Chapter 5

To study the effect of subclinical dose of cadmium on steroidogenesis in insulin resistant human luteinized granulosa cells.

5.1 Introduction

Reproductive disorders are important health issues. Many couples desire children but cannot achieve pregnancy through natural means. The causative factors of infertility may be identified in either of the partner, however, the cause of subfertility remains unknown in about 50% of these cases. It is now believed that a combination of environmental and endocrine factors may be responsible for the same.

In previous chapter we have already discussed the effect of both cadmium and insulin resistance in isolation and combination as cited from various animal studies with respect to HPO axis. Our result from earlier chapter clearly demonstrated that cadmium and insulin resistance together mediate more deleterious effects at different level of reproduction affecting granulose cell receptors, steriodogenesis, higher rate of apoptosis and dysregulation from hypothalamus in the form of decreased GnRH and gonadotrophins. We further wanted to study the combined effect of IR and Cd in *in vitro* condition in human model for which we chose human luteinized granulosa cells.

The presence of insulin receptor in human luteinized granulosa cells (hLGC's) identifies ovary as a target of insulin activity (Poretsky et al. 1999b). Alterations in insulin functioning due to sedentary life style and diet rich in carbohydrate leads to insulin resistance which is associated with abnormalities leading to infertility. Amongst several endocrine factors, insulin resistance is more prevalent and is present in 50-70% of PCOS women contributing to its pathogenesis (Mukherjee and Maitra 2010a). Increase in the fatty acid content during IR affects granulosa cells and developmental competence of oocyte possibly by influencing its lipid metabolism ultimately leading to poor ovulations, poor pregnancy outcomes and frequent miscarriages (Jakubowicz and Sharma 2007). *In vitro* experiments with hLGC's from anovulatory PCOS subjects have demonstrated increased steroid accumulation with physiologically high levels of insulin accompanied by gonadotropins (Willis et al. 1996). On the contrary, resistance has been

observed to insulin dependent glucose metabolism in human granulosa-luteal cells from anovulatory women with polycystic ovaries indicating inhibition of the metabolic activities (Rice et al. 2003).

Cd is one of the reproductive toxicant exposed usually as a result of waste from human activities in the environment (Frydman et al. 2010). It's long half-life *in vivo* facilitate its bio-accumulation and results in bio magnification in the food chains, thereby exposing females through diet (Satarug et al. 2011). Presence of Cd has been identified in follicular fluid as well as oocytes of female smokers undergoing in vitro fertilization (IVF) therapy leading to reduction in fertility and fecundity (Zenzes et al. 1995; Thompson and Bannigan 2008; Jackson et al. 2011). Studies have demonstrated increased accumulation of Cd in the ovary with an increase in age thus leading to failure of progression of oocyte development and ovulation (Frydman et al. 2010). *In vitro* administration of Cd to cultured human ovarian granulosa cells decreased preovulatory LH surge and progesterone secretion (Paksy et al. 1997b). Cd accumulation in gonads of foetus decreases the number of germ cells and in the embryos it leads to degeneration, apoptosis and breakdown in cell adhesion thus inhibiting its progression to the blastocyst stage. (Frydman et al. 2010).

Nowadays the risk of infertility is enhanced by variety of environmental as well as lifestyle factors. Owing to this there has been an increase in the process of *in vitro* fertilization for the couples willing to have a child. hLGC's obtained from IVF patients after controlled stimulation and retrieved from the follicles during aspiration of the ovulated egg represent a homogeneous population of luteinized cells. Several such layers of cumulus and mural granulosa cells are generally discarded at the time of IVF as shown in the Fig 5.1. These granulosa luteal cells being the most abundant cell type inside the follicle undergo substantial differentiation, interact with oocytes, mediate the effect of gonadotropins on the follicular maturation and are easily accessible for studying the overall quality of the follicles in response to gonadotropins (Albertini 2004). Hence these discarded granulosa luteal cells are used for extensive research purpose (Tripathi et al. 2013).

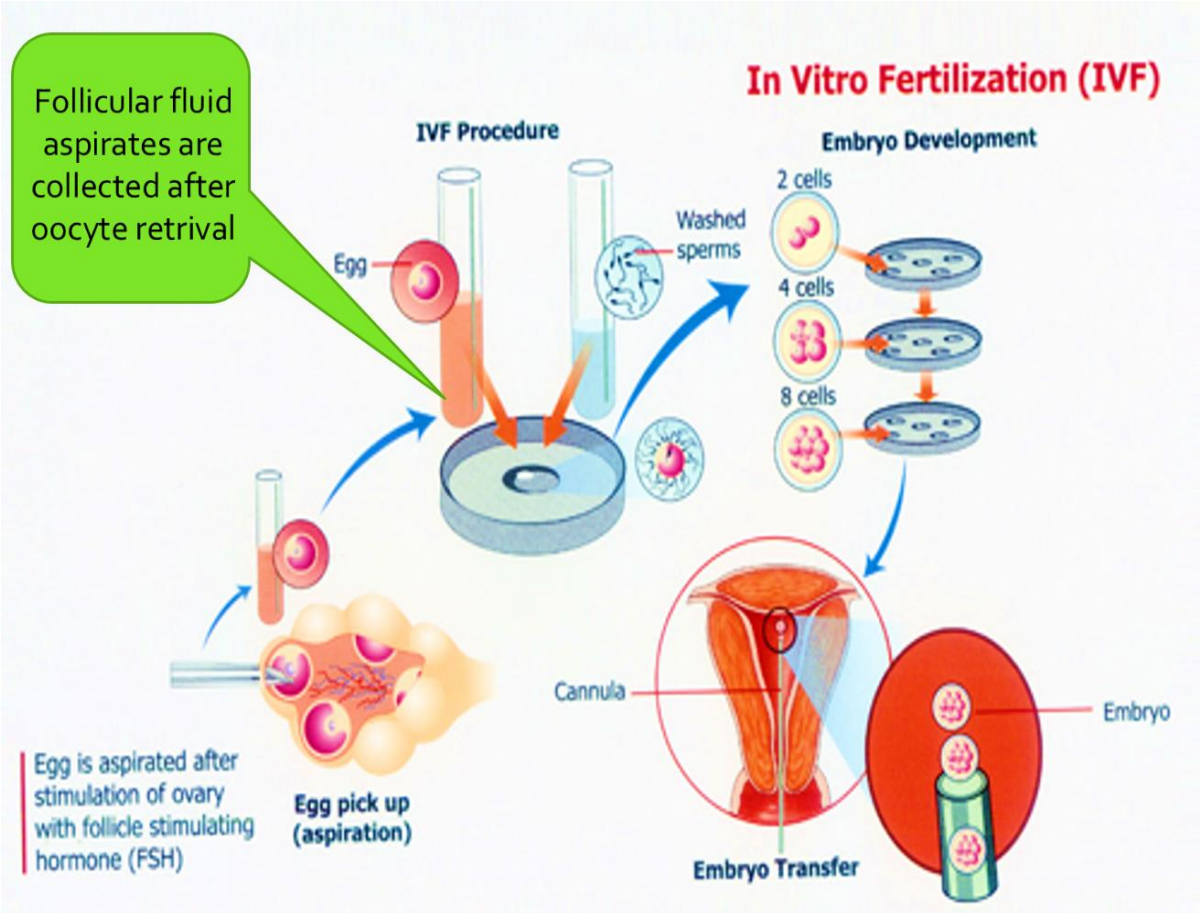
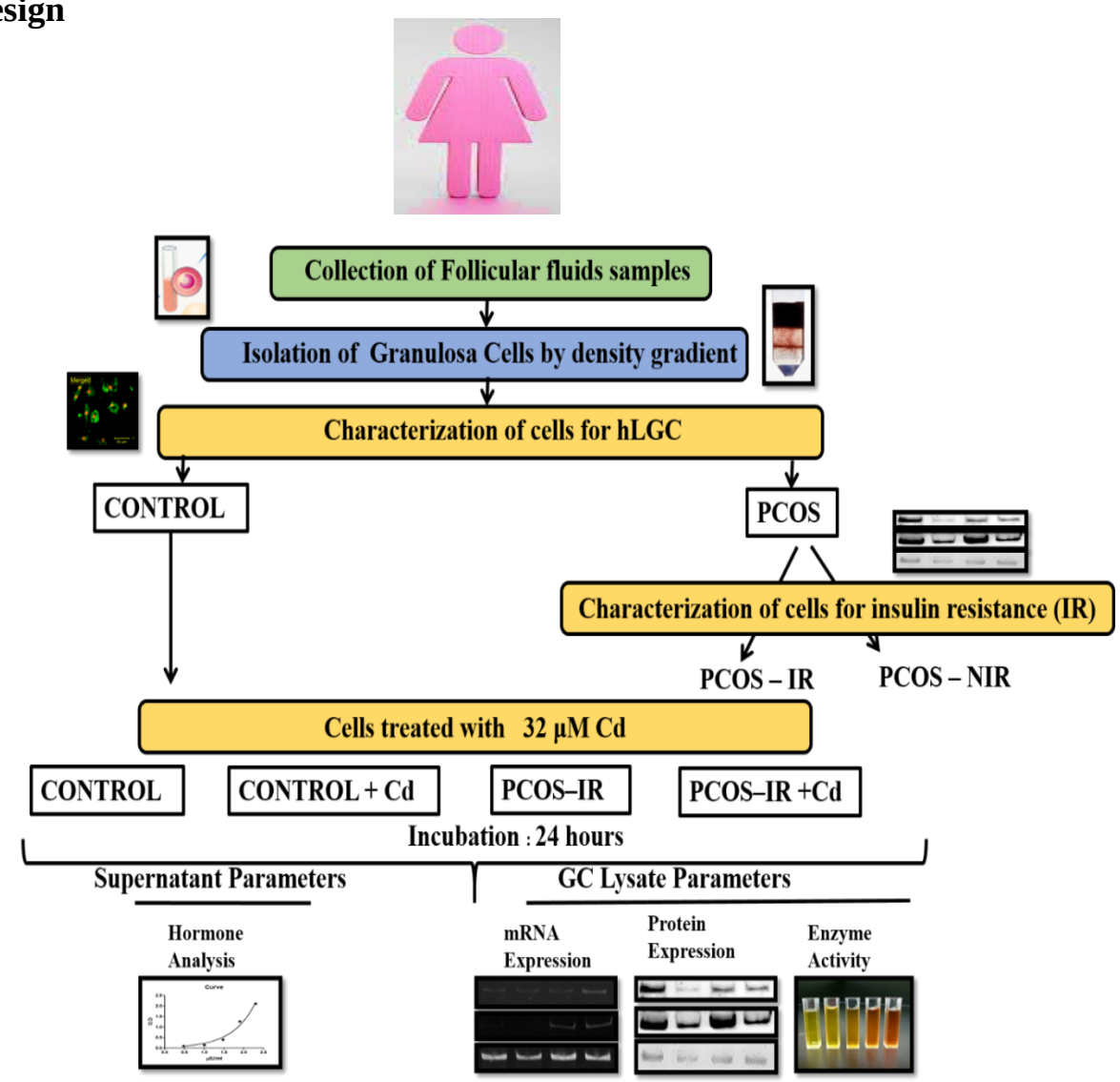


Figure 5. 1: Schematic diagram of IVF. After the retrival of oocyte, the remaining follicular fluid is discarded. It is this follicular fluid that is rich in granulosa cells and is used for research purposes.

In this chapter, based on our prior work, we made an effort to understand *in vitro* effect of combined exposure of heavy metal Cd and insulin resistance in human luteinized granulosa cells (hLGC's) isolated from follicular fluid of PCOS and non-PCOS patients undergoing IVF. The study is novel as there are no results for effect of Cd on naturally IR human luteinized granulosa cells mimicking today's scenario. Thus in the present study the combined effect of IR and Cd was evaluated on granulosa cell death parameters along with other physiological, biochemical and molecular parameters such as mRNA and protein expression of StAR for cholesterol transport and CYP11A1, 3β-HSD, CYP19A1 and 17β-HSD responsible for steroid synthesis. Apart from this estimation of Cd from the follicular fluid samples of control and PCOS has been done to correlate the *in vitro* results of combined effect of IR and Cd with that of *in vivo*.

5.2 Experimental Design



5.3 Results

Follicular fluid sample from IVF patients were collected as per the inclusion and exclusion criteria as mentioned in section 3.3.8 and classified as control and PCOS based on hospital record.

5.3.1 Characterization of cells isolated from follicular fluid

Granulosa cells were isolated from follicular fluid sample of IVF patients with and without PCOS. These cells were characterised by immunostaining with antibodies against steroidogenic enzymes. Isolated cells were positive for CYP19A1, 17β-HSD and 3 β-HSD with DAPI as the nuclear stain (Fig: 5.2).

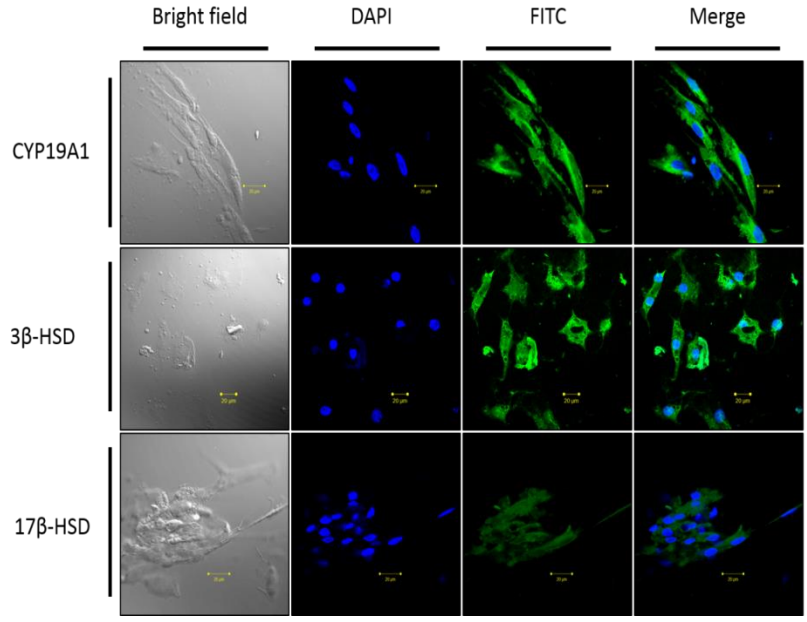


Figure 5. 2: *Characterization of hLGC's isolated from follicular fluid. Blue color indicates presence of nucleus and green color reflects the steroidogenic enzymes.*

5.3.2 Insulin Resistance confirmation in PCOS-hLGC's

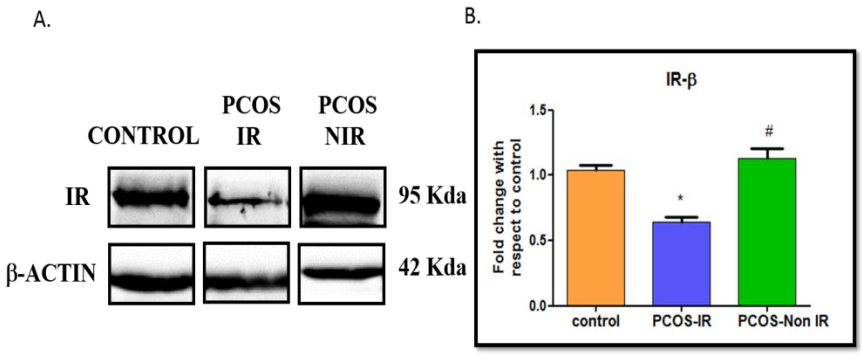


Figure 5. 3: IR in hLGC's from PCOS samples. A) Western blot of INR-β using β- actin as endogenous control. B) Densitometry analysis. Data represented as Mean ± SEM of n=18 control, n= 24 PCOS-IR and n= 14 PCOS-NIR. * P <0.05 vs control, # P as compared to PCOS-IR. (20 µg protein).

Although IR is known to be associated with PCOS, its presence in PCOS patients is not universal. Thus to distinguish PCOS-IR from PCOS-NIR, hGC's from 30 PCOS and 18 control, samples were analysed for protein expression of INR-β employing β-actin as an internal control. hGC's isolated from few PCOS samples demonstrated a significant decrease ($P < 0.05$) in expression of INR- β when compared to control and rest PCOS samples. Based on these results we grouped the 30 PCOS follicular fluid as 24- PCOS –IR (insulin resistant) and 14 PCOS-NIR (non insulin resistant) (Fig: 5. 3).

5.3.3 Cd concentration in follicular fluid

Cd concentrations were analysed in the follicular fluid samples from control and PCOS-IR by atomic absorption spectroscopy (AAS). Presence of Cd in the follicular fluid samples was not detected as shown in Figure 5.4. The sensitivity of AAS instrument was 0.0043 ppm.

Sample	Results	
	ppm	µg/ml
Control	Not detected	Not detected
Control	Not detected	Not detected
Control	Not detected	Not detected
PCOS-IR	Not detected	Not detected
PCOS-IR	Not detected	Not detected
PCOS-IR	Not detected	Not detected

Figure 5. 4: Cd concentration as estimated in control and PCOS by AAS in few representative samples.

5.3.4 Effect of Cd exposure on viability of control and PCOS-IR hLGC's

As the presence of Cd was not detected in follicular fluid samples, we proceeded further with *in vitro* experiments wherein Cd concentration was optimized in control hLGC's. Treatment of hLGC's with Cd ranging from 0-64µM showed dose dependent decrease in the % cell viability. The decrease was observed to be significant ($P < 0.01$ and $P < 0.001$) at concentrations ranging from 8-64 µM when compared to 0 µM. % granulosa cell viability was observed to be 56.8% at 16 and 32 µM doses of Cd. Based on this, control and PCOS-IR hLGC's were treated with or without 32 µM. Cell viability was highy compromised in alone Cd group ($P < 0.01$) as well as PCOS-IR ($P < 0.01$) with respect to control when observed by trypan blue exclusion dye after 24 hours of *in vitro* incubation in optimum culture condition. However, decrease in the viability was more significant ($P < 0.001$) in PCOS-IR+Cd as compared to control. This indicated that

the survival of hLGCs was negatively affected with Cd treatment during insulin resistant condition (Fig 5.5).

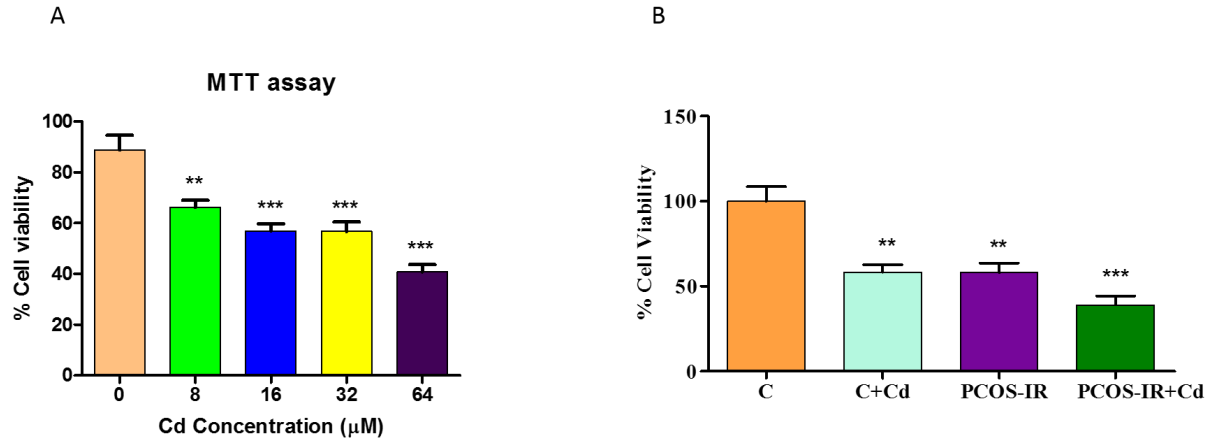


Figure 5. 5: A. Dose dependent study of Cd on hLGC's viability (in vitro). B. Effect of IR and Cd either alone or in combination on hLGC's viability. Data represented as Mean ± SEM of 3 independent experiments. *** P < 0.001, ** P < 0.01 as compared to C.C = control

5.3.5 Effect of Cd and IR on cell death parameters in hLGC's.

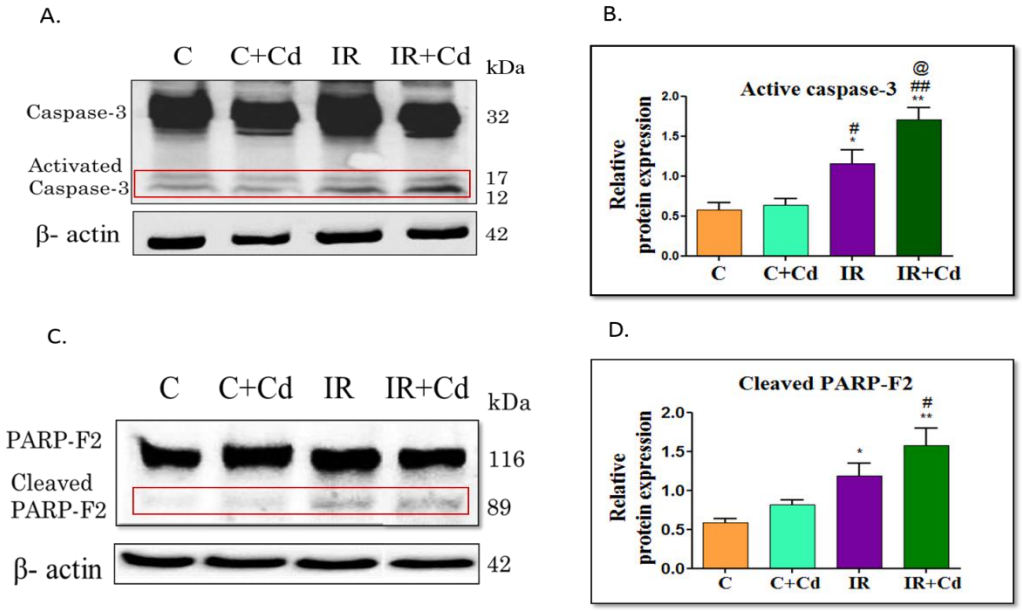


Figure 5. 6: Effect of IR and Cd either alone or in combination on protein expression of A). actived caspase-3 B). Densitometry for actived caspase-3 C). PARP-F2 D) Densitometry for cleaved PARP-F2. Data represented as Mean ± SEM of 3 independent experiments. * P < 0.05 and ** P < 0.01 as compared to C, # P < 0.05 as compared to C+Cd (20 μg protein).

To assess the effects of Cd, IR and their co-exposure on the genes involved in cell death, protein expression of cleaved PARP-F2, actived caspase-3 and binding of AnnexinV/PI in hLGC's was

analyzed by Immunoblotting and confocal microscopy respectively. There was a significant increase in protein expression of activated caspase-3 in PCOS-IR ($P < 0.05$) and PCOS-IR+Cd ($P < 0.01$) as compared to control and alone Cd group. Within the groups, significant increase ($P < 0.05$) was observed in PCOS-IR+Cd group as compared to PCOS-IR. Surprisingly no difference was observed in expression of activated caspase-3 in Cd alone group as compared to control. Similar results were obtained in the protein expression of cleaved (activated) PARP F2 where there was a remarkable increase in activated PARP in PCOS-IR and PCOS-IR exposed to Cd (Fig 5.6). In AnnexinV/PI staining, C+Cd, PCOS-IR and PCOS-IR + Cd were observed to be positive for the staining of Annexin V as well as PI as compared to control (Fig 5.7).

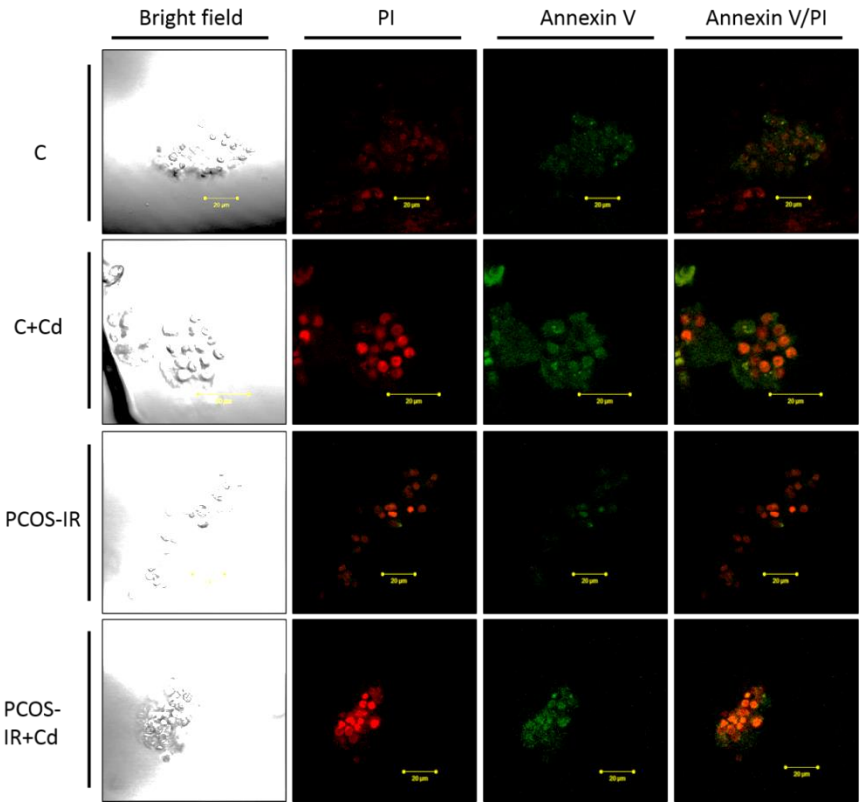


Figure 5. 7: Effect of IR and Cd either alone or in combination on Annexin V and PI staining in hLGC's. Annexin V-FITC is indicated by green color, PI is indicated by red color.

5.3.6 Effect of Cd and IR on steroid concentrations in hGLC's

Estradiol and progesterone concentration were estimated in cell culture supernatant by ELISA. Significant decrease was observed in C+Cd ($P < 0.01$), PCOS-IR ($P < 0.01$) and PCOS-IR+Cd ($P < 0.05$) groups in estradiol concentration as compared to control . Progesterone also

demonstrated significant decrease in C+ Cd ($P < 0.001$), PCOS-IR ($P < 0.001$) and PCOS-IR+Cd ($P < 0.01$) as compared to control (Fig. 5.8).

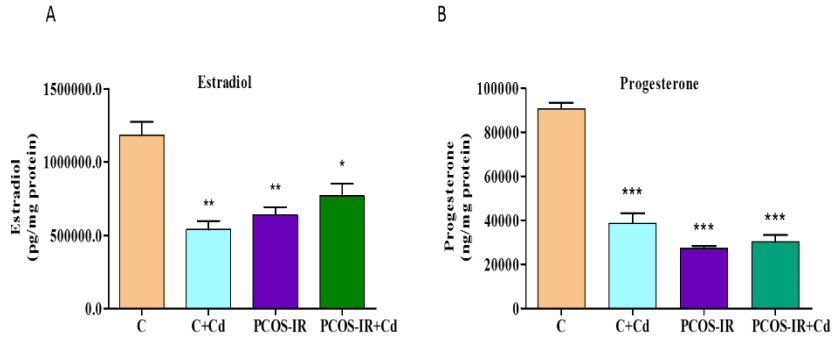


Figure 5. 8: Effect of IR and Cd either alone or in combination on steroid hormone concentrations in hLGC cell culture supernatant. A). estradiol and B). progesterone * P < 0.05, **P < 0.01, *** P < 0.001 as compared to C. Data represented as Mean ± SEM of 3 independent experiments.

5.3.7 Effect of Cd and IR on expression of steroidogenic genes and proteins in hLGC's

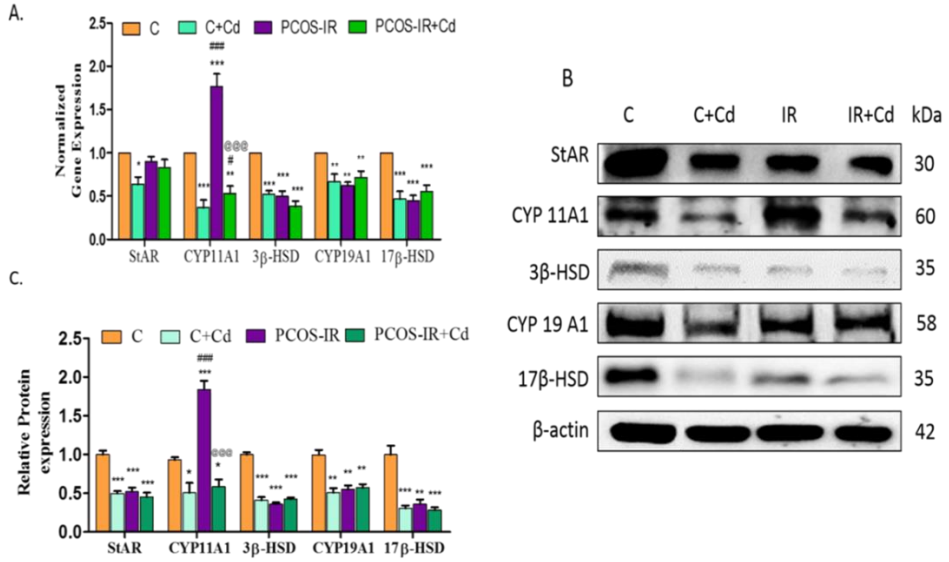


Figure 5. 9: Effect of IR and Cd either alone or in combination on hLGC's. (A) mRNA expression of genes involved in steroidogenesis (B) protein expression of genes involved in steroidogenesis (C) densitometric analysis for protein. The normalized expression values are represented as mean ± SEM of three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. C, # P < 0.05, ## P < 0.01, ### P < 0.001 vs. Cd, @ P < 0.05, @@ P < 0.01, @@@ P < 0.001 vs. PCOS-IR.

Gene and protein expression of StAR, CYP11A1, 3β-HSD, CYP19A1, and 17β-HSD involved in steroidogenic machinery were assessed in hLGC's from control and PCOS-IR treated with or without Cd. The mRNA expression of StAR revealed significant decrease ($P < 0.05$) only in C+Cd group as compared to control whereas its protein expression demonstrated significant decrease (P < 0.001) in all the three groups as compared to control. mRNA and protein expression of CYP11A1 revealed a considerable decrease in C+ Cd ($P < 0.001$) group with

respect to control. To our surprise mRNA and protein expression of CYP11A1 showed a drastic increase ($P < 0.001$) in PCOS-IR which was significantly decreased in the presence of Cd ($P < 0.01$) when compared to control. A noteworthy decrease ($P < 0.001$ and $P< 0.001$) was observed in CYP11A1 protein expression in C+Cd and IR+Cd group respectively as compared to control. mRNA and protein expression of 3β-HSD, CYP19A1 and 17β-HSD showed significant decrease in all the three groups as compared to control with no difference within the groups (Fig: 5.9).

5.3.8 Effect of Cd and IR on hydroxy steroid dehydrogenase activity in hLGC's.

Control and PCOS-IR hLGC's were treated with 32 μM Cd for 24 hrs followed by which 17β-HSD and 3β-HSD enzyme activity were analysed from the granulosa cell lysate. hLGC from PCOS-IR+Cd, PCOS-IR and C+Cd group showed significant decrease ($P < 0.001$, $P < 0.001$ and $P < 0.01$) in 17β-HSD activity as compared to control. Similarly C+Cd, PCOS-IR and PCOS-IR+Cd groups demonstrated a significant decrease ($P < 0.01$) in the activity of 3β-HSD as compared to control (Fig 5.10).

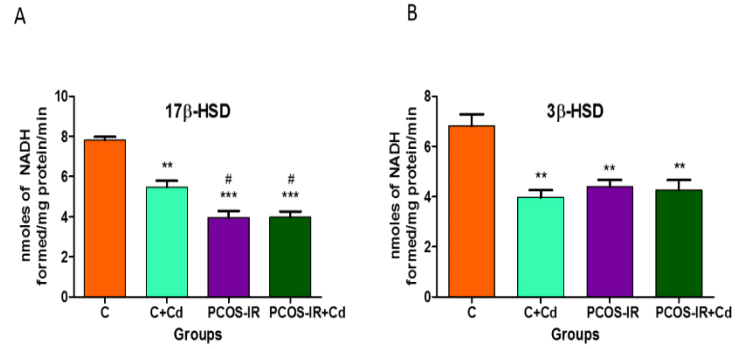


Figure 5. 10: Effect of Cd and IR either alone or in combination on A. 17β-HSD and B. 3β-HSD activity in hLGC's. **P <0.01, *** P <0.001 as compared to C, # P as compared to C+Cd. Data represented as Mean ± SEM of 3 independent experiments.

5.3.9. Effect of IR and Cd on expression of gonadotropin receptors genes in hLGC's.

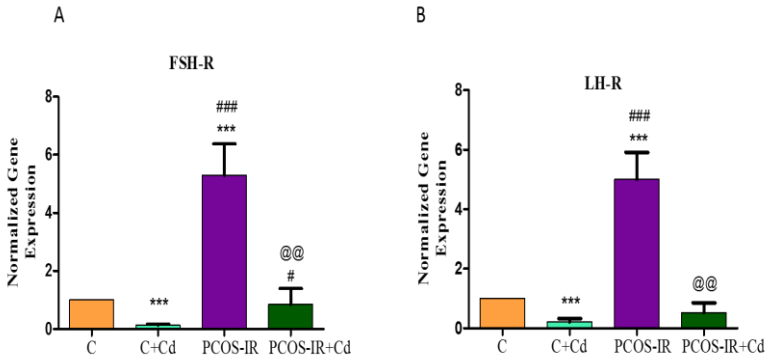


Figure 5. 11: Effect of IR and Cd either alone or in combination hLGC's. Effect of IR and Cd either alone or in combination in hLGC's on (A) mRNA expression of FSH-R (B) mRNA expression of LH-R The normalized expression values are represented as mean ± SEM of three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001 vs. C, # P < 0.05, ## P < 0.01, ### P < 0.001 vs. Cd, @ P < 0.05, @@ P < 0.01, @@@ P < 0.001 vs. IR.

Gene expression of FSH-R and LH-R were analysed by quantitative real time PCR in control and PCOS-IR granulosa cells treated with Cd for 24 hrs. Real time analysis revealed significant

decrease ($P < 0.001$) in mRNA expression of FSH-R and LH-R in C+Cd as compared to control. mRNA levels of FSH-R and LH-R were significantly higher in PCOS-IR ($P < 0.001$) as compared to control. However treatment with Cd demonstrated an antagonistic effect by significantly decreasing ($P < 0.001$) FSH-R and LH-R mRNA levels as compared to IR (Fig 5.11).

5.4 Discussion

Although as an individual agents Cd, an environmental disruptor and IR have affected the fertility of women, their combination might be more deleterious. In previous chapter we demonstrated that combined effect of IR and Cd in animal model caused varied effects at different levels ultimately leading to disruption in steroidogenesis. To make the study clinically more relevant, it was further studied in hLGC's isolated from follicular fluid during invitro fertilization. Positive staining for CYP19A1, 17β-HSD and 3β-HSD indicated the presence of steroidogenic proteins, thus characterizing the cells isolated from human follicular fluid as luteinized granulosa cells (Kossowska-Tomaszczuk et al. 2009).

The molecular basis for development of IR has been established where the potential mechanism for insulin resistance involves phosphorylation of serine/threonine (Ser/Thr) causing down regulation of INSR (Werner et al. 2004; Shanik et al. 2008). In the present study protein expression of INSR-β was down regulated in 70% of LGC's isolated from follicular fluid aspirates of PCOS whereas rest 30% did not show INSR-β down regulation. This was in line with the literature claiming 70-80% of PCOS as IR and other PCOS as non insulin resistant (NIR) (Mukherjee and Maitra 2010b; Kaur et al. 2012). We then estimated Cd concentration in follicular fluid samples from control and PCOS patients where the concentration of Cd was undetectable. In light of our previous study with rats (chapter 3), IR and Cd demonstrated a deleterious effect. Hence we intended to understand for the same condition in hLGC's. We therefore exposed them to Cd in dose dependent concentrations and chose 32 μM for further study. This correlates with literature where the same concentration was used to define environmental, occupational and smoking risk factors in female reproductive life span (Paksy et al. 1997b). The dose also correlated with the concentrations observed in follicular fluid of cigarette smoking females (Varga et al. 1993). Thus with 32μM concentration of Cd and PCOS-IR hLGC's, we further intended to study their combined effect on steroidogenesis and cell death

parameters. Amongst the somatic cells of the ovary, granulosa cells provide nutrients and maturation-enabling factors to ensure successful maturation, developmental competency and protection of oocytes (Tripathi et al. 2013). Along with our previous study in rat granulosa cells, other reports from literature have demonstrated decrease in granulosa cell number and change in its morphology with cadmium exposure (Paksy et al. 1997a; Smida et al. 2004; Nampoothiri and Gupta 2006). Hyperinsulinemia in PCOS implicated to decrease granulosa cell numbers relative to follicle size (Poretsky et al. 1999a). Our data for reduced cell viability implies that Cd exposure along with IR alters granulosa cell viability more deleteriously in co-exposed group.

Cleavage of PARP-1 into two fragments by execution caspases-3 is an important terminal event and is strongly implicated in the process of follicular atresia. Moreover increased reactivity of cleaved caspase-3 has been observed in the granulosa cells from ovarian cysts in the PCOS patients (Banu et al. 2011; Wei and Shi 2013). These findings from the literature confirm the basal expression of activated caspase-3 in luteinized granulosa cells from control group in the present study. Cd induced apoptosis through activation of caspase-3 and cleaved PARP has been observed in many different species, primary cultures and cell lines (Zhao et al. 2015). In insulin signaling, PI3K is an essential regulator of apoptotic pathways. Hence during IR low levels of insulin reduce the activity of PI3K leading to activation of caspase-3 (Du et al. 2004; Banu et al. 2011). Results of the present study demonstrate significant increase in activation of caspase-3 in PCOS-IR+Cd and C+Cd and PCOS-IR groups when compared to control thus indicating apoptosis as the type of cell death. In the process of apoptosis the up regulation of caspase-3 is accompanied by elevated phosphatidylserine (PS) externalization and an increase in membrane permeabilization (McComb et al. 2010; Dunai et al. 2012). The study observed granulosa cells in C+Cd , PCOS-IR and PCOS-IR+Cd to be positive for both annexin V and PI dual staining confirming apoptosis as the type of cell death. These results correlate with literature where, in PCOS ovaries activation of p38MAPK, ERK1/2, and c-Jun N-terminal kinase signaling induce proinflammatory cytokines such as TNF further inducing apoptosis (Jansen et al. 2004).

Luteinizing granulosa cells from PCOS ovaries lose the capacity of secreting progesterone and estradiol as compared to the normal ovaries (Doldi et al. 1998; Chang 2007). Cadmium is known to have endocrine disruptive effects on sexual steroid synthesis even at very low concentrations (Knazicka et al. 2015). In agreement with these studies we found decrease in concentrations of

estradiol and progesterone in cell culture supernatants in all the groups as compared to the control.

The StAR protein performs a critical function by delivering cholesterol to the inner mitochondrial membrane (Park et al. 2015). mRNA and protein levels for StAR were decreased in Cd alone when compared to control group. This is supported in the literature with a finding that free radicals generation by Cd could be a reason for decrease in expression of StAR, thus revealing anti-steroidogenic property of Cd (Gunnarsson et al. 2004; Gupta et al. 2004). Many studies have also demonstrated StAR as a potent target for Cd to bind and alter steroidogenesis (Paksy K 1992; Paksy K 1997; Zhang and Jia 2007; Pillai et al. 2010). IR alone failed to demonstrate any effect on expression of StAR mRNA but showed a significant decrease in its protein expression in IR alone and IR+Cd group supported by earlier reports (Jakimiuk et al. 2001a) (Petrescu et al. 2001). CYP11A1 and 3 β-HSD play a key role in the biosynthesis of progesterone. In the present study decreased expression of 3 β-HSD mRNA and protein was observed in all the groups as compared to control. The gene and protein expression of CYP11A1 demonstrated a significant decrease in C+Cd group and an increase in PCOS-IR group. Furthermore the increase in CYP11A1 expression by PCOS-IR was significantly down regulated by Cd as could be seen in PCOS-IR+Cd group. Cd is known to affect directly CYP11A1 and 3β-HSD by interfering with the DNA binding zinc finger motif through the substitution of Cd^{2+} for Zn^{2+} or by the cAMP-protein kinase A-dependent pathway which is the downstream pathway or by interfering with the phosphorylation of protein kinases (Kawai et al. 2002). Further, binding of Cd to the active site of enzyme decreases its enzyme activity as observed in the present study (Sengupta 2013). IR condition decreases the activity of enzymes involved in steroidogenesis (Mukherjee and Maitra 2010b). Studies with human luteinized granulosa cells from PCOS have reported increased expression of CYP11A1 and decreased expression along with activity of 3 β-HSD (Doldi et al. 2000; Jakimiuk et al. 2001b). All the above findings together indicate these as the possible mechanism for the decrease in progesterone synthetic pathway as observed in the PCOS-IR+Cd group in the present study.

CYP19A1 is a key enzyme for estradiol biosynthesis and for maintaining homeostasis balance between androgens and estrogens (Wang et al. 2011). 17-β-HSD plays an important role in conversion of estrone to estradiol, the principle ovarian estrogen (Glister et al. 2012). In the

present study, we demonstrated significant decrease in protein expression of 17β HSD and CYP19A1 in the IR+Cd group as compared with control. In a normal physiological condition expression of CYP19A1 increases in a dominant follicle with a diameter of ~9 mm, whereas in PCOS it is very low as the growth of follicle stops beyond 4-7mm due to hyper responsiveness of granulosa cells to LH (Jonard and Dewailly 2004). Other than this presence of several proteins in PCOS follicular fluid such as high molecular weight FSH receptor binding inhibitor, inhibin-a subunit precursor, insulin-like growth factor binding proteins (IGFBPs), epidermal growth factor (EGF), tumour necrosis factor-a (TNF-a) and 5a-androstane-3,17-dione reflect that the physiological microenvironment is insufficient to induce the expression of CYP19A1 and 17β-HSD (Jakimiuk et al. 1998; Naessen et al. 2010). Moreover expression and activity of CYP19A1 and 17 β-HSD are found to be decreased *in vivo* in smokers and *in vitro* by exposure to Cd (Mukherjee and Maitra 2010b). All these findings confirm the decreased expression of CYP19A1and 17β-HSD as observed in PCOS-IR+Cd group in the present study.

FSH-R plays a major role during folliculogenesis by involving in the growth of antral follicles and LH-R plays a major role in ovulation for the release of oocyte. In the present study Cd concentration led to decrease in the gene expression of FSH-R and LH-R. Our this data was in agreement with the previous studies demonstrating that GC's exposed to Cd showed decrease in the gene expression of FSH-R and LH-R along with the decrease in levels of cAMP in the testis of Cd treated rats (Gunnarsson et al. 2003; Yang et al. 2008; González-Fernández et al. 2011). Cd is known to alter the gene expression and directly interfere with LH-R in a way that makes it non-functional or less functional than normal thus indicating more than one site for its drastic action (Gunnarsson et al. 2003). GC's from PCOS express high amounts of FSH-R and are highly responsive to FSH hormone in culture because of the amplification of the physiological phenomenon (Catteau-Jonard et al. 2008; González-Fernández et al. 2011). An increase in LH-R in GC's along with an exaggerated responsiveness to LH compared with granulosa cells in control follicles of similar diameter has also been observed in PCOS (Jakimiuk et al. 2001b). These findings support our observation of an increase in gene expression of FSH-R and LH-R in PCOS GC's as compared to control cells. Further decrease in gene expression of FSH-R and LH-R in the combined group as compared to IR group could be a result of more indicated adversity of Cd over IR.

Based on these studies, we propose that infertility observed is due to granulosa cell apoptosis with decreased steroidogenesis and deregulated responsiveness as observed in our previous results with rat granulosa cells. Thus IR and cadmium both make PCOS patients more prone to follicular atresia leading to reduced rate of healthy oocyte production and thus fertilization. Moreover the study could not be taken further in the direction of Cd and IR at the clinical level due to absence of Cd levels in the follicular fluid from controls as well as PCOS. We therefore further focused our study on understanding the insulin and steroidogenic mechanism with respect to IR in human luteinized granulosa cells from PCOS.

5.5 References:

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