., 2019).

The administration of eCG has been used in synchronization treatments with intravaginal sponges to decrease the variation in timing of expression of behavioral estrus and ovulations (Cueto and Gibbons, 1995). Similarly, there has been inclusion of treatment with eCG in the double-PG treatment regimen with this being a possible alternative for improving reproductive results when there is use of these PG-based treatment regimens, although there have been very few studies in which there were these types of evaluations (Hasani et al., 2018). In the Patagonia region of South America, synchronization of estrus using two doses of PG administered 14 days apart and subsequent FTAI with fresh semen has resulted in acceptable fertility rates in ewes (Gibbons et al., 2019), however, lesser than those with use of progestin sponges. To achieve greater pregnancy rates with this treatment regimen, the inclusion of an eCG dose is an alternative approach which should be evaluated because there have not been studies conducted where there was the use of a 14 day-interval PG treatment regimen with the addition of eCG for conducting FTAI in both reproductively mature ewes and ewe lambs.

The hypothesis for the present study was that the addition of eCG treatment at the end of a long-interval PG treatment regimen would result in an increase in the reproductive efficiency of ewes compared with the use of the PG treatment regimen alone. It was also hypothesized that the use of the long-interval PG-eCG treatment regimen would result in similar reproductive outcomes as compared with the use of the treatment regimen in the progestin sponge for conducting FTAI.

2. Materials and methods

2.1. Animals and experimental design

The experiment was conducted in the Farm Research Unit of the INTA, located in the Río Negro province, Argentine (41° 7´ 23´´ S, 70° 43´ 12´´ W). The experimental procedures were approved by the Animal Ethics Regional Committee of the INTA (Comité Institucional de Cuidado y Uso de Animales de experimentación [CICUAE INTA–PATNOR- Res. 533/16 Disp. CRPN 66/17]).

A total of 210 Merino ewes and 182 ewe lambs, in moderate body condition (> 2.5 of five; scale from 0, emaciated, to 5, obese; Russel et al., 1969) and moderate body weight (ewes, 43.4 ± 4.8 kg; ewe lambs, 39.9 ± 4.5 kg) were used during the breeding season (May, southern hemisphere). The animals were maintained outdoors in natural grazing conditions and had free access to water.

The females were randomly assigned to three experimental groups: Long-interval PG (*n* = 71 ewes and 40 ewe lambs), timing of estrus was synchronized using two injections i.m. of PG 14 days apart (125 µg sodium cloprostenol, Estrumate®, InterVet, Argentine). Ewes *(n* = 64 ewes and 48 ewe lambs) of another group were assigned to a long-interval PG+eCG treatment regimen with time of estrus being synchronized using the same treatment regimen as group long-interval PG combined with 200 IU i.m. of eCG (Novormon®, Zoetis, Argentine) administered concomitantly with the second PG injection. There was a third group of ewes (*n* = 75 ewes and 94 ewe lambs) assigned to a MAP+eCG treatment regimen with there being synchronization in time of estrus by the insertion of intravaginal progestin sponges (60 mg of MAP; Progespon®, Zoetis, Argentine) for 14 days combined with 200 IU i.m. of eCG (Novormon®, Zoetis, Argentine) administered at the time of sponge removal.

2.2. Semen collection and artificial insemination

Fresh semen was collected with an artificial vagina as described by Evans and Maxwell (1987) from five clinically healthy adult Merino rams (breeding soundness examination and test for Brucella ovis). The identification of the ram was necessary because the ewes are part of a breeding genetic program. Semen was assessed for progressive motility (80%) for approval for its use and ejaculates with a minimum concentration of 3 x 10^9 spermatozoa/ml and a minimum mass motility of 3 (scale from 0 to 5) were used.

Cervical FTAI was performed at 54 ± 2 h after the second PG administration or after sponge withdrawal, as far as possible in the external cervical os and was performed using a speculum equipped with a light source and a multidose insemination device (Walmur® Veterinary Instruments, Montevideo, Uruguay) by one technician, as described by Evans and Maxwell (1987). The insemination dose per ewe was 0.03 ml containing 100 x10^6 spermatozoa approximately. All the ewes were inseminated at random but taking into consideration the identification of each unit of ram semen used for AI.

2.3. Ultrasonographic evaluation and reproductive rates

Pregnancy rate (number of pregnant ewes/number of inseminated ewes x 100) and prolificacy rate (number of fetuses/number of pregnant ewes x 100) were diagnosed on Day 35 following FTAI using trans-rectal ultrasonography (6 MHz linear probe, Aquila Vet, Philipsweg, Netherlands) as described by Viñoles et al. (2010). Fecundity rate was defined as the number of fetuses/number of inseminated ewes x 100. There was a typical length gestation period before birth of all lambs with lamb birth date being recorded at the time of parturition of each ewe.

2.4. Statistical analyses

Analysis of data was conducted using the R Commander (R Core Team, 2016). Pregnancy and prolificacy data were analyzed using a glmer model with treatment, age and interaction in a 2 x 2 factorial and ram as a random effect, with a binomial distribution. Fecundity was analyzed in a similar model using a mixed procedure. There were considered to be mean differences when there was a $P<0.05$.

3. Results

Among the three treatment groups, timing of estrus was synchronized among 392 females of which 369 were inseminated (Table 1). The other ewes were not inseminated because they did not have vaginal responses consistent with those that occur during behavioral estrus at the time of insemination (mucous flow, edema, vaginal hyperemia). The number of females inseminated with semen from each ram was: A = 72, B = 25, C = 66, D = 191, E = 15.

There was no interaction between treatment and age of the females (*P*>0.05). Additionally, there was no effect of the age of females on pregnancy, prolificacy, and fecundity rates (*P*>0.05, Table 1).

The percentage of pregnancy for ewes reached in the MAP+eCG group was greater than those of ewes in the long-interval PG and long-interval PG+eCG groups (*P*<0.05, Table 1). Furthermore the prolificacy rate was greater in the ewes of the long-interval PG+eCG group compared to the other groups (*P*<0.05). For fecundity rate, ewes of the long-interval PG+eCG and MAP+eCG groups had greater values than that of ewes in the long-interval PG group (*P*<0.05).

4. Discussion

In the present study, the hypothesis was that the addition of an eCG treatment at the end of a long-interval PG treatment regimen would result in an increase in the reproductive efficiency of ewes as compared with the ewes with which there was only the long-interval PG treatment regimen imposed. There is partial acceptance of this hypothesis. The results of the present study indicate the 14-day PG treatment regimen combined with eCG administration is an useful estrous synchronization method for conducting cervical FTAI in Merino sheep. This treatment regimen would be effective in extensive rearing production during the breeding season with there being improvements in the prolificacy and fecundity compared with the use of long-interval PG treatment regimen without inclusion of eCG. This PG-eCG combination treatment regimen, however, was not as effective in achieving the fertility rates as those that occurred with imposing of the MAP-eCG treatment regimen.

The addition of eCG to the long-interval 14-day PG treatment regimen results in an increase in prolificacy and fecundity compared with when there is imposing of long-term PG and MAP+eCG treatment regimens for FTAI. This could be due to stimulation of the pre-ovulatory follicular development and greater ovulation synchrony (McNatty et al., 1982) with the outcome being an improvement in these reproductive outcomes after FTAI. In contrast, there was no effect of eCG on the prolificacy rate in another study (Vilariño et al., 2017) in which there was evaluation of a short-interval PG-based treatment regimen. Hasani et al. (2018) also reported similar findings when there were comparisons with a 12-day-interval PG treatment regimen with and without eCG administration. These differences in results among studies could be due to the hormonal status prior to fertilization as a result of the different intervals between PG administrations (Fierro et al. 2016). Due to the longer interval between PG administrations, the time-interval is longer in which the dominant follicles have relatively greater progesterone concentrations in the follicular milieu, improving reproductive performance compared to short- and mid-interval treatment regimens (Fierro et al., 2013). The lesser prolificacy rate when there is imposing of the MAP+eCG compared with the long-interval PG+eCG treatment regimen may be explained by the negative effect of relatively greater concentrations of progestin on oocyte and embryo quality (Viñoles et al., 2001). The relatively greater prolificacy rate when there was imposing of long-interval PG+eCG compared with long-interval PG treatment regimen and the relatively similar pregnancy rate when there was imposing of the long-interval PG+eCG and

MAP+eCG treatment regimens is of great interest to sheep producers. Each technician, however, should evaluate application of these treatment regimens that result in an increase in the rate of twinning that could lead to greater perinatal mortality if lambing is not carefully monitored and if dystocia problems are not immediately addressed at the time of lambing.

In the experimental conditions of the present study, the administration of eCG at the end of PG treatment regimen did not affect pregnancy rate. This result is similar to that reported by Vilariño et al. (2017), but is inconsistent with that reported by Boland et al. (1978), in a study where there was an increased conception rate (71.4% compared with 42.1% with or without eCG) when using a larger eCG dose (500 IU) at the end of a long-interval 14-day PG treatment regimen when there was natural mating. An explanation for the lack of effect of the eCG on pregnancy rate in the present study could be the distribution of estrus after imposing of the long-interval 14-day PG combined with eCG treatment regimen, which was not evaluated in this experiment, and would allow for estimating with greater accuracy the optimal time for insemination. More studies, therefore, are required to evaluate the distribution of estrus to determine the optimal timing of FTAI for maximizing pregnancy rate.

When comparing the pregnancy rates with the use of the long-interval PG and MAP treatment regimens in the present study, there was a greater percentage pregnancy rate with use of the MAP+eCG (76.4%) compared with the long-interval PG (52%) and the PG+eCG (62.5%) treatment regimens. There are some findings that are inconsistent regarding percentage pregnancy rate in previous studies with the use of similar treatment regimens as those evaluated in the present study for synchronizing the time of estrus. Results from other studies indicate that the use of the PG treatment regimen leads to a lesser fertility as a result of FTAI compared to the conventional P4-eCG treatment regimen (Olivera-Muzante et al., 2011; Viñoles et al., 2011). In this regard, Mahmoud and Senosy (2019) reported that progestin treatment improved the synchronization in time of ovulation among ewes when there was a comparison with results when there was FTAI after imposing the PG treatment regimen when the injections of PG were given at a 10- day interval. Acritopoulou-Fourcroy et al. (1982), however, reported that there was similar fertility when there was imposing of a 12-day interval PG and P4-eCG treatment regimens for conducting FTAI. Similarly, Fierro and Olivera-Muzante (2017) reported that there were no differences in the reproductive outcomes when there was imposing of long-interval (15 or 16 d) compared with P4-eCG treatment regimen when there was cervical FTAI. Differences in the results from these various studies may be attributed to the differences in experimental designs because in many of the studies the initial PG injection of the PG treatment regimen occurred during the luteal phase, therefore, with these evaluations there were not ewes in random stages of the estrous cycles at the time of the initial PG administration. Furthermore, there were different time intervals between PG injections and different procedures used for insemination in these various studies which likely resulted in the variation in ewe responses among these various studies (Fierro and Olivera-Muzante, 2017).

In the present study, the relatively greater reproductive efficiency when there was imposing of the MAP+eCG treatment regimen is consistent with the long-interval progestin treatment regimens being effective as a consequence of pregnancy rates being greater than 75% with these results being consistent with those from previous studies (Allaoui et al., 2014; Fierro and Olivera-Muzante, 2017; Martinez-Ros et al., 2019). Even though there are inconsistencies in results among studies regarding the effects of the long-interval progesterone treatments on oocyte and embryo quality (Viñoles et al., 2001; Evans et al., 2001; Nakafeero, 2018), results from the present study indicate that fertility outcomes are similar when there is use of the long-interval PG combined with eCG treatment regimen compared with that when there was imposing of the MAP-eCG treatment regimen. The long-interval progestin-treatment regimen, however, has been associated with negative effects related to the development of vaginitis and retention of sponges that may affect animal welfare, due to proliferation of bacteria (Cortés-López et al., 2013). Progestin sponges also have the potential for environmental contamination due to residual P4 in used devices and the addition of antibiotics to prevent vaginitis (Fierro et al., 2013).

In summary, when there are random stages of estrous cycle at the time of the initial PG administration when imposing a long-interval PG treatment regimen, there are acceptable pregnancy percentages as a result of FTAI, however, these are less than those that occur when there is imposing of the MAP+eCG treatment regimen for conducting FTAI. Even though there are greater reproductive rates with use of the MAP+eCG treatment regimen, it is important to consider that with use of the long-interval PG treatment regimen there is lesser environmental impact, a greater sanitary methodology that is associated with a greater animal welfare conditions and there is no need to dispose of milk or meat that would normally be used for human consumption because of the required withdrawal period before milk or meat from MAP-treated animals can be used in human food sources (Fierro et al., 2013). A major advantage of the PG-based treatment regimens is the ease of implementation making it a suitable technique for extensive sheep production systems. Furthermore, the 14-day PG treatment regimen combined with eCG is an important alternative to the use of the conventional progestin sponge combined with eCG administration because there is similar in fecundity to that when there is use of the MAP-eCG treatment regimen.

When considering the age of the ewes and ewe lambs in the present study, there were no differences in pregnancy, fertility and prolificacy rates as a result of age differences. It should be taken into account that the values for these fertility variables are affected to a great extent by the nutritional status and body size of females, therefore, the findings in these regards in the present study are likely due to the good body condition and size of the ewe lambs at the time the study was conducted. Based on results from the present study, the long-interval PG based treatment regimens can also be recommended for use in ewe lambs, providing that these lambs are in good body condition before imposing the treatment regimens and conducting FTAI.

5. Conclusions

The 14-day interval PG treatment regimen combined with an eCG treatment is an alternative to the use of the conventional progestin sponge-eCG treatment regimen. The rationale for this is that there is a greater fecundity rate with use of the long-interval PG treatment regimen combined with eCG administration than there is when there is use of the long-interval PG treatment regimen without administration of eCG. Furthermore, the similarity in values for fertility variables with the use of the long-interval PG-eCG as compared with the

MAP-eCG treatment regimen further supports the rationale for the PG+eCG treatment regimen as an efficacious option to MAP-eCG treatment regimen.

Author Contribution Statement

We would like you to consider our article for publication in Animal Reproduction Science. This article provides original information about the alternative of adding eCG to a long interval PG treatment in order to achieve worthy fecundity rates in Merino sheep without the generation of environmental residues.

We hope that our manuscript will accomplish the criteria and the standard of the journal. The authors are aware of the contents manuscript and agree to be part of it.

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The authors are aware of the contents manuscript and agree to be part of it.

Involvement of the authors

Marcela Cueto: participated in the experimental design, analyzed data and drafted paper.

Macarena Bruno-Galarraga: participated in the experimental design, on the field trials and drafted the paper.

Jimena Fernandez: participated on the field trials, analyzed data and drafted the paper.

Sergio Fierro: participated in drafted the paper.

Alejandro Gibbons: Senior research. Participated in the experimental design, on the field trials and drafted the paper.

Competing interest statement

The authors declare that there is no conflict of interest that could affect the integrity of the currently reported results.

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Table 1

Number (*n*) of females oestrus synchronized, inseminated and pregnant, pregnancy rate (%), number (*n*) of fetuses, prolificacy (%) and fecundity (%) when there was imposing of different estrous synchronization treatment regimens (PG, 14-days PG regimen; PG+eCG, 14-day-interval PG regimen plus 200 IU eCG; MAP+eCG, 14-days progestin plus 200 IU eCG) and in different age of the females (Ewes, Ewe lambs), after cervical FTAI with fresh semen.

	Estrous synchronization treatment			Age of female	
	PG	PG+eCG	MAP+eCG	Ewes	Ewe lambs
Synchronized ewes (*n*)	111	112	169	210	182
Inseminated ewes (*n*)	100	104	165	202	167
Pregnant ewes (*n*)	52	65	126	103	110
Pregnancy (%)	52.0[A]	62.5[A]	76.4[B]	65.8[a]	65.9[a]
Fetuses (*n*)	52	74	130	144	112
Prolificacy[1] (%)	100[A]	114[B]	103[A]	108[a]	102[a]
Fecundity[2] (%)	52.0[A]	71.2[B]	78.8[B]	71.3[a]	67.1[a]

[a, b]Different letters indicate differences between the age of the females within groups ($P<0.05$)

[A, B]Different letters indicate differences among synchronization treatment regimens ($P<0.05$)

[1]Number of fetuses/Number of pregnant ewes x 100

[2]Number of fetuses/Number of inseminated ewes x 100