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Progesterone exposure of the preovulatory follicle in the seasonally anestrous ewe alters the expression of angiogenic growth factors in the early corpus luteum

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Abstract

Gonadotrophin releasing hormone (GnRH)-induced ovulation in seasonally anestrous ewes is associated with a high incidence of defective corpora lutea (CL), which can be completely eliminated by priming ewes with progesterone before GnRH treatment, but the physiological basis of this has remained elusive. This study tested the hypothesis that progesterone priming eliminates defective luteal function by altering the expression of Vascular Endothelial Growth Factor (VEGF), its receptor VEGFR-2, and angiopoietin (ANG)-1, ANG-2 and their receptorTIE-2 in the early CL. Fifteen seasonally anestrous ewes were treated by i.m. injection with 20 mg of progesterone 3 days before the start of GnRH treatment, while another 15 animals served as controls. Intravenous injections of 500 ng GnRH were given to all the ewes every 2 h for 28 h, followed by a 300 μg GnRH bolus injection to synchronize the preovulatory luteinizing hormone (LH) surge. Corpora lutea were collected 1, 2 and 4 days after ovulation and analyzed for protein and mRNA expression of VEGF, VEGFR-2, ANG-1, ANG-2 and Tie-2 using Western Immunoblotting and in situ hybridization. VEGF, VEGFR-2 and ANG-1 expression was significantly higher ($P \leq 0.05$) in the CL of progesterone-primed animals compared to non-primed ones. However, no differences were observed in the ANG-2 or Tie-2 expression levels between the two treatment groups. These data suggest that progesterone priming of the preovulatory follicle alters the expression of some angiogenic growth factors in the early CL, leading to greater vascular stability and thereby normal luteal function.

Keywords: Progesterone; GnRH; Angiogenic growth factors; Corpora lutea; Sheep

1. Introduction

Infertility is a major issue in both human and animal medicine [1]. Pregnancy failure has important economic consequences in farm animals, and in human IVF/ET programs high wastage of embryos increases both distress to patients and cost of the treatment. The lack of extensive understanding of the factors involved in follicular maturation and luteal development [2] is an obvious limitation for the development of fertility protocols and innovative strategies to increase reproductive efficiency in both humans and livestock [1,3,4]. Transient inadequate luteal function is one of the main causes of subfertility that occurs naturally in ruminants at puberty, the early *post-partum* period and during the

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transition from seasonal anestrus to the breeding season [5,6].

Seasonally anestrous ewes induced to ovulate with small dose multiple injections of gonadotrophin releasing hormone (GnRH) have been widely used as a model for the study of luteal dysfunction [7]. The abnormal corpus luteum (CL) often formed in most of such animals [8,9] results in an increase in plasma progesterone concentrations between Days 1 and 4 after ovulation similar to that of functional CL, before returning to baseline levels by Day 6 [10]. Defective and functional CL are morphologically indistinguishable soon after ovulation but differences become apparent on Day 5 after ovulation [9,10], with defective CL showing reduced growth and signs of early regression [11]. However, progesterone pretreatment administered before the start of the GnRH injections results in normal luteal function in all the animals [12–14].

The exact mechanism by which progesterone pretreatment ensures normal luteal function has not yet been resolved. Previous studies do not indicate any differences in luteal weight or luteinizing hormone (LH) receptor content between functional and defective CL up until the period immediately preceding premature regression [9,10,15]. Moreover, no differences were observed between animals with normal and defective CL regarding the timing of the LH surge and therefore the time period for which the preovulatory follicles were exposed to pulsatile LH secretion [9,15–17]. Furthermore, neither synchronization of follicular development before GnRH treatment [18] nor hysterectomy completely eliminates the subnormal luteal function in non-primed animals [8]. Collectively, these studies suggest that the early demise of CL is neither a function of premature generation nor increased sensitivity to uterine $PGF_{2\alpha}$, rather it is suggestive of defective luteinization and hence establishment of the mature CL.

A high correlation between systemic levels of progesterone and blood flow to the luteal ovary suggests that an adequate vascular supply is important for the luteal function [19] and an inadequate supply in the growing CL may result into defective luteal function [8,20,21]. Development of the vascular bed in the early CL depends on the formation of new blood vessels from already existing ones [22–24]. Follicular blood supply is confined to the theca layer and it is only at ovulation that thecal-derived pericytes and endothelial cells migrate into the granulosal region to form the capillary bed of the developing CL [25]. Subsequent CL development is a rapid process [26] and requires an extensive vascular network to be established to adequately support the morphologic and functional changes taking place soon after ovulation [27,28].

Many factors have been implicated in the regulation of angiogenesis, including vascular endothelial growth factor (VEGF) and the angiopoietins [22,29]. VEGF is responsible for stimulating endothelial cell proliferation and migration, maintaining immature blood vessel viability [30] and ensuring endothelial cell survival through the phosphorylation of its main receptor VEGFR-2 (Flk-1) [31]. The angiopoietins (ANG)-1 and -2 act in association with VEGF to regulate blood vessel formation and maturation [32]. ANG-1 regulates vascular maturation [32] while ANG-2 destabilizes existing vessels [33]. Both angiopoietins bind to the Tie-2 receptor, and ANG-2 blocks ANG-1 activity by competing for the same receptor [34]. The ANG-2: ANG-1 ratio is used as an index of vascular stabilization in conjunction with VEGF levels [35]. When ANG-2: ANG-1 ratio and VEGF levels are high, new blood vessel networks are formed. However, when ANG-2: ANG-1 ratio is high but VEGF levels are low, regression of blood vessels happens and low ANG-2: ANG-1 ratio along with relatively low levels of VEGF result in stabilization of blood vessels [36].

From the studies cited above it seems that VEGF and angiopoietins play an essential role throughout luteal development, stimulating proliferation, maturation and finally regression of luteal blood vessels [24,30,32,34]. The fact that progesterone priming ensures normal luteal function in seasonally anestrous ewes induced to ovulate with small dose multiple injections of GnRH, led us to hypothesize that progesterone priming of the preovulatory follicle eliminates subsequent defective luteal function by altering the expression of VEGF, VEGFR-2, ANG-1, ANG-2 and Tie-2 in the early CL. To test this hypothesis corpora lutea from both progesterone primed and non-primed seasonally anestrous ewes, induced to ovulate with small dose multiple injections of GnRH, were compared for the expression of the afore-mentioned angiogenic growth factors.

2. Materials and methods

2.1. Animals and treatments

The experiment was conducted in August 2006 during the non-breeding season at Aberystwyth University, UK (latitude 52°25′ N) on 30 adult seasonally anestrous Welsh Mule ewes (*Ovis aries*). These ewes