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Expression of genes for oestrogen and progesterone receptors in the cervix of anoestrous ewes treated with gonadotrophin releasing hormone with or without progesterone priming

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ABSTRACT

The aim was to determine the oestrogens receptor alpha (ERα) mRNA and the binding capacity of oestrogens (ER) and progesterone receptor (PR) in the cervix of anoestrous ewes treated with gonadotrophin-releasing hormone (GnRH) with or without progesterone (P) priming, at the expected time of induced ovulation and early luteal phase. In Experiment 1, ewes were treated with P for 10 days ($n = 4$), with nine micro-doses of GnRH followed by a GnRH bolus injection ($n = 4$), or with P plus GnRH treatments ($n = 3$), and tissues were harvested either without treatment ($n = 4$), when P was removed, or 24 h after the GnRH bolus injection. In Experiment 2, ewes were treated with the same GnRH or P plus GnRH treatments and tissues were harvested on Day 1 ($n = 12$) or Day 5 ($n = 10$) after the GnRH bolus injection. In the cranial cervix, the P treatment decreased and the GnRH treatment (after P treatment) increased the ERα mRNA, ER and PR concentrations ($P < 0.002$). The ERα mRNA and ER concentrations were greater on Day 1, than on Day 5 in P plus GnRH treated ewes ($P < 0.0005$). In the caudal cervix, lesser ERα mRNA, ER and PR concentrations than cranial cervix were found ($P < 0.0001$). In conclusion, the ERα transcriptional activity and ER and PR binding capacity were strongly influenced by P and/or GnRH treatments in the cranial cervix, while the steroid receptors binding capacity remained unchanged in the caudal cervix of anoestrous ewes at the expected time of induced ovulation and early luteal phase.

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1. Introduction

Corriedale is the predominant sheep breed in Uruguay, being two thirds of the total ewes. Corriedale ewes have a marked seasonal reproductive behaviour, with a breeding season that extends from February through June (South America 35°SL) (Rodriguez Iglesias et al., 1993). Consequently, several techniques for out-of-season induction of ovulation and fertilization have been developed to improve the wool, lamb-meat, and milk sheep productivity (Durán del Campo and Durán del Campo, 1955; Ungerfeld and Rubianes, 1999; Ungerfeld et al., 2005).

Hormonal induction of ovulation has been performed in domestic sheep during the non-reproductive season (Thimonier, 1981; Robinson, 1988; Scaramuzzi et al., 1988; Hunter, 1991; Safranski et al., 1992). A well established protocol for the induction of ovulation in anoestrous ewes consists of treating ewes with gonadotrophin-releasing hormone (GnRH) that results in the physiological surge of luteinizing hormone (LH) (Hunter, 1991). The GnRH induction of ovulation during anoestrus is accompanied by a greater than typical incidence of short luteal phases (Hunter, 1991; Liu et al., 2007) that can be avoided by pre-treatment with P (P + GnRH) (McLeod et al., 1982; Husein

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and Kridli, 2003). This P + GnRH combined treatment effectively synchronizes ovulation, and induces physiologic release of LH and high incidence of healthy corpus luteum. Thus P + GnRH treatment has been used as an experimental model in different breeds (Bartlewski et al., 2004; Bramley et al., 2005), including the Corriedale (Tasende et al., 2005a).

The cervix of the ewe acts as a barrier against the passage of the transcervical instruments used for artificial insemination (AI) (Halbert et al., 1990; Campbell et al., 1996), and for embryo transfer (ET) procedures (Armstrong and Evans, 1983; Kraemer, 1989). In oestrous cycling ewes, the depth of cervical penetration was greater in the non-luteal than in the luteal phase, indicating that a degree of natural cervical relaxation at oestrus could be under the influence of the ovarian steroid hormones (Kershaw et al., 2005). The genomic actions of oestrogens (E) and progesterone (P) depend on their circulating concentrations and on the sensitivity of the target tissues in terms of the concentration of their specific and high-affinity nuclear receptors (ER and PR, respectively) (Clark et al., 1992; Meikle et al., 2004). Two subtypes of ER (ERα and ERβ) have been described in the cervix of rats and human, of which ERα is the predominant (Wang et al., 2000; Gorodeski and Pal, 2000; Stygar et al., 2001). In sheep, the immunoreactive ERβ has been found in uterus, but not in cervix of lamb ewes (Morrison et al., 2003)

Greater binding of ER and PR at oestrus than in the luteal phase was reported in cervix of ewes (Rodríguez-Piñón et al., 2008). In a previous study, conducted during the postpartum anoestrus in the breeding season, the cervical ER and PR binding capacity was less after parturition and increased in the late postpartum (Rodríguez-Piñón et al., 2000). This result was probably due to the presence of large follicles in the ovarian surface (Tasende et al., 1996), presumably oestrogens-secreting follicles (Driancourt and Avdi, 1993). The pattern of regulation described in the cervix of both cycling and postpartum ewes during the resumption of the cyclic ovarian activity is consistent with the accepted up regulation of uterine ER and PR expression by E, and down regulation of both receptors by P (Ing et al., 1993; Couse et al., 2006). In addition to the variation in the concentration of ER and PR during the oestrous cycle, a differential gene expression for both receptors along the longitudinal axis of the cervix was also found (Rodríguez-Piñón et al., 2008). This probably reflects the coexistence of systemic and local regulatory mechanisms on the expression of steroid receptors in the ovine cervix.

Although the hormonal induction of ovulation during the anoestrous season has been widely studied, there is little information about cervical steroid receptor gene expression. At present, there is no report of cervical gene expression for ER and PR in seasonally anoestrous ewes hormonally treated to induce ovulation. The aim of the present research was to determine the ERα transcriptional activity and ER and PR binding capacity in the cervix of anoestrous ewes treated with GnRH with or without P priming. These measurements were performed in the cranial and caudal cervical regions at the expected time of the induced ovulation and early luteal phase.

2. Materials and methods

2.1. Animals

Two experiments were conducted during the anoestrous season (September, mean 11.5 h of natural light) of adult Corriedale ewes in Uruguay (35°SL). In both experiments, ewes were under natural day length, grazed on native pastures and had *ad libitum* access to water. To confirm the anoestrous condition of the ewes, vasectomized rams fitted with colour-marking harnesses were kept with the ewes for 2 months before the beginning of the study. All animal manipulations and treatments were performed at the experimental field of the Veterinary Faculty, University of Uruguay (UdelaR) and in compliance with regulations of the Ethical Committee of our Institution.

2.2. Experiment 1

The aim of Experiment 1 was to determine the effect of GnRH treatment with or without P priming on the ER and PR cervical gene expression in anoestrous ewes at the expected time of the induced ovulation. Fifteen anoestrous ewes (body weight, mean ± pooled s.e.m., 42.0 ± 1.2 kg) were randomly assigned to four groups as follows: Group A (anoestrus, $n = 4$) without treatment; Group P ($n = 4$) treated with 0.33 g of P (Controlled Internal Drug Release (CIDR), EASI-BREED CIDR, Hamilton, New Zealand) for 10 days; Group GnRH ($n = 4$) treated every 2 h for 16 h with 6.7 ng (i/v) of GnRH (Busereline acetate; Receptal; Hoechst, Buenos Aires, Argentina) followed by a bolus injection (i/v) of GnRH (4 μg Receptal) at 18 h (Day 0), and Group P + GnRH ($n = 3$) treated with the combined treatments of groups P and GnRH. The GnRH treatment started immediately after CIDR removal. Tissues from the ewes were harvested at the beginning of the experiment (Group A), immediately after CIDR removal (Group P) or 1 day after the GnRH bolus injection (groups GnRH and P + GnRH). In the GnRH and P + GnRH groups all ewes had a synchronized timing for the LH surge with maximum LH concentrations at 2 h after the GnRH bolus injection and with 8 h of duration, confirming the effectiveness of the GnRH treatment (Tasende et al., 2002).

2.3. Experiment 2

The aim of Experiment 2 was to determine the cervical expression of ER and PR in the early luteal phase as compared with the expected time of ovulation in anoestrous ewes treated with GnRH with or without P pretreatment. Twenty two anoestrous ewes (body weight, mean ± pooled s.e.m., 43.1 ± 1.3 kg) were randomly assigned to two treatments, GnRH ($n = 11$) and P + GnRH ($n = 11$). GnRH and P + GnRH treatments were identical to Experiment 1. The treated ewes were randomly assigned to two days for tissue harvest after the GnRH bolus injection as follows: Day 1, at the expected time of ovulation (groups GnRH 1, $n = 6$ and P + GnRH 1, $n = 6$) or Day 5, in the early luteal phase (groups GnRH 5, $n = 5$ and P + GnRH 5, $n = 5$). All ewes had a synchronized timing for the LH surge with maximum LH

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concentrations at 2 h after GnRH bolus injection and duration of 8 h (Tasende et al., 2005a).

2.4. Tissue samples

Cervices were dissected into three equal length segments named cranial, middle and caudal region. The cranial (next to the uterus) and caudal (next to the vagina) regions were selected. The samples were frozen in liquid nitrogen and stored at −80 °C until transcript solution hybridisation and receptor binding assays were conducted.

2.5. ERα mRNA by solution hybridization assay

The hybridisation probes were derived from plasmids containing 360 bp cDNAs from the ovine ERα (Ing et al., 1996) and previously used in sheep cervix (Rodríguez-Piñón et al., 2008). Total nucleic acids (TNA) were obtained by digesting homogenised (Polytron® homogenizer PT-10 Kinematica AG, Littau Luzern, Switzerland) cervical tissues (200–250 mg) with proteinase K in a buffer containing sodium dodecyl sulfate and subsequent extraction with phenol–chloroform. The TNA content in the samples was determined spectrophotometrically at 260 nm and expressed as absorbance relative units (ARU). The ^{35}S-UTP-labeled cRNA was hybridised overnight at 70 °C to TNA samples. The hybridisations were performed in duplicates at two different volumes (5 and 15 μL) in 40 μL of hybridisation formamide-buffer under two drops of paraffin oil. To digest unhybridized RNA after hybridisation, samples were treated with 1 mL of RNase buffer containing 40 μg RNase A, 118 U RNase T1 (Boeringer-Mannheim, Mannheim, Germany), and 100 μg calf thymus DNA for 45 min at 37 °C. Labeled hybrids protected from RNase digestion were precipitated with trichloroacetic acid and collected on filters (Whatman GF/C, Whatman Nederland B. V., Hertogenbosch, The Netherlands). Radioactivity was determined in a liquid scintillation counter. The concentration of ERα mRNA (amol ARU^{-1}) was calculated using a calibration curve with seven increasing concentrations (7–450 amol/tube) of the labeled probe. All samples from the experiment were processed in the same assay, with a sensitivity of 5 amol ARU^{-1} and 12% of the intra-assay CV.

2.6. Steroid receptors by binding assay

Ligand-binding assays for ER and PR determinations were performed in soluble fraction of cervix as previously described (Garófalo and Tasende, 1996; Rodríguez-Piñón et al., 2000). The term 'soluble fraction' refers to the supernatant fractions of tissue homogenates after a high-speed centrifugation, and does not imply cellular receptor localisation. Unless otherwise stated, the reagents were obtained from Sigma Chemicals (St. Louis, MO, USA). The frozen cervical samples (300–500 mg) were sliced and homogenised in Tris buffer with a Polytron homogenizer (Polytron® homogenizer PT-10 Kinematica AG, Littau Luzern, Switzerland). The soluble fractions were separated by a first centrifugation at 1000 × *g* for 15 min and then at 40,000 × *g* for 90 min. All these and subsequent procedures were carried out at 0–4 °C. The soluble fractions, in duplicate, were incubated with five to six increasing concentrations of ^{3}H-E$_2$ (86 Ci/mmol; 0.15–15 nM) or ^{3}H-ORG-2058 (40 Ci/mmol; 0.5–30 nM) (Amersham International, Buckhinghamshire, England), for determination of the total bound ^{3}H-labeled ligands. Identical duplicate samples were incubated with 200-fold molar excess of either unlabeled diethylstilbestrol or unlabeled ORG-2058, for determination of non-specifically bound ^{3}H-labeled ligands. After 18 h incubation free hormones were removed and radioactivity was measured by liquid scintillation counting. Specific binding data were obtained by subtracting non-specific from total binding. A linear regression test of inverse Scatchard model analysis (Braunsberg, 1984) was used to obtain the apparent dissociation constant (K_d, nM) and the concentration of receptor sites expressed as fmol mg^{-1} proteins. The concentration of protein was determined in the soluble fractions by the method of Lowry et al. (1951). The concentration of protein was positively correlated with the amount of tissue used in the receptor assay, showing that the protein extraction procedure was similar in the different cervical samples.

2.7. Statistical analysis

The concentration of ERα mRNA, ER and PR and the K_d values was analysed by analysis of variance (ANOVA) using the MIXED Procedure of Statistical Analysis Systems (SAS Institute, Cary, NC, USA), including the fixed effects of treatment (A, P, GnRH and P + GnRH), cervical region (cranial and caudal) and interactions for Experiment 1, or effects of day and treatment (groups GnRH 1, P + GnRH 1, GnRH 5 and P + GnRH 5), cervical region (cranial and caudal) and their interactions for Experiment 2. Correlation analysis (SAS) was applied to determine the relationship between the concentration of ERα mRNA compared to ER, and ER compared to PR. The results were expressed as the least square mean ± pooled s.e.m. The level of significance was $P < 0.05$, except where otherwise specified.

3. Results

3.1. Experiment 1

There was an effect of treatment ($P < 0.0001$) and cervical region ($P < 0.0001$) on ERα mRNA concentrations, as well as an interaction between them ($P < 0.0001$) (Fig. 1A). ERα mRNA was undetected (under the assay sensitivity) in all caudal cervical samples of groups A and P, and one cranial cervical sample of Group P. In the cranial region, the concentration of ERα mRNA was greater in groups GnRH and P + GnRH than in groups A and P, respectively. Group P had the lesser concentration of ERα mRNA and P + GnRH the greatest concentration of ERα mRNA. In the caudal region, groups GnRH and P + GnRH had similar concentration of ERα mRNA. In all groups, the concentration of ERα mRNA was greater in the cranial than in the caudal region.

Single, saturable and high affinity-binding sites for E and P were found in all samples. The K_d values were not affected by treatment or cervical region. The K_d means (±pooled s.e.m., nM) were 0.70 ± 0.04, $n = 30$ and 1.08 ± 0.07, $n = 30$ for ER and PR, respectively.

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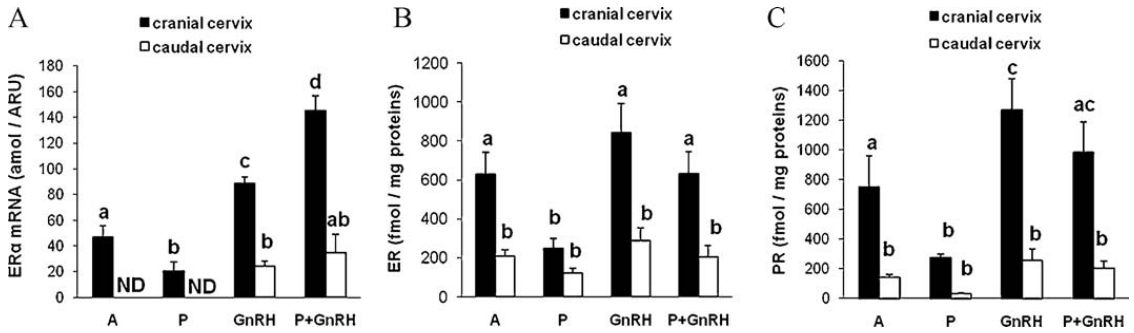

Fig. 1. Concentrations (mean ± pooled s.e.m.) of oestrogen receptor α messenger (ERα mRNA, amol ARU^{-1}, panel A), oestrogen (ER, panel B) and progesterone (PR, panel C) receptors (fmol mg^{-1} proteins) in the cranial and caudal cervical regions of anoestrous non-treated ewes (Group A, $n = 4$) and ewes treated with progesterone (Group P, $n = 4$), GnRH (Group GnRH, $n = 4$) or progesterone plus GnRH (Group P + GnRH, $n = 3$). The tissues from ewes were harvested at the start of the experiment (Group A), immediately after progesterone removal (Group P) or 24 h after GnRH bolus injection (Groups GnRH and P + GnRH). ND, non-detected. Bars with different letters differ within groups and regions ($P < 0.05$).

There was an effect of treatment ($P < 0.002$) and cervical region ($P < 0.0001$) on ER concentrations (Fig. 1B). In the cranial region, the concentration of ER was less in Group P than in the other groups. No differences between groups were found in the caudal region. The concentration of ER was greater in the cranial than in the caudal region in all the groups, except Group P. There was a positive correlation between the concentration of ERα mRNA and ER, $r = 0.5940$, $n = 21$, $P < 0.005$.

There was an effect of treatment ($P < 0.001$), cervical region ($P < 0.0001$), and interaction between these variables ($P < 0.05$) for concentration of PR (Fig. 1C). In the cranial region, concentration of PR was less in the Group P than in the other groups and greater in Group GnRH than in the Group A. No differences between groups were found in the caudal region. The concentration of PR was greater in the cranial than in the caudal region for Groups A, GnRH and P + GnRH. There was a positive correlation between the concentration of ER and PR ($r = 0.78$, $n = 30$, $P < 0.002$).

3.2. Experiment 2

There was a significant effect of Day and treatment ($P < 0.0002$), cervical region ($P < 0.0001$), and interaction between these variables ($P < 0.003$) on concentration of ERα mRNA (Fig. 2A). In the cranial region, the concentration of ERα mRNA in the Group P + GnRH was greater on Day 1 than Day 5, whereas no differences between days were found in Group GnRH. No differences between treatments were found on Day 1, whereas on Day 5, concentration of ERα mRNA was greater in the Group GnRH 5 than in the Group P + GnRH 5. In the caudal region, there was no difference in the concentration of ERα mRNA between days in Group P + GnRH, whereas in Group GnRH it was greater on Day 1 than Day 5. The concentration of ERα mRNA was greater in the cranial than caudal region in Groups GnRH 1, GnRH 5 and P + GnRH 1.

Binding sites of high affinity for E and P were found in all samples. The K_d values were not affected by day, treatment, or cervical region. The K_d means (mean ± pooled s.e.m., nM) were 0.67 ± 0.06, $n = 44$ and 0.85 ± 0.08, $n = 44$, for ER and PR, respectively.

There was an effect of Day and treatment ($P < 0.0005$), cervical region ($P < 0.0001$), and interaction between these variables ($P < 0.002$) for ER concentration (Fig. 2B). In the cranial region, the concentration of ER was greatest in Group P + GnRH 1, and the least in Groups GnRH 5 and P + GnRH 5. The concentration of ER was lesser in Group GnRH 1 than in the Group P + GnRH 1, but no differences between groups were found on Day 5. In the caudal region, there were no differences between groups. The concentration of ER was greater in the cranial than in the caudal region in all the groups. There was a positive correlation between the concentration of ER and ERα mRNA ($r = 0.70$, $n = 44$, $P < 0.001$).

The concentration of PR was influenced by Day and treatment ($P < 0.0001$), cervical region ($P < 0.0001$) and the interaction between these variables ($P < 0.001$) (Fig. 1C).

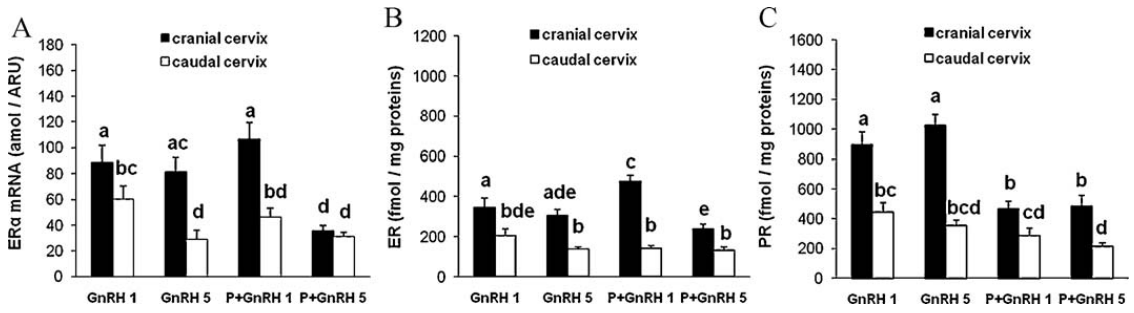

Fig. 2. Concentrations (mean ± pooled s.e.m.) of oestrogens receptor α messenger (ERα mRNA, amol ARU^{-1}, panel A), oestrogens (ER, panel B) and progesterone (PR, panel C) receptors (fmol mg^{-1} proteins), in the cranial and caudal cervical regions of anoestrous ewes treated with GnRH (Groups GnRH) or progesterone plus GnRH (Groups P + GnRH). Tissues from ewes were harvested on Day 1 (Groups GnRH 1, $n = 6$ and P + GnRH 1, $n = 6$) or 5 (Groups GnRH 5, $n = 5$ and 5 P + GnRH 5, $n = 5$) after GnRH bolus injection (Day = 0). Bars with different letters differ within groups and regions ($P < 0.05$).

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In the cranial region, the concentration of PR was grater in Group GnRH than Group P + GnRH, whereas there was no difference between Days 1 and 5. In the caudal region, there was no difference between groups. The concentration of PR was greater in the cranial than caudal region in all the groups. There was a positive correlation between concentration of ER and PR ($r = 0.51$, $n = 44$, $P < 0.003$).

4. Discussion

In the present study, there was greater gene expression for steroid receptor in the cranial cervix of anoestrous ewes, and ERα transcriptional activity and ER and PR binding capacity were influenced by GnRH treatments, with or without progesterone priming. In addition, in the caudal cervix of ewes in anoestrus at the expected time of the induced ovulation (Day 1) and early luteal phase (Day 5), the steroid receptor gene expression in terms of concentration of ERα-mRNA, ER and PR was less than in the cranial cervix.

There were high affinity receptor binding proteins for oestrogens and progesterone in all cervical samples. The K_d values for ER and PR in all cervical regions and all groups of treated and untreated anoestrous ewes were similar. This finding suggests that variations in the responsiveness of the anoestrous cervix to E and P may not depend on changes of affinity, but rather on binding capacity (concentration of ER and PR). The cervical ER and PR K_d values were similar to those found in the cervix of adult ewes (Rodríguez-Piñón et al., 2000, 2008) as well as in other E and P target tissues of sheep (Garófalo and Tasende, 1996; Meikle et al., 2004; Rodríguez-Piñón et al., 2005; Tasende et al., 2005b).

In seasonally anoestrous ewes, concentration of ER and PR was similar to that in the cervix of Corriedale oestrous cycling ewes in the breeding season (Rodríguez-Piñón et al., 2008). Results of the present study are not consistent with those of Zhao et al. (1999) who found no ER and few PR immune-staining positive cells in the endometrium of the cervix in seasonally anoestrous ewes. These differences could be due to the methodology used to measure ER and PR, as the binding assay permits detection of minimal receptor quantities. The concentration of cervical steroid receptor during the anoestrous season in ewes could be due to a constitutive receptor gene expression and/or to a stimulatory effect of the basal circulating concentrations of E with a lack of the P inhibitory effect, as was suggested by Garófalo and Tasende (1996) for the uterus of prepubertal lambs. This explanation is consistent with the ER and PR concentrations in uterus of seasonal anoestrous ewes (Tasende et al., 2002).

Interestingly, the concentration of ERα mRNA, ER and PR was greater in the cranial than in the caudal cervix in both Experiments (1 and 2). This suggested that the cervical zones could be molecularly and functionally different.

Progesterone treatment decreased ER and PR binding capacity in the cranial cervix of anoestrous ewes. This is consistent with the inhibitory effect of greater than basal circulating concentrations of P on the concentration of ER and PR, as was demonstrated in cervix during the luteal phase of oestrous cycling ewes (Rodríguez-Piñón et al., 2008). The P treatment also decreased the ERα mRNA, suggesting a down-regulatory effect of transcription, as was described in the uterus during the luteal phase of oestrous cycling ewes (Ott et al., 1993). After P priming, GnRH treatment increased the cranial cervical ER and PR binding capacity. This could be due to P withdrawal and/or the stimulatory effect of the increment in E_2 induced by the GnRH treatment previous to tissue collection (Tasende et al., 2002). In the P + GnRH treated ewes, increment of the ER binding capacity was associated with an increment in the concentration of ERα mRNA, suggesting an up-regulatory effect on transcription. However, GnRH treatment without P priming stimulated ERα transcription without having any effect on the ER binding capacity, indicating induction of the transcript – even if commonly used to predict protein concentration – do not always reflect the steady-state amounts of receptor protein (Meikle et al., 2000). The PR binding capacity was greater in the GnRH-treated ewes with or without P priming than in Groups A and P. Consistent with the concept that PR is a marker of oestrogenic action; the PR and ER binding capacities were positively correlated, indicating interdependence between the expression of the genes for both steroid receptors.

After the P + GnRH treatment, the ER binding capacity and the concentration of ERα mRNA in the cranial cervix of anoestrous ewes were greater on Day 1 than Day 5 after the GnRH bolus injection. These results are consistent with the stimulatory effect of the greater than basal concentrations of E_2 around the ovulation period (Day 1) induced by GnRH and with the inhibitory effect of P found at the early luteal phase (Day 5) as was described in uterus (Tasende et al., 2005a). The positive correlation found between concentration of ER and ERα mRNA suggests at least in part, a transcriptional control of the ER gene expression. In ewes treated with GnRH alone, no differences in the concentration of ERα mRNA and ER were found between Day 1 and 5. This could be due to the lesser concentration of circulating P in GnRH than in P + GnRH treated ewes on Day 5 (Tasende et al., 2005a), that would not be enough to down-regulate the expression of ER. The circulating P in ewes treated with GnRH alone was similar to those found in ewes with induced subnormal corpus luteum (Tasende et al., 2005a). In addition, there was no difference in the PR binding capacity from Day 1 to Day 5 after GnRH and P + GnRH treatments, probably due to a delay in the inhibitory effect of P at early luteal phase. The cranial cervical pattern of variation of the expression of genes for ER and PR was similar to that described for the uterus of anoestrous ewes treated with GnRH or P + GnRH (Tasende et al., 2002, 2005a).

Interestingly, the caudal cervix of anoestrous ewes showed non-detectable ERα transcriptional activity and lesser ER and PR binding capacity as compared with the cranial region. This finding suggests that the caudal region has diminished sensitivity and capacity of response to oestrogens and progesterone. Consequently, the P treatment had no effect on expression of genes for ER and PR, probably due to a lesser PR binding capacity, which would limit the response to P in the caudal cervix. The GnRH treatment, with or without P pre-treatment (Experiment 1), had no effect on ER and PR binding capacity in the caudal cervix, but increased the concentration of ERα mRNA at the expected time of induced ovulation. This result indicates

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an oestrogens stimulatory effect – during transcription or post-transcriptionally (Ing and Ott, 1999; Ing, 2005) – and the result is a reduced rate, or a delay in the synthesis of the ER protein. Independent of treatments in Experiment 2 of the present study, there was not any difference in ER and PR gene expression in the caudal cervix during the expected time of the induced ovulation (Day 1) and early luteal phase (Day 5). The lesser amount of steroid receptors in the caudal cervix of anoestrous ewes and its lack of response – in terms of ER and PR expression – to P and GnRH treatments differed from the results found in the cranial cervix. In oestrous cycling ewes, there were no marked differences in ER and PR gene expression in cranial and caudal cervix, except for an increase in ERα gene expression and PR binding capacity in the caudal cervix around ovulation (Rodríguez-Piñón et al., 2008). In non-pregnant cows, independent of hormonal status, immunostaining activity of ER and PR was greater in the caudal than the cranial cervix (Breeveld-Dwarkasing et al., 2000, 2002). These findings in oestrous cycling and anoestrous ruminants might indicate that the seasonal anoestrous condition of ewes differentially alters the constitutive gene expression and systemic and/or local regulatory mechanisms of steroid receptors along the longitudinal axis of the cervix.

It has been proposed that the increment in oestrogens during the follicular phase of oestrous cycling ewes is associated with the natural cervical relaxation at oestrus (Kershaw et al., 2005), through a mechanism that involve the ciclooxygenase-2 (COX-2) and prostaglandin E_2 system (Kershaw et al., 2007; Kershaw-Young et al., 2009). The increment in COX-2 mRNA (Kershaw et al., 2007), prostaglandin E receptor EP_2 mRNA and hyaluronan cervical content (Kershaw-Young et al., 2009) could induce the cervical relaxation and dilatation around the oestrus. In addition, in a hypogonadotrophic ovariectomized ewe model, the estradiol treatment increased COX-2 mRNA in the cranial but not in the caudal cervix (Kershaw-Young et al., 2010). The lesser ER and PR gene expression in the caudal region of the ovine cervix in the anoestrous condition could be related to a reduced capacity of response to steroid hormones in seasonal anoestrus. Considering the caudal region as the first and more difficult step for the transcervical canulation in ewes (Halbert et al., 1990; Kershaw et al., 2005), the present findings should be taken into account to develop hormonal treatments to increase reproductive effectiveness in AI and ET programs out of the breeding season.

In conclusion, there was greater steroid receptor gene expression in the cranial cervix in anoestrous ewes and a greater effect of treatments with GnRH, with or without progesterone priming, on ERα transcriptional activity and ER and PR binding capacity. In contrast, steroid receptors were not detected or were minimal in the caudal cervix of anoestrous ewes, and binding capacity remained unchanged during the expected time of the induced ovulation and early luteal phase. The lesser concentration of steroid receptors could limit the steroid hormone response in the caudal cervix and make it difficult for transcervical canulation in seasonally anoestrus ewes induced to ovulate.

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