Exploring the role of cadmium, an endocrine disruptor and insulin resistance: mechanisms and implications on ovarian cells

Ph.D. Thesis, 2015

Chapter 2
Aims and Objectives

2.1 Aim and Rationale for the study

Pregnancy is a complex, heterogeneous, biological phenomenon in which the embryo develops into a fetus within the female uterus. However, due to various etiological factors, the females nowadays either are not able to conceive or the growing embryo is unable to survive after conception ultimately leading to infertility/ pregnancy loss. Despite of many reasons for infertility such as endocrine, genetic, environmental, immunologic, many infertile couples continue to be labelled with the diagnosis of idiopathic infertility. Advances in the field of diagnosis and treatment of idiopathic infertility have remained relatively stagnant. Moreover, the couples are given descriptive diagnosis and often their infertility is bypassed with assisted reproductive technology (ART). The situation hence calls for a proper diagnosis to avoid idiopathic infertility.

It is quite possible that association between any two factors such as endocrine and environmental could lead to idiopathic infertility. The change in the present life style inculcating in it sedentary style and carbohydrate rich diet has led to increase in insulin resistance condition. Apart from affecting the peripheral organs such as liver, muscle and adipocytes, insulin resistance affects ovary. IR is associated with multi follicular development, oocytes with lower fertilization rates after IVF, abnormal endometrial development and embryos that are unable to implant thus potently contributing to miscarriages and ultimately infertility.

Environmental endocrine disruptors, such as heavy metals, dioxins, bis phenol A (BPA) and phthalates accumulate in reproductive organs and eventually become causative risk factors for pregnancy defects. Recently, increase in smoking trends and industrial processes have led to increase in levels of endocrine disruptor-cadmium, which is a non-essential heavy metal. Studies in our lab revealed adverse effects of cadmium on granulosa cell steroidogenesis, the hypothalamus-pituitary axis and different developmental stages leading to transgenerational effects. In today's scenario, females are exposed to various factors simultaneously leading to idiopathic infertility. Owing to the fact that only few reports on association between any two factors on infertility are available; a positive or negative association between insulin resistance and cadmium might be- one of the very strong reasons for predisposing women towards

infertility. Unravelling the mechanism of association between these two factors in granulosa cells would help in developing intervention strategies that are urgently needed to avoid idiopathic infertility.

Apart from idiopathic infertility, insulin resistance (IR) plays a pathogenic role in 70-80% of women with polycystic ovary syndrome (PCOS). PCOS accounts for 8-10% of infertility cases. It is exceptionally a common disorder of premenoausal women and is characterized by hyperandrogenism, hyperinsulinemia and chronic anovulation. The statistic claims that 20-30% of PCOS are non-insulin resistant (NIR). Till date only one study has described difference between PCOS-IR and PCOS-NIR at physiological level. Higher doses of gonadotropin stimulation are required for PCOS-IR women as compared to PCOS-NIR regardless of body weight. Luteinized granulosa cells derived from PCOS-IR women undergoing IVF, release less progesterone in vitro than cells from PCOS –NIR. There is a decrease in the expression of key proteins involved in insulin as well as steroidogenic signaling in luteinized granulosa cells of PCOS-IR patients and the same is still unexplored in granulosa cells of PCOS-NIR patients. In spite of these available differences between PCOS-IR and NIR conditions, the infertile patients are diagnosed for PCOS and not for IR at the clinical level. Moreover, these patients are prescribed insulin sensitizers such as metformin, thiazolidinediones and D-chiro Inositol as the main therapeutic choice irrespective of IR or NIR condition. The insulin sensitizer's work with the goal of increasing insulin efficacy thus have no role to play in PCOS-NIR condition. This suggests that the diagnosis for association of PCOS with IR or without IR should be given recognition. Hence, extensive investigation for unravelling differences in the signaling network in luteinized granulosa cells from PCOS- IR and PCOS-NIR conditions need to be undertaken for better diagnosis and appropriate interventions for achieving a faster clinical pregnancy.

The unwanted side effects of the synthetic drugs for treatment of PCOS have reinforced the value of herbal medicine. Many active principal compounds from herbal plants have been reported for antidiabetic, anti-inflammatory and anti-lipidemic activity. Swertiamarin from *Enicostemma littorale* (EL) and curcumin from *Curcuma longa* are known for their best anti-diabetic and anti-inflammatory effects. Our lab has focused on EL's hypoglycemic, anti-oxidant, hypolipidemic and diabetic complications related diseases in various diabetic animal models. Our lab has also demonstrated improvement in insulin sensitivity along with inhibition of adipogenesis thereby restoring glucose and lipid metabolism in peripheral tissues by

swertiamarin, an active lead from EL. Curcumin, an active lead from *Curcuma longa* has been demonstrated for hypo glycemic activity, decreased peripheral tissue insulin resistance and anti-inflammatory activity. However, the role of swertiamarin and curcumin is hidden with respect to their action on insulin and steroidogenic signaling in luteinized granulosa cells from PCOS-IR or without IR.

Thus lack of understanding of the causes for infertility along with a need for targeted interventions led to the following objectives for the thesis.

2.2 Objectives

2.2.1: To study the effect of cadmium on cell death and expression of steroidogenic and major candidate genes in insulin resistant rat ovarian cells.

2.2.2: To study the effect of sub-clinical dose of cadmium on steroidogenesis in insulin resistant human granulosa cells.

2.2.3: To elucidate mechanism underlying insulin and steroidogenic signaling in insulin resistant and non-insulin resistant human granulosa cells.

2.2.4: To identify insulin sensitizer bioactive compound to ameliorate insulin resistance and steroidogenic dysfunction in human granulosa cells.