Concentrations of steroid hormones, estrous, ovarian and reproductive responses in sheep estrous synchronized with different prostaglandin-based protocols

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ABSTRACT

To determine estrous, ovarian and reproductive responses after different prostaglandin (PG)-based protocols, ewes were assigned to groups PG10, PG12, PG14 or PG16 (two PG injections administered 10, 12, 14 or 16 days apart; respectively). Experiment I (n = 132) was conducted to evaluate the estrous response, ovulation rate (OR), conception and fertility. Experiment II (n = 24) was conducted to evaluate ovarian follicle growth, steroid concentrations and the interval from the second PG injection to estrus (PG-estrus) and ovulation (PG-ovulation). Estrous response was less with the PG16 (P<0.05) treatment, and the extent of estrous synchrony was greater with the PG10 and PG12 treatments. Ovarian follicle growth and the intervals for the variables PG-estrus, PG-ovulation and OR were similar among groups (P>0.05). From 8 to 4 days before estrus, progesterone (P4) concentrations were greater for the PG14 and PG16 than for the PG10 and PG12 (P<0.05) groups. There were more days where concentrations of P4 were above 3.18 nmol/L with the PG14 and PG16 than PG10 and PG12 (P<0.05) treatments. Use of the PG14 and PG16 treatments resulted in greater estradiol (E2) at estrus and 12 h later than use of the PG10 and PG12 treatments. A positive correlation was observed between the duration of the luteal phase and maximum E2 concentrations, and between duration of the luteal phase and days with E2 concentrations above 10 pmol/L. Conception and fertility were greater with use of the PG14 compared with PG10 and PG12 (P<0.05) treatments. The administration of two PG injections 10, 12, 14 or 16 days apart resulted in different durations of the luteal phase that were positively associated with E2 concentrations and the reproductive outcome. The shorter luteal phases were associated with greater synchrony in time of estrus. The intervals for the variables PG-estrus, PG-ovulation and OR were similar among groups.

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1. Introduction

Timed artificial insemination (TAI) represents a practical tool in genetic programs, but requires hormonal treatments that ensure a synchronized time of ovulation and acceptable pregnancy rates (Menchaca and Rubianes, 2004). Progestagen-based protocols are the
preferred option by technicians and farmers to manage flock reproduction (Gordon, 1999) even though there are potentially environmental and tissue contamination risks due to residues of progestagen devices, as well as risks with use of eCG, or the addition of antibiotics to avoid vaginitis (Gonzalez-Bulnes et al., 2005; Viñoles et al., 2011). Also, progestagen based estrous synchronization protocols have been associated with alterations in oocyte quality that can result in lesser fertilization rates and impaired embryo development (Gonzalez-Bulnes et al., 2005; Berlinguer et al., 2007). Because consumers demand foods produced by “clean, green and ethical” guidelines (Martin et al., 2004), progestalndins became a desirable alternative because lungs rapidly metabolize the drug hence it does not accumulate in tissues of treated animals (Piper et al., 1970; Davis et al., 1980). Furthermore, progestalndin-based protocols are easily applied by intramuscular injection, thus improving animal management and welfare (Abeca et al., 2012), and are more economically feasible compared with intravaginal devices plus eCG in sheep production enterprises.

Progestaglin F2α and its synthetic analogues (PG) have been widely studied since its discovery in 1970 as a pouWalful luteolitic agent (McCracken et al., 1970). Different alternatives of PG-based protocols have been used to synchronize time of estrus in sheep for TAI (rev: Fierro et al., 2013). However, use of most of these treatments is associated with lesser pregnancy rates compared with use of progesterone-eCG based protocols (Boland et al., 1978; Olivera-Muzante et al., 2011a; Viñoles et al., 2011).

Traditional PG-based protocols consist of two PG injections administered 9–14 days apart (Fierro et al., 2013), however, there is considerable variability in timing of estrus onset and ovulation (Acrítropoulou et al., 1978; Loubser and van Niekerk, 1981; Houghton et al., 1995; Viñoles and Rubianes, 1998), that limit the practicality of use of these protocols for TAI programs (Menchaca and Rubianes, 2004). When a PG-based protocol of two injections given 7 days apart is used (Rubianes et al., 2004), a highly synchronized time of estrus and timing of ovulation are observed (Rubianes et al., 2003; Menchaca et al., 2004), but undesirable pregnancy rates are often achieved that are related to an altered profile and lesser progesterone (P4) concentrations that in turn result in a lesser ovulation rate (OR), fertility and prolificacy compared with what occurs when inseminations occur as a result of spontaneous estrus (Fierro et al., 2011). Attempts to develop alternatives to improve this protocol (two injections given 7 days apart) have not been successful (Olivera-Muzante et al., 2011b, 2013; Fierro et al., 2013, 2014).

Lesser fertilization rates were reported when the interval between PG injections was reduced from 14 to 8 days (Fairnie et al., 1977). Furthermore, treatment with a supplemental P4 source provided via an intra-vaginal impregnated device that is inserted 8 days before the PG injection increased the number of ewes in estrus (93.4% compared with 82.0%), and pregnancy rate (84.9% compared with 75.3%) than occurred with untreated ewes (Loubser and van Niekerk, 1981). The interval between PG injections has been defined as important by Fairnie and Wales (1980), and other reports indicated that the inter-
and 41.0 ± 2.4 kg, respectively, were used. Ewes were maintained under field conditions, grazing pastures that are typically used for sheep production in the region where the studies were conducted (500 kg DM available per hectare, 90.6% DM, 13.8% CP and 9.2 MJ ME/kg DM), 0.4 kg/ewe/day of supplement (91.9% DM, 20.5% CP and 11.3 MJ ME/kg DM), and water was provided ad libitum.

2.2. Experimental design

Two experiments were conducted with the objective to determine estrous, OR and reproductive response (Experiment I; n = 132), concentrations of steroid hormones, ovarian follicular growth and time of ovulation (Experiment II; n = 24) in ewes in which stage of the estrous cycle was synchronized with different PG-based protocols. Ewes were assigned by body condition and weight (Experiments I and II), and by age (Experiment II) to four groups: time of estrus was synchronized with two injections of Belprostestante i.m. (160 µg per injection, Glandinex®, Universal Lab, Montevideo, Uruguay), administrated 10, 12, 14 or 16 days apart (PG10, PG12, PG14 or PG16, respectively; n = 33 and n = 6 in each group, Experiment I or II, respectively; Fig. 1).

2.3. Estrous detection

Estrous detection was performed using Corriedale androgenized wethers (given 100 mg of Ciclopentipropionate, on three occasions, 7 days apart; Testosterona Ultra Fuerte®, Laboratorio Dispert, Uruguay), with marker paint at a rate of six wethers/100 ewes. In Experiment I, estrous response (ewes in estrus/total ewes) was evaluated every 24 h from Days-4 (~96 h) to 0 (day of second PG injection; Hour 0), and then every 12 h until 120 h. In Experiment II, estrous detection was performed every 12 h after the second PG injection until ovulation, considering the intervals from PG administration to estrous detection (PG-estrous) and PG administration to the time of ovulation (PG-ovulation).

2.4. Semen collection, evaluation and dilution

Semen from five adult Corriedale rams, assessed for breeding soundness, was collected using an artificial vagina and assessments occurred as described by Evans and Maxwell (1987a). Two consecutive ejaculates from each ram were collected, evaluated and pooled. Soon after pooling, the semen was extended with UHT skim milk and antibiotics (250 mg of enrofloxacin/1 L of extender; Baytril®), and assessed for progressive sperm motility (80%) before its use.

2.5. Artificial insemination

Cervical AI was performed using a speculum equipped with a light source and an insemination device (Walmur® Veterinary Instruments, Montevideo, Uruguay) after estrous detection, as described by Evans and Maxwell (1987b). The insemination dose was 0.08 mL and contained 100 × 10⁶ sperm cells, slowly released into the cervix at the time of insemination. Extended semen was maintained at room temperature and protected from sunlight until AI.

2.6. Ultrasonography evaluation

In both experiments, the ewes’ estrous cyclicity (presence of a corpus luteum –CL-) was evaluated before the first PG injection (Viñoles et al., 2004). In Experiment I, OR (CL/ewes having ovulations) was evaluated 10 days after estrous detection in ewes that showed estrous behavior after the second PG injection (n = 32, 33, 31 and 24, PG10, PG12, PG14 and PG16 respectively). Conception (pregnant ewes/inseminated ewes × 100) and fertility rates (pregnant ewes/estrous synchronized ewes × 100) were evaluated 35 days after estrous detection. Values for all variables were determined by trans-rectal ultrasonography using a 7.5 MHz rigid linear array transducer (ALOKA SSD-500, Overseas Monitor Corp., Ltd., Richmond, BC, Japan) using the methodology described by Viñoles et al. (2010).

In Experiment II, pre-ovulatory follicle growth was evaluated every 24 h from the day of the second PG injection (Day 0, Hour 0) to the time of estrous detection, and time of ovulation was assessed every 12 h after estrous detection until the disappearance of the largest follicle (ovulation) by trans-rectal ultrasonography. The number, diameter and relative position of all ovarian follicles with a diameter of ≥2 mm and CL on both ovaries were mapped in each ultrasonography session. Follicle definitions used in this study were: the maximum diameter was the largest diameter of the pre-ovulatory follicle (mm), final diameter was the diameter detected the day before ovulation (mm), rate of follicular growth was calculated as the size difference from the first ultrasonic evaluation to ovulation, divided by the number of days it took to attain the maximum diameter (mm/d).

2.7. Blood collection and hormone concentrations

Jugular blood was collected into glass tubes with heparin using disposable needles. Blood samples were collected once a day (in the morning after a 12 h fasting period) from Day-10 to estrous detection, each 12 h after estrus to ovulation, and at 5 and 12 days after estrous detection (Experiment II; Fig. 1). Blood samples were stored at 5 °C until centrifugation at 2100g for 15 min; plasma was pipetted and stored at −20 °C until hormonal concentrations were analyzed. Progesterone and 17-β estradiol concentrations (E2) were quantified by radioimmunoassay (RIA) as described by Meikle et al. (1997). Progesterone concentrations were determined by a direct solid-phase RIA using Siemens kits (Siemens, Los Angeles, CA, 90045 USA). The RIA had a sensitivity of 0.32 nmol/L. The intra-assay coefficients of variation for the lesser (1.59 nmol/L) and greater (31.8 nmol/L) concentration control samples were 5.7% and 3.4%, respectively. The inter-assay coefficients of variation for the lesser and greater concentration control samples were 5.6% and 3.7%, respectively. Estradiol 17β concentrations were determined by a liquid phase RIA (DPC kits; Diagnostic Product Co., Los Angeles, CA, USA). Samples were analyzed in duplicate in the same assay. The sensitiv-
2.8. Statistical analyses

All analyses were performed using the Statistical Analysis System (SAS, version 9.1.3, 2004). Differences in the variance in time of estrus, OR, conception and fertility were analyzed by GENMOD. Follicle growth and the intervals for the variables PG-estrus and PG-ovulation, P4 and E2 concentrations in plasma were compared by analysis of variance using PROC Mixed. The model included the fixed effects of the group, ewe age, day and the interactions of these variables. Ewe within group was considered as the random effect. The covariance was modelled to consider the correlation between successive measurements of the same animal, with the option autoregressive order 1 (AR(1)). The duration of the luteal phase was analyzed using PROC GLM, including in the model the age of ewe, group and the interaction as fixed effects. Differences were considered significant if $P<0.05$ and trends if $P<0.1$ and $>0.05$.

3. Results

3.1. Experiment I

Fig. 2 depicts data for estrous behavior observed from –48 to 96 h after the second PG injection. Overall, the estrous response from 0 to 96 h after the second PG injection was similar for the PG10, PG12 and PG14 (97%, 100%, 94%; $P>0.05$) groups, but greater compared to the PG16 group (73%; $P<0.05$). More ewes from the PG16 group were in estrus 48 and 24 h prior to the second PG than the other groups ($P<0.05$). Estrous response was greater in the PG14 group at 24 h, PG10 group at 48 h, and PG12 group at 72 h ($P<0.05$). No differences were detected in OR among groups ($P>0.05$, Table 1). Conception and fertility rates for the PG14 group were greater compared with the PG10 and PG12 groups ($P<0.05$), but similar to the PG16 group (except that fertility tended to be less for the PG16 than PG14 group, $P=0.08$). The PG10, PG12 and PG16 groups had similar conception and fertility rates ($P>0.05$, Table 1).
3.2. Experiment II

Data for P4 concentrations relative to time of estrous detection are presented in Fig. 3. The effects of group, day and the interaction were significant \((P<0.05)\), but not ewe age \((P>0.05)\). Progesterone concentrations were greater in the PG14 and PG16 groups from 8 to 4 days prior to estrus compared to ewes from the PG10 and PG12 groups \((P<0.05; \text{Fig. 3})\). However, the decrease in P4 concentrations after the second PG injection and concentrations at 5 and 12 days after estrus were similar among groups \((P>0.05)\) except that ewes from the PG10 group had greater concentrations than those from the PG12 group on Day 12 \((P<0.05)\). The number of days with P4 above 3.18 nmol/L was similar between the PG14 \((10.6 \pm 1.3 \text{ d})\) and PG16 groups \((12.5 \pm 0.8 \text{ d}; P>0.05)\), and both were greater compared with the PG10 \((4.3 \pm 1.6 \text{ d})\) and PG12 groups \((6.8 \pm 2.6 \text{ d}; P<0.05)\).

Data for E2 concentrations from \(-48\) to \(36\) h after estrus are depicted in Fig. 4. The effects of group and day were significant \((P<0.05)\), but not that of ewe age and the interaction of group and day \((P>0.05)\). At estrus and 12 h later, E2 concentrations were greater in ewes from the PG14 and PG16 groups compared with ewes from the PG10 and PG12 groups \((P<0.05; \text{Fig. 4})\). Ewes from the PG14 and PG16 groups had more days with E2 concentrations greater than 10 pmol/L \((1.40 \pm 0.89 \text{ and } 2.83 \pm 0.75 \text{ d}; \text{PG14 and PG16 groups, respectively})\), compared with ewes from the PG10 and PG12 groups \((0.83 \pm 0.75 \text{ d}; P<0.05)\). A positive correlation was observed between the duration of the luteal phase and maximum E2 concentrations \((r=0.5; P<0.01)\) and between the duration of the luteal phase and the number of days with E2 concentrations greater than 10 pmol/L \((r=0.78; P<0.001)\).

Data on the intervals for the variables PG-estrus and PG-ovulation, final and maximum diameter of the ovulatory follicle, and follicular growth rate are presented in Table 2.

No significant differences were detected among groups for any of these variables \((P>0.05)\).

4. Discussion

The working hypothesis in the present research that the administration of two PG injections administered 10, 12, 14 or 16 days apart affected differences in time of estrous response, ovarian response, concentrations of steroid hormones and reproductive performance in ewes was supported by some of the results from these studies. The use of the protocols evaluated resulted in differences in P4 and E2 concentrations, estrous response up to \(96\) h after the second PG administration, and in conception and fertility. However, the morphological changes in the pre-ovulatory follicle, and intervals for the variables PG-estrus and PG-ovulation, and OR were similar among groups.

The PG-based protocols evaluated in the present experiments were associated with different estrous responses until \(96\) h after the second PG administration. The intervals between PG injections determined that at the moment of the second PG administration the age of the CLs (in ewes that responded to the first PG injection) varied from 7 to 14 days (mid to late luteal phases) so they were all sensitive to the luteolytic effect of PG \((\text{Houghton et al., 1995; Rubianes et al., 2003; Conterras-Solis et al., 2009})\). Estrous response variables were reported previously using the two PG injection protocols with an interval of 9 days \((95\%, \text{Acritopoulo-Fourcroy et al., 1982; 76.9\%, Boland et al., 1978; 100\%, Haresing and Acritopoulo, 1978}; 10 days \((71.4\%, \text{Godfrey et al., 1997; 88\%, Das et al., 1999}); 11 days \((82\%, \text{Loubser and van Nierkerk, 1981}; 100\%, \text{Oyediji et al., 1990}); \text{and 14 days (73\%, Boland et al., 1978})\). Nevertheless, the use of two PG injections administered 16 days apart (PG16 group) resulted in a lesser overall estrous response compared with the values for the PG10, PG12 and PG14 groups. This could be explained by some ewes in the PG16 group, expressing estrous behavior 48 h prior
to the administration of the second PG injection because natural luteolysis had already occurred (Rubianes et al., 2003). The PG16 group was, nevertheless, initially included in the present experiment because the interval between PG injections was expected to facilitate the induction of a physiologic luteal phase (duration and hormonal profile), and be associated with an enhanced reproductive performance. Most of the ewes from the PG10 and PG12 groups expressed estrous behaviour between 48 to 72 h after the second PG injection; demonstrating a concentrated estrous response when these protocols are applied, a result that is very important for TAI programs. In summary, under the conditions of the present study, all the protocols were determined to provide for an acceptable estrous response after treatment; however, the use of the PG10, PG12 and PG14 protocols resulted in a greater number of ewes in estrus than use of the PG16 protocol and the PG10 and PG12 groups had a greater synchrony in time of estrus than the other groups.

The intervals for the PG-estrus and PG-ovulation variables were similar among groups, although the variation was greater for these variables with the PG14 and PG16 groups. These results were unexpected because the interval PG-estrus variable has been associated with the age of the CL (Houghton et al., 1995) and with the follicular status of each ewe at the time of the PG administration (Viñoles and Rubianes, 1998). Nevertheless, this result was supported by a similar rate of P4 decrease for all groups in the present study after PG administration. There are previous reports for the PG-estrus interval variable under different experimental conditions with results for 7 day (48 ± 2.3 h, Rubianes et al., 2003; 36.6 ± 3.6 h, Contreras-Solís et al., 2009); 9 day (38.8 ± 1.3 h, Acritopoulou et al., 1978; 38.6 ± 0.8 h, Haresign and Acritopoulou, 1978; 43.5 ± 6 h, Acritopoulou, 1979; 45.8 ± 1.1 h, Acritopoulou-Fourcroy et al., 1982); 10 day (51.6 ± 2.4 h, Das et al., 1999); and 11 day (41.7 ± 2.2 h, Oyediji et al., 1990) intervals between PG injections being reported, however, longer intervals (14 and 16 d) between injections were not evaluated in previous studies and the comparisons have not been made under the same experimental conditions as occurred in the present study. The interval between when basal P4 concentrations are achieved to estrous detection (Wiley et al., 1997), and between estrous onset and LH peak to ovulation (Cuming et al., 1973) has been reported to be similar. Fairnie et al. (1977) observed a similar mean time from treatment to ovulation after two PG injections were administered at 8 compared with 14 day intervals. Even though there were non-significant differences among PG-ovulation interval variables of the groups in the present study, the differences observed with some groups may need to be considered in determining the optimal AI time in TAI programs, mainly when chilled or frozen semen are used, due the shorter lifespan of sperm cells stored in these conditions compared with use of fresh semen (Salamon and Maxwell, 2000). Furthermore, it is possible that the experimental design in the present study was not appropriate to detect differences among groups in these variables due to the extended time between estrous observations (12 h).

Follicular growth rate, as well as final and maximum follicle diameter were similar among groups. These results were not expected, because the final stage of growth of a follicle is related to LH pulse frequency that is down-regulated by P4 (Ginther et al., 1995). In the present study, LH pulse frequency was not assessed but there were differences in P4 concentrations detected and in the number of days with P4 above 3.18 nmol/L (longer in PG14 and PG16 groups). An altered P4 profile was associated with altered ovarian follicular dynamics and poor reproductive performance when a short PG-based protocol was used (Fierro et al., 2011). Furthermore, similar follicular diameters were related to different E2 concentrations among groups. Ewes from the PG14 and PG16 groups had greater E2 concentrations compared with ewes from the PG10 and PG12 groups around the time of estrus. It is well known that the secretion of E2 by the follicles is associated with the LH pulse frequency (Sirois and Fortune, 1990; Stock and Fortune, 1993; Viñoles et al., 1999), reinforcing the existence of physiologic differences in the dominant follicles among groups. There was a similar diameter for pre-ovulatory follicles in all groups, and the lesser steroidogenic function of the follicles of ewes in the PG10 and PG12 groups may indicate that these ewes had an impaired steroidogenic capacity (White et al., 1987) even though there was greater estrous synchrony for these groups. An altered steroidogenic function of follicles has been described as a consequence of an inadequate P4 priming (Coleman and Dailey, 1983), and to lesser numbers of LH receptors in granulosa cells (McNatty et al., 1984). Furthermore, a premature exposure to LH inhibited the potential of human granulosa cells to secrete steroids in vitro (McNatty and Sawers, 1975). Moreover, Deaver et al. (1986) reported that differences in the patterns of gonadotropin secretion before the pre-ovulatory surge of LH might be caused by differences in P4 or the P4-E2 ratio when luteal regression is induced on different days of the estrous cycle.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>PG-estrus (h)</th>
<th>PG-ovulation (h)</th>
<th>Maximum diameter (mm)</th>
<th>Final diameter (mm)</th>
<th>Growth rate (mm/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG10</td>
<td>50.0 ± 6.0a</td>
<td>720 ± 6.6a</td>
<td>6.3 ± 0.5a</td>
<td>6.0 ± 0.9a</td>
<td>0.7 ± 0.4a</td>
</tr>
<tr>
<td>PG12</td>
<td>44.0 ± 4.9a</td>
<td>700 ± 6.2a</td>
<td>6.5 ± 0.8a</td>
<td>6.3 ± 0.8a</td>
<td>1.0 ± 0.5a</td>
</tr>
<tr>
<td>PG14</td>
<td>37.2 ± 23.4a</td>
<td>58.8 ± 21.8a</td>
<td>6.2 ± 0.4a</td>
<td>6.0 ± 0.7a</td>
<td>1.1 ± 0.7a</td>
</tr>
<tr>
<td>PG16</td>
<td>44.0 ± 15.9a</td>
<td>64.0 ± 9.0a</td>
<td>6.5 ± 1.2a</td>
<td>6.3 ± 1.0a</td>
<td>1.3 ± 0.5a</td>
</tr>
</tbody>
</table>

Maximum diameter: the largest diameter reached by the pre-ovulatory follicle; Final diameter: the diameter reached before ovulation; Growth rate: calculated as the size difference from the first ultrasonic evaluation to ovulation, divided by the number of days it took to reach the maximum diameter; Data are presented as LS means ± pooled SEM. *In the same column: P > 0.05.
Conception and fertility rates were related to the hormonal profiles in the different groups in the present study. The ewes of the PG14 group had greater conception and fertility rates after estrous detection compared with the PG10 and PG12 groups. The greater concentrations and numbers of days with elevated P4 concentrations observed in the PG14 group prior to mating may be responsible for the greater conception and fertility rates for this group (Folman et al., 1973; Fairnie et al., 1977; Loubser and van Niekerk, 1981). The alteration of the steroidogenesis, as observed in ewes of the PG10 and PG12 groups (lesser P4 and E2 concentrations compared to groups with longer periods between PG injections) have been reported to affect oocyte development and transport, fertilization rate and early embryo development in cattle (Gustafsson and Plöen, 1986; Greve et al., 1995). Estradiol induces the development of the secretory cells in the oviduct, which affects fertilization and early embryo development (Murray, 1992; Nacarrow and Hill, 1995), and lesser E2 concentrations have been associated with a decreased fertilization rate in estrous synchronized sheep (Gonzalez-Bulnes et al., 2005). Furthermore, E2 and P4 concentrations regulate the expression of the E2 and P4 receptor genes in the uterus (Clark and Mani, 1994; Ing et al., 1996; Ing and Ott, 1999), and it was suggested that reproductive failure in P4 estrous-synchronized ewes may be related to a decrease in the expression of E2 and P4 receptor genes (Garcia-Palencia et al., 2007). It is possible that these altered physiological functions when P4 was used to synchronize the time of estrus may have contributed to the reproductive performance achieved by ewes of the PG10 and PG12 groups. Detailed morphological and functional studies of the follicles, oocytes and embryo quality are needed to determine the reasons of this altered steroidogenic function when protocols with different intervals between PG injections are used. In general, the use of protocols with longer periods between PG injections resulted in enhanced reproductive performance after estrous detection, although conception and fertility of the PG16 group in the present study was intermediate. The tendency for a lesser fertility in the PG16 compared with the PG14 group could be due to the lesser number of ewes that expressed estrous behavior and were inseminated after the second PG injection in this group.

Regarding the P4 profile after mating, there was a similar P4 profile and concentrations at 5 and 12 days after estrous detection in all groups, except the PG10 group had greater concentrations than PG12 group on Day 12 subsequent to ovulation. This may indicate a normal lifespan of the CL after PG administration (Fierro et al., 2011, 2013) even when there were differences in follicle functionality observed during the follicular phase. Moreover, findings of the present study reinforce the importance of the hormonal milieu during the follicular phase preceding ovulation on oocyte quality and the uterine environment so as to support early embryo development (Gustafsson and Plöen, 1986; Garcia-Palencia et al., 2007). This effect cannot be overcome by having greater P4 concentrations during the mid-luteal phase following ovulation. This rationale is supported by the lesser conception and fertility rates in PG10 and PG12 groups of the present study, even though there were greater P4 concentrations on Day 12 in ewes from the PG10 group.

Ovulation rate is a variable associated with inconsistent results when PG-based estrous synchronization protocols are used (Fierro et al., 2013). No differences were detected for OR among groups in the present study, and this was unexpected because an increase in OR has been reported when PG was administered in the mid-luteal phase of the estrous cycle probably because of the development of a “less dominant” pre-ovulatory follicle with a lesser steroidogenic capacity that contributed to maintaining FSH concentrations above the threshold so as to stimulate the selection of multiple ovulatory follicles from a single follicular (Letelier et al., 2011), or from two consecutive follicular waves (Bartlewska et al., 1999; Gibbons et al., 1999). Considering the findings with the present study on the E2 production by the dominant follicles in the different groups, it is speculated that the development of follicles with a longer dominant phase occurred in PG14 and PG16 groups compared with the PG10 and PG12 groups. This is inconsistent with results when PG was administered in the mid-luteal phase (Letelier et al., 2011) where with this treatment it would be expected to result in a greater OR. To the best of our knowledge, this is the first report where OR was compared with use of different PG-based protocols for estrous synchronization in sheep under the same conditions. However, it is important to note the small number of ewes used to evaluate this variable, thus, more research is needed to determine whether the findings of the present study can be confirmed.

The experimental designs used in the present study allowed for the enhanced understanding of the effects of different PG-based protocols for estrous synchronization in sheep under similar conditions. In brief, the PG14 treatment resulted in a desirable estrous response, lesser time to estrous synchrony, and greater P4, and E2 concentrations as well as reproductive performance after estrous detection compared with the results with use of the PG10 and PG12 treatment protocols. Ewes of the PG16 group have a lesser estrous response and intermediate reproductive outcome, but similar hormonal profiles as the PG14 group. It is important to note that the more desirable reproductive performance of the PG14 group may not necessarily indicate a greater fertility after AI at fixed time. When a TAI protocol is applied, a greater estrous response and synchrony are important for desirable reproductive outcomes. Future trials need to be performed to determine the reproductive success with use of the protocols applied in the present study in TAI programs.

5. Conclusion

It is concluded that the administration of two PG injections 10, 12, 14 or 16 days apart contributed to the different durations of the luteal phase that were positively associated with E2 production by the pre-ovulatory follicles and the reproductive outcome in the present study. However, the shorter intervals between PG injections in the present study were associated with an enhanced estrous synchrony which is a requisite for TAI programs. There were no differ-
ences in the variables of intervals PG–estrus, PG–ovulation and OR among groups in the present study.

**Conflict of interest**

None.

All authors have no financial or personal relationship with organizations or people that could influence or bias the study.

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