Dev. Reprod. Vol 5, No 1, 2001(Suppl). pp 7-9

# Apoptosis and suppression of luteal steroidogenesis by GnRH agonist in pregnant rat

**Hyunwon Yang**
Cardiovascular Genome Center, Cardiovascular Research Institute,
Yonsei University College of Medicine

Gonadotropin-releasing hormone plays an important role in regulating the secretion of gonadotropins by which the production of gonadal steroids is controlled. However, recent studies have reported that an intrinsic GnRH system with ligand, receptor, and biological response, exists in the ovary [1, 2]. In particular chronic administration of GnRH or its highly potent agonist is known to have paradoxical inhibitory effects on a variety of reproductive functions that include ovarian steroidogenesis, follicular development, and ovulation [3, 4, 5]. GnRH has also been shown to increase apoptotic fragmentation of DNA in rat granulosa cells in a time- and dose-dependent manner, thus demonstrating that GnRH administration *in vivo* induces apoptosis in ovarian follicles [6].

Recent studies from our laboratory have shown that continuous administration of a gonadotropin-releasing hormone agonist (GnRH-Ag) *in vivo* suppressed progesterone production and induced apoptosis in the corpus luteum (CL) of pregnant rats [7, 8]. Furthermore, we demonstrated that levels of peripheral-type benzodiazepine receptor (PBR) and steroidogenic acute regulatory protein (StAR), both of which are involved in the mitochondrial cholesterol transport, and levels of P450 side chain cleavage enzyme (P450scc) transiently decreased after GnRH-Ag treatment [9]. In addition, GnRH-Ag treatment increased Bax, but not Bcl-2, gene expression in the luteal mitochondrial preparations, whereas Bcl-XL gene expression was reduced [8, 10].

NO, a highly reactive free radical molecule generated in a biological system, is synthesized from L-arginine by nitric oxide synthase (NOS) isozymes [11]. To date, three different isoforms of NOS have been characterized, two of which were first identified in the endothelium (eNOS) and brain (nNOS) as constitutively expressed isoforms [12]. An inducible isoform (iNOS) has been found in many cells and is correlated with cytostatic and cytotoxic events [13]. NO is now recognized as an important intracellular and intercellular messenger molecule known to have diverse physiological and pathological roles in multiple systems [14]. Recently, NOS isozymes have been found in follicles and CL of human, rat, and rabbit [15, 16, 17]. NO has been known to be associated with a variety of female reproductive functions that include follicular development [18, 19], ovulation [20, 21], and oocyte meiotic maturation [22]. Specifically, NO has emerged as a potential regulator of ovarian function suppressing the production of estradiol and progesterone in the follicle and corpora lutea [15, 23].

The similar effects of GnRH and NO on the ovarian function lead us to postulate that NO might act as a messenger molecule for GnRH to suppress progesterone production and induce apoptosis in the CL of the pregnant rat. In the present study, we investigated serum progesterone and intraluteal NO levels, PBR production, and apoptosis after GnRH-Ag treatment *in vivo* and *in vitro* to test this hypothesis.

Rats were treated individually on day 8 of pregnancy with 5g/day of GnRH-Ag for 4, 8,

and 24 h. GnRH-Ag decreased the production of progesterone and pregnenolone 8 and 24 h after the administration, whereas apoptosis in the CL increased. Corresponding with the reduction in these steroid hormones, intraluteal NO levels decreased at 8 and 24 h. Western blotting and immunohistochemical results of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in the CL showed that administration of GnRH-Ag was associated with a marked decrease in eNOS and iNOS compared to sham controls at 4, 8, and 24 h. In addition, we demonstrated, for the first time, the presence of neuronal nitric oxide synthase (nNOS) protein in the CL of the pregnant rat, but nNOS did not change throughout the experimental period. The results of the present study demonstrate that suppression of NO synthesis by GnRH-Ag correlates with the reduction in progesterone and pregnenolone production and the increase in apoptosis suggesting that GnRH could induce luteolysis in pregnant rats via NO.

To confirm the mechanism(s) by which GnRH-Ag treatment leads to the suppression of progesterone and the induction of apoptosis *in vitro*, corpora lutea of day 8 pregnant rats were enzymatically dissociated and mixed population of luteal cells were incubated in medium 199 at 37C. After 90 min, they were treated with various doses of GnRH-Ag or saline control. They were terminated 4, 8, 12, or 24 h after the commencement of treatment and the media, and cells were removed and stored in the freezer. GnRH-Ag, at a dose of 10-6 M, consistently suppressed progesterone levels at 8 and 12 h and thus this dose was chosen. GnRH-Ag had no effect on luteal GnRH receptors at any time period. GnRH-Ag treatment suppressed luteal production of nitric oxide (NO) at 8 and 12 h; and that was due to the luteal suppression of NO synthases, iNOS and eNOS at 4 and 8 h. Further, GnRH-Ag treatment suppressed peripheral-type benzodiazepine receptor (PBR) binding sites at 12 h and this was confirmed by Western blot. GnRH-Ag treatment induced prostaglandin F2 (PGF2) production and release by the luteal cells into the medium. These data suggest that mechanism(s) by which GnRH suppresses luteal production of progesterone could be due to a fall in luteal NO, PBR, and/or an increased luteal production of PGF2. We hope to utilize this model system, where the action of GnRH is reversible on steroidogenesis, to delineate a role for PBR in cholesterol transport as well as a role for GnRH in fertility regulation.

## REFERENCES


Clayton RN, Eccleston L, Gossard F,Thalbard JC, Morel G. Rat granulosa cells express the gonadotropin-releasing hormone gene: Evidence from in situ hybridization histochemistry. J Mol Endocrinol 1992; 9:189-195.

Kaiser HB, Zhao D, Cardona GR and Chin WW. Isolation and characterization of cDNAs encoding the rat pituitary gonadotropin-releasing hormone receptor. Biochem Biophys Res Commun 1992; 189:1645-1652.

Rivier C, Rivier J, Vale W. Chronic effects of {D-Trp6-Pro9-NET}luteinizing hormone -releasing hormone on reproductive processes in the female rat. Endocrinol 1978; 103:2299-2305.

Cusan L, Auclair C, Belanger A, Ferland L, Kelly PA, Seguin C, Labrie F. Inhibitory effects of long-term treatment with a luteinizing hormone-releasing hormone agonist on the pituitary gonadal axis in male and female rats. Endocrinol 1979; 104:1369-1376.

Yoshinaga K. Suppression of ovarian function by LHRH and its analogues in pregnant rats. Adv Exp Med Biol 1979; 112:729-734.

Billig H, Furuta I, Hsueh AJW. Gonadotropin -releasing hormone directly induces apoptotic cell death in the rat ovary: Biochemical and in situ detection of deoxyribonucleic acid fragmentation in granulosa cells. Endocrinol 1994; 134:245

-252.

Sridaran R, Lee MA, Haynes L, Srivastava RK, Ghose M, Sridaran G, Smith CJ. GnRH action on luteal steroidogenesis during pregnancy. Steroids 1999; 618-623

Sridaran R, Hisheh S, Dharmarajan AM. Induction of apoptosis by a gonadotropin-releasing hormone agonist during early pregnancy in the rat. Apoptosis 1998; 3:51-57.

Sridaran R, Philip GH, H Li, Culty M, Liu Z, Stocco DM, Papadopoulos V. GnRH agonist treatment decreases progesterone synthesis, luteal peripheral benzodiazepine receptor mRNA, ligand binding and steroidogenic acute regulatory protein expression during pregnancy. J Mol Endocrinol 1999; 22:45-54.

Papadopoulos V, Dharmarajan AM, Li H, Culty M, Lemay M, Sridaran R. Mitochondrial peripheraltype benzodiazepine receptor expression: Correlation with gonadotropin-releasing hormone (GnRH) agonist-induced apoptosis in the corpus luteum. Biochem Pharmacol 1999; 58:1389-1393.

Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43:109 -142.

Lamas S, Marsden PA, Li GK, Tempst P, Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci USA 1992; 89:6348-6352.

Xie Q, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Ding A, Troso T, Nathan C. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science 1992; 256:225-228.

Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 1991; 43:109-142.

Van Voorhis BJ, Dunn MS, Snyder GD, Weiner CP. Nitric oxide: an autocrine regulator of human granulosa-luteal cell steroidogenesis. Endocrinol. 1994; 135:1799-1806.

Jablonka-Shariff A, Olson LM. Hormonal regulation of nitric oxide synthases and their cell-specific expression during follicular development in the rat ovary. Endocrinol. 1997; 138:460-468.

Hesla JS, Preutthipan S, Maguire MP, Chang TS, Wallach EE, Dharmarajan AM. Nitric oxide modulates human chorionic gonadotropin-induced ovulation in the rabbit. Fertil. Steril. 1997; 67:548-552.

Zackrisson U, Mikuni M, Wallin A, Delbro D, Hedin L, Brannstrom M. Cell-specific localization of nitric oxide synthases (NOS) in the rat ovary during follicular development, ovulation and luteal formation. Hum Reprod 1996; 11:2667-2673.

Dixit VD, Parvizi N. Nitiric oxide and the control of reproduction. Anim Reprod Sci 2001; 65:1-16.

Bonello N, McKie K, Jasper M, Andrew L, Ross N, Braybon E, Brannstrom M, Norman RJ. Inhibition of nitric oxide: effects of interleukin-1ß-enhanced ovulation rate, steroid hormones, and ovarian leukocyte distribution at ovulation in the rat. Biol Reprod 1996; 54:436-445.

Shukovski L, Tsafriri A. The involvement of nitric oxide in the ovulatory process in the rat. Endocrinology 1994; 135:2287-2290.

Jablonka A, Olson LM. The role of nitric oxide in oocyte meiotic maturation and ovulation: meiotic abnormalities of endothelial nitric oxide synthase knock-out mouse oocytes. Endocrinology 1998; 139:2944-2954.