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Reproductive outcome with GnRH inclusion at 24 or 36 h following a prostaglandin F2α-based protocol for timed AI in ewes

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ABSTRACT

The objective of this experiment was to study the reproductive performance obtained after a short-interval prostaglandin (PG) F2α-based protocol for timed artificial insemination (TAI) in sheep (Synchrovine®: two injections of PG 7 d apart), including a GnRH analogue at 24 or 36 h after the second PG injection. The experiment involved 296 Corriedale ewes (206 multiparous and 90 nulliparous) grazing natural pastures during the breeding season (March-April; UTU "La Carolina", Flores Uruguay, 33° S-57° W). Ewes were assigned to three treatment groups: a) Synchrovine® (Control, $n = 101$): two injections of D-Cloprostenol 75 μg, 7 d apart, b) Synchrovine® + GnRH24 ($n = 98$): Synchrovine® plus GnRH (busereline acetate 8.4 μg) 24 h after the second PG injection, and c) Synchrovine® + GnRH36 ($n = 97$): Synchrovine® plus GnRH 36 h after the second PG injection. All ewes were subjected to cervical TAI (Day 0), 44 to 47 h after second PG injection, with fresh extended semen pool from six rams. Reproductive performance of ewes having ovulations and ovulation rate on Day 10, estrous cycle length in ewes that returned to estrus and non-return rate to estrus up to Day 22, fertility, prolificacy and fecundity on Day 70 were analyzed. Ewes having ovulations, ovulation rate, estrous cycle length and prolificacy did not differ between groups ($P > 0.05$). However, non-return to estrus, fertility and fecundity was decreased in Synchrovine® + GnRH24 ($P < 0.05$) and similar between Synchrovine® and Synchrovine® + GnRH36 ($P > 0.05$). It was concluded that the reproductive performance obtained by Synchovine® TAI protocol was impaired by GnRH at 24 h and not improved by GnRH administered at 36 h after the second PG injection.

1. Introduction

Timed artificial insemination (TAI) is an important tool when estrus detection is not feasible. Currently, widespread application of these biotechnologies under commercial field conditions requires easy implementation procedures and acceptable pregnancy rates (Menchaca and Rubianes, 2004). Prostaglandin F2α or its analogues (PG) are potent luteolytic agents in ruminants (McCracken et al., 1970). A short-interval PG-based protocol that included two injections of PG given 7 d apart (Synchrovine® MIEM – Cámara Nacional de Registros, Montevideo Uruguay) was developed for TAI in ewes (Menchaca and Rubianes, 2004; Rubianes et al., 2004). Although this protocol induced synchrony of estrus and ovulation (Rubianes et al., 2003; Menchaca et al., 2004), it yielded lesser fertility after cervical or intrauterine TAI compared to the conventional P4-eCG protocol (Olivera-Muzante et al., 2011a; Viñoles

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et al., 2011) or spontaneous estrus (Fierro et al., 2011). Alternatives to improve this protocol such as reduction in the dose of PG administered, an increase in the interval between PG injections (7 compared with 8 d), or insemination of ewes that showed estrous behaviour after the treatment have not been successful (Olivera-Muzante et al., 2011b), evidencing the needs for more research.

The male effect has been used after PG-based protocols inducing an earlier onset of estrus, preovulatory LH surge, and ovulation (Contreras-Solis et al., 2009; Ungerfeld, 2011), thus improving fertility compared to a P4-based protocol (Contreras-Solis et al., 2009). These observations support the hypothesis of an alteration in patterns of LH release and ovulation as one of the sources of reproductive failure when time of estrus in ewes is synchronized with PG (Barrett et al., 2002). A protocol including a GnRH treatment 36 h after PG, however, resulted in the induction of an LH surge, ovulation within 48 h, and a fully functional corpus luteum (Rubianes et al., 1997). Therefore, administration of GnRH around mating would be a practical option to improve the endogenous pre-ovulatory LH surge and ovulatory synchrony (Walker et al., 1989; Eppleston et al., 1991; Reyna et al., 2007), fecundity (Fernandez Abella et al., 2004), or prolificacy (Türk et al., 2008; Martemucci and D'Alessandro, 2011), as demonstrated in ewes using P4-based protocols. However, when a single GnRH dose was administered at the time of TAI (*e.g.* 42 h after the second PG injection), prolificacy tended to be greater, but it did not improve the final reproductive outcome resulting from use of the Synchrovine® protocol (Olivera-Muzante et al., 2011b). It was theorized that the GnRH was administered too late to promote an adequate endogenous LH surge and a synchronized time of ovulation.

In the present study, the hypothesis was tested that the reproductive outcome of the Synchrovine® protocol might be improved by the inclusion of a GnRH injection before TAI, improving the number of ovulatory follicles per ewe, the timing of ovulatory synchrony and, therefore, the fecundity of the flock. The objective of the present study was to compare the effect of two times of GnRH administration, 24 or 36 h after a second PG injection on the reproductive outcome.

2. Materials and methods

All procedures were approved by the Universidad de la República's Animal Ethics Committee (CUEA-Universidad de la República).

2.1. Location and animal care

The experiment was conducted at "Escuela Agraria La Carolina" (33° S-57' W, Flores, Uruguay) during the breeding season (March to April, 2011); the animals were managed under field conditions (forage allowance of 1.64 kg of dry matter/kg live weight; 8.3% CP, 7.9 MJ ME/Kg, and water *ad libitum*). Clinically healthy Corriedale ewes ($n = 296$; 206 multiparous over 2.5 y old, and 90 nulliparous 1.5 y old), in a moderate body condition (3.2 ± 0.3 and 3.4 ± 0.2; respectively; scale 0 to 5, Russel et al., 1969) and weight (55.7 ± 5.6 kg and 42.6 ± 4.6 kg; mean $\pm$ SD; respectively), were used in the study. Six healthy Corriedale rams (1.5 to 3.5 y old, approved after a breeding soundness examination) were used as semen donors.

2.2. Experimental design

Ewes were blocked on the basis of parity, body condition and weight, and randomly assigned to three experimental groups: a) Synchrovine® (Control group, $n = 101$): treated with two injections of D-Cloprostenol (75 μg im, Sinchron D®, Laboratorio Uruguay S.A) given 7 d apart, starting on Day −9 (Day 0: TAI); b) Synchrovine®+GnRH24 ($n = 98$): Synchrovine® plus GnRH (busereline acetate 8.4 μg im, Gonaxal® Laboratorio Biogénesis-Bagó, Argentina) 24 h after the second PG injection; c) Synchrovine®+GnRH36 ($n = 97$): Synchrovine® plus GnRH 36 h after the second PG injection. A schematic representation of the experimental design is shown in Fig. 1.

2.3. Semen collection and processing

Semen from six Corriedale rams was collected by artificial vagina and evaluated macro and microscopically (Evans and Maxwell, 1987). Two consecutive ejaculates from each ram were collected, pooled and extended at 35 °C with UHT skim milk (supplemented with 2.5% egg yolk, 100,000 IU sodium penicillin and 100 mg of dihydrostreptomycin/100 mL) to a final concentration of 1600×10^6 spermatozoa/mL; and maintained at room temperature (maximum for 3 h) before TAI.

2.4. Artificial insemination procedures

Cervical AI was performed with an insemination instrument and a vaginal speculum equipped with a light source (Walmur® Veterinary Instruments, Montevideo, Uruguay) by two technicians (Evans and Maxwell, 1987). Ewes were inseminated randomly between groups and technicians. The insemination dose (0.1 mL, 160×10^6 spermatozoa) was slowly released as deep as possible into the cervix, at a fixed time between 44 and 47 h after the second PG injection (Fig. 1).

2.5. Reproductive performance

The ewes having ovulations (number of ovulated/total ewes × 100) and ovulation rate (number of corpora lutea/ewes having ovulations) were evaluated on Day 10 in all ewes by trans-rectal ultrasonography using a 7.5 MHz linear array transducer designed for examination of the human prostate (ALOKA SSD-500, Overseas Monitor Corp. Ltd., Richmond, BC, Japan) as described by Viñoles et al. (2010). The estrous cycle length in ewes that returned to estrus (d), and the non-return rate to estrus up to Day 22 (ewes non returning to estrus/total ewes × 100) was observed from Day 13 to 22 using marker vasectomized rams (rate of 6 rams/100 ewes) once a day (18 h to 7 h next morning). Fertility (pregnant/total ewes × 100), prolificacy (foetus/pregnant ewes) and fecundity rate (foetus/total ewes × 100) were evaluated by ultrasonography using a

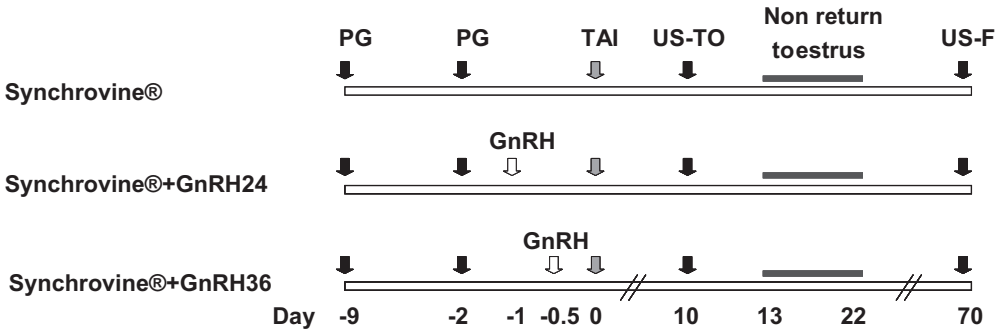

Fig. 1. Diagram of the experimental design. Synchrovine®: ewes synchronized with two injections of D-Cloprostenol (PG, 75 μg each), 7 d apart, starting on Day −9; Synchrovine® + GnRH24: Synchrovine® plus GnRH at 24 h after second PG injection (busereline acetate 8.4 μg im); Synchrovine® + GnRH36: Synchrovine® plus GnRH at 36 h after second PG injection; TAI (timed artificial insemination, Day 0): cervical TAI 44–47 h after second PG injection (fresh semen); US-OR: ovulation rate evaluated by transrectal ultrasonography (on Day 10); Non return to estrus: non return to estrus evaluated with vasectomized rams (Day 13 to Day 22); US-F: fertility evaluated by transabdominal ultrasonography (on Day 70).

trans-abdominal 3.5 MHz convex array transducer on Day 70 (Fig. 1).

2.6. Statistical analyses

Differences in number of ewes having ovulations, ovulation rate, estrous cycle length, non-return rate to estrus, fertility, prolificacy and fecundity among groups were analyzed by ANOVA for categorical and non categorical variables, respectively (SAS). Two ewes in the Synchrovine®+GnRH36 group had a greater than optimal ovulation rate (four and five ovulations respectively) and were eliminated from the statistical analyses. Results are presented as means, with $P < 0.05$ considered significantly different.

3. Results

Differences in numbers of ewes having ovulations, ovulation rate, estrous cycle length or prolificacy were not significant between groups ($P > 0.05$; Table 1). However, non-return to estrus, fertility and fecundity were less in Synchrovine®+GnRH24 treated ewes ($P < 0.05$), and not improved in the Synchrovine®+GnRH36 treated ewes in comparison with the Control group ($P > 0.05$).

4. Discussion

The hypothesis in the present study that the reproductive outcome with the Synchrovine® protocol may be improved with use of a GnRH analogue before TAI was rejected. Firstly, number of ewes having ovulations, ovulation rate and prolificacy were not improved with the inclusion of a GnRH analogue at 24 or 36 h after the second PG injection. These results are somewhat inconsistent with those reported by Olivera-Muzante et al. (2011b) where there was administration of a GnRH analogue at the time of TAI (42 h after second PG injection). Although fertility was decreased, prolificacy tended to increase compared to the Control group (Olivera-Muzante et al., 2011b). In other studies, there was a tendency for an increase in litter size as a result of the GnRH treatment at the time of AI in ewes where time of estrus was synchronized with a P4-eCG-PG (Türk et al., 2008), or 30 h after a P4-PG or P4-PG-eCG treatment (Martemucci and D'Alessandro, 2011). The increase in lambing rate was attributed to the effect of GnRH on ovulation rate (Nancarrow et al., 1984; Khan et al., 2003; Türk et al., 2008) or the synchronous ovulation achieved (Walker et al., 1989; Eppleston et al., 1991; Reyna et al., 2007). Whether treatment with GnRH increases ovulation rate or prolificacy is not clear, and is most probably dependent on the rate of follicle growth induced by the synchronization protocol used.

Secondly, considering the similar number of ewes having ovulations and ovulation rate between groups, but the lesser non-return rate to estrus, fertility and fecundity of the Synchrovine®+GnRH24 group may indicate GnRH administration at this time promoted luteal dysfunction or an asynchrony between ovulation and AI. A premature induction of ovulation from immature follicles, compromising the granulosa proliferative cell events and consequently the formation of the corpus luteum have been observed in ewes treated with GnRH 12 h after a PG injection (Murdoch and Van-Kirk, 1998). As a consequence of decreased progesterone production, the oviductal and uterine environments may not be adequate to sustain embryo development, explaining the decreased fertility observed in the Synchrovine®+GnRH24 group. Similar results were reported in estrous cyclic and anestrous beef cows induced to have ovulations from small or immature dominant follicles (Atkins et al., 2010a,b). If ovulation induced by the Synchrovine® protocol occurs about 60 h after the second PG (Rubianes et al., 2003; Contreras-Solís et al., 2009), and the physiological LH-surge occurs about 24 h before ovulation (Cumming et al., 1973) the endogenous LH surge would occur 36 h after the last PG injection. Considering that GnRH injections promote a LH surge 2 to 4 h after its administration (Eppleston et al., 1991; Rubianes et al., 1997), it would be possible that the GnRH administered 24 h after the second PG injection was given too early, thus advancing ovulation and consequently the TAI was done too late for optimal fertility. In addition, although the GnRH dose was given at about the time of the physiological LH-surge in the Synchrovine®+GnRH36 treatment group, reproductive outcomes were not improved. Despite that in some studies there was altered LH release patterns as one of the sources of reproductive failure in ewes in which time of estrus was synchronized with PG (Barrett et al., 2002),

Table 1
Reproductive outcome in ewes estrous synchronized with two injections of prostaglandin 7 d apart, with or without GnRH at 24 or 36 h after the second prostaglandin injection, and inseminated cervically with fresh semen.

	Synchrovine®	Synchrovine®+GnRH24	Synchrovine®+GnRH36
Ewes having ovulations (%)	100[a] (101/101)	97[a] (95/98)	99[a] (94/95)
Ovulation rate	1.22[a] (123/101)	1.15[a] (109/95)	1.22[a] (115/94)
Estrous cycle lenght (d)	16.6 ± 1.8[a]	17.3 ± 2.3[a]	17.0 ± 2.0[a]
Non-return rate to estrus (%)	42.6[a] (43/101)	15.3[b] (15/98)	34.7[a] (33/95)
Fertility (%)	42.6[a] (43/101)	10.2[b] (10/98)	33.7[a] (32/95)
Prolificacy	1.09[a] (47/43)	1.00[a] (10/10)	1.13[a] (36/32)
Fecundity (%)	46.5[a] (47/101)	10.2[b] (10/98)	37.9[a] (36/95)

Synchrovine® ($n = 101$): two PGF2α (PG) injections 7 d apart (75 μg D-Cloprostenol im) and TAI (timed artificial insemination, Day 0) 44 to 47 h after second PG; Synchrovine®+GnRH24 ($n = 98$): Synchrovine® plus GnRH (busereline acetate 8.4 μg im) 24 h after second PG injection; Synchrovine®+GnRH36 ($n = 95$): Synchrovine® plus GnRH 36 h after second PG injection; Ewes having ovulations: ewes having ovulations/total ewes × 100; Ovulation rate: number of corpora lutea/ewes having ovulations by trans-rectal ultrasonography at Day 10; Estrous cycle length: estrous cycle length in ewes that returned to estrus (Mean ± SE), and Non-return rate to estrus: non returning to estrus/total ewes × 100 detected with vasectomized rams from Day 13 to 22; Fertility (pregnant/total ewes × 100), Prolificacy (foetus/pregnant ewes), and Fecundity (foetus/total ewes × 100) evaluated by transabdominal ultrasonography at Day 70.

[a] Compared with.

[b] In the same row denote differences ($P < 0.05$).

previous results including GnRH at the time of TAI (Olivera-Muzante et al., 2011b) seems to reinforce that the LH surge may not be the cause of the poor reproductive outcome obtained when applying the Synchrovine® protocol.

Similar non-return rates to estrus and fertility observed among groups in the present study suggests that reproductive failures obtained with this PG-based protocol may occur prior or at maternal recognition of pregnancy. Results of the present study do not support the existence of serious disturbances in the ovulation process itself (similar number of ewes having ovulations and ovulation rate among treatments). Previously, similar fertilization rate, embryo quality or progesterone profiles during the induced estrus were reported after the application of PG-based protocols compared to progesterone-based protocols in ewes (González-Bulnes et al., 2005), or in ewes expressing a spontaneous estrus (Fierro et al., 2011). However, basal but increasing progesterone concentrations during the growth phase of the pre-ovulatory follicle that stimulated a faster growth and a larger follicular size, were associated with a lesser ovulation rate, prolificacy and fecundity in ewes that received the Synchrovine® TAI protocol (Fierro et al., 2011). In this sense, to extend the interval between PG injections (Fierro et al., 2013), thus promoting adequate progesterone plasma concentrations during the development of the pre-ovulatory wave, are alternatives that may improve the reproductive outcome of the PG based protocols for timed AI.

5. Conclusion

It is concluded from results in the present study that the reproductive performance obtained after applying the Synchovine® TAI protocol was decreased by the administration of GnRH 24 h after the second PG injection, and was not improved by the administration of GnRH at 36 h after the second PG injection.

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