Chapter 8

Summary and Conclusion

8.1 Summary

There is a mounting evidence regarding increase in female infertility during the last decades, particularly in industrialized nations. The pivotal roles of etiological factors such as genetic, immunologic, endocrinologic, environmental pollutants and life style have been explored in pathological conditions like Polycystic Ovarian Syndrome (PCOS), endometriosis, tubal factor infertility, pre-mature ovarian failure etc. accounting for 50% of infertility however, in remaining 50%, the cause remains unknown (idiopathic infertility). In present scenario females are eventually exposed to both Cd and are highly prone to life style problems leading to IR which ultimately cause altered reproductive performance and fertility related problems. In this regard, in the present study an effort has been made to understand effect of IR and environmental endocrine disruptor Cd. We first created dexamethasone (3mg/kg/b.w for 28 days) induced IR rat model and exposed it to Cd (0.05mg/kg/b.w for 15 days) which mimicks present environment (Chapter 3). In the ovary, granulosa cells have an inherent capacity to proliferate, undergo steroidogenesis, secrete growth factors for maintenance of oocytes and undergo apoptosis thus playing a pivotal role. Hence we chose granulosa cells as the model system for investigating cell death along with other physiological, biochemical and molecular parameters related to steroidogenesis and its feedback regulators. The results clearly demonstrated that Cd and IR together in the rat granulosa cells decreased gene and protein expression of steroidogenic factors along with the activity of their enzymes, steroid hormone concentrations and gonadotropin receptors. The decrease in steroidogenesis was attributed to dysregulation at hypothalamus pituitary axis in the form of decreased protein expression of GnRH, CYP19A1, FSH-β and LH-β along with increased rate of luteinized granulosa cell apoptosis. Overall the study demonstrated that IR and Cd together mediated deleterious effect which was observed at different levels of reproduction.

The results of the in vivo study in rat model motivated us to design a similar study in human model in order to make it clinically more relevant. We availed follicular fluid samples **(**control (donor, male factor, tubal factor) and PCOS from an IVF clinic and isolated human luteinized granulosa cells (chapter 4a). Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine-

metabolic disorder affecting 17.8% of reproductive aged women and is characterized by hyperandrogenism, chronic anovulation, and/or polycystic ovaries. IR is one of the most significant metabolic aberration in PCOS and is present in almost 60-70% of the cases, indicating it as a common but not the universal feature. At the very first place, we confirmed isolated cells as luteinized granulosa cells and then identified for the protein expression of insulin receptor (INR-β) in hLGC's and Cd levels in the follicular fluid of control and PCOS. As expected we observed down regulation of INR-β in 70% of hLGC's from PCOS indicating them as PCOS-IR, but strikingly the Cd levels were undetectable in follicular fluid samples. We performed an *in vitro* experiment and on the basis of dose dependent study in normal hLGC's we selected 32μM Cd concentration and exposed IR hLGC's with it for 24 hrs. The study reports for the first time that effect of Cd on naturally IR human luteinized granulosa cells mimick today's scenario with increased granulosa cell apoptosis and decreased steroidogenesis thus leading to infertility at the pre conception stage itself as observed in our previous studies with rat.

In spite of the finding that IR is not present in all PCOS patients, insulin sensitizing drugs (ISD)'s are prescribed blindly to PCOS patients irrespective of the diagnoses for IR which has led to adverse effects. This practice at the clinical level thus calls for understanding the insulin and steroidogenic signaling in IR and NIR PCOS to unravel the candidate molecules responsible for such aetiologies. We segregated PCOS-IR follicular fluid samples from PCOS-NIR based on the down regulation of INSR-β in hLGC's (chapter 4b). Our study revealed IR in 70% of PCOS as observed in the literature. We then targeted insulin and steroidogenic signalling mechanism in control, PCOS-IR and PCOS-NIR. Decreased levels of key insulin signalling proteins along with increased expression of the key enzymes like SREBP-1c, PPAR-γ, ACC-1, FAS and CPT-1 depicted insulin resistance condition along with excessive fat accumulation in PCOS-IR as compared to PCOS-NIR and control. The process of steroidogenesis demonstrated dramatic changes wherein expression of CYP11A1 increased in PCOS-IR and PCOS-NIR as compared to decrease in expression of StAR, CYP19A1, 3β HSD and 17β HSD. These changes were further accompanied by decrease in follicular fluid progesterone in PCOS-IR as well as PCOS-NIR whereas drastic increase in the follicular fluid testosterone was observed only in PCOS-IR. Thus hyper androgenemia and hyper insulinemia as observed in PCOS-IR group along with increased expression of gonadotropin receptors and IGF receptors indicate metabolic disruption as the

culprit in PCOS-IR condition as compared to intrinsic ovarian disturbances as the probable cause for PCOS-NIR condition.

Recognition of IR for playing a pivotal role in the pathogenesis of PCOS revolutionized the use of insulin sensitizing drugs (ISD's) such as metformin, thiazolidinediones (TZD's) and D-chiro Inositol. However, in spite of their approach towards improved insulin sensitivity, ovarian morphology and intra ovarian androgen levels their use is under controversy if prescribed to normo insulinemic PCOS subjects. Hence the medical management has shown concern for better therapy, herbal medicine as an alternative approach in this regard. To address this, we made an attempt to unravel the role of swertiamarin and curcumin, the principal compounds from the herbs *Enicostemma Littorale Blume* and *Curcuma Longa* respectively on insulin signalling and steroidogenesis in *in vitro* hLGC's from PCOS-IR and PCOS-NIR (chapter 5). Our lab has already observed insulin sensitizing effects of swertiamarin and curcumin has been reported in literature for its PCOS alleviating effects. Treatment with swertiamarin (66μM) and curcumin (33μM) markedly enhanced cell viability and insulin sensitivity accompanied by decrease in stress by restoring protein expression of INSR-β, p-IRS (ser307), PI(3)K, pAkt, PKCζ, PPARγ, pP38 MAPK, ERK1/2 and genes involved in IGF system to normal levels with a similar potential as compared to metformin (1mM) in PCOS-IR samples. Further amelioration of aberrant steroidogenesis by restoring gene and protein expression of StAR, CYP11A1, 3β HSD, CYP19A1 and 17β HSD along with their enzyme activity, steroid hormone secretion and gonadotropin receptors was a result of treatment with both bio actives. Our results for the first time report significant role of swertiamarin and curcumin in improving steroidogenesis in *in vitro* naturally IR hLGC's. The findings further recognize swertiamarin as a better candidate compared to curcumin for PCOS-IR patients aiming to improve fertility at the pre conception stage.

The positive results of the bio actives in PCOS-IR inquisted us to observe for their effects in PCOS-NIR. Surprisingly both the bio actives in PCOS-NIR could only restore pP38 MAPK and ERK1/2- the genes involved in stress kinases followed by increase in cell viability only. Swertiamarin and curcumin could not revert back the decreased steroidogenesis in PCOS-NIR indicating role of other unidentified factors which might not be targeted by them.

In conclusion, association between two factors IR and Cd present ubiquitously in today's scenario can be attributed to increase in infertility. We further report swertiamarin as a fertility drug by majorly targeting aberrant steroidogenesis through insulin signalling and thus more effective therapy in PCOS-IR patients. This study also suggests a need for diagnosing IR in PCOS from PCOS-NIR at the clinical level.