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Multiple roles of hypoxia in bovine corpus luteum

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Abstract. There has been increasing interest in the role of hypoxia in the microenvironment of organs, because of the discovery of hypoxia-inducible factor-1 (HIF1), which acts as a transcription factor for many genes activated specifically under hypoxic conditions. The ovary changes day by day during the estrous cycle as it goes through phases of follicular growth, ovulation, and formation and regression of the corpus luteum (CL). These phenomena are regulated by hypothalamic and pituitary hormones, sex steroids, peptides and cytokines, as well as oxygen conditions. Hypoxia strongly induces angiogenesis via transcription of a potent angiogenic factor, vascular endothelial growth factor (VEGF), that is regulated by HIF1. A CL forms with a rapid increase of angiogenesis that is mainly induced by HIF1-VEGF signaling. Hypoxia also contributes to luteolysis by down-regulating progesterone synthesis and by up-regulating apoptosis of luteal cells. This review focuses on recent studies on the roles of hypoxia- and HIF1-regulated genes in the regulation of bovine CL function.
Key words: Corpus luteum, Hypoxia, Hypoxia-inducible factor-1 (HIF1), Luteal formation, Luteolysis

Introduction

The corpus luteum (CL) develops after ovulation and is accompanied by active angiogenesis. Its primary role is the establishment and maintenance of pregnancy in mammals. When conception does not occur, the CL regresses with a decrease in progesterone (P4) synthesis and increased apoptosis of luteal cells. Variable blood flow to the ovary during the ovarian cycle [1] causes changes in the transport of nutrients, hormones, and gases, including O_2, to the ovary. Ovarian blood flow in cows decreases during luteal regression and is kept at low levels during luteal formation after ovulation [1]. Thus, during luteal regression and formation, the intra-luteal environment is characterized by low oxygen (hypoxic) conditions due to decreased blood supply.

The discovery that cellular responses to hypoxic conditions are mainly regulated by hypoxia-inducible factors (HIFs) was the basis of the study that won the 2019 Nobel Prize in Physiology or Medicine [2]. HIFs are hypoxia-specific transcription factors. HIF1 was first identified as an inducer of erythropoiesis in the kidney [2, 3] and was later found to be involved in inducing many physiological processes including angiogenesis, glycolysis, apoptosis and autophagy [4]. Recently, it was discovered to be a factor in the regulation of ovarian functions [5, 6].

This review focuses on the roles of hypoxia and HIF signaling in the development and death of the CL in cattle.

Luteinization

Luteinization is the process by which the follicular granulosa and theca cells are transformed into luteal cells. It begins in the developing follicle, whose interior is under hypoxic conditions [7–10]. Moderate hypoxia, such as the hypoxia in the peri-ovulatory follicles, has been shown to stimulate P4 production in granulosa cells (GCs) [11–13]. Culturing murine GCs [12] and bovine luteinized GCs [11, 13] under 10% O_2 conditions increased the transcription of steroidogenic acute regulatory protein (STAR) as well as production of P4. In mice, HIF1-induced autophagy was found to be vital for GC proliferation through the selective degradation of damaged mitochondria during follicle-stimulating hormone (FSH) -mediated follicular development [14, 15]. This autophagy-related action has been observed in luteinization as well as luteal formation after ovulation [16]. BCL2/adenovirus E1B 19 kilodalton protein-interacting protein 3 (BNIP3) is a cell death factor that induces autophagy of the mitochondria (mitophagy) [17]. The finding that BNIP3 is abundantly expressed in the early CL supports the idea that hypoxia-induced autophagy is necessary for luteinization and luteal formation [16]. These studies suggest that the hypoxia generated in the developing follicle induces luteinization by up-regulating P4 production in GCs and this luteinization continues during early luteal formation after ovulation.

Luteal Formation

VEGF
Vascular endothelial growth factor (VEGF) – a strong angiogenic factor [18] – is the most essential factor for angiogenesis during luteal formation [5, 8, 19–22]. Soon after HIF1 was discovered [23],

it was found that it profoundly induced the transcription of VEGF [24]. The early luteal tissue just after ovulation is presumed to be under hypoxic conditions, since the vasculature and the structure of follicular wall are destroyed by ovulation [25] and the blood flow to the ovary is low around the time of ovulation [1]. In bovine luteal endothelial cells, the mRNA expressions of the α subunit of HIF1 (HIF1A) and VEGF were not significantly different between normoxic (20% O_2) and hypoxic (1% O_2) cultures [26]. In the porcine CL, *HIF1A* mRNA expression was high at the early luteal stage suggesting that HIF1 assists in luteal formation [27]. The amount of HIF1 inside the cells is tightly regulated at the protein level [28]. Under normoxic conditions, HIF1A protein is rapidly degraded by the ubiquitin-proteasome pathway. In contrast, hypoxic conditions slow the degradation of HIF1A, leading to its accumulation. Consequently, HIF1 forms a dimer with the β subunit (HIF1B, also called aryl hydrocarbon receptor nuclear translocator; ARNT) to become a functional transcription factor [28]. Immunostaining of the primate ovary showed that HIF1A protein was highly expressed in the early CL in the primate ovary [29]. HIF1A protein expression was also high in the early and developing bovine CL, and along with VEGF protein, was significantly up-regulated under hypoxic conditions (3% O_2) [30]. These studies suggest that the vascular and structural changes caused by ovulation lead to an acute oxygen shortage locally in follicular walls, which, in turn, strongly activates HIF1. This induces the transcription of VEGF, which is necessary for angiogenesis that occurs during luteal formation.

BNIP3

BCL-2 family proteins are well known regulators of apoptosis. BNIP3 is a member of this family that was first identified as an apoptosis promoter [31]. Subsequently, BNIP3 was found to regulate the induction of autophagy, especially mitophagy [17]. Recently, BNIP3-related autophagy was discovered in the ovarian functions. In the murine ovarian follicles, FSH induced the autophagy of murine GCs via HIF1, the latter being necessary for follicular development and atresia [15]. BNIP3 expression was also detected in bovine follicles and CLs and was found to be up-regulated by hypoxic conditions in GCs and luteal cells [16]. BNIP3 has also been suggested to have roles before ovulation, because of its increased expression in the GCs of bovine large follicles [16]. Furthermore, in the bovine CL, BNIP3 expression at the early luteal stage is much higher than that at other luteal stages. These results suggest that BNIP3 regulates mitophagy and autophagy in the early CL in order to form and establish the CL. They also suggest that the activation of BNIP3 is induced by HIF1 and hypoxia that occur during ovulation.

GLUT1

HIF1 is also known to induce genes related to glycolysis, one of which is the facilitative glucose transporter 1 (GLUT1) [4]. The expression of GLUT1 in CL has been reported in canine [32] and bovine [33, 34] ovaries. In the canine CL, GLUT1 expression was positively correlated with the plasma P4 concentration and expression of HIF1A [32]. In the bovine CL, GLUT1 is expressed throughout the estrous cycle [33] and is most highly expressed at the early luteal stage [34], when the expression of HIF1A is also high [30]. Culturing bovine luteal cells under hypoxic conditions induced GLUT1

expression, while inhibiting GLUT1 decreased P4 production [16]. These results support the idea that GLUT1 is needed for luteal P4 production, which luteal cells need to take up glucose. They also support the idea that GLUT1 expression at the early luteal stage is induced by HIF1, after HIF1 is activated by the hypoxic conditions during ovulation.

Luteal Regression

Functional luteolysis

Luteal regression is characterized by a decrease in P4 production (functional luteolysis), followed by a decrease in luteal size (structural luteolysis), which is largely achieved by apoptosis [35–39]. In cows, ovarian blood flow (as measured by electromagnetic probes) is low just after ovulation, increases gradually toward the luteal stage, and then decreases during luteal regression [1, 40]. Measurements with color-doppler ultrasound also show that intra-luteal blood flow decreases simultaneously with the decrease in plasma P4 concentrations during luteolysis, suggesting that the decreased blood supply is related to functional luteolysis in cows [41, 42]. Vascular occlusion was found to occur following the sloughing of endothelial cells into the lumina of small blood vessels during luteolysis, suggesting that vascular occlusion is the cause of the decreased blood supply and hypoxic conditions in the CL [43]. We found that hypoxic conditions decreased P4 production in mid luteal cells by inhibiting the expression and activity of the enzyme P450scc (cytochrome P450 side-chain cleavage enzyme), which converts pregnenolone into P4 by cleaving the side-chain [44]. This inhibitory effect was evident in the mid CL, while it was not detected in the early CL [45]. Hypoxia has been suggested to inhibit the process of side-chain cleavage of cholesterol, since molecular oxygen is required for this process [46], and the importance of oxygen has been reported in different types of cells [47, 48]. Hypoxia is also known to generate reactive oxygen species in mitochondria, thereby damaging the mitochondria [49]. The damage to mitochondria could be one of the reasons for the hypoxia-induced inhibition of P450scc activity by hypoxia. Because the expression of HIF1A in bovine CL is low during the regressed stage [30], hypoxia-induced functional luteolysis seems to occur without the activation of HIF1. However, the relationship between P450scc activity and HIFs is not known, and needs to be explored in future studies.

Structural luteolysis

Apoptosis, which is essential for structural luteolysis [36], was also induced in cultured luteal cells under hypoxic conditions. In cultured luteal cells, hypoxic conditions induced caspase-3 – an effector caspase in the apoptotic cascade [50]. BNIP3, which facilitates apoptosis [31] and mitophagy [17] under hypoxic conditions, was also induced in bovine luteal cells by hypoxia. These findings suggest that the oxygen deficiency in the CL is one of the factors that accelerate luteolysis principally induced by uterine prostaglandin F2α and other luteolytic factors, such as cytokines, peptides and gases [37].

Conclusion

The findings cumulatively suggest that hypoxia plays multiple
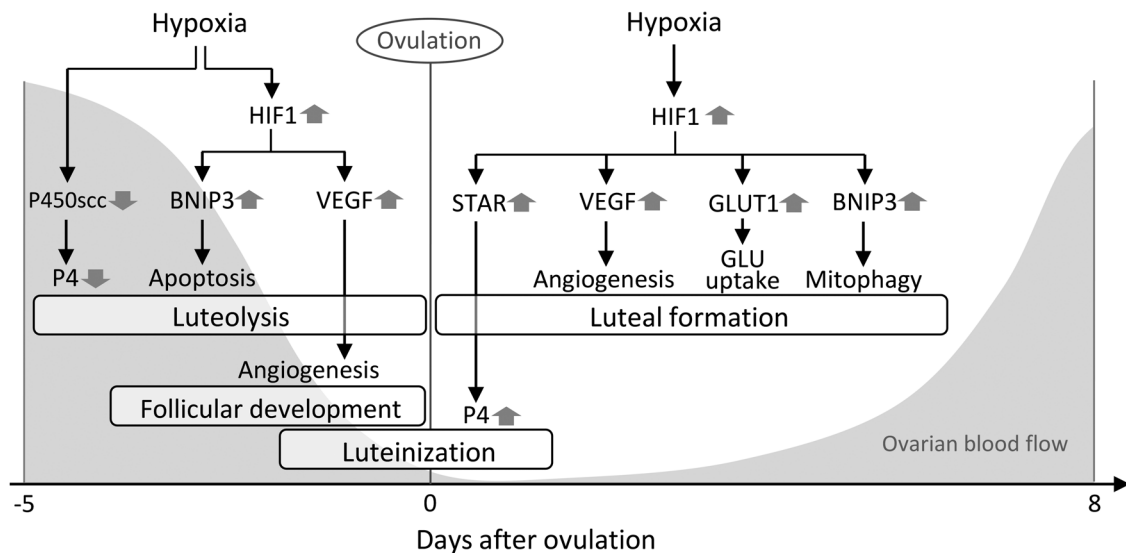
Fig. 1. Possible roles of hypoxia in the ovary. Hypoxia generates multiple signals related to several ovarian processes. <Before ovulation> 1) In luteolysis, hypoxia down-regulates progesterone (P4) production (functional luteolysis) via cytochrome P450 side-chain cleavage enzyme (P450scc) inhibition and induces luteal cell apoptosis (structural luteolysis) via hypoxia-inducible factor-1 (HIF1)-BCL2/adenovirus E1B 19 kilodalton protein-interacting protein 3 (BNIP3) signaling. 2) In follicular development, hypoxia induces angiogenesis via HIF1-vascular endothelial growth factor (VEGF) signaling. <During the peri-ovulatory period> 3) In luteinization, moderate hypoxia stimulates P4 production via HIF1-steroidogenic acute regulatory protein (STAR) signaling. <After ovulation> 4) In luteal formation, acute hypoxia activates HIF1, leading to angiogenesis by VEGF, glucose (GLU) uptake by glucose transporter 1 (GLUT1) and mitochondrial autophagy (mitophagy) by BNIP3. Gray-colored parts show the dynamics of bovine ovarian arterial blood flow based on a previous report [1].

roles in both the formation and regression of the bovine CL. During CL formation, hypoxia promotes luteinization and induces angiogenesis, glucose uptake, and mitophagy, while during CL regression, it decreases P4 synthesis and promotes apoptosis (summarized in Fig. 1). Further studies on how the length and degree of hypoxia determine the fate of cells in each luteal stage and what other factors, such as hormones, regulate HIF1 signals will contribute to a better understanding of the roles of hypoxia in ovarian physiology.

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