1. Introduction:

Obesity is rapidly becoming a worldwide epidemic affecting both children as well as adults. Obesity is a condition in which the natural energy reserve is stored in the tissue of humans and other mammals. It is an abnormal or excessive accumulation of body fat, usually 20% or more over an individual's ideal body weight **(**WHO, 2000**).** The principle cause of obesity is an imbalance state of the body's energy intake and expenditure, wherein the energy consumed exceeds the energy expended (Galgani and Ruvassinm, 2008). Excessive weight or obesity can result in many serious potentially life threatening health problems including hypertension, type-II diabetes mellitus, coronary disease, unexplained heart attack (Mah *et al.*, 2010), hyperlipidemia, infertility and a higher prevalence of colon, prostrate, endometrial and possibly breast cancers. Approximately 30,000 deaths a year were attributed globally to obesity. The body mass index (BMI), uses the height-weight relationship to calculate an individual's ideal weight and risk of developing obesity-related health problems. BMI is a good marker of obesity and it is calculated by person's weight in kilograms divided by the square of their height in meters (kg/m^2). If BMI is within the range of 18.5 to 24.9 the person is said to be normal, if it is below 18.5 is underweight, above 25 is overweight and above 30 is considered as obese (WHO, 2000). BMI in rats is calculated as the ratio of body weight (g) to square of body length (cm^2) measured from tip of the nose to the anus (Remmers *et al.*, 2008). Studies have also suggested that, genetic, physiological and behavioral factors also play a significant role in the etiology of obesity (Wellborn *et al.*, 2005). Both over-nutrition and under-nutrition affect the reproductive system (Bray, 1997). Though some studies have shown a relationship

between obesity and infertility, still it remains controversial (Fernandez *et al.,* 2011). Obesity causes a variety of alterations in male (Hammoud *et al.,* 2008a) and female reproductive system (Rich-Edwards *et al.,* 1994, Metwally *et al.,* 2007).

Obesity and male reproduction:

Several studies have been conducted in humans as well as in animal models to know the effects of obesity on male reproductive organs, semen quality and fertility.

Shafik and Olfat (1981) reported that, the number of Sertoli cells is low in obese persons than non obese subjects might result in lower sperm count permanently. In men testicular size remains normal, but serum concentration of testosterone decreases as obesity increases (Kley *et al*., 1980). Obesity is considered as one of the risk factors to affect fertility and increased BMI is associated with poor semen quality (Magnusdottir *et al.,* 2005), decreased sperm concentration (Jensen *et al.,* 2004; Hammond *et al.,* 2008b), decreased normal-motile sperm cells and increased DNA fragmentation index (Kort *et al*., 2006). Kasturi *et al* (2008) reported that obesity affects sperm concentration, motility and morphology and increases sperm DNA damage in humans. Obesity is possibly related to oxidative stress (OS) in the epididymis. Likewise obesity could also be related to other impairments such as erectile dysfunction which is a problem in obese human males (Feldman *et al*., 2000). There is a higher prevalence of oligozoospermia (<20 million spermatozoa/mL) in overweight and obese men compared with normal weight men (Jensen *et al,* 2004). Men with higher BMI (>25) have quantitatively and qualitatively low sperm, lower serum testosterone concentration and higher in levels of estrogen and FSH than men with BMI ranging between 20 and 25 (Sallmen *et al.,* 2006; Hammoud *et al.,* 2008b). Inhibin B is a good marker for spermatogenesis and it is reported that reduction in

inhibin B results in lower sperm count in obese males (Jensen *et al.,* 2004; Myers *et al.,* 2008; Pauli *et al*., 2008)**.** Increasing BMI was positively correlated with the DNA fragmentation Index (DFI) as overweight and obese men had higher DFI, i.e. 25.8% and 27% respectively, compared with 19.9% in normal-weight men (Kort *et al*., 2006). There is a higher probability of prevalence of abnormal spermatozoa and infertility in obese men (Du Plessis *et al.,* 2010). Reduction in testosterone levels (Bray, 1997) and higher levels of estradiol were observed in obese men (Schneider *et al.*, 1979; Strain *et al.*, 1982; Tsai *et al.*, 2004). In obese men, higher levels of estrogen affects spermatogenesis by direct action on the testis as well as by alteration in gonadotropin secretion (Schneider *et al.,* 1979; Tsai, *et al*., 2004; Hammond *et al*., 2006).

Leptin is a main product of body fat (Considine *et al,* 1996). Serum concentration of leptin is increased in obese people and causes decrease in the testosterone synthesis in the Leydig cells, *leading* to inhibition of maturation of spermatozoa (Margetic *et al*., 2002). Leptin appears to act as a direct inhibitory signal for testicular steroidogenesis (Tena-Sempere and Barreiro, 2002).

In addition deposition of fat around the scrotal blood vessels leads to impaired blood cooling and elevated testicular temperature and thereby adversely affects spermatogenesis in men (Sharpe, 2010). Lipophilic contaminants are associated with decreased sperm production and thus reduction in male reproductive potential, even if fat and fat soluble toxins are not localized in the scrotal area (Aggerholm *et al.,* 2008). Obesity is thought to increase DNA damage in spermatozoa through a variety of mechanisms such as increasing reactive oxygen species levels, testes temperature and

levels of fat-soluble toxins and altering hormone levels (Du Plessis *et al.,* 2010; Sharpe, 2010)

The impact of obesity on reproduction has been studied in animal models to understand the reproductive alterations, as obesity can be induced in normal animals. Studies in animals also reveal adverse effects of obesity. High fat diet can induce nutritional obesity in pubertal rats, which in turn may lead to the under development of the testis (Wang *et al*., 2007). Obesity caused reduction in germ layers in the seminiferous tubules was observed in rats (Wang *et al.,* 2007) and sperm count in mice (Ghanayem *et al.*, 2010, Bakos *et al*., 2011, Palmer *et al.,* 2011, 2012) and rats (Fernandez *et al.,* 2011). In Sprague–Dawley rats high fat diet (HFD) induced obesity resulted in impaired glucose tolerance and insulin sensitivity (Sheau-Fang *et al*., 2010). Decrease in sperm motility as well as increase in DNA damage and sperm intracellular ROS have been observed in diet- induced obese mice (Ghanayem *et al*., 2010; Bakos *et al*., 2011). Further, diet induced male obesity reduced sperm motility, sperm counts and the percentage of sperm with normal morphology concomitant with increase in epididymal sperm transit time was observed in rats (Fernandez *et al*., 2011; Duale *et al.,* 2014). Rise in sperm DNA damage, as a result of increased adiposity has also been observed in other studies (Bakos *et al*., 2011; Palmer *et al*., 2012; Chen *et al*., 2013; Duale *et al.*, 2014; Vendramini *et al.,* 2014; Zhao *et al.,* 2014). Obese rabbit testis showed expanded spaces between seminiferous tubules and reduction in seminiferous tubule diameter, lumen and proportion of sperm (Soltani *et al.,* 2013). In mice, high fat diet had reduced the sperm binding with oocytes, which was directly related to reduced sperm capacitation and fertilization (Bakos *et al*., 2011; Palmer *et al.*, 2012).

In addition, obesity effects on reproductive organ weights have also been reported. The obese rats had significantly smaller epididymis and seminal vesicles, larger prostates and underdeveloped testes compared with lean rats (Suh *et al.,* 2011). High fat diet feeding for 15, 30 and 45 weeks in rats caused similar alterations in reproductive organ weights and sperm counts compared to standard diet group at all time points studied (Fernandez *et al.,* 2011). Induction of high fat diet induced obesity in pre-pubertal rats showed increase in the body weight, BMI and Lee index and decrease in the relative testicular weight in mature rats (Hussain *et al.,* 2015). However, the testis and epididymis weight did not change in diet induced obese mice compared to normal mice (Ghanayem *et al.,* 2010).

In the genetic models of obesity, there is a marked impairment in reproductive functions in males (Bray, 1997). Reproductive behavior has been shown to be abnormal in obese male Zucker rats (Edmonds and Withyachumnarnkul, 1980). In obese male Zucker rats, the testis morphology showed hypertrophy of the Leydig cells which contained many lipid droplets (Young *et.al.,* 1982). Plasma testosterone was reduced in obese male Zucker rats when compared with non-obese male Zucker rats (Young *et al.,* 1982). However, in Zucker rats, the genetic model of obesity, the testis and epididymis weight of obese rats did not differ from lean rats (Edmonds *et al.,* 1982).

Obesity is also known to cause hormonal alterations. Obesity is known to affect androgen secretion and thereby androgen dependent processes. The preputial separation, an external sign of puberty in male rats is androgen dependent process (Korenbrot *et al.,* 1977). Under feeding of male mice before weaning lead to a substantial delay in onset of puberty (Jean -Faucher *et al.,* 1982) whereas obesity

caused early onset of puberty in rats (Yen *et al.,* 1994). In men testicular size remains normal, but serum concentration of testosterone decreases as degree of obesity increases (Kley *et al.,* 1980). Male obesity is associated with reduction in sperm concentration, counts of progressively motile sperm and serum levels of total and free testosterone compared to normal individuals (Mokdad *et al.,* 2001; Magnusdottir *et al.,* 2005; Sallmen *et al.,* 2006; Hammoud *et al.,* 2008a). High fat diet can induce nutritional obesity in pubertal rats, which in turn may lead to the abnormal levels of gonadal hormones (Wang *et al*., 2007). For instance, male rats fed with high fat diet had significantly lower levels of testosterone compared with control diet fed male rats (Sanaa *et al*., 2014).

Despite good number of reports cited above, there are some lacunae in our understanding of influence of obesity on male reproduction. For instance, influence of obesogenic environment due to mother's obesity during pregnancy and lactation followed by HCD during pre-pubertal period, on age dependent appearance of different categories of germ cells viz. spermatogonia, spermatocyte, spermatids and spermatozoa is not known. In addition whether reduction in sperm count in obese males of both men and experimental animals, is due to effect of obesity on early or advanced stages of spermatogenesis needs to be understood.

Obesity and Female reproduction:

Obesity is an important risk factor in obstetrics and gynecology and affects female reproductive functions in several respects. The presence of anovulatory cycles, oligoamenorrhoea and hirsutism, either separately or in association were significantly higher in obese than in normal weight women (Hartz *et al.,* 1979).

A significant association is seen between excess body fat (particularly abdominal obesity) and irregular menstrual cycles (Kirschner, 1982). In addition, menstrual disorders are more frequent in girls with excess body weight during onset of puberty than in those who were obese during infancy (Lake *et al*., 1997). Obesity in childhood and in the early twenties increased the risk of menstrual problems (Lake *et al*., 1997).

Maternal obesity may have a greater deleterious effect on pre-term parturition (Lucas *et.al.,* 1988). Prolonged duration of labor (Garbaciak *et al.,* 1985; Edwards *et al*., 1996; Weiss *et al*., 2004), early pregnancy loss (Wang *et al*., 2002), increased risk of miscarriage (Millis, 1992; Metwally *et al.,* 2008) and still birth (Chu *et al.,* 2007) are associated with obesity in women. Complications during pregnancy, childbirth, menstrual disturbance and infertility are consequences of female obesity (Pettigrew and Hamilton-Fairley, 1997). There is an increased risk for gestational diabetes mellitus in obese women. This association increases hypertension during pregnancy and there is also increased risk for macrosomic fetus, hypoglycemia and hyperinsulinemia at birth (Glueck *et al.,* 2002; Andreasen *et al.,* 2004; Linne, 2004).

The pubertal development in females involves changes in body weight and/or body composition, which are critical factors for regulating puberty (Frisch and McArthur, 1974; Frisch, 1980). Rate of weight gain during very early childhood may be more critical determinant of the timing of puberty in both boys and girls (Mills *et al*., 1986; Cooper *et al*., 1996). Overweight condition at younger ages is associated with earlier menarche (Moisan *et al.,* 1990, Maclure *et al.,* 1991). It is opined that obesity is an important contributing factor to the onset of puberty in girls

(Kaplowitz *et al.,* 2001). Leptin, a hormone secreted by adipose tissue plays an important role in onset of puberty (Farooqi *et al.,* 1999). Girls have increased leptin concentration compared with boys, independent of adiposity (Sandra *et al.,* 1996). Obese children and adults have higher levels of leptin, insulin, androgen and estrogen levels than lean individuals (Klein *et al*., 1998; Di-Vall and Radovick, 2008). In obese people, leptin levels are more than sufficient to suppress the appetite which may result in resistance to leptin. Obese children have high leptin levels which plays a role in earlier onset of puberty in them (Shalitin and Phillip, 2003). The menarche occurring at early age in obese girls (Kaplowitz, 2008; Keim *et al*., 2009; Boynton-Jarrett *et al*., 2011, Deardorff *et al.,* 2013) is associated with high leptin concentration that causes the gonadotropin-releasing hormone pulsatile secretion leading to menses (Blum *et al.,* 1997; Wong *et al.,* 1998). Normal leptin levels are needed for maintenance of menstrual cycles (Clarke and Henry, 1999; Mantozors, 2000).

Obesity also affects other aspects of female reproduction. For instance, women with central obesity have higher testosterone production rates than those with peripheral obesity (Kirschner *et al*., 1990). Obesity is associated with low oocyte count and reduced rate of embryo quality (Millis, 1992). Onset of ovarian senescence and increased production of FSH at menopause occurs several years earlier in obese than in normal weight women (Bray, 1997; Norman and Clark , 1998). On the other hand , a higher BMI than normal, delayed the age at menopause (Akahoshi *et al.,* 2002). Obesity caused increased levels of gonadotropin and androgens results in an abnormal ovulation (delayed /early) (Wilborn *et al.,* 2005). Obesity may play a role in the development of the polycystic ovary syndrome in

susceptible women (Akamine *et al*., 2010). Mechanisms by which obesity affects fertility are complex and still not completely understood (Pasquali *et al.,* 2003).

Studies in animal models also revealed adverse effects of obesity on female reproduction. Obesity due to overfeeding is associated with prolonged estrous cycles (Innami *et al*., 1973; Rolls and Rowe, 1982). Genetically obese female rats show delayed puberty (delayed vaginal opening) and abnormal estrous cyclicity (Bivens and Olster, 1997). Food restriction in female rats delays pubertal onset, induces abnormal estrous cycles and inhibits reproductive behaviour (Bivens and Olster, 1997). High fat diet fed obese female rats show an extended estrous cycle (Akamine *et al*., 2010). Leptin is a main product of body fat (Considine *et al*., 1996) which regulates the gonadotropin surge that initiates the development of pubertal stages (Farooqi *et al.*, 1999). Mice injected with leptin had earlier onset of three classic pubertal parameters viz. vaginal opening, estrous and cycling (Ahima *et.al*., 1997). On the other hand in leptin-deficient ob\ob mouse the reproductive system remains pre-pubertal (O'Railly, 1998).

In many experimental animals with obesity, particularly the genetic forms of obesity, there is complete infertility in females (Bray, 1997). Genetically obese female rats have enlarged ovaries and undeveloped uteri (Bivens and Olster, 1997). Feeding of high fat diet in rats reduces capacity to conceive and ability to maintain litter during peri-natal period (Shaw *et al.*, 1997) and increases serum leptin levels and decreases estradiol levels (Balasubramanian *et al.,* 2012). In mice high fat diet caused anovulation and reduction in fertilization rate (Gouveia and Franci, 2004) and irregular estrous periodicity and reduction in number of corpora lutea (Sharma, *et al*., 2013) were observed. Robker *et al*., (2011) showed that excessive accumulation of

lipids leads to oxidative stress and inflammation that mediate ovarian dysfunction in rats. Further, obesity of mothers might also affect ovarian function of offspring. In C57BL/6J mice, high fat diet prior to conception and maternal obesity during pregnancy and lactation had deleterious effect on follicular growth in adult offspring ovaries (Cheong *et al.*, 2014).

The ovarian follicular development, a crucial event in producing viable oocytes involves a sequence of events to convert primordial follicle into pre-ovulatory follicle. High fat diet interferes with these events. High fat diet fed obese female rats showed altered ovarian morphology (Akamine *et al.,* 2010). High fat diet for 4 months caused increase in apoptosis of ovarian follicles, smaller and fewer oocytes, decrease in embryonic IGF-IR staining, smaller fetuses, increase in placental Igf2r mRNA and smaller pups in C57BL/6J mice (Jungheim *et al.,* 2010). Cafeteria diet induced obesity caused reduction in number of oocytes and preantral follicles (Sagae *et al.,* 2012) and increase in follicular atresia (Wang *et al.,* 2014) in rats. On the other hand, an increase in number of primordial follicles following high fat diet for 120 days after weaning period was observed in rats (Tsoulis. 2014).

The ovarian follicular development is initiated during post-natal period, which follows a sequence of age dependent events, converting first time, the primordial follicles into pre-ovulatory follicles. This event is completed within first 4-5 weeks of life in rat, then the sequence of events repeat until follicular reserve exhausts (McGee and Hsueh, 2000). In women the pattern of follicular development is similar to rodent but for the fact that first development of pre-ovulatory follicles takes a few years, compared to a few weeks in rodents and the duration required for maturation and ovulation of follicle during each ovarian cycle in women is longer than rodents

(McGee and Hsueh, 2000). Thus far there is no information as to whether or not obesity during pre-pubertal period alters these age dependent sequence of events of ovarian follicular development, i.e. whether obesity advances or delays the process is not known. In addition, alterations if any in number of each category of follicles at different age intervals corresponding to land marks of ovarian follicular development in obese individuals need to be understood. Studies on these lines gain importance due to fact that incidence of childhood obesity is increasing globally in human population. Hence, there is a need to study the impact of *in-utero* and post-natal exposure to obese condition on early follicular development in animal models.

Obesity, fertility and reproductive outcome:

Maternal obesity has well-recognized short-term complications for both mother and child (Garbaciak *et al*., 1985) and it is commonly associated with fetal overgrowth rather than growth restriction (Catalano *et al*., 2012). Children of obese parents have an increased risk of becoming obese compared to children of parents of normal weight (Lyra *et al*., 2003). Obese fathers are more likely to father an obese child (Li *et al.*, 2011).

Pregnancy complications viz., hypertensive disorders, gestational diabetes, prolonged duration of labor, increased need of operative delivery, macrosomia, increased blood loss occur due to obesity (Garbaciak *et al.*, 1985; Edwards *et al.*, 1996; Weiss *et al.*, 2004). In obese women, pregnancy rate and live birth rate is consistently decreased especially due to an increased miscarriage rate (Wang *et al.*, 2002; Lintsen *et al.*, 2005; Maheshwari *et al.*, 2007). Ovulatory and anovulatory subfertile women are overweight or obese (Koning *et al*., 2010). Fertility is affected in

obese men due to decrease in count of spermatozoa (Jensen *et al*., 2004, Magnusdottir *et al.,* 2005, Hammound *et al.,* 2008b).

Studies in animal models also reveal impaired fertility and poor reproductive outcome due to obesity. Overfeeding induced obesity is associated with reduced rates of pregnancy in rodents (Innami *et al*., 1973; Rolls and Rowe, 1982). Increased pup mortality was found among those born to dams fed with high fat diets (Bue *et al*., 1989) or cafeteria diet (Rolls *et al*., 1980; Rolls and Rowe, 1982). Consuming a high fat diet reduces a rat's capacity to conceive and ability to maintain her litter during the perinatal period (Shaw *et al.,* 1997). Obese rats experiences greater difficulty in delivering their pups and more number of their pups die in the first few days of life compared to controls (Rasmussen, 1998). Diet-induced (60% of fat in diet) obesity in male C57BL/6J mice caused a significant reduction in fertility after natural mating (Ghanayem *et al.,* 2010). High fat diet induced paternal obesity in mice results in oxidative stress, sperm DNA damage and reduction in fertilizing ability (Bakos *et al*., 2011). Likewise, paternal obesity leads to intergenerational transmission of a decline in fertility, particularly in males, through both F_1 parental lines to F_2 generations (Fullston *et al*., 2012). Continued feeding of high fat diet for longer duration reduces pup's weight and increases mortality rate with early age of puberty in rats (Suker *et al*., 2013).

However effects of exposure to obesogenic environment during prenatal development due to dam's obesity or early post-natal development due to high calorie/high fat diet on fertility and reproductive outcome have not been studied.

It is evident from above literature review, that there are several lacunae in our understanding of influence of obesity on reproduction, as mentioned at the end of each section. There is a dire need to understand the impact of obesity during the pre-natal (impact of mother's obesity) and pre-pubertal development, as gonads develop, differentiate and mature during these periods. Studies on these lines gain importance due to fact that prevalence of childhood obesity is increasing globally. In the present day human societies, though prevalence of overweight and obese conditions in adults and children are increasing, studies conducted in animal models thus far do not mimic this situation, because reproductive effects of induced obesity either in adults (Akamine *et al.,* 2010; Balasubramanian *et al*., 2012) or effects of paternal or maternal obesity on offspring reproduction when they become adults have been studied (Fullston *et al*., 2012; Suker *et al*., 2013) whereas an animal model wherein effects of obesity on developing gonads could be understood, has not been developed. In view of the above mentioned facts, the present study was planned with objectives mentioned below:

Objectives:

To find out whether or not exposure to obesogenic environment during *in utero* development and pre-pubertal period affects,

i) age related development of different stages of germ cells, counts of germ cells, morphology and concentration of spermatozoa and onset of puberty in male offspring of obese mother rats,

,

ii) age related ovarian follicular development, counts of ovarian follicles belonging to different categories, follicular atresia and onset of puberty in female offspring of obese mothers rats, and

iii) reproductive performance of male and female offspring born to obese mothers