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BRIEF COMMUNICATION: Uterine expression of oestrogen receptor alpha in Suffolk and Cheviot ewes at Day 19 of pregnancy, following embryo transfer

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Introduction

Whilst it is recognised that maternal uterine capacity has a marked influence on offspring birth weight and size (Walton & Hammond 1938; Allen et al. 2002; Jenkinson et al. 2007, 2012; Sharma et al. 2012), Sharma et al. (2010) demonstrated in reciprocal embryo transfer experiments that the maternal uterine environment exerts an effect on embryonic growth by Day 19 in early gestation, when maternal uterine capacity is not a limiting factor.

Regulation of the growth and development of the peri-implantation conceptus consisting of the embryo and associated extraembryonic membranes, is primarily controlled by histotrophic secretions (Satterfield et al. 2006). Progesterone from the corpus luteum is one such secretion that plays a key role in pregnancy establishment and maintenance (Clemente et al. 2009). Differences in circulating progesterone concentrations in the early post-conception period have been associated with advanced conceptus elongation in sheep (Satterfield et al. 2006). The action of progesterone is mediated through its nuclear receptor, a progesterone receptor. Sequeira et al. (2012) demonstrated that immunohistochemical localisation and semi-quantification of a PR in the deep intercaruncular stroma and deep caruncular stroma of Suffolk dams appears to be affected by embryo genotype with reduced PR expression in the deep stroma of the large genotype Suffolk dams carrying small genotype Cheviot embryos, whilst a PR in the deep intercaruncular stroma and deep caruncular stroma of Cheviot dams appeared to be unaffected by embryo genotype. Oestrogens are one of the main regulators of a progesterone receptor uterine expression. These act through oestrogen receptor α (ER α ; Meikle et al. 2004), thus making the action of ER α in endometrial tissue of particular interest.

The hypothesis of our overall study is that differences in early conceptus growth, such as those observed by Sharma et al. 2010, are due to differential signalling between the conceptus and the maternal uterine environment as reflected in the endometrial expression of specific proteins. The objective of this specific study was to examine the immunohistochemical location and semi-quantification of ER α at Day 19 (D19) of pregnancy in the endometrium of Suffolk and Cheviot dams carrying Suffolk and Cheviot embryos transferred within and reciprocally between breeds.

Materials and methods

The animals used in this study have been described previously by Sequeira et al. (2012). Briefly, Cheviot (C) and Suffolk (S) sheep breeds were used to provide genotypes with dissimilar mature body size according to previously established protocols for modifying uterine environment (Jenkinson et al. 2007; Sharma et al. 2010, 2012). The animals were maintained under commercial farming conditions at Massey University's Keeble Farm, Palmerston North, New Zealand.

Donors were four year-old ewes, whilst the recipient ewes were of mixed ages (three to six years old) and parities. Purebred-embryos were transferred using standard commercial embryo transfer procedures within and reciprocally between breeds of sheep to create four treatment groups: SinS (Suffolk embryo in Suffolk dam – large genotype control), SinC (Suffolk embryo in Cheviot dam- large genotype embryo in small genotype dam), CinS (Cheviot embryo in Suffolk dam- small genotype embryo in large genotype dam) and CinC (Cheviot embryo in Cheviot dam – small genotype control) (Sequeira et al. 2012). On D19 of gestation, nine recipient ewes from each treatment group were slaughtered and endometrial samples were taken from the maternal uterus for investigation of ER α localisation and staining intensity.

Immunoreactive ER α was visualised in transverse 5 μ m sections from the middle one third of the uterine horn ipsilateral to the corpus luteum using an avidin–biotin–peroxidase immunohistochemical technique (Meikle et al. 2000). The primary antibody used was mouse monoclonal anti-ER α (Santa Cruz, California, USA) diluted 1:25 in phosphate buffered saline. A negative control was generated by replacing the primary antibody with a homologous non-immune IgG at an equivalent concentration (Santa Cruz Biotechnology, Santa Cruz, California, USA). After primary antibody binding, sections were incubated with a biotinylated secondary antibody (horse anti-mouse IgG; Vector Laboratories, Burlingame, California, USA) diluted 1:200 in normal horse serum. A VectastainABC anti-mouse kit (Vector Laboratories, Burlingame, California, USA) was used for the protein detection. The location of the bound enzyme was visualised by 3,3-diaminobenzidine in H₂O₂ (DAB kit; Vector Laboratories, Burlingame, California, USA) and the sections counterstained with haematoxylin and dehydrated before they were mounted.

Figure 1 Example of immunohistochemical localization of (a) oestrogen receptor α (ER α) on Day 19 of gestation in maternal uterine tissue ipsilateral to the corpus luteum; and, (b) negative control lacking positive staining for ER α .

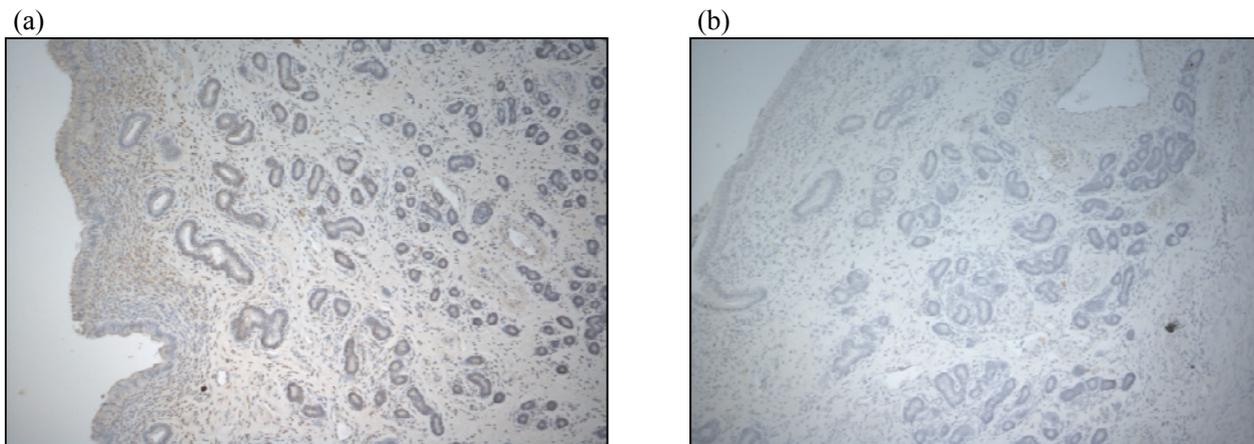
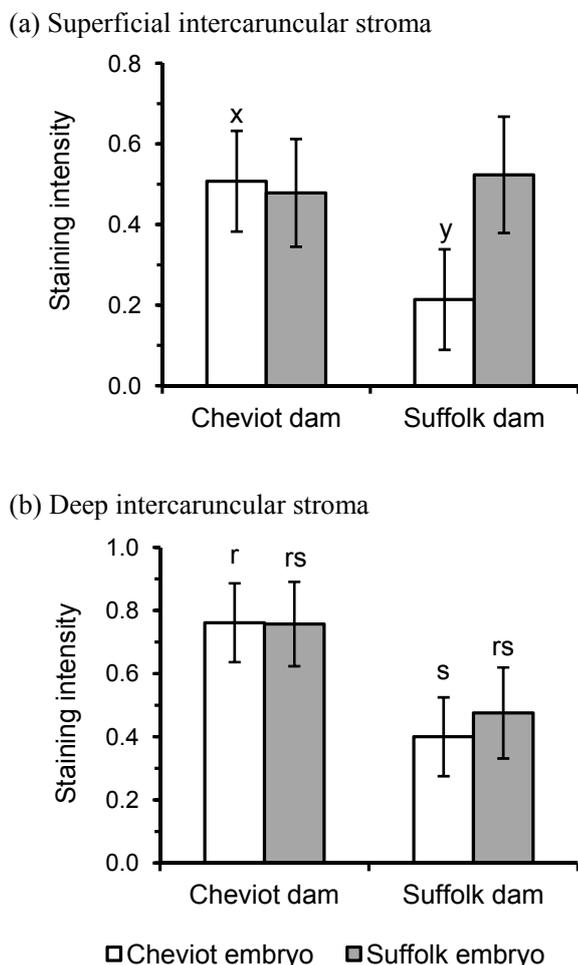


Figure 2 Endometrial staining intensity of oestrogen receptor α (ER α) in (a) superficial intercaruncular stroma and (b) deep intercaruncular stroma of Cheviot dams carrying Cheviot embryos, Suffolk dams carrying Cheviot embryos, Cheviot dams carrying Suffolk embryos and Suffolk dams carrying Suffolk embryos. Within cell type ‘r’ versus ‘s’ indicates significantly different at $P < 0.05$ and ‘x’ versus ‘y’ indicates approaching significance with a P value between 0.05 and 0.10.



Similar to the PR evaluation of Sequeira et al. (2012), ER α expression was detected in five endometrial compartments: the luminal epithelium, glandular epithelium (arbitrarily divided in two portions, the superficial glandular epithelium, next to the uterine lumen, and the deep glandular epithelium, next to the myometrium), and the intercaruncular stroma (also divided into superficial and deep regions). The amount of immunoreactive protein in the different cell types was estimated subjectively by an independent observer who was blinded to the treatment groups. Ten fields were analysed for each cell type at a magnification of $\times 1000$ for all ewes. The staining of the nuclei was scored as negative (-), faint (+), moderate (++) or intense (+++) and the extent of staining of each cell type was expressed over a scale of 0–10, where 0 is the absence of staining and 10 is the maximum staining intensity (Thatcher et al. 2003). The average staining intensity was calculated as $(1 \times n^+) + (2 \times n^{++}) + (3 \times n^{+++})$, where n is the number of cells in each field exhibiting faint (n^+), moderate (n^{++}) and intense (n^{+++}) staining (Boos et al. 1996).

Staining intensity was analysed using a mixed procedure (SAS 2008) that included the fixed effects of dam breed (S and C), embryo breed (S and C), cell type (luminal epithelium, glandular epithelium, and stroma), cell location (superficial and deep) and their interactions. Data are presented as least square means \pm pooled standard errors. Tukey Kramer tests were conducted to analyse differences among groups.

Results and discussion

Positive staining of ER α was localised to the nuclei of the endometrial cell types studied (Figure 1a). No positive staining was detected in the negative control for ER α (Figure 1b). No positive staining was observed for ER α in the luminal epithelium and very low staining was observed in the superficial glandular epithelium and deep glandular epithelium with no differences found among groups (M Sequeira, Unpublished data).

There was positive ER α staining observed in both the superficial stroma and deep stroma. Whilst the main effects of embryo and dam genotype were not significant and nor was there a significant interaction between dam genotype and embryo genotype, Tukey Kramer tests show that staining intensity of ER α in the superficial stroma tended to be higher ($P < 0.10$) in Cheviot dams carrying Cheviot embryos (CinC) compared with Suffolk dams carrying Cheviot embryos (CinS) (Figure 2). Additionally staining intensity of ER α in the deep stroma was greater ($P < 0.05$) in Cheviot dams carrying Cheviot embryos (CinC) compared to Suffolk dams carrying Cheviot embryos (CinS) (Figure 2).

The positive ER α staining observed in the superficial stroma and deep stroma, coupled with the lack of ER α staining in the luminal epithelium and the low levels of ER α staining in the superficial glandular epithelium and deep glandular epithelium, supports findings from previous studies in ruminants that show ER α receptor staining becoming undetectable or low around D11 to D13 of pregnancy in the uterine luminal epithelium and glands respectively, while it remains present in the stroma (Spencer et al. 1995; Kimmins & MacLaren 2001; Sosa et al. 2009). Low staining intensity is indicative of reduced receptor expression, thus it appears that large genotype Suffolk dams carrying a small genotype Cheviot embryo have reduced intercaruncular stromal ER α expression compared to Cheviot dams carrying a Cheviot embryo (small control). Interestingly, it was in the CinS group that Sharma et al (2010) observed increased embryo length and heart bulge width, suggesting that uterine sensitivity to oestrogens may be negatively associated with embryo growth.

In the same endometrial tissue as that investigated in the study reported here, Sequeira et al. (2012) found that at D19 of gestation there was a reduced expression of a progesterone receptor in the deep intercaruncular stroma of CinS when compared to all other groups, indicative of reduced PR expression and reduced stromal sensitivity to progesterone. In conjunction with the results found by Sequeira et al. (2012) and those reported here, the lower progesterone receptor and ER α expression in the endometrial intercaruncular stroma in Suffolk dams carrying Cheviot embryos (CinS) compared to Cheviot dams carrying Cheviot embryos (CinC), may be associated with the increased embryo growth observed by Sharma et al. (2010) in their similar Cheviot-Suffolk reciprocal embryo transfer experiment.

This study demonstrates that Suffolk dams carrying Cheviot embryos appear to have reduced uterine ER α expression compared to Cheviot dams carrying Cheviot embryos, however it remains unclear if this is due to altered signalling from the Cheviot conceptus. Uterine expression of ER α in Cheviot dams appears to be unaffected by embryo type. The mechanisms via which the maternal uterine environment regulates early embryonic growth are

complex and a better understanding of uterine-conceptus interactions is necessary to identify those mechanisms that promote successful implantation and enhance the growth and viability of the conceptus.

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