BRIEF COMMUNICATION: Expression of uterine progesterone receptor in Suffolk and Cheviot ewes at Day 19 of pregnancy, following embryo transfer

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Introduction

Many animal studies have shown that uterine capacity has a marked influence on birth weight and size (Walton & Hammond 1938; Allen et al. 2002; Jenkinson et al. 2007; Sharma et al. 2012). However, in reciprocal embryo transfer experiments, Sharma et al. (2010) demonstrated that the maternal uterine environment can also regulate embryonic growth very early in gestation when uterine capacity is not a limiting factor. Sharma et al. (2010) showed that pure-bred Suffolk (large genotype) embryos were shorter and narrower when developing in Cheviot (small genotype) dams, compared to pure-bred Suffolk embryos developing in Suffolk dams. Further, pure-bred Cheviot embryos were longer and wider when developing in Suffolk dams, compared to pure-bred Cheviot embryos developing in Cheviot dams. Sharma et al. (2010) also found that Suffolk embryos developing in Cheviot dams had fewer trophoblastic binucleate cells and these cells tended to be smaller than those of Suffolk embryos developing in Suffolk dams. However, Cheviot embryos developing in Suffolk dams tended to have more trophoblastic binucleate cells and these were larger compared to Cheviot embryos developing in Cheviot dams. Sharma et al. (2010) suggested that the trophoblast mediates conceptus-maternal interactions which influence the development of the peri-implantation embryo.

The growth and development of the peri-implantation conceptus, which includes the embryo and associated extraembryonic membranes, is primarily regulated by uterine secretions collectively referred to as histotroph (Satterfield et al. 2006). Differences in early embryonic growth may, in part, be explained by differential signalling between the embryo and its uterine environment due to increased or decreased numbers and/or specificity of receptors. Progesterone plays a key role in pregnancy establishment and maintenance (Clemente et al. 2009) and high concentrations of circulating progesterone in the immediate post-conception period have been associated with an advancement of conceptus elongation in sheep (Satterfield et al. 2006). The action of progesterone is mediated through its nuclear progesterone receptor (PR).

The objective of this preliminary study was to examine the expression of PR at Day 19 (D19) of pregnancy in the endometrium, particularly in the stroma, 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 were maintained under commercial farming conditions at the Massey University Keeble Farm, Palmerston North, New Zealand.

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; Sharma et al. 2012).

All donors were four year-old ewes, the recipient ewes were of mixed ages (three to six year-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) (n = 16), SinC (Suffolk embryo in Cheviot dam – large genotype embryo in small genotype dam) (n = 17), CinS (Cheviot embryo in Suffolk dam – small genotype embryo in large genotype dam) (n = 17) and CinC (Cheviot embryo in Cheviot dam – small genotype control) (n = 18). On D19 of gestation, nine ewes from each treatment group were sacrificed and endometrial samples taken from the maternal uterus for investigation of PR localisation and staining intensity.

Immunoreactive PR were visualised in transverse 5 μm thick 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-PR (Zymed, South San Francisco, CA, USA) diluted 1:100 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, CA, USA). After primary antibody binding, sections were incubated with a biotinylated secondary antibody (horse anti-mouse IgG; Vector Laboratories, Burlinham, CA, USA) diluted 1:200 in normal horse serum. A VectastainABC anti-mouse kit (Vector Laboratories,

Burlinham, CA, USA) was used for the protein detection. The location of the bound enzyme was visualised by 3,3-diaminobenzidine in H_2O_2 (DAB kit; Vector Laboratories Burlinham, CA, USA) and the sections counterstained with haematoxylin and dehydrated before they were mounted.

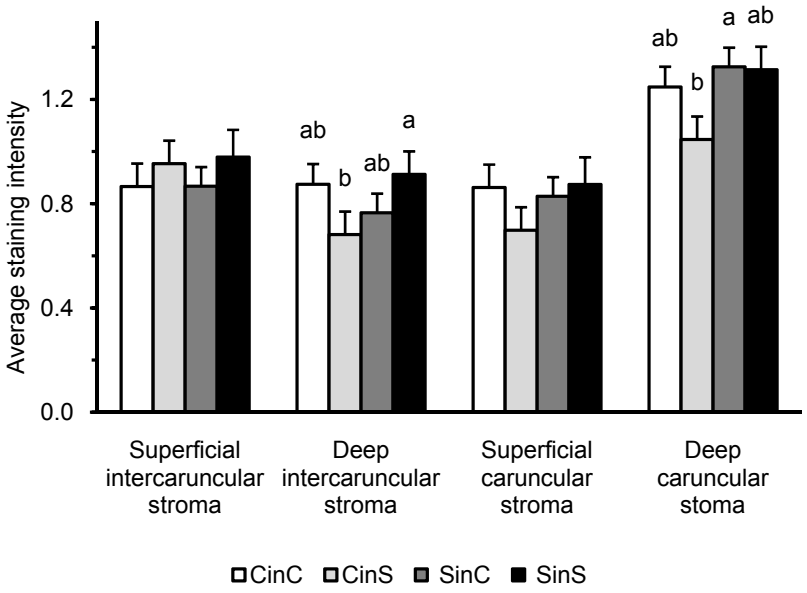
PR expression was evaluated immunohistochemically in seven endometrial compartments, namely the luminal epithelium, glandular epithelium (arbitrarily divided in two portions, the superficial glandular epithelium, next to the uterine lumen, and the deep glandular epithelium, next to the myometrium), and the caruncular stroma (divided into superficial (SCS) and deep (DCS) regions), and the intercaruncular stroma (also divided into superficial (SIS) 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 ×1000 for all ewes. The staining of the nuclei was scored as being negative (−), faint (+), moderate (++) or intense (+++) if nuclei exhibited no, light brown, brown or dark brown tinction, respectively. The frequencies of different staining intensities (-, +, ++, +++) within the various endometrial cell types were assessed and expressed on a scale of 0–10 (Thatcher et al. 2003), where 0 was an absence of staining and 10 was maximum (100%) staining. The average staining intensity for each cell type was calculated as $(1 \times n^{+}) + (2 \times n^{++}) + (3 \times n^{+++})$, where n is the proportion of cells per field exhibiting faint (n^{+}), moderate (n^{++}) and intense (n^{+++}) staining (Boos et al. 1996).

Staining intensity was analysed using a mixed procedure (SAS 2002) that included the 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.

Results and discussion

No positive staining was detected in the negative control. No positive staining was observed in the luminal epithelium. Low levels of gland staining were observed in the superficial glandular epithelium and deep glandular epithelium but with no differences in staining intensity between the treatment groups (SinS, CinS, SinC, CinC) (SJ Pain, Unpublished data). These results support findings from previous studies in ruminants (Kimmin & MacLaren 2001; Spencer et al. 2004; Sosa et al. 2009) that show after approximately D11 to D13 of pregnancy PR staining becomes low or undetectable in the uterine luminal and glandular epithelium and is only present in the stroma.

Figure 1 Staining intensity of progesterone receptor (PR) in different uterine endometrial stroma (superficial and deep intercaruncular stroma and superficial and deep caruncular stroma) from Cheviot dams carrying Cheviot embryos (CinC); Suffolk dams carrying Cheviot embryos (CinS); Cheviot dams carrying Suffolk embryos (SinC); and Suffolk dams carrying Suffolk embryos (SinS). Within cell type, different superscript letters indicate $P < 0.05$. Error bars indicate the standard error of the mean.



There was positive PR staining observed in both the superficial and deep stroma. No differences ($P > 0.05$) in staining intensity were found between treatment groups within either the superficial intercaruncular or caruncular stroma. However, treatment group differences were found in the PR staining intensity in both the deep caruncular and intercauncular stroma. CinS had reduced ($P < 0.05$) PR staining intensity in the deep intercaruncular stroma and tended to show reduced ($P < 0.10$) PR staining intensity in the deep caruncular stroma, compared to SinS (Fig. 1). There were no differences observed between CinC or SinC in either the deep intercaruncular stroma or the deep caruncular stroma. CinS tended ($P < 0.10$) to show reduced PR staining intensity in the deep intercaruncular stroma compared to CinC. This trend was not observed in the deep caruncular stroma. No differences were observed in PR staining intensity between SinC or SinS in either the deep intercaruncular stroma or the deep caruncular stroma.

Lower staining intensity is indicative of reduced PR expression. Exposure of the uterus to progesterone in very early pregnancy causes down regulation of PR expression (Spencer et al. 2004) and a loss of PR from the uterine luminal epithelia before

implantation (Spencer et al. 1995), as was observed in the current study.

This study has demonstrated that expression of PR in the deep intercaruncular stroma and deep caruncular stroma of Suffolk dams appears to be affected by embryo type with reduced PR expression in the deep stroma of the CinS. It remains unclear if this reduced PR expression is due to altered signalling from the Cheviot conceptus. Expression of PR in the deep intercaruncular stroma and deep caruncular stroma of Cheviot dams appears to be unaffected by embryo type. Further investigation of uterine gene expression is needed, and embryos and trophoblasts collected from the current study will also be assessed for gene expression. Better understanding of uterine-conceptus interactions is necessary to identify those genes and pathways associated with creating a uterine environment that promotes successful implantation and growth of the conceptus.

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