Roles of reactive oxygen species in the regulation of luteal function

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Ephemerality and prolongation of luteal function have been matters of great concern in reproduction for many years. However, their control mechanisms are very complex and differ among mammals. Recently, evidence has indicated that reactive oxygen species may play important roles in the regulation of luteal function. Reactive oxygen species are present in most somatic cells and are involved in apoptosis, or 'physiological cell death'. In the corpus luteum, reactive oxygen species also exert luteolytic effects as well as some paradoxical luteotropic effects. This paper discusses the possible roles of reactive oxygen species in the control of luteal function.

Recently, much attention has been focused on reactive oxygen species (ROS) in the regulation of luteal function. The corpus luteum produces progesterone, which is essential for maintaining the embryo in the reproductive tract in mammals. Reactive oxygen species are generated in the corpus luteum and influence progesterone synthesis (Behrman et al., 1991; Gatuzli et al., 1991; Sugino and Aten, 1993a; Carlson et al., 1995). However, regulation of progesterone production involves many factors and differs among mammals (reviewed by Rothchild, 1981). For example, in dogs, the lifespan of the corpus luteum is long enough to cover the duration of pregnancy even when the female does not become pregnant. In rats, the lifespan of the corpus luteum is too short (2-3 days) to prepare the uterus for implantation, but it is prolonged for approximately 10 days by prolactin surges induced by copulation, and is maintained further by the synergistic action of oestriadiol and prolactin-like luteotrophins of the decidual tissue and, after implantation, of the placenta. The luteal phase in humans continues for 2 weeks, which is long enough for implantation to occur. After implantation, the function of the corpus luteum is prolonged by placental luteotrophins. Rothchild (1981) proposed that the rising phase in the secretion of progesterone by the corpus luteum is maintained primarily by progesterone itself, but once the intracellular progesterone concentration reaches a critical value, PGF$_{2\alpha}$ may begin to act as a luteolysin. The prevention of the luteolytic effects of PGF$_{2\alpha}$ would be a key step in prolonging the lifespan of the corpus luteum.

Generation of reactive oxygen species in the corpus luteum

Reactive oxygen species include superoxide radical (O$_2^-$), hydroxy radical (OH$^-$) and hydrogen peroxide (H$_2$O$_2$) (Fig. 1). Superoxide radicals are generated in the mitochondria and are converted to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide in the presence of superoxide radical and iron forms a hydroxy radical, a more reactive form, which is converted further to lipid peroxide. Superoxide radicals are scavenged to H$_2$O by glutathione peroxidase or catalase. Lipid peroxide is scavenged to H$_2$O by glutathione peroxidase or glutathione-S-transferase. There are three types of SOD, Cu,Zn-SOD, Mn-SOD and extracellular SOD. Generally, Cu,Zn-SOD acts in the cytosol, while Mn-SOD is present in the mitochondria and converts superoxide radicals to hydrogen peroxide. Antioxidants also include vitamins A, C and E. It is well known that LH decreases ascorbic acid concentrations in the ovary, and ascorbic acid scavenges the hydroxy radical.

Reactive oxygen species are generated in the steroidogenic cells and mononuclear phagocytes in the corpus luteum. Reactive oxygen species increase in the corpus luteum from day 3 to day 13 of pseudopregnancy in rats, while they increase from day 15 to day 21 in pregnant rats (Sugino et al., 1993a; Shimamura et al., 1995). Superoxide dismutase increases until day 9 in pseudopregnant rats, but increases until day 12 and gradually decreases thereafter in pregnant rats (Sugino et al., 1993a,b; Shimamura et al., 1995). The increases in SOD and glutathione peroxidase during the mid-luteal phase in pregnant rats may be due to the increase in concentrations of placental luteotrophins (Agrawal and Leloraya, 1977; Aten et al., 1992). The rapid increase in ROS at the luteal phase may be due to the increase in serum LH concentrations (Sawada et al., 1996) or to the increase in the production of ROS by PGF$_{2\alpha}$ in the corpus luteum (Riley and Behrman, 1991; Sawada and Carlson, 1991; Aten et al., 1992; Shimamura et al., 1995). Decrease in blood flow in the corpus luteum during the regression phase also activates the xanthine–xanthine oxidase system and produces ROS in the presence of oxygen (Sugino et al., 1993b). The mononuclear phagocytes produce ROS in the corpus luteum throughout the luteal phase. However, the production of ROS in mononuclear phagocytes was inhibited during the mid-luteal phase by high concentrations of progesterone (Sugino et al., 1996).

Roles of reactive oxygen species in progesterone production

The pathway of progesterone synthesis in the corpus luteum includes incorporation of esterified cholesterol from the lipoproteins in blood, transport of cholesterol into the mitochondria, where it is converted to pregnenolone by the cytochrome P450 side chain cleavage (SCC) enzyme, and the conversion of...
**Fig. 1.** Generation of reactive oxygen species. Superoxide radical is converted to hydrogen peroxide by superoxide dismutase (SOD) and further scavenged to H₂O by glutathione peroxidase or catalase. Superoxide radical also produces hydroxy radical, which is further converted to lipid peroxide. Hydroxy radical is scavenged by vitamin E and ascorbic acid, and lipid peroxide is scavenged by glutathione peroxidase or glutathione-S-transferase. Hypoxanthine and xanthine oxidase are produced during the ischaemic period. Hypoxanthine in the presence of xanthine oxidase and oxygen produces superoxide radical.

**Fig. 2.** Roles of reactive oxygen species (ROS) in the regulation of luteal function. LH or placental luteotrophins increase ROS and antioxidants. Reactive oxygen species interfere with progesterone synthesis by impairing LH receptors, inhibiting the translocation of cholesterol to the mitochondria or cytochrome P450, enzyme activity. Antioxidants are increased by luteotrophins and generally decrease ROS activity. Progesterone also decreases ROS generation, and low concentrations of hydrogen peroxide exert luteotropic effects. Prostaglandin F₂α is luteolytic and increases generation of ROS. Reactive oxygen species also increase the production of PGF₂α, but the luteolytic effects of ROS may not be mediated by PGF₂α.
pregnenolone to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD) in the microsomes. Progesterone is further metabolized to an inactive form, 20α-hydroxyprogesterone (20αOHP), in rats (reviewed by Gibori et al., 1988). LH stimulates formation of cAMP to activate protein kinase A, which liberates cholesterol from its esterified form (review by Niswender et al., 1994). Prolactin prevents the metabolism of progesterone to 20αOHP, and increases LH receptor sites or cytochrome P450sec activity in the corpus luteum (review by Gibori et al., 1988). Prostaglandin F₂α increases the free intracellular calcium concentration, activates protein kinase C, and affects progesterone production by inhibiting cholesterol transport or 3β-HSD expression (Niswender et al., 1994). Prostaglandin F₂α also increases ROS production (Riley and Berhman, 1991; Sawada and Carlson, 1991; Aten et al., 1992; Shimamura et al., 1995), while progesterone inhibits ROS production (Sugino et al., 1996).

Reactive oxygen species damage the luteal cell membrane, but also affect progesterone production by impairing LH receptors (Gatzuli et al., 1991; Vega et al., 1995) or by inhibiting the translocation of cholesterol to the mitochondria (Behrman and Aten, 1991) or cytochrome P450sec enzyme activities (Carlson et al., 1995). Helmer et al. (1979) reported that ROS stimulate the synthesis of PGF₂α, but luteolytic effects of ROS are not blocked by indomethacin (Sugino et al., 1993b). Sawada and Carlson (1996) demonstrated that low concentrations of LH increased superoxide radicals and progesterone production in the corpus luteum in rats, while high concentrations of LH further stimulated ROS but decreased progesterone production. These authors demonstrated further that both of these biphasic effects of LH on progesterone production were blocked by SOD and catalase. In addition, low concentrations of hydrogen peroxide activate transcriptional factors (Nose et al., 1991). Since serum LH concentrations in rats remain low between day 13 and day 18 of pregnancy and increase rapidly thereafter (Morishige et al., 1973), it is likely that concentrations of ROS remain low and exert luteotropic effects during midpregnancy in rats. Biphasic effects of ROS on progesterone production have also been demonstrated in humans (Vega et al., 1995), in that treatment of mid-luteal cells with a low concentration of hydrogen peroxide stimulated progesterone secretion, while high concentrations of hydrogen peroxide inhibited hCG-stimulated progesterone secretion. We also found that treatment of the corpus luteum with low concentrations of hCG in vitro increased SOD activity in the corpus luteum but higher concentrations of hCG decreased it (S. Takiguchi, K. Hashida, N. Sugino, Y. Nakamura and H. Kato, unpublished). It is notable that luteal function in humans declines by week 7 of pregnancy when serum hCG concentrations increase rapidly. Although there is no evidence for this, hCG may also exert biphasic effects on luteal function in humans.

Collectively, currently available results indicate that ROS do not initiate luteolysis but mediate other factors that regulate luteal function. Reactive oxygen species may also have slight luteotropic effects. Many questions remain about how such complex systems evolved in the corpus luteum.

**References**

Key references are indicated with asterisks.


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