REVIEW OF LITERATURE

2.1 Review of Literature

For over last 3 decades, researchers focused on EDCs and their impact on human health. EDCs have ability to act as agonist or antagonist to endogenous hormones. Diseases and disorders like reduced fertility, diabetes, obesity and polycystic syndrome have been linked to the exposure of EDCs (59). The mechanism of causing disorder is not clear and still has uncertainty on dose level of EDCs. However, low dose of EDC can cause serious diseases at later stage of life (49).

BPA is one the most ubiquitous endocrine disruptor on earth since 1950 because of high resistant to heat, temperature, visual clarity and electrical resistance. Polycarbonate plastics are found in plentiful products such as dental products, plastic baby bottles, food, canned drink and medical products. Leaching rate of BPA from polycarbonate plastic is very low but due to large amount of polycarbonate in the environment this ratio has increased. The impact of BPA on human health is widely disputed. The amount of BPA leaches from polycarbonate bottles is very low, less than threshold value suggested by United States Environmental Protection Agency (USEPA) safety norms. Furthermore, there is lot of literature present which links BPA exposure and polycystic ovary syndrome in women (105).

PCOS can be characterized on the basis of high level of androgenism, irregular menstrual and multiple follicles and insulin resistant among PCOS (106). Women with PCOS have elevated level of LH and low level of FSH which results in hirstusim (106). In experimental studies, the effect of BPA on reproductive organs such as ovary, uterus and estrus cycle was also shown.

2.2 BPA Effect on Oogenesis

The impact of BPA on oogenesis has been studied mainly in animal models and in vitro. These studies revealed that BPA has an impact on two stages of oogenesis which includes the beginning of meiosis in fetal and breakdown of germ cell and follicle formation. Richter et al., 2007 reported that gestational exposure affects the beginning of meiosis and induces nondisjunction in fetal ovary does not cause aneuploidy (59). In a study it was observed that when a low dose (<1 ng/ml in mother serum) was introduced, it caused disrupted synapsis and
homologous recombination in meiosis (107, 108). Previous findings proved that gestational low dose BPA exposure on mice results in changes of gene expression in cell line and meiosis. Lawson et al., 2007 proved that low dose exposure elevated the expression of Stra8 gene and various meiotic genes C57BL/6 mice (109). One year later of Lawson study, it was found that longer exposure to BPA down regulate the Stra8 gene, Dazl gene and Nobox gene in CD-1 mice (110). These studies clearly indicate that low or high dose BPA exposure regulate the gene expression at meiosis level. Further in vitro studies also provide evidence in support that BPA has affected the onset of meiosis. In cultured human fetal oocyte it is observed that BPA exposure increased degeneration in oocyte by impede in meiosis. Similar effects were observed in animal study like increased level of recombination at the prophase stage in human fetal oocyte studied in mouse model by BrieñoEnriquez et al. (111). Trapphoff et al., 2013 showed that BPA exposure increased methylation error in genes of oocytes of C57/BL6xCBA/Ca in mice (113).

In animal models it has been identified that BPA interferes with germ cell nest breakdown and is responsible for increased multiioocyte follicles (114). Similarly when macaque diet was exposed with low dose BPA, it showed increased follicles in secondary and elevation in incidence of unenclosed oocytes due to continuous exposure to BPA (108). Zhang et al 2012 revealed the pattern of oocyte on dose dependent manner with increased number of unenclosed oocyte at low dose whereas high dose decreased the primordial follicles (110). Above studies have collectively shown that low and high doses of BPA has affected gestational stages of oocyte. Multiple oocytes in ovary is a pathological condition and may lead to ovulatory problem at later stage (115). In an animal model it was identified that BPA exposure accelerated the follicle transition, reduction in primordial follicles and rising follicles without upsetting follicle numbers (114). Rodriguez et al., 2010 found same results in Wistar rats when exposed to BPA (116). Whole data suggested that BPA enhances the recruitment and growth of primordia in all studied animal models. But it is not clear that BPA exposure at gestational level have deleterious effect on human health or not. Because all the study has been done on animal models and have been cross designed, so low dose deleterious effects of BPA cannot be measured in humans. Animal studies suggest that low dose initiate the gene expression while high dose inhibit gene expression.
2.3 Steroidogenesis in female

A lot of investigation was done to identify the relation between BPA exposure and steroid hormone production in females. In three different studies it was observed that prior to oocyte retrieval, the estradiol level decreases when exposed to BPA (117, 118, 119). Kandaraki et al., 2011 showed that women with PCOS have high level of testosterone and androstenedione (105). Ehrlich et al., 2013 collected data from 60 women undergoing *in vitro* fertilization (IVF) and observed that BPA was not responsible for negative regulation of linear dose response for expression of steroidogenic enzyme Cyp19 of granulosa cell, although the BPA response in granulose cell was associated with non-monotonic dose (120). In contradiction, in Italy a prospective population based study identified that BPA was not associated with elevated level of estradiol and testosterone (121). Mostly studies have been done on IVF population but to correlate or to know the side effects of BPA on steroidogenesis, study must be conducted on the general population.

Animal models were also used to explain the BPA impact on steroidogenesis, for instance, in a study on rodents, it was proved that BPA is associated with increased level of estradiol (122, 123). Research conducted on pregnant mice and SD rats show increased level of progesterone and testosterone (122, 123). It was also identified in a research that only prenatal exposure did not alter hormone level in mice model in parallel study shown that gestational and gestational plus neonatal BPA exposure in mice, rats and lambs model had no effect on steroidogenesis (114, 124, 125,126). The dose used in above study was lower than the previous study which may be reason for different results in the same model. Berger et al., 2008 conducted study on pregnant mice and BPA dose did not affect estradiol but decreased the level of progesterone in early stages of pregnancy (127). Moreover, in adult rats low dose BPA (<0.1 mg/kg/day) decreased expression of aromatase (Cyp 19), Steroidogenic acute regulatory protein (Star) and reduce the level of estradiol and testosterone (128). It was also observed that low dose of BPA in adult rat causes reduction in estrogen and progesterone receptor but did not affect hormonal level (129). Difference in low dose response may be due to the variation in dose level, time and choice of animal model for study, which makes this problem more complex to understand it.
The inhibition of estradiol, testosterone, estrone, dehydroepiandrosterone, androstenedione and progesterone has been reported in various in vitro studies. Researcher found the decreased expression of StAR and Cyp11a1 in in vitro study of cultured intact murine antral follicles (130, 131). Zhou et al., 2008 reported opposite effect of BPA on rat theca-interstitial cells (132). Another study shows that higher dose of BPA inhibit the estradiol production in granulosa cells (133). Above studies indicate the impact of BPA exposure on steroidogenesis, but the effect of dose response varies between the model and study style.

2.4 Effect on Oocytes

Very few studies are available to analyze the association between fertilization and BPA level in urine or serum. In a human study, Fujimoto et al., 2011 included 58 infertile women and 37 male partners to correlate association between BPA level and oocyte maturation and fertilization rate. Fujimotto et al., found relation of BPA level associated in Asian women (134). In two studies conducted on same prospective, it was revealed that the number of retrieved oocyte, mature and normally fertilized oocytes were reduced in women those have higher BPA concentration in urine (118, 119). Above results in human study point out that decreased number of oocyte or mature oocyte have association with BPA level in humans. Further studies need to be conducted to clarify this scenario and to improve success rate in IVF.

Previous findings supported the new research in which it was proved that BPA affects the meiosis in preovulatory oocytes (135). Germinal vesicle breakdown in hybrid female mice inhibited by low dose of BPA confirmed the previous results of BPA effect on germinal cell (136). Eichenlaub-Ritteret el., 2008 administered low dose BPA at postnatal (22-28 days) in hybrid mice and observed no effect on germinal vesicle breakdown and spindle aberrations (137). Similarly in superovulated female mice low dose BPA does not affect oocyte number and maturation of oocytes (138). Although, variation among above studies are unknown and there could be numerous reasons behind these variations.

Eichenlaub-ritter et al., 2008 conducted in vitro test of BPA on cultured oocytes. It was found that BPA (43.8 µl) induce congressional failure in mouse via altering spindle formation and disturbing pericentriolar material (137). Another experiment showed that 30µl BPA
concentration causes impaired spindle alignment in meiosis and arrest meiosis before germinal vesicle breakdown (139). In 2013, Machtinger et al., showed BPA effect on *in vitro* maturation of oocytes in the presence of different range of BPA concentration (0.02, 0.2, 20µg/ml). Results showed that with increased concentration of BPA, the incidence of meiotic arrest also increases by causing disturbance in spindle alignment, formation and activation of oocytes (140). From above researches it is concluded that BPA have adverse effects on maturation of oocytes.

### 2.5 Effect of BPA on Oviduct

There is only one study available which shows the effect of BPA dose on oviduct. Newbold et al., 2009 exposed CD-1 mice with low dose of BPA; it induces proliferation of lesions in oviduct (141). It is believed that morphology of oviduct is critical for fertilization and transportation of embryo. Further studies need to be conducted which are required to understand the effects of various doses on oviduct.

### 2.6 Effect of BPA on uterus

There is no evidence present regarding the impact of BPA during gestational period in human because of tremendous difficulties to measure effects on adults of parental exposure to BPA. However some animals studies showed that BPA exposure at gestational stage disturb the morphology of uterus in adults. Newbold et al., 2009 reported that low dose of BPA may induce benign and malignant lesion in uterus and accumulation of mass in inner lining of uterus (endometrial polyp) in mice (141). Study of BPA exposure on hens revealed that BPA is associated with reduction in thickness of tunica mucosa and density of uterine glandular structure as compared to non-exposed hen (142). From above research it is concluded that BPA has adverse effects on uterine morphology leads to deleterious effects on health.

### 2.7 Effects on Uterine Endometrium

Uterine endometrium can be defined as the inner lining or inner mucous membrane of mammalian uterus. Uterine endometrium is essential for implantation of embryo and morphology of this layer changes with change in sexual hormones. A limited data is available in relation with exposure of BPA and effect on uterine endometrium. Earlier studies showed that BPA level in serum are associated with endometriosis. Buck Louis et al., 2013 conducted a study
on 495 persons and found no correlation between BPA exposure and endometriosis (143). However Buck Louis experimental design has lapse and not designed for BPA exposure. In animal study it was identified that low dose exposure during prenatal and neonatal stage in Balb-c mice induces an endometrial like structure with stroma and gland of adipose tissue present around genital tract. It was found that endometrial like structure expressed Hoxa 10 that is responsible for proliferation of stromal tissue (144). This finding supported by other research in which adult mice CD-1 and ICR were exposed at prenatal stage with low and high BPA dose which induced expression of Hoxa 10 (145, 146). In rodent study of BPA exposure varying with dose, time and route, it was observed that these factors caused decreased expression of estrogen receptor α (Esr 1) responsible for endometrial proliferation in uterine (129; 145, 147).

Mendoza et al., 2011 note reduction in uterine epithelium proliferation at low dose in adult rats with respect to hormone treatment (125,147). Aldad et al., 2011 reported that progesterone level was not affected by BPA exposure in non-human primates and in another study it was reported that BPA is associated with impairment of apoptosis in uterine epithelium (125, 148).

Aghajanova and Giudice 2010 checked the BPA (50 and 100µl) effect on human endometrial and found that BPA inhibit the proliferation of human endometrial stromal fibroblast and endometrial endothelial cells (149,150). Collaboratively, the data suggested that BPA could damage the uterine cell proliferation.

### 2.8 Effects on Embryo Development

Researchers tried to find out the effect of BPA exposure on embryo development and little evidences are present which supports this theory. In group of 174 women with similar characteristics, the urinary BPA level was measured and it was concluded that BPA is associated with decreased rate of blastocyst formation (118). Bloom et al., 2011b studied 27 couples undergoing IVF treatment and found elevated concentrations of BPA in male urine but female partner don’t have elevated level of BPA in their urine samples. This study showed that BPA in men is associated with decreased probability of high fragmentation of embryo suggesting that embryos are good enough for IVF (151). In vivo studies of BPA exposure also gave interesting results. In a study it was found that BPA with high dose is associated with delay in embryo development in mice (152). Susiarjo et al., 2013 suggested that low dose BPA exposure
disturbed the expression of imprinted genes of placenta and midgestation embryo (153). To get precise result of BPA exposure and their impact on embryo development, it needs more research in this field.

2.9 Effect on Placenta

Although the epidemiological study on placenta and BPA is absent, however Tan et al., 2013 found a relation between BPA and placenta but this relation indirectly effects placenta. It is concluded that low and high dose of BPA elevates the mRNA expression of corticotrophin-releasing hormone and activate protein kinase C and δ in placenta which is responsible for increase in estradiol, testosterone and corticotrophin releasing hormone (CTRH) levels in plasma (123). In an in vitro study, it was observed that BPA (1-10µM) causes apoptosis in trophoblast cell of trimester placenta while at 10µM concentration of BPA cell viability was decreased (154). Susiarjo et al., 2013 suggested that BPA alters the placental gene (153). However, above studies are not enough to conclude that whether BPA has adverse effect on placenta or not, so further research need to clarify the impact of low and high dose on placenta.

2.10 Effect on pregnancy outcomes

Pregnancy outcomes and BPA relations are well studied in humans. It was observed that premature delivery and BPA exposure positively associated with one another (155). Mostly data available is on the basis of animal study related to pregnancy outcomes and association of BPA. In mice study it was noticed that BPA exposure at low dose do not change the length of gestation period in mice (156; 157, 158, 159). Cabaton et al., 2011 suggested that BPA exposure at the time of gestation decreased the rate of successful delivery in mice (156). BPA has also been reported for declining the percentage of hatching among chicken when they were exposed on day 4 to BPA (134 ng/kg) (160). In a study it was concluded that maternal exposure may affect delivery but paternal exposure to BPA don’t have any adverse effect on pregnancy (161). Many in vivo experiments showed that number of live pups and delivered pups in mice and rats were not changed after BPA exposure at low dose (157, 158, 159, 161, 162,163,164, 165). While Cabaton et al., reported depreciation in number of live pups in CD-1 mice when exposed at parental or neonatal stage to BPA (156, 166). In pregnancy condition animal data showed that BPA exposure at the time or before pregnancy gives different results.
2.11 BPA effects on male reproductive system

In two studies; one group of male was those who were exposed during work and in second group, the males selected from Infertility Centre and it was observed that results in higher BPA level in urine leads to decrease in sperm count and motility (167, 168). However semen parameter in a fertile man was not associated with BPA exposure while it is linked with markers of testosterone (169). It is early to conclude that semen quality or quantity in humans have association with BPA exposure. But still a few evidences indicate that BPA exposure lowers the quality of sperm in male. In a study it was observed that BPA exposure at prenatal and postnatal stage affects the spermatogenesis in adult rats. At the time of development of testis, BPA exposure has adverse effect on testis development in rats. However study design like exposure route, time, concentration, and choice of animal, strain of animal, species of animal and life stage of animal factors affects the outcomes of study. Recently in ICR mice, it was noticed that longer exposure to low dose at gestational stage affect the number of elongated spermatids and sperm number (170, 171). Studies also revealed that at the time of puberty, BPA exposure in rats and mice reduced spermatogenesis and increased apoptosis (172, 173, 174, 175). Adult rats were also reported with reduced number in sperms after 48 hr long exposure (176, 177). In a research it was observed that any route of exposure to BPA causes reduction in sperm number and increased motility (177-181).

Minamiyama and co-workers experimentally proved that when antioxidant (n-acetyl cystein) was administrated during BPA exposure in rats, it prevented sperm motility (179). This effect produced may be due to increased level of reactive oxygen species that scavenged by antioxidant motility of sperm decreased. This study concluded that BPA exert oxidative stress among species and leads to adverse effects. Spermatogenesis is regulated by the androgens and as discussed earlier, the BPA level affects the endogenous hormone by agnostic or antagonistic actions. BPA and endocrine related changes have been reported in many cases. Rodents exposed with BPA at gestational time leads to impaired spermatogenesis, reduced number of steroid receptor and decreased expression of Star. In many studies DNA damage was also reported which is associated with BPA and reactive oxygen species (ROS). Longer exposure to BPA affects the production of ROS and ultimately DNA damage in rats and mice (173, 178, 179, 182, 183; 184,
Tiwari et al., 2013 found that oral administration of BPA can cause sperm DNA damage in rats at low doses (177).

2.12 Relation between BPA and PCOS

PCOS is well thought as the utmost endocrine irregularity in women of the reproductive age group, occurring in 6-8% of the females and is characterized by elevated serum LH, testosterone, and insulin; low or normal levels of FSH; abnormalities of estrogen secretion; and premature pubic hair growth (pubarche).

Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide which is crucial for normal reproduction in mammals. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are synthesized and released upon stimulation of gonadotropes by GnRH (186). The secretion and frequency of GnRH varies during the development and menstrual cycle of females as well. The GnRH peaks are well followed by the Increases in plasma gonadotropins, thereby causing disturbance in the normal pulsatile secretion and hence causing reproductive disorders in humans, such as PCOS (187).

Limited studies are available representing the relation between the BPA levels in blood and urine samples of PCOS and healthy females. Kandaraki et al. 2011 determined the BPA levels in women with PCOS and were compared to controls, age and body mass index (BMI) matched and moreover, the relationship between BPA levels and hormonal and metabolic parameters of studied subjects were also observed (105). A cross-sectional study was done involving 71 PCOS females and 100 healthy subjects with age- and body mass index–matched. PCOS women were found to have higher BPA levels as compared to the healthy counterparts. Moreover, a statistically significant positive relationship amongst androgens and BPA pointed towards the likely role of this endocrine disruptor in PCOS pathophysiology.
Furthermore, it has also been observed by Fernandez et al. 2010 in animal studies that during the period of brain sexual differentiation, altered the hypothalamic–pituitary–gonadal axis was observed in female Sprague-Dawley rats when they were exposed to high doses of BPA (188). These finding potentially associate the development of poly-cystic ovarian syndrome to the neonatal exposure to high doses of BPA in rats. Moreover, it has been established that the oocyte development is highly influenced by the exposure of experimental animals to BPA adversely resulting in ovarian cystic morphology.