Localization and Physiological Actions of Adiponectin and Kisspeptin in Normal and Polycystic Ovaries of Mice and Human

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ABSTRACT

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Preface and Consolidated Abstract

The polycystic ovary syndrome (PCOS) is one of the leading causes of infertility due to anovulation in women. It affects upto 10% women of reproductive age and over 75% of all cases of anovulatory infertility. It is a multifactorial disorder characterized by chronic anovulation, hyperandrogenism (HA), hyperinsulinemia (HI), insulin resistance (IR), polycystic ovary and recurrent miscarriage. About half of women with PCOS are obese. The mechanism of anovulation is uncertain, but there is evidence of incomplete development of antral follicle, which might be due to abnormal interaction of insulin and luteinizing hormone (LH) on granulosa cells. Decreased fertility in PCOS may also be due to changed levels of gonadotropin- releasing hormone, gonadotropins, HA and HI. Despite the prevalence of PCOS worldwide, a clear understanding of the aetiology, pathophysiology and treatment of this syndrome remains inconclusive. The heterogeneous pathophysiology of PCOS results in a number of constraints for its management. If PCOS is not corrected, it may lead to other serious consequences such as Type II diabetes mellitus, cardio-vascular diseases, ovarian and endometrial cancer, depression etc. Thus, PCOS is a major issue for women's health with ramifications well beyond the reproductive age.

HA is considered as a core feature of PCOS since it can be induced by treatment with large dose of androgen. About 70-80% of women with HA are diagnosed with PCOS. HA is also the major factor behind most clinical manifestation of PCOS. Insulin resistance followed by HI is regarded as the second most important factor responsible for development of HA and other PCOS-like features. Besides HA and abnormal insulin secretion and action, defects in neuro-endocrine regulation lead to hyperpulsatility of GnRH secretion and obesity related metabolic dysfunctions. These are the two important factors responsible for manifestation of PCOS. Because the primary cause of PCOS is not yet precisely known, treatment is mainly directed to the symptoms of the disorder. Currently, reversing HA and IR or inducing ovulation in women with PCOS constitute the fundamental approach for the management of PCOS. Although the drugs such as anti-androgen-spironolactone; insulin sensitizers-metformin and ovulation inducer-clomiphene citrate are often being used to induce ovulation and pregnancy in PCOS, but effective treatment to manage PCOS is still a challenge. The obesity factor and neuro-endocrine/hypothalamic regulator are relatively understudied factors that may play important role in the development and manifestation of the PCOS.

Therefore, in the present study the obesity factor, adiponectin and hypothalamic factor, kisspeptin, are selected to evaluate their role in development of PCOS-like features in the mouse. The information obtained through studies on mouse-model may help in developing suitable therapeutics for PCOS treatment in humans. Further, it is thought out to examine the physiological role of adiponectin and kisspeptin in the ovary of cyclic mice. In order to confirm the pathophysiological role of adiponectin and kisspeptin in women with PCOS, a preliminary study is also performed in the ovaries of normal and PCOS women.

The study incorporated in this thesis is broadly divided into three sections. **Section-I** describes the role of obesity factor, adiponectin on ovarian activities of normal and PCOS-induced mice. This section is divided into two chapters. **Chapter 1** incorporates the effects of adiponectin on ovarian activities of normal mice. **Chapter 2** describes the effect of adiponectin on ovarian activities in PCOS mice. This chapter is subdivided into part 2.1 and 2.2 describing the effect of adiponectin on ovarian activities in PCOS-induced mice *in vitro* (Part 2.1) and *in vivo* (Part 2.2). **Section-II** incorporates the studies demonstrating the role of hypothalamic factor, kisspeptin on ovarian activities of normal and PCOS-induced mice. This section is also divided into two chapters. **Chapter 3** deals the effects of kisspeptin on ovarian activities of normal mice. **Chapter 4** describes the effect of kisspeptin on ovarian activities of PCOS-induced mice. **Section-III** includes **Chapter 5** which incorporates the comparative studies demonstrating the immunolocalization of GnRH, GnRH-R, Kisspeptin and Adiponectin in the ovary of normal and PCOS women.

Chapter 1

Effect of adiponectin on ovarian steroidogenesis and folliculogenesis in adult mice: an *in vivo* study

The aim of present study was to evaluate role of adiponectin in ovarian activities in adult cyclic mice. Mice were treated *in vivo* with two different doses of adiponectin (2 µg/animal/day and 20 µg/animal/day) for 15 days. The treatment of low dose of adiponectin caused a significant increase in the expression of AdipoR1 in the ovary when compared to the control, whereas, high dose of adiponectin caused a significant decrease in the expression of AdipoR1 in the ovary as compared with the low dose. But changes in the ovarian concentration of AdipoR1is positively correlated with the circulating level of estradiol. The change in the ovarian expression of AdipoR1 is positively correlated with the changes in

expression of insulin receptor protein, together with expression of steroidogenic markers and serum estradiol levels. These findings suggest that the adiponectin affects ovarian steroidogenesis by influencing insulin receptor. Similar to steroidogenesis, the effect of adiponectin on follicular growth, apoptosis and ovulation may also be mediated through insulin receptor. The adiponectin treatment may affect steroidogenesis and follicular development by modulating ovarian expression of AdipoR1 and insulin receptor in cyclic mice.

Chapter 2 (Part 2.1)

Effect of adiponectin on ovarian activities in PCOS-induced mice (an *in vitro* study)

The aim of the current study was to evaluate the direct role of adiponectin either alone or together with luteinizing hormone (LH) on sex hormone synthesis and changes in insulin receptor (IR) in the ovary of dehydroepiandrosterone (DHEA) treated PCOS-mice. Immunohistochemical study showed decreased expression of adiponectin receptor (AdipoR1) in PCOS-ovary which correlated with increased synthesis of androgen and decreased expression of IR. The treatment with adiponectin with or without LH to PCOS-ovary *in vitro* caused significant decline in androgen synthesis and increase in IR. This study showed the direct role of adiponectin in ameliorating hyperandrogenism and reducing IR in the ovary of PCOS-mice. Thus, adiponectin treatment may be a novel therapeutic strategy for combating PCOS.

Chapter 2 (Part 2.2)

Effect of adiponectin on ovarian activities in PCOS-induced mice: (an *in vivo* study)

The objective of the present study was to comprehensively examine the role of adiponectin in regulating (reversing) the reproductive, metabolic and fertility status of mice with polycystic ovary syndrome (PCOS). PCOS was induced in prepubertal (21 to 22 day old) mice using dehydroepiandrosterone (6mg/ 100 g body weight for 25 days), after which the mice were administered either a low or high dose of adiponectin (5 or 15 µg/animal/day; subcutaneously). PCOS mice exhibited typical features, including the presence of numerous cystic follicles, increased circulating androgens, increased body mass, altered steroidogenesis, decreased insulin receptor expression and increased serum triglycerides, serum glucose, Toll-like receptor (TLR)-4 (a marker of inflammation) and vascular endothelial growth factor (VEGF; a marker of angiogenesis). These parameters were significantly correlated with a

reduction in adiponectin in PCOS mice compared with vehicle-treated control mice. Exogenous adiponectin treatment to PCOS mice restored body mass and circulating androgen, triglyceride and glucose levels. Adiponectin also restored ovarian expression of steroidogenic markers (LH receptors, steroidogenic acute regulatory protein and 3β-hydroxysteroid dehydrogenase), insulin receptor, Toll-like receptor (TLR-4) and vascular endothelial growth factor (VEGF) levels in control mice. Adiponectin restored ovulation in PCOS mice, as indicated by the presence of a corpus luteum and attainment of pregnancy. These findings suggest that adiponectin effectively facilitates fertility in anovulatory PCOS. Thus, systemic adiponectin treatment may be a promising therapeutic strategy for the management of PCOS.

Chapter 3

Effect of kisspeptin-10 on ovarian steroidogenesis and folliculogenesis in adult mice: an *in vivo* study

This study was conducted to evaluate the effects and underlying mechanism of *in vivo* treatment of kisspeptin (KP) on ovarian activities in the cyclic mice. Mice were treated *in vivo* with two different doses of KP (10 pg/animal/day and 100 pg/animal/day) for 10 days. KP treatment depending upon dose caused changes in body mass, circulating steroid levels, follicular development and ovulation in cyclic mice. Treatment with the low dose of KP caused increased follicular development by increasing cell proliferation and induced ovulation as indicated by the presence of many newly formed corpora lutea. The treatment of KP stimulated estradiol synthesis by increasing ovarian expression of G-protein coupled membrane receptor (GPR54) and steroidogenic markers (LH-R, StAR and 3β-HSD proteins). This study further demonstrated that the stimulatory effect of KP in the ovary may be mediated through increased expression of GnRH-R. Whether KP and GnRH interact with each other during various ovarian activities requires further investigation.

Chapter 4

Effect of kisspeptin-10 on ovarian activities in PCOS-induced mice: *In vivo* and *in vitro* studies

In this segment, it was aimed to evaluate the role of Kisspeptin-10 (KP-10) in improving PCOS-like features in the dehydroepiandrosterone (DHEA)-treated ovary of PCOS mice. The result showed significant decrease in expression of G-protein coupled membrane receptor (GPR54) protein in the ovary of PCOS mice which correlated with increased synthesis of androgen and decreased expression of insulin receptor. The treatment of KP-10 restored

normal reproductive activity in PCOS mice by decreasing body mass, inducing follicular development, increasing insulin receptors and GPR54. The *in vitro* study showed the direct action of KP-10 in suppressing androgen synthesis and reducing insulin receptor in the ovary of PCOS mice. Thus, KP-10 treatment in future may be a promising therapeutic strategy and target for the management of PCOS.

Chapter 5

Immunolocalization of Gonadotropin releasing hormone (GnRH), Gonadotropin releasing hormone receptor (GnRH-R), Kisspeptin and Adiponectin in the ovary of control and PCOS human females

The aim of this study was to determine the localization and distribution of GnRH, GnRH-R, Kisspeptin and Adiponectin in the ovary of normal (control) and PCOS women and to determine whether or not immunostaining of these neuropeptides and adipokine varies in the ovaries of PCOS women. The most abundant immunoreactivity of these neuropeptides was found in the thecal cells of large antral or cystic follicles. Immunoreactivity of these neuropeptides was also noticed in the luteal cells of control ovary. Significant ($p<0.05$) increase in immunostaining of GnRH, GnRH-R and Kisspeptin was noticed in the ovary of PCOS when compared with the control. Significant ($p<0.05$) decrease in immunostaining of Adiponectin was noticed in the ovary of PCOS when compared to the control. This study provides evidence for the presence of GnRH, GnRH-R, Kisspeptin and Adiponectin in the human ovary. A close association between GnRH and Kisspeptin in the ovary was noticed through this study. Increased immunostaining of GnRH and Kisspeptin in the thecal layer of antral or cystic follicles suggest involvement of these neuropeptides in increased thecal androgen synthesis in PCOS. Low immunolocalization of adiponectin in PCOS ovary suggests the involvement of adiponectin during PCOS condition.