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Long interval prostaglandin-based treatment regimens do not affect ovulatory or prolificacy rates of multiparous ewes after cervical fixed timed AI

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ABSTRACT

To evaluate effects of a longer, than conventional, interval between time of prostaglandin $F_{2\alpha}$ (PG)-based administrations in a PG-based treatment regimen for fixed timed AI (FTAI) on ovulation rate (OR), non-estrous return rate on Day 21 subsequent to the time of AI (NRR21), as well as conception, prolificacy and fecundity rates, ewes were assigned to two groups. Ewes of treatment group (PG15) were estrous-synchronized using two PG doses 15 days apart and FTAI was conducted at 56 h after the second PG administration (Day 0). Ewes of the Control group (SE) had imposed a pre-estrous synchrony treatment regimen with two PG doses 7 days apart and AI was conducted after detection of spontaneous estrus from 17 to 19 days after the second PG administration (Day 0). Ovulation rate on Day 8, NRR21, conception, prolificacy and fecundity rates on Day 60 were evaluated. There were no differences ($P > 0.05$) between ewes of the PG15 and SE groups in OR (1.47 ± 0.50 and 1.54 ± 0.50, respectively) or prolificacy (1.42 ± 0.80 and 1.33 ± 0.62, respectively), however, there were lesser values ($P < 0.05$) in the PG15 than SE group for NRR21 (65.2% and 91.3%, respectively), conception (59.8% and 91.3%, respectively) and fecundity (84.8% and 120%, respectively). The longer interval with the PG-based treatment regimen does not affect OR and prolificacy, but there is a lesser NRR21, conception and fecundity rate in comparison to ewes of the Control group.

1. Introduction

Fixed timed artificial insemination (FTAI) in sheep represents a practical technique in commercial flocks that shorten the AI breeding period, because there is no estrous detection and there is greater use of genetically superior rams, access to nutritional resources and more effective use of labor resources (Menchaca and Rubianes, 2004), however, it involves hormonal treatments to ensure synchronization of timing of ovulation among ewes and acceptable pregnancy rates (Abecia et al., 2012). The application of these biotechnologies in commercial farming enterprises requires ease in implementation of procedures, acceptable reproductive outcomes and a lack of effects on the environment as a result of imposing treatments (Martin and Ferasyi, 2016). Because of the rapid rate of metabolization (Piper et al., 1970; Davis et al., 1980), ease of application and relatively lesser cost, the use of prostaglandin

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F2α represents a more desirable option than treatment regimens including intravaginal devices impregnated with progesterone or progestogens plus an i.m. dose of equine chorionic gonadotrophin at time of device withdrawal (P4-eCG) for the reproductive management of sheep (Martemucci and D'Alessandro, 2011; Viñoles et al., 2011; Abecia et al., 2012; González-Bulnes et al., 2020).

Prostaglandin F2α and its synthetic analogues (PG) are potent luteolytic agents in ruminants when there is FTAI (Fierro et al., 2013), however, lesser than desirable reproductive outcomes have occurred when there was use of short-interval (7–8 days) or conventional PG-based regimens (9–12 days apart between doses) are used, have resulted in a lack of use of these treatment regimens (Gordon, 1983; Menchaca and Rubianes, 2004; Olivera-Muzante et al., 2011a). Extending the interval between PG administration times for as long as 14–16 days between administrations ("long interval" treatment regimens), increase the time that pre-ovulatory follicles develop when there are luteal-phase progesterone concentrations, resulting in more desirable reproductive outcomes after FTAI (Fierro et al., 2016, 2017). Furthermore, the use of these treatment regimens with longer, than conventional, periods between PG administrations resulted in non-estrous return rates by Day 21 following AI (NRR21) and conception rates comparable to what occurs with use of P4-eCG based treatment regimens (Fierro and Olivera-Muzante, 2017). The ovulation rate (OR), prolificacy and conception rate, however, when there is use of PG FTAI-based treatment regimens may be less than those when there is AI of ewes after detections of a spontaneous estrus, an important factor to be considered in flocks where there is commercial lamb production or in those focused on genetic improvement.

Results from studies in which there were evaluations of OR, prolificacy and conception rate after the use of PG are inconsistent. There was no detrimental effect on OR when PG was administered during the mid-luteal phase of the estrous cycle (Bindon et al., 1979; Houghton et al., 1995), however, Fierro et al. (2011) reported there was a decrease in OR, prolificacy and conception rate when PG was administered during the early luteal phase of the estrous cycle when treatments were imposed on multiparous Corriedale ewes. The OR in multiparous Corriedale ewes tended to be greater when there was a relatively longer, as compared with a shorter or conventional interval between PG administrations in the estrous synchrony treatment regimen (Fierro et al., 2017). When there was a focused feeding regimen imposed on multiparous Merino ewes using a relatively longer, than conventional, interval between PG administrations or when there was AI after a detection of a spontaneous estrus there was a lesser OR, NRR21 and conception rate, but no difference in prolificacy in pregnant ewes of control and treated groups (Errandonea et al., 2018). The result from this previous study leads to the question of whether the problem related to fertility when there is imposing of the relatively longer, that conventional, interval between PG administrations is the lesser variability in OR and prolificacy of the breed of sheep or when there is a PG-based treatment regimen used in the comparison to what occurs when ewes are AI after detection of a spontaneous behavioral estrus.

The aim of the present experiment, therefore, was to evaluate the effect of a relatively longer, than conventional, interval between PG administrations in a PG-based treatment regimen when there was cervical semen placement with FTAI on OR, NRR21, conception, prolificacy and fecundity rate in multiparous Corriedale ewes. It was hypothesized that OR and prolificacy of ewes would not be affected, however, NRR21, conception and final fecundity rates would be less with use of this treatment regimen.

2. Materials and methods

The experiment was conducted during the breeding season (April–June) at the School of Agriculture "La Carolina", Universidad del Trabajo del Uruguay (Ismael Cortina, Flores. Uruguay; 33 °S–57 °W). The experimental procedures were approved by the Animal Ethics Committee of the Faculty of Veterinary-Universidad de la República (CUEA-FVet).

2.1. Animals

Multiparous Corriedale ewes (2.5–7.5 years of age; 24% with multiple births in previous spring; n = 295), with recommended health management, body condition scores (3.5 ± 0.3, mean ± SD; score 1–5, Russel et al. (1969); BCS), and body weight (57.1 ± 6.6 kg, fasted overnight; Q&F AD-4406 weighing scale, Japan; BW) at the beginning of the experiment were used to conduct the present study (Day -25; AI = Day 0).

2.2. Experimental design

Based on initial BCS, age, BW and reproductive performance the previous spring (one or more lambs produced), ewes were randomly assigned on Day -25 to one of two experimental groups. The treatment group (PG15) consisted of ewes that were estrous-synchronized with PG and conducting of an FTAI (three sub groups: PG15a, PG15b and PG15c respectively; n = 132, mean ± SEM; 3.5 ± 0.03 points, 4.7 ± 0.11 years of age, 56.9 ± 0.60 kg, 1.24 ± 0.04 lambs born/ewe lambing). The Control group (SE) consisted of ewes that were pre-estrous synchronized and AI was conducted based on time of detection of spontaneous estrus (three sub groups: SEa, SEb and SEc respectively; n = 115, from 163 originally pre-estrous synchronized ewes; 3.5 ± 0.04 points, 4.7 ± 0.11 years of age, 57.2 ± 0.63 kg, and 1.23 ± 0.04 lambs born/ewe lambing, BCS, age, BW and reproductive performance respectively). A schematic representation of the experimental design is depicted in Fig. 1.

2.3. Estrous synchronization treatment regimens

The timing of estrus among ewes was synchronized in the PG15 group using two PG administration 15 days apart (Delprostenate; 160 μg/dose, Glandinex®, Universal Lab, Uruguay) and FTAI occurred at 56 h after second PG administration. To perform FTAI on 3

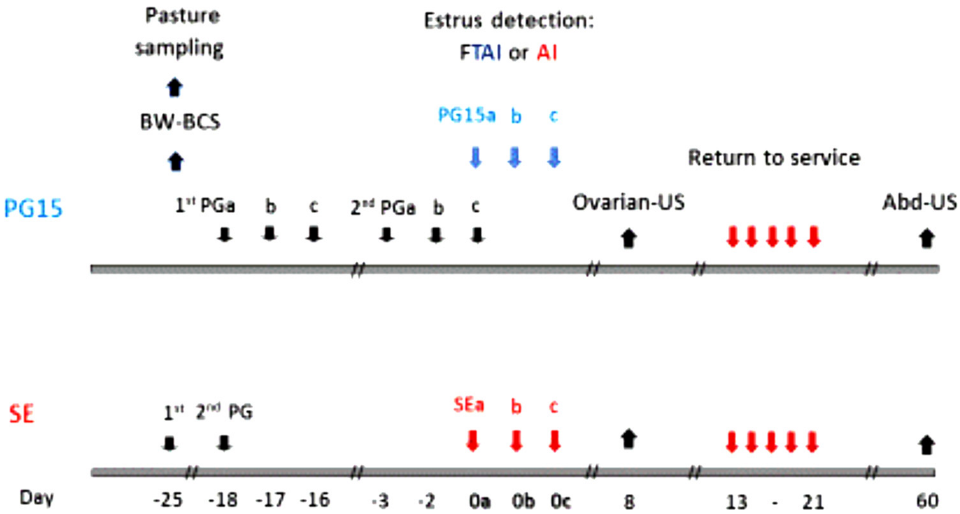

Fig. 1. Experimental design. PG15a, b, c: ewes estrous synchronized with two prostaglandin (PG) administrations 15 days apart and cervical fixed timed insemination (FTAI) with fresh semen three consecutive days (Days 0a, 0b and 0c respectively; Treatment group). SEa, b, c: ewes that were pre-estrous synchronized with spontaneous estrous detection and cervical artificial insemination (AI) with fresh semen on the same three consecutive days (Days 0a, 0b and 0c respectively; Control group); Pasture sampling: to evaluate quality and forage mass. BW: body weight; evaluated using an electronic scale with ewes fasted overnight; BCS: body condition score; Estrous detection: assessed using chest painted marker rams with the ductus deferens section to render the rams infertile; Ovarian-US: ovarian trans-rectal ultrasonography to evaluate ovulation rate; Return to estrus: assessed using chest painted marker rams; Abd-US: trans-abdominal ultrasonography to evaluate conception, prolificacy and fecundity.

different days, the first and the second PG administration were conducted on 3 consecutive days, therefore, there were three subgroups of 40, 46 and 46 ewes/day/group: PG15a, P15Gb and PG15c respectively (Fig. 1). Ewes of the Control group had imposed a pre-estrous synchrony treatment regimens which consisted of two administrations of PG 7 days apart on Day -25 and -18, respectively, so that there was a large number of ewes in the Control group to be bred in a short period of time based on the time behavioral estrus was detected. Estrous behavior was detected in both groups twice a day (from 8:00 to 17:00 and from 18:00 to 07:00 h; AM and PM, respectively) for 6 days in each PG15 sub-group (from -48 to 72 h after the second PG administration = 0 h; Days 5 to 2 respectively), and for 3 days (17–19 days after the second PG administration; Days 0a, 0b and 0c, respectively) in the ewes of the Control group, utilizing chest-painted marker rams in which the ductus deferens had been sectioned to render the rams infertile at a rate of three rams/100 ewes. On each day, the ewes (cumulative number detected in estrus at the AM and PM periods) of the Control group were AI each morning while FTAI was conducted on the ewes of the PG15 sub-groups. Visual-physical state of vaginal mucus and the time of the AI relative to the time of estrous detection was evaluated for each ewe at the time of AI (Classifications were Grade 1–6 based on vaginal mucus characteristics and timing of AI relative to time of estrous detection = clear and scarce – beginning of estrus, clear and copious, cloudy and copious, thick, creamy or caseous – end of or post estrus – respectively; Restall, 1961).

2.4. Nutritional management and composition of the pasture

Forage mass of the experimental paddock was sampled at Day -25 as described by Haydock and Shaw (1975). Pasture content was analyzed for percentage of dry matter (DM), crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) at the Laboratory of Nutrition of INIA "La Estanzuela". Dry matter and CP (N*6.25) percentage were determined using the Official Methods 934.01 and 955.04 of the AOAC System (AOAC, 1990), respectively. The ADF and NDF were determined using the protocols described by Van Soest et al. (1991). The metabolic energy (ME) was estimated using the NRC recommendations (2007). Ewes grazed natural pastures in a paddock of 12.5 ha with a forage allowance of 8 kg of DM/100 kg of BW (initial availability of 2100 kg/ha of DM; CP: 7.4%, ADF: 42.4%, NDF: 70.5%, ME: 2.0 Mcal/kg of DM), and fresh water were available *ad libitum*.

2.5. Semen collection, evaluation and dilution

On all days, that AI was conducted, semen was collected from five sexually mature Corriedale rams (assessed for normal breeding soundness), using an artificial vagina and assessments of semen quality was evaluated as described by Evans and Maxwell (1987). Two consecutive ejaculates from each ram were collected, evaluated (approved for use if there was > 80% sperm subjective progressive motility) and pooled according to the individual sperm concentration, so that each ram contributed similar numbers of spermatozoa to the semen pool. Soon after pooling, semen was extended with UHT skim milk to the final sperm concentration (mean of 1500×10^6 spermatozoa/mL). Extended semen was maintained at room temperature and protected from sunlight until the AI were conducted. On each day of AI, the same semen pool was used for PG15 and SE ewes.

2.6. AI procedures

Cervical AI insemination was performed by two technicians randomly assigned, using a speculum equipped with a light source and an insemination instrument (Walmur® Veterinary Instruments, Montevideo, Uruguay), as described by Evans and Maxwell (1987). The insemination dose was 0.10 mL, containing a mean of 150×10^6 spermatozoa that were slowly released after placement of the insemination pipette as far as possible into the cervix before there was a significant resistance to further insertion incurred. Ewes of the PG15 group were inseminated at a fixed time (56 ± 1.5 h after the second PG administration, Fierro et al., 2016), at the same time that there was AI of the ewes of the Control group on each day that AI were conducted.

2.7. Estrous and ovarian response, estrous-non-return and fertility evaluations

Estrous response (number of ewes in estrus/number of ewes on which estrous synchrony was imposed × 100) and variation in the time of estrous onset in the PG15 group was determined. Ovulation rate (number of corpus luteum/number of ewes having ovulations) was evaluated on Day 8 using trans-rectal ultrasonography (7.5 MHz linear array, ALOKA SSD-500, Overseas Monitor Corp. Ltd., Tokyo, Japan) as described by Viñoles et al. (2010). Estrous non-return rate on Day 21 following AI (number of ewes not expressing behavioral estrus subsequent to AI /number of ewes inseminated × 100; NRR21) was assessed from Day 13–21 using chest painted markers placed on Corriedale rams at a rate of three rams/100 ewes. Conception (number of pregnant ewes/number of inseminated ewes × 100), prolificacy (number of fetuses/number of pregnant ewes), and fecundity (number of fetuses/number of ewes inseminated x 100) were evaluated on Day 60 using trans-abdominal ultrasonography utilizing a 3.5 MHz convex array transducer and the same ultrasonic device that was previously described in this section of this manuscript.

2.8. Statistical analyses

Data were analyzed using an analysis of variance for categorical variables utilizing the CATMOD procedure of SAS Institute Inc. (2000), considering a categorical response (0 and 1 for NRR21 and conception rate, or 0, 1 -single- and 2 -multiple- for OR and prolificacy). The statistical model included the effect of "Day of AI", the type of estrus (PG induced or spontaneous), and the interactions. Data for OR and prolificacy are presented as means ± SD, while NRR21, conception and fecundity data are presented as percentages. There were considered mean differences when there was a $P < 0.05$.

3. Results

There was no observed "Day of AI" effect on the reproductive outcomes for each sub-group of ewes ($P > 0.05$); therefore, the data from the different days of AI were pooled for each group. There were no differences ($P > 0.05$) in OR and prolificacy ($P > 0.05$), but NRR21, conception and fecundity rate were less in ewes of the PG15 compared with Control group ($P < 0.05$; Table 1).

A total of 91.6% (range 85.1–94.6) of PG15 ewes expressed behavioral estrus after the second PG administration (0.0%, 2.3%, 6.1%, 9.8%, 64.4% and 17.4% at −48, −24, 0, 24, 48 and 72 h relative to the time of the second PG administration, respectively). There were 20.5% of the ewes in the PG15 group at the time of FTAI with vaginal mucus characterized as being of Grade 5 or 6 (creamy or caseous), of which 60% expressed behavioral estrus prior to 24 h relative to the time of the second PG administration. Conception rates in ewes of the PG15 group with a vaginal mucus Grade of 5 and 6 was less than that of ewes with a Grade 1–4 vaginal mucus score (33.3% and 79.5%, respectively; $P < 0.05$). The ovulation (2.00, 1.51, 1.31, 1.48, 1.44) and prolificacy (1.00, 1.00, 1.43, 1.40, 1.53) rates were not different ($P > 0.05$) in ewes of the PG15 group that expressed behavioral estrus at −24, 0, 24, 48 or 72 h relative to the time of the second PG administration, respectively. Conception (33.3%, 12.5%, 53.8%, 64.7%, 65.2%) and fecundity 33.3%, 12.5%, 76.9%, 90.6%, 100%) rates as a result of FTAI (tended to greater ($0.05 > P < 0.1$) in ewes of the PG15

Table 1
Reproductive outcomes of multiparous Corriedale ewes after imposing a prostaglandin $F_{2\alpha}$-based treatment regimen or a Control regimen with spontaneous estrus followed by AI with fresh pooled semen.

Group (*n*)	OR	NRR21 (%)	Conception (%)	Prolificacy	Fecundity (%)
PG15 (132)	1.47 ± 0.50 [a]	65.2 [b]	59.8 [b]	1.42 ± 0.80 [a]	84.8 [b]
Control (115)	1.54 ± 0.50 [a]	91.3 [a]	91.3 [a]	1.31 ± 0.58 [a]	120 [a]

PG15: ewes estrous synchronized with two prostaglandin (PG) administrations 15 days apart and cervical FTAI with fresh semen on 3 consecutive days; Control: ewes in pre-synchronized group with ewes being AI based on time of detection of a spontaneous estrus and with AI occurring with cervical deposition of fresh semen on the same three consecutive days; OR: ovulation rate measurement by trans-rectal ultrasonography on Day 8 (number of corpus luteum/number of ewes with ovulations); NRR21: non-estrous return rate after FTAI between Day 13 and 21 assessed using chest painted rams (number of ewes not returning to estrus/number of ewes inseminated × 100); Conception (number of pregnant ewes/number of ewes inseminated × 100); prolificacy (number of fetuses/number of pregnant ewes); fecundity (number of fetuses/number of ewes inseminated × 100) evaluated on Day 60 following AI using trans-abdominal ultrasonography; Cervical AI = Day 0; Data for OR and prolificacy are presented as means ± SD; NRR21, conception and fecundity are presented as percentages; [a, b] Mean values in the same column with different superscripts are considered to be different, $P < 0.05$.

group that expressed behavioral estrus at −24, 0, 24, 48 or 72 h relative to the time of the second PG administration, respectively.

4. Discussion

The hypothesis that, after a relatively longer interval, than that conventionally used, between times of PG administration when imposing a PG-based treatment regimen for cervical FTAI, the OR and prolificacy would not be affected, however, NRR21, conception and final fecundity rates of ewes would be less, was accepted based on the results from the present study. Firstly, the results of this experiment indicate that OR and prolificacy rate as a result of FTAI is not altered when there are longer, than conventionally imposed intervals, between times of PG administrations when imposing a PG-based treatment regimen as compared with the values for these variables in ewes of the Control group that were AI subsequent to detection of spontaneous estrus. This finding leads to the question of whether the problems that arise with use of this PG-based protocol is the duration of interval between PG injections, rather than the use of PG in the treatment regimen. In this regard, Fierro et al. (2016) when conducting a study with the same breed as those used in the present study, evaluated the most efficacious durations in periods between PG administrations when considering similar variables as those evaluated in the present study. Findings in this previous study indicated that the longer the interval between PG administrations, the longer there were adequate blood progesterone concentrations to modulate gonadotropin secretions. There, therefore, was a longer growth phase for follicular development resulting in greater estradiol concentrations at the time of estrus. This subsequently resulted in greater reproductive outcomes when ewes are AI after imposing an estrous synchrony treatment regimen with longer, as compared with when there were shorter, intervals between the time of PG administrations when imposing a PG-based estrous synchrony regimen in ewes. With the relatively longer interval between PG administrations for estrous synchrony of ewes, there may be development of a “less dominant” preovulatory follicle which has a lesser steroidogenic capacity, therefore, there is maintenance of gonadotropin concentrations above the threshold to stimulate selection of multiple follicles from which ovulations occur during the ovarian wave of follicular dynamic changes (Letelier et al., 2011), consequently, not altering the natural OR prolificacy when AI occurs as a consequence of this induced estrus.

The reproductive outcomes after imposing a relatively longer, than conventional, interval between PG administrations in a PG-based estrous synchrony treatment regimen is not consistent among ewes of different breeds. Errandonea et al. (2018) reported that there was a similar final prolificacy, however, a lesser OR when there was imposing of the PG15 treatment regimen using in the present study before FTAI of multiparous Merino ewes compared with the prolificacy rate of ewes in a Control group that were AI after detection of a spontaneous estrus. Perhaps the lesser OR and prolificacy of this breed as compared with that of many other sheep breeds, or the relatively lesser average BW of ewes of this breed which is associated with the OR of ewes (Smith et al., 1979) affected the OR response when there was the longer, than conventional, interval between PG administrations when imposing an estrous synchrony treatment regimen in Merino ewes. In this regard, the efficacy of the estrous synchrony regimen imposed on ewes in the present study could be evaluated by conducting studies with breeds that have a greater body size and BW, variability of OR and prolificacy such as the Corriedale that was used in the present study (McGuirk and Scarlett, 1966; Iwan et al., 1971; Fernández Abella, 2008; Fierro et al., 2011). Furthermore, it is possible that at the time of the second PG administration in the present study when there was imposing of the longer, than conventional, intervals between time of PG administrations there were some ewes in which natural luteolysis was occurring or that had already occurred resulting in these ewes being in the follicular, rather than luteal, phase of the estrous cycle at the time of the second PG administration. There were likely ewes in the follicular phase of the estrous cycle, based on time of expression of behavioral estrus in many ewes relative to time of the second PG administration and this could lead to different OR and prolificacy results as compared with all ewes being in the luteal phase of the estrous cycle at the time of the second PG administration. This occurrence of ewes being in the follicular phase at the time of the second PG administration should not be a problem when imposing this estrous synchrony treatment regimen and should be an advantage when the regimen is imposed for conducting FTAI.

Secondly, results from the present study indicate that NRR21, conception and final fecundity rate were markedly less when there was FTAI subsequent to imposing the longer, than conventional, interval between time of PG administrations as compared with when there was AI of ewes in the Control group after detection of spontaneous estrus. These results from the present study are consistent with those from previous studies with Corriedale or Merino ewes when there were relatively shorter (Fierro et al., 2011) or longer (Errandonea et al., 2018) intervals between PG administrations when conducting FTAI. Olivera-Muzante et al. (2011b), however, reported that with Corriedale and crossbred Corriedale ewes there was not a lesser conception rate when there was imposing of a relatively shorter interval for PG administrations in an estrous synchrony treatment regimen, however, in these studies ewes were inseminated at 12 h after estrous detection as compared with the FTAI procedure that was conducted in the other studies. In the present study, although there was an acceptable estrous response after the second PG administration, conception and fecundity rates were greater in ewes where the timing of the FTAI was more closely aligned with the time of onset of estrus (48–72 h; 82% of total), and with mucus Grade 1–4. When there is the premise that FTAI will continue to be an important technique for the breeding of ewes, there needs to be a focus in future research when using FTAI on evaluation of sperm transport in the reproductive tract (Hawk et al., 1981); achieving a greater behavioral response and concentration of ewes in estrus after the second PG administration; altering the insemination time so that an optimal timing of FTAI can be ascertained; and/or performing cervical FTAI two times to enhance reproductive outcomes. There should, therefore, be a continued focus on investigating how to increase the NRR21, conception and fecundity rates of ewes when imposing the relatively longer, than conventional, interval between PG administrations when utilizing this estrous synchrony treatment regimen for FTAI of ewes.

5. Conclusion

A relatively longer, than conventional, interval between times of PG administration when imposing the PG based estrous synchrony treatment regimen does not affect OR and prolificacy, however, there were lesser NRR21 rates, conception rates and fecundity when imposing this regimen for cervical FTAI with fresh semen in comparison to what occurs in ewes of the Control group that were FTAI based on timing of detection of spontaneous estrus.

Autor statement

We would like you to consider our article for publication in Animal Reproduction Science. This article provides original information about the effect of a long interval prostaglandin F2α-based protocol for cervical fixed timed AI on ovulation rate, non-return rate to service, conception, prolificacy, and fecundity in comparison to detected spontaneous estrus ewes

We hope that our revised manuscript will accomplish the criteria and the standard of your journal. The authors: Sergio Fierro, Mauro Minteguiaga and Julio Olivera are aware of the content's manuscript, and agree to be part of it.

Involvement of the authors:

1 Sergio Fierro (DMV-MSc-PhD): participated in the experimental design, on the field trials, and drafted the paper.
2 Mauro Minteguiaga (MSc): participated on the field trials, analyzed data, and drafted the paper.
3 Julio Olivera-Muzante (DMV-PhD): Senior research. Participated in the experimental design, on the field trials, analyzed data, and drafted the paper.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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