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Review

The significance of estradiol metabolites in human corpus luteum physiology

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ABSTRACT

The human corpus luteum (CL) is a temporary endocrine gland derived from the ovulated follicle. Its formation and limited lifespan is critical for steroid hormone production required to support menstrual cyclicity, endometrial receptivity for successful implantation, and the maintenance of early pregnancy. Endocrine and paracrine-autocrine molecular mechanisms associated with progesterone production throughout the luteal phase are critical for the development, maintenance, regression, and rescue by hCG which sustains CL function into early pregnancy. However, the signaling systems driving the regression of the primate corpus luteum in non-conception cycles are not well understood. Recently, there has been interest in the functional roles of estradiol metabolites (EMs), mostly in estrogen-producing tissues. The human CL produces a number of EMs, and it has been postulated that the EMs acting via paracrine-autocrine pathways affect angiogenesis or LH-mediated events. The present review describes advances in understanding the role of EMs in the functional lifespan and regression of the human CL in non-conception cycles.

1. Introduction

The secretion of estradiol (E_2) throughout the ovarian cycle in women depends upon follicle recruitment, selection of a single dominant follicle, followed by the LH surge that ends the program of FSH-dependent steroidogenesis in granulosa cell (GCs) and initiates the differentiation of follicular cells into granulosa lutein and theca lutein cells. The two cell, two gonadotropin model of E_2 biosynthesis in the preovulatory follicle encompasses androgens production by theca cells followed by their aromatization by P450arom in GCs of the follicle. The theca lutein and granulosa lutein cells (GLCs) of the human CL carry out similar steroidogenic functions to generate E_2 in the CL [1].

Ovarian estrogens have a variety of biological effects on different human tissues, and many of these effects are mediated by nuclear E_2 receptor (ER) [2,3]. Estradiol is metabolized by diverse metabolic pathways including hydroxylation, glucuronidation, sulfonation and methylation to form estrogen metabolites (EMs). These transformations take place mainly in the liver and specific extra-hepatic tissues e.g., (brain, kidney, ovaries, and testis). While a number of the EMs are inactive, others have important effects on the physiology of different tissues, particularly in E_2 producing tissues, acting through diverse pathways not associated with classical nuclear ER. [4,5]. It is important to emphasize that the human CL produces high levels of E_2 (leading to blood levels of 150–200 pg/ml) during mid-luteal phase [6], and expresses cytochrome P4501A1 (CYP450) and catechol-O-methyl transferase (COMT), such that luteal E_2 synthesized by GLCs could be converted into multiple EMs catechol and methoxy estrogens [7–9].

The prominent role of the human CL in the elaboration of steroid hormones requires a complex vascular network established through neovascularization after ovulation [10,11]. The capillary expansion requires endothelial cell proliferation, which is associated with a decreasing resistance index to blood flow as assessed by traditional Doppler ultrasound [12]. However, conventional Doppler does not quantify blood flow accurately through small capillary structures such as the network formed in the CL. Use of contrast reagents combined with destroy-replenishment imaging dynamic contrast-enhanced ultrasound (DCE-US) increases the accuracy of blood flow assessment and blood volume measurements in the CL of primates and confirms the low resistance index of early and mid-luteal phase CL [13]. The vascular features of human luteal regression include increased in blood flow impedance [12]. Based on these findings, and the known action of catecholestrogens, 4-hydroxyestrone (4-OHE_1) and 2-hydroxyestradiol (2-OHE_2), which are pro-angiogenic [14,15]; and methoxyestrogens, 2-methoxyestradiol (2-ME_2) and 2-methoxyestrone (2-ME_1), which are

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anti-angiogenic [16–19], we examined the potential role of EMs in the regulation of angiogenesis in the human CL.

2. Laboratory and clinical trials of the investigation

Human CL were collected at mini-laparotomy from women aged 30–33 years who requested surgical sterilization at our institution. The participants were healthy with normal body mass index an regular menstrual cycles. The surgical procedure was scheduled at varying times during the luteal phase. The day of ovulation and the histological criteria were used to confirm the age of the CL as previously reported [20].

Laboratory techniques: A biopsy of CL tissue for histology and immunohistochemistry was fixed in paraformaldehyde [21]. Another portion of CL (100 mg) was homogenized, and steroid extraction was performed with ethyl-acetate. The EMs were quantified by high performance liquid chromatography-mass spectrometry [22].

3. 4-Hydroxyestrone (4-OHE_1) and 16-ketoestradiol (16-ketoE$_2$) in the human corpus luteum throughout the luteal phase

Angiogenesis in the early CL has its origins in the vasculature of the developing follicle. Capillary expansion appears to be related to endothelial cell proliferation, and the vast majority of dividing cells in the early developing CL are microvascular endothelial cells [10,11]. It is thought that the mid-cycle LH surge increases VEGF production by the GLC in the developing CL [23]. Concomitant with these vascular changes, we found that 4-OHE_1 and 16-ketoE$_2$ concentrations in the human early and mid-luteal phase CL are significantly greater than in late luteal phase CL. Interestingly, *in vivo* administration of hCG in the late luteal phase increased significantly the luteal tissue levels of 4-OHE_1 and 16-ketoE$_2$, observations that are consistent with a paracrine role of EMs in angiogenesis or maintenance of the capillary network in the CL (Fig. 1A) [24].

To expand our understanding of pro-angiogenic activities of these EMs, we measured the VEGF concentrations in conditioned media (CM) from GLCs cultures in the presence of physiological concentrations of 4-OHE_1 and 16-ketoE$_2$, showing that both EMs increased significantly VEGF secretion by GLCs and the pro-angiogenic potential of the CM (Fig. 1B and C). These findings suggest an important role for these EMs in the formation and development of the CL [24].

It is important to mention that local factors such as insulin-like growth factors (IGF)-1 and -2 may synergize with LH to promote VEGF production [25]. However, the usual stimulator of VEGF production in most tissues is hypoxia [26]. The hypoxic microenvironment that occurs in rapidly differentiating tissues like the human CL is a major contributor to its ability to survive via the induction of an intricate vascular network. Cellular responses to hypoxia are mediated by hypoxia inducible factor-1A (HIF-1A), a heterodimer consisting of a constitutively-expressed β subunit an oxygen-regulated α subunit, which binds to the hypoxia responsive cis elements present in the promoter regions of responsive genes [27]. Several genes are critical for the angiogenic process in the developing CL: vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF-2), prokinectin receptor 2 (PK-R2), and endothelin-2 [28].

4. 2-Methoxyestradiol in the human corpus luteum throughout the luteal phase

2-Methoxyestradiol (2-ME$_2$) is a biologically active metabolite of E$_2$ that has antiangiogenic and anti-proliferative effects [16–19]. Its anti-angiogenic actions are believed to be the result of inhibition of HIF-1A action [29]. In the non-fertile cycle, the CL of the human undergoes a process of regression known as luteolysis, which encompasses loss of functional and structural integrity of the gland [30]. The molecular events involved in human luteal regression in non-conception cycle remain to be elucidated. It is thought that a decline in LH levels and LH receptor mRNA do not account for luteal regression in primates [31], but that regression of the CL is determined by factors downstream from the LH receptor. Several molecule including prostaglandin PGF2-α, TNF-α, IL-1β, estrogens, and reactive oxygen's species have been implicated in the luteolytic process [1–32]. In exploring the roles of

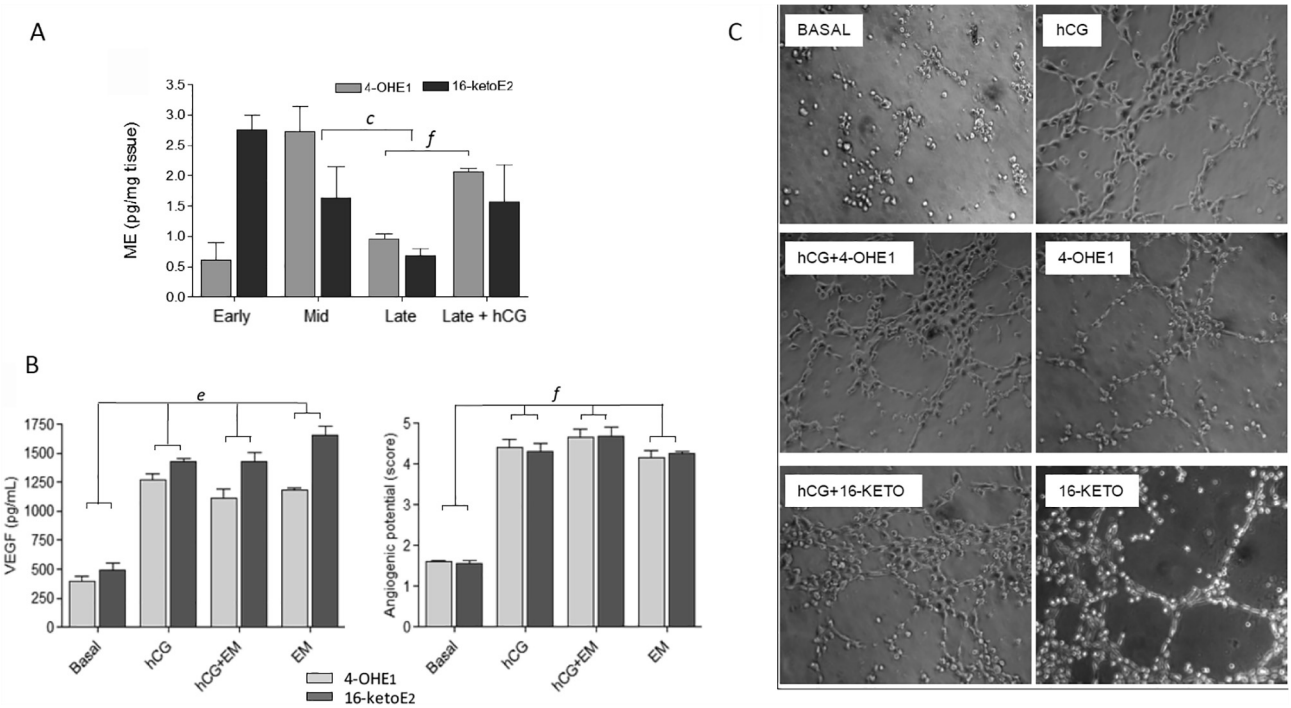

Fig. 1. Tissue levels of 16-ketoE$_2$ and 4-OHE_1 in the CL throughout the luteal phase and after administration of hCG. Effect on VEGF production and angiogenic activity by GLCs cultures. Panel A, late 16-ketoE$_2$ and 4-OHE_1 v/s mid and late plus hCG ($^{c,f}P < 0.05$) n = 5 per CL stage. (B) 16-ketoE$_2$ and 4-OHE_1 increased VEGF production by LGCs compared to basal conditions ($^{e}P < 0.05$). (C) Photomicrograph of the angiogenic assay, both EM increased tube formation compared to basal conditions ($^{f}P < 0.05$).