Chapter 1

Introduction And Review of Literature

1. Introduction

A drastic decline in the world total fertility rate (TFR) in the last couple of decades has now reached alarming proportions making infertility the fifth highest global disability in the young population by World Health Organization (WHO) and is associated with increased risk of subsequent chronic health conditions. Infertility is considered as one of the most important unappreciated health problems, particularly in developing countries including India (WHO/Infertility/2016).Worldwide higher prevalence of female factor infertility than that of male factor is a fallout of rapid economic development, urbanization and westernization as well as industrialization (Unuane *et al.*, 2011, Sohrabvand *et al*., 2015; Cyriac *et al*., 2017; Elhussain *et al.*, 2019). Amongst the various causes of female infertility, the disorders due to endocrine dysfunction are the leading cause and of these the most prevalent are thyroid disturbances. Hypothyroidism is the most common thyroid disorder and this has been shown to be an important cause of infertility in women of reproductive age (Krassas *et al.*, 2000; Poppe *et al*., 2007). Globally, overt/clinical hypothyroidism (OH) has been extensively studied for its role in causing female infertility, while subclinical hypothyroidism (SCH) as cause of female infertility is not documented well especially for western Indian population. Thus, a study finding prevalence and incorporating contributions from the most common causes such as autoimmune, environmental and genetic factors towards subclinical hypothyroidism is considered necessary. It would also be worthwhile to look at the resulting alterations in reproductive hormones, lipid profile as well as oxidative stress levels and subsequently verify correlation of these factors with female infertility, either independent or as a cumulative effect. This will contribute to our understanding of the etiology of this multi factorial disorder, which can further be used for a diagnostic approach in the management and treatmwent of primary infertility in reproductive age women.

1.1 Female Infertility

On the earth human beings are the least fertile creatures. It is not that easy to become pregnant even for people who do not have fertility problems and there is only 25% chances of conception each month since there is a very small window within the menstrual cycle when conception is possible. It is estimated that 10% of normally fertile couple fail to conceive within their first year of attempt and 5% after two years (Gnoth *et al.*, 2005**).** The reproductive health is described as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system (WHO/Reproductive health indicators/2006). Reproduction is a

fundamental evolutionary process necessary to sustain life and fertility is the key element for reproduction. “Fertility” is the ability to conceive and produce offspring. On the other hand, “Infertility” is the inability to reproduce by natural means and it is not the natural state of a healthy adult. A clinical definition of infertility is “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse”. In contrast, demographers define it as the inability of women in their reproductive age (15–49years) to become pregnant after exposure to regular unprotected sexual intercourse for five or more years (Zegers *et al*., 2009; Gurunath *et al*., 2011). While the WHO’s epidemiologic definition of infertility is given as “women of reproductive age at risk of becoming pregnant who report inability to get pregnant after more than two years” (WHO/Reproductive health indicators/2006). It can also be defined as failure of couple to conceive after 12 months of regular intercourse without the use of contraception in women <35 years; and after 6 months of regular intercourse without the use of contraception in women ≥35 years (Practice Committee of the American Society for Reproductive Medicine, 2013).

1.2 Epidemiology/ Prevalence of female infertility

Female infertility is a serious health concern affecting 15% of couples worldwide (WHO/Infertility/2016; Gurunath *et al.*, 2011; Mascarenhas *et al.*, 2012; Direkvand *et al.*, 2014)). Fertility is one of the prime determinants of population dynamics and among the key indices for measuring the development of any nation (Population Reference Bureau-PRB, 2016). The fertility rate of a country is the average number of children that women from that country will have throughout their reproductive years and thus the total numbers of children born in the year gives total fertility rate (TFR). World TFR has declined drastically from 4.9 in the year 1950 to 2.6 in 2010 showing almost 50% decrease; figure 1.1A and B (UN World Population Projects, 2008). And in the year 2020, it was estimated to be near to the replacement level of fertility which is 2.1(figure 1.2) according to the “Population Reference Bureau’s 2020 World Population Data Sheet” (Kaneda *et al.*; 2020). According to the United Nations World Fertility Prospects there is a drastic and rapid decline in the world TFR during last 6-7 decades and is expected to further fall below to 2.1 by the year 2050 for many countries including India, figure 1.3 (United Nations World Fertility prospects, 2008).

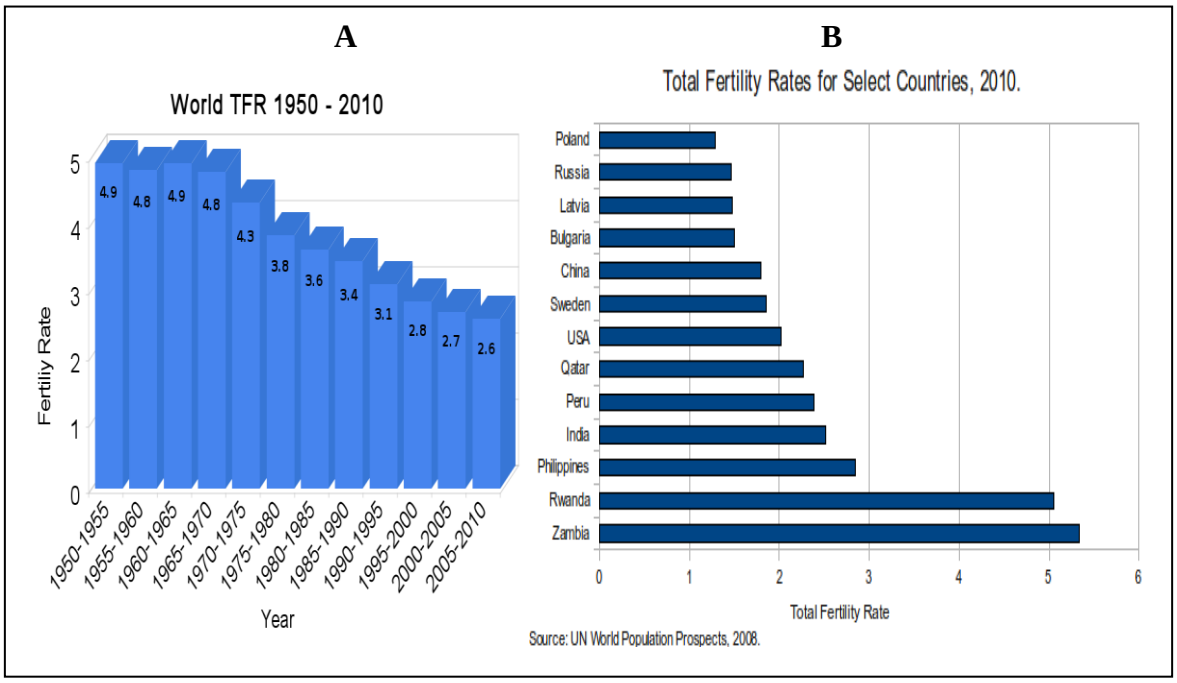

Figure1.1A.World TFR 1950-2010: The world total fertility rate (TFR) has declined from 4.9 to 2.6 during the years 1950-2010. **B. TFR for selected countries, 2010:** The TFR for selected countries shows the fertility rates of different countries including India near to the replacement level in the year 2010 (UN World Population Projects,2008).

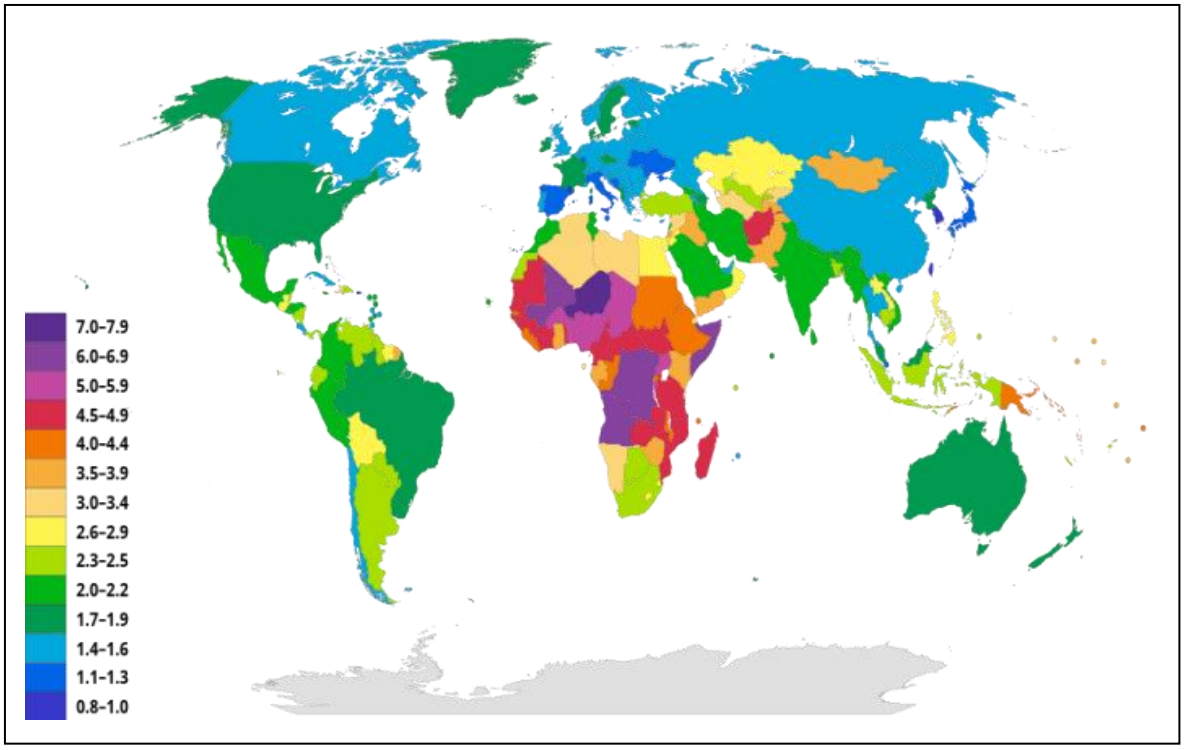

Figure 1.2World TFR-2020: The world TFR data in the year 2020 indicates a drastic decrease in fertility rates of different countries including india which is at the replacement level with the TFR rate of 2.0-2.2.TFR, Total Fertility Rate (Population Reference Bureau's 2020 World Population Data Sheet).

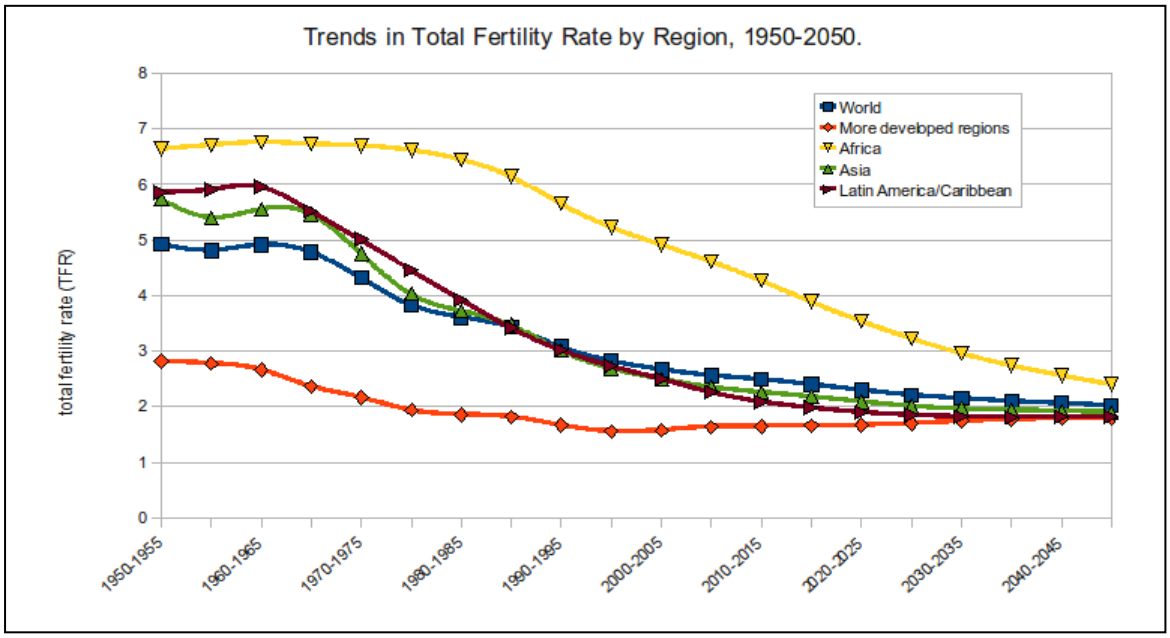

Figure 1.3 Trends in TFR by region, 1950-2050: The world TFR is expected to fall below 2.1, which is considered as the replacement level of fertility by the year 2050 in most of the countries worldwide including the developing countries like India. TFR, Total Fertility Rate (United Nations World Fertility prospects, 2008).

To find out the global burden of disease (GBD) for infertility a recent study by Sun *et al.* (2019) was conducted in 195 countries from 1990 to 2017. The study provides comprehensive estimates of the global, regional, and national burden of infertility concluding that the global disease burden of female infertility has been increasing throughout the period from 1990 to 2017 (Sun *et al.*, 2019).The Indian TFR has declined significantly in the second half of the twentieth century, and the prevalence of primary infertility at national, regional levels has increased from 1992-93 to 2005-06 with a further remarkable increase in 2015–16. The decrease in the rate of fertility follows a common correlation between quality of life, where the fertility rate decreases as the standard of living improves. Post-independence socio-economic improvements in India led to a decreased fertility rate, which has fallen down further to 2.2 in 2020s (niti.gov.in Total fertility rate 2000-2016). It is estimated that there are 60–80 million infertile couples worldwide out of which 25– 28% (15–20 million) are in India alone (WHO/ Reproductive health indicators for global monitoring/2001; Boivin *et al.*, 2007; Purkayastha *et a.*., 2021). Figure 1.4A shows the data of TFR in different Indian states in the year 2012 depicting the Gujarat state fertility rate at 2.2-2.6, which was near to the replacement level of fertility. As per the data collected by the Sample Registration Survey (SRS), conducted by the Registrar General of India, the country's official source of birth and death data, 2013 the TFR in eight states has fallen below the replacement level of fertility.

West Bengal at 1.8 has India's lowest fertility, while Bihar with a TFR of 3.2 is the highest. Comparative Data on the Indian states for the years 2006-07 and 2015-2017 depicts a drastic decline in the TFR, with Gujarat state at the fertility rate at 2.2, figure 1.4A and B (SRS, 2013).

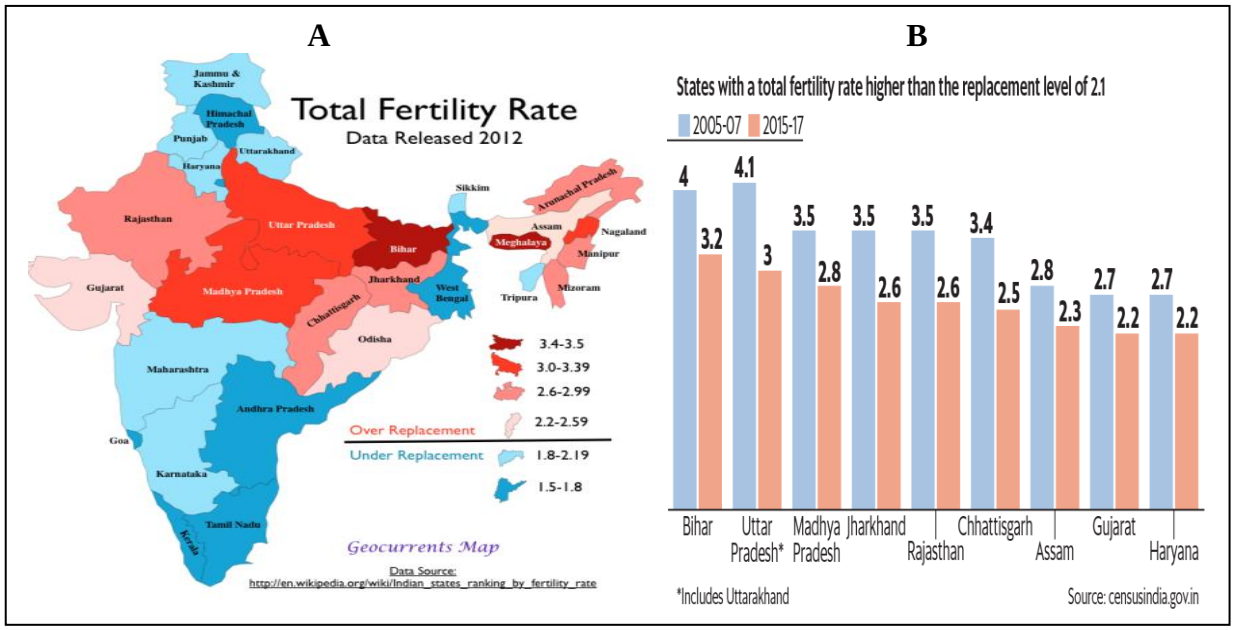

Figure1.4A. In dian TFR: TFR in different Indian states in the year 2012 (http://en.wikipidia.or/wiki/indian_states_ranking_by_fertility_rate). **B.** Fertility rates of Indian states: Comparative Data on the Indian states for the years 2006-07 and 2015-2017 TFR**,** Total fertility rate (SRS, 2013).

According to the data of surveys of the National Family Health Survey (NFHS), NFHS I-IV women in the reproductive age group, the prevalence of women experiencing primary infertility has shown a remarkable increase during the most recent decade in India,figure 1.5 (Ganguly *et al.*, 2010).

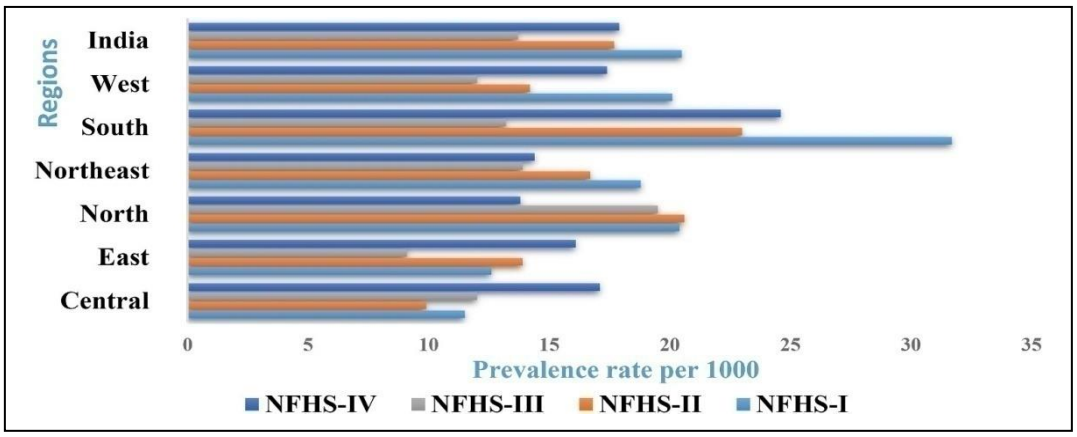

Figure 1.5 Prevalence of primary infertility among currently married women in India, NFHS I –IV. NFHS, National Family Health Survey (Ganguly *et al.*, 2010).

The NFSH I-IV data shows prevalence of primary infertility in currently married women in different parts of the India for all four rounds of NFHS, with western region also showing a high increase in the primary infertility rate in females in recent years.

1.3 Classification of female infertility

Female infertility can be classified into primary and secondary infertility. **Primary infertility** refers to the inability to conceive or give birth because of not being able to become pregnant. A woman who is unable to bear a child is classified as having primary infertility. **Secondary infertility** refers to the inability to conceive or give birth when there was a previous pregnancy or live birth. A woman who has previously conceived and successfully given birth but is unable to do so subsequently is classified as having secondary infertility (WHO/Infertility/2016). Globally, most infertile couples suffer from primary infertility (WHO/Report of the second interagency meeting/2001; Inhorn *et al*., 2003; Tabong *et al*., 2013; Masoumi *et al*., 2015; Allow *et al.*, 2016; Benksim *et al.*, 2018). In India also, the overall prevalence of primary infertility is 3.9% to 16.8% (Zagar *et al.*, 1997; Talwar *et al.*, 1986; Unisa *et al.*, 1999; WHO/ Infecundity, infertility and childlessness in developing countries/2004; Kumar *et al.*, 2007; Ganguly *et al.*, 2010; Adamson *et al*., 2011; Patel *et al*., 2016; Katole *et al.*, 2019; Purkayasthaa *et al.*, 2021), while Deshpande *et al.* (2019) reported the prevalence of primary infertility at 57.5%. The prevalence of primary infertility has shown a remarkable increase in 2015–16 (niti.gov.in Total fertility rate 2000-2016; Purkayasthaa *et al*., 2021). The present study also aims to find out to the prevalence of primary infertility in female population in the western part of India, specifically Gujarat region.

1.4 Etiology of female infertility

Historically, the onus of infertility has always been on women (Olu*et al*., 1999), though both the partners can contribute to it equally. Etiology of infertility prevalence and patterns of causes of infertility in different regions are diverse (Macaluso*et al.*, 2010). The majority of the studies in literature reveals that 30 to 50% of infertility is due to female factors while 20 to 40% are related to male factors, 20 to 40% relate to both male and female factors and up to 20% remained unexplained (Unuane *et al.*, 2011; Chandra *et al.*, 2013; Sohrabvand *et al.*., 2015; ESHRE, 2016; Chaudhary *et al.*, 2017; Elhussein *et al.*, 2019), while a study reported by Some *et al.* in 2017 gives a slightly different statistics by attributing 62.5% of infertility to unexplained causes (Cyriac*et al.*, 2017). Unexplained infertility is a diagnosis of exclusion

after evaluation of the male and female factors fail to identify a specific cause for infertility (Fritz *et al.*, 2011; Gelbaya *et al.*, 2014). Fertility declines with age in both men and women, but the effects of age are much greater in women. In their 30s, women are about half as fertile as they are in their early 20s, and women's chance of conception declines significantly after age of 35 years (Sudha *et al.*, 2013; Practice Committee of the American Society for Reproductive Medicine, 2015; Datta *et al.*, 2016). Since the fertility potential of the female partner decreases after 35 years of age, most authorities recommend initiating an infertility evaluation after 6 months of attempting conception in women 35 to 40 years of age and after 3 months in women over 40 years of age. Causes of infertility are difficult to diagnose and as cases of female infertility are increasing it is now imperative to look for hither to undiscovered underlying risk factors that contribute to this problem. The present study is a step in that direction and focuses on the female infertilityin the back drop of thyroid dysfunction.

1.5 Risk factors and causes of female infertility

Infertility is a fall out of multiple factors hence proper evaluation of this disorder should involve a multipronged approach. Many of the risk factors for both male and female infertility are the same which include age at marriage (Talwar *et al.*, 1997; Mokhtar *et al.*, 2006; Deshpande *et al.*, 2019), reproductive system damage or abnormalities (Talwar *et al.*, 1997; Elussein *et al.*, 2008; Farhi *et al.*, 2011), sexually transmitted diseases and hormonal disorders (Verma *et al.*, 2012), sperm/ egg quality and quantity (Shamila *et al.*, 2011; Kumar *et al.*, 2015), genetic disorders, as well as congenital defects. Apart from these, conditions such as diabetes, hypertension, lack of proper nutrition, change in diet, lack of exercise and in some cases intense exercise, lifestyle diseases such as weight loss/gain (obesity) (Freund *et al.*, 2003; Hruska *et al.*, 2000), environmental pollution (Dhaibar *et al.*, 2021), immune response, stress (Soltani *et al.*, 2014), exposure to radiation, increased mobile phone use (Ashok*et al.*, 2011), sexual violence and anxiety are accepted as influencers of pregnancy outcomes. Smoking, tobacco and alcohol abuse as well as addictions in the young has also shown to contribute to the problem of infertility (Barbieri RL, 2001; Deyhoul *et al.*, 2017). Infertile couples in the developing world have an additional disadvantage of less participation in societal activities (Shah *et al.*, 2010). A marked trend for delaying the timing of first birth has been seen in developed as well as developing countries (Mathews *et al.*, 2009; Sharma *et al.*, 2011).

Female fertility can be limitedin a number of ways. WHO task force on Diagnosis and Treatment of Infertility conducted a study of 8500 infertile couples using a standardized diagnostic protocol and reported that diseases in the female most often identified were ovulatory disorders, unexplained infertility, endometriosis, pelvic adhesions, tubal occlusion, other tubal abnormalities, and hyperprolactinemia (WHO Technical Report Series: Recent Advances in Medically Assisted Conception, 1992; Unuane *et al.*, 2020). In a review of 21 published reports containing a total of 14,141 infertile couples, Collins *et al.* reported that the primary diagnoses in the couples were ovulatory disorders, abnormal semen parameter, tubal defect, unexplained, endometriosis, and other causes (Collins *et al.*, 2016). In another data set of 2198 infertile couples, the distribution of primary diagnoses was unexplained, abnormal semen parameter, tubal disease, ovulatory disorders, endometriosis, and other (Smith *et al.*, 2003). While, Healy *et al.* has divided the causes of female infertility into 10 broad categories which are (i) Endocrine disturbances (ii) Anomalies of uterus (iii) Endometriosis (iv) Ovulatory failure (v) Tubal dmage (vi) Immunologic factor (vii) Urogenital infection (viii) Acquired factors (ix) Congenital anomalies and (x) Unexplained infertility as depicted in the figure 1.6 (Healy *et al.*, 1994).

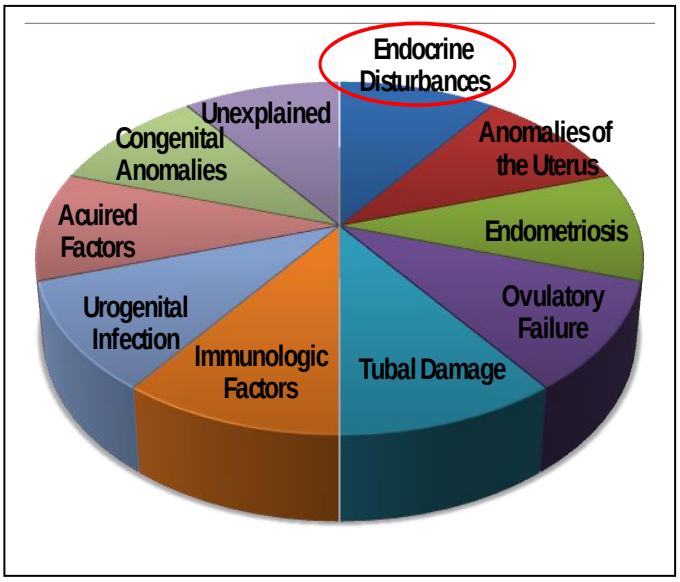

Figure 1.6 Causes of female infertility (Healy *et al.*, 1994).

The possibility of experiencing infertility is higher among people with poor diet, obesity and thyroid dysfunction. Role of diet, Body Mass Index (BMI), and thyroid as important factors influencing infertility has also been addressed extensively (Grodstien *et al.*, 1994; Poppe *et al.*, 2008; Purkayastha *et al.*, 2021) and they have been shown to have a significant influence on primary infertility. The incidence of infertility is experienced more in urban, rich and educated women as compared to that of rural women due to the association of different environmental and lifestyle causes (Admson*et al.*, 2011; Ganguly *et al.*, 2010). Among all the

above risk factors and causes of female infertility the present study mainly focuses on the endocrine disturbances as a cause of female infertility.

1.6 Female infertility of endocrine origin

Endocrine disturbances are amongst the leading causes of infertility in females. The interplay between various hormones important for reproduction is the key to successful pregnancy. Different hypothalamic, pituitary, thyroid, adrenal, and ovarian disorders may affect fertility, figure 1.7 (Wiess *et al*., 2014). Thyroid disorders are reported to be as the most prevalent endocrine disturbances in the reproductive age women with a consequence of infertility worldwide.

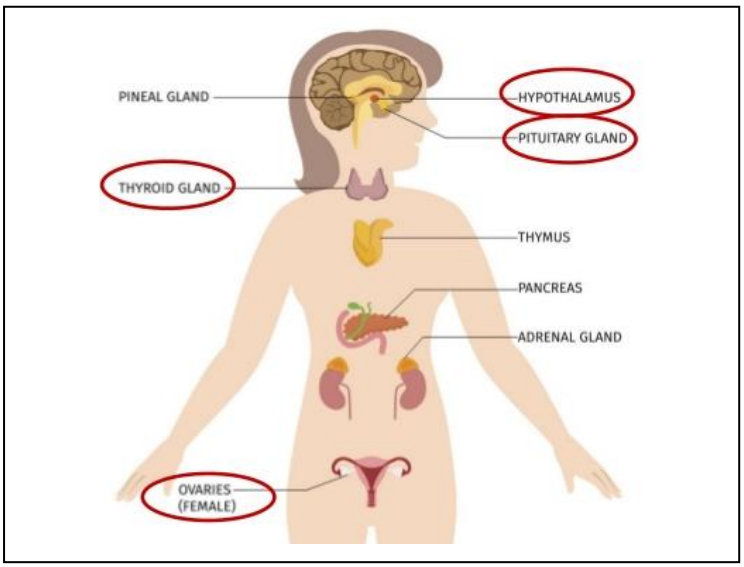

Figure 1.7 Endocrine glands that participate in female fertility (Wiess *et al.*, 2014).

1.6.1 Thyroid disorders and female infertility

Thyroid is the most important endocrine organ next to pituitary that regulates the growth, metabolism and all other functions of the body including the reproductive system. At every stage starting from maturation of ovarian follicle up to implantation of the embryo, a convenient endocrine environment including normal thyroid hormone levels is of utmost importance. Fertility maintenance involves a complex interplay between the hypothalamo-pituitary axis and the thyroid gland (HPT axis), figure 1.8 (Guyton & Hall, 11th Edition, 2006). Thyrotropin releasing hormone (TRH) from the hypothalamus and thyroid-stimulating hormone (TSH) from the anterior pituitary stimulate synthesis and release of thyroid hormones. Low blood levels of T3 and T4 or low metabolic rate stimulate the hypothalamus to secrete TRH. TRH enters the hypophyseal portal veins and flows to the anterior pituitary, where it stimulates thyrotrophs to secrete TSH. TSH stimulates nearly every activity of thyroid follicular cell such as iodide trapping, hormone synthesis and secretion and growth of

the follicular cells. The thyroid follicular cells release T_3 and T_4 into the blood. An elevated level of T3 inhibits release of TRH and TSH (negative feedback inhibition), figure 1.9 (Guyton & Hall, 11th Edition, 2006).

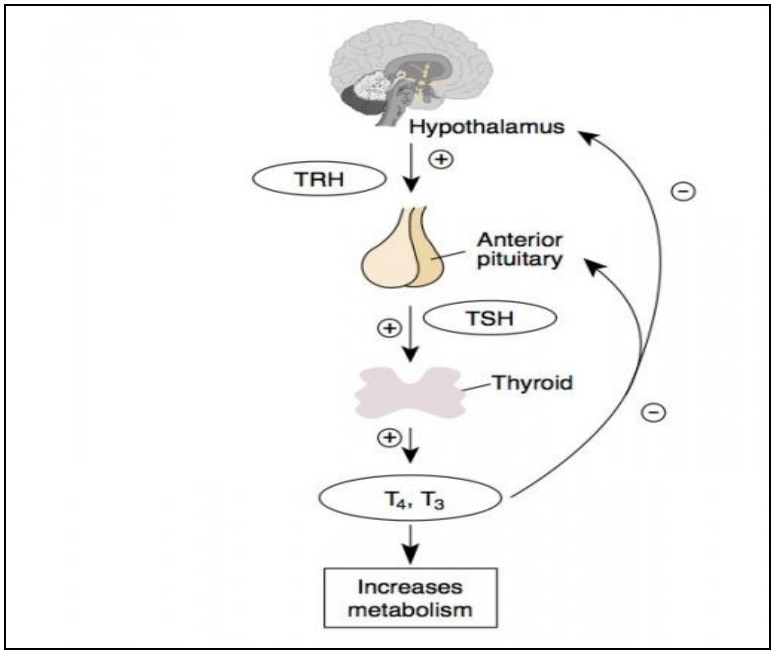

Figure1.8Hypothythalamus-Pitutory-Thyroid [HPT] axis (Guyton & Hall, 11th Edition, 2006).

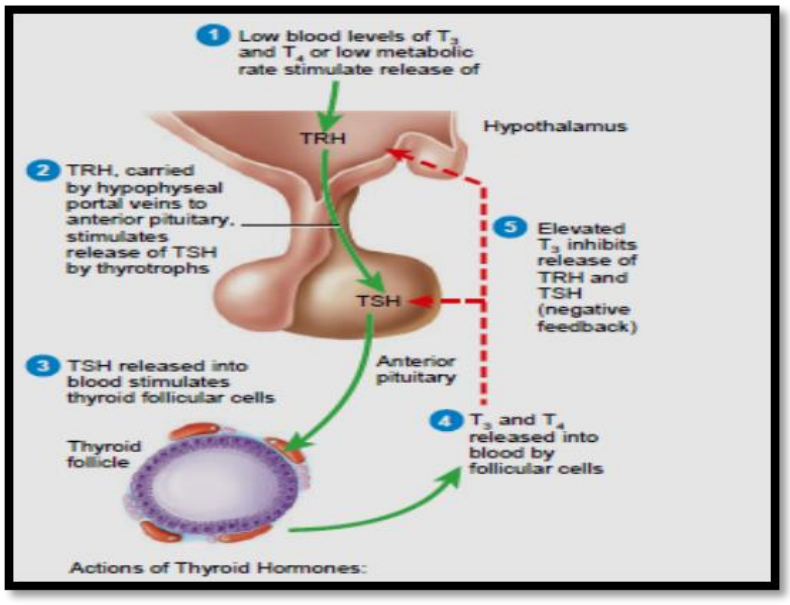

Figure1.9Thyroid Hormone Actions (Guyton & Hall, 11th Edition, 2006).

1.6.1.1 Thyroid disorders

In normal thyroid condition which is called euthyroid state, the serum Thyroid Stimulating Hormone

(TSH) levels as well as the levels of free Triiodothyronine (fT_3) and free Thyroxine (fT_4) are within normal reference range. Any alteration in thyroid homeostasis leads to the thyroid disorders. Thyroid disorders are classically divided into two types which are (i)

Hyperthyroidism with low serum TSH and high fT4 levels (ii) Hypothyroidism with high serum TSH and low fT4 levels in the circulation as shown in figure 1.10 (Ross *et al.*, 2001).

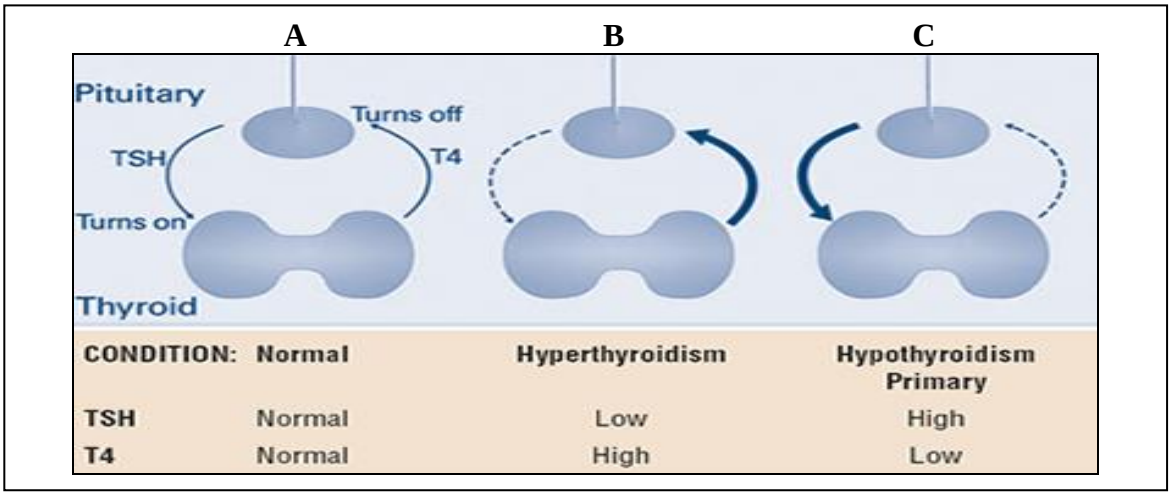

Figure 1.10 Different thyroid conditions: A. Euthyroid (normal) condition with normal TSH and fT_4levels in the circulation. **B. Hyperthyroidism** with low TSH and high fT_4levels in the circulation. **C. Hypothyroidism** with high TSH and low fT_4 levels in the circulation.fT_4, free Thyroxine; TSH, Thyroid Stimulating Hormone (Ross *et al.*, 2006).

Hyperthyroidism and hypothyroidism are further classified into (i) Overt/clinical hypothyroidism and (ii) Subclinical Hypothyroidism. The prevalence of infertility is more common in hypothyroid females as compared to hyperthyroid subjects. The present study focuses on the subclinical hypothyroidism as a cause of female infertility. Overt and subclinical hypothyroidism "**Overt Hypothyroidism** (OH)- can be defined as an elevated TSH and a decreased thyroxine level, in the presence of clinical symptoms (TSH <10μIU/ml)" and "**Subclinical Hypothyroidism** (SCH)-Is a state in which an asymptomatic person has normal serum T4 levels but increased concentration of TSH (4.0-10μIU/ml)". It is a state before actual clinical disorder where there are no apparent clinical symptoms, but it can further progress to a clinical condition. The biochemical markers in hypothyroidism as discussed by Ross *et al.* (2006) are listed in table 1.

Table 1 Biochemical Markers in Hypothyroidism (Ross *et al.*, 2006)

TSH level	Free T_4	Free T_3	Likely diagnosis
High	Low	Low	Primary hypothyroidism
High (>10 µU per mL [10 mU per L])	Normal	Normal	Subclinical hypothyroidism with high risk for future development of overt hypothyroidism
High (6 to 10 µU per mL [6 to 10 mU per L])	Normal	Normal	Subclinical hypothyroidism with low risk for future development of overt hypothyroidism
High	High	Low	Congenital absence of T4-T3converting enzyme; amiodarone (Cordarone) effect on T4-T3 conversion
High	High	High	Peripheral thyroid hormone resistance
Low	Low	Low	Pituitary thyroid deficiency or recent withdrawal of thyroxine after excessive replacement therapy

TSH, Thyroid stimulating hormone; free T_4, free thyroxine; free T_3, free triiodothyronine.

1.6.1.1.1 Risk factors and causes of thyroid dysfunction

Some of the risk factors for the thyroid dysfunction include history of thyroid dysfunction/thyroid surgery, family history of thyroid disease, goitre, thyroid auto antibodies, clinical symptoms/signs of hypothyroidism, diabetes type I, history of miscarriage/preterm delivery, other autoimmune disorders, history of subfertility, history of therapeutic head or neck irradiation, age ≥30 years, previous treatment with amiodarone, previous treatment with lithium, recent exposure to iodinated radiological contrast agents, genetic factors etc (Ross *et al.*, 2006).

1.6.1.1.2 Hypothyroidism and female infertility

Thyroid diseases are the most common endocrine disease in females at reproductive age, while infertility is common in women with thyroid dysfunction. Fertility is maintained by the balance between the hypothalamic pituitary adreno-genital axis. Clinical and experimental studies have suggested a close relationship between the Hypothalamic-Pituitary-Thyroid axis

(HPT) and the Hypothalamic-Pituitary Ovarian axis (HPO). The clinical/ overt hypothyroidism via feedback loop causes an increase in TRH which stimulates excess prolactin (PRL) secretion. Increased TSH also causes increased PRL levels and this is due to increased production of TRH. Increased TRH causes decrease in dopamine due to which PRL levels increase leading to hyperprolactinaemia. Hypothyroidism followed by hyperprolactinaemia, may cause ovulatory dysfunction, luteal phase defects, and even oligomenorrhea and amenorrhea. Such condition can cause infertility. Thyroid disorders are associated with various problems in the reproductive age group like the delayed onset of puberty, menstrual abnormality and recurrent miscarriages. The data in the literature studies report that the most common endocrine disorder causing infertility is hypothyroidism (Poppe *et al.*, 2007; Jeffery *et al.*, 2015; Habbul *et al*., 2016; Naz *et al.*, 2020). Thyroid dysfunction is associated with a number of hormonal changes important for normal reproduction and furthermore leads to menstrual disturbances through a number of mechanisms. Women with thyroid dysfunction often have menstrual irregularities, infertility and increased morbidity during pregnancy (Krassas *et al.*, 2000; Poppe *et al.*, 2007; Unuane *et al.*, 2011; Dosiou *et al.*, 2020). The menstrual pattern is influenced by thyroid hormones directly through impact on the ovaries and indirectly through impact on sex hormone binding globulin (SHBG), PRL and gonadotrophin releasing hormone (GnRH) secretion and coagulation factors. The impact of hypothyroidism on the menstrual cycle has been identified since the 1950s and leads to changes in cycle length and blood flow (Goldsmith *et al.*., 1952). Joshi *et al.* (1993) found 68% of menstrual abnormalities in women with hypothyroidism. In the study by Krassas *et al.* (1999), the prevalence of menstrual irregularities (mainly oligomenorrhoea) was 23% with 12% of women having amenorrhoea in the hypothyroid group. The authors also showed an association between the severity of menstrual abnormalities and higher serum TSH levels. In women of reproductive age prevalence of hypothyroidism is 2–3% while hyperthyroidism has 1–2% prevalence rate (Krassas *et al*., 2000). The prevalence of overt hypothyroidism in the developed world is 4-5% and that of subclinical hypothyroidism is 4- 15%. According to Lincoln *et al.* (1999) the prevalence of hypothyroidism in the reproductive age group is 2–4% and has been shown to be the cause of infertility and habitual abortion. According to Wang *et al.* (1997), studies show that women with both overt and subclinical hypothyroidism are at risk of increased incidence of preeclampsia, early and recurrent pregnancy loss, stillbirth, failure of lactation and adverse neonatal outcomes (Gillette *et al.*, 2004; Surks *et al.*, 2004; Maraka *et al.*, 2016). Women

presenting with subfertility appear to have raised mean serum TSH levels (Poppe *et al.*, 2002) and increased rates of subclinical hypothyroidism (Abalovich *et al.*, 2002).

1.6.1.1.3 Subclinical hypothyroidism (SCH) and female infertility

Subclinical hypothyroidism (SCH) as cause of female infertility mostly remains undiagnosed in females of reproductive age due to its subtle symptoms. SCH has been challenged as data have indicated that physiological free T_4 (fT_4) variations are narrower in one individual than those observed within the reference range of a population. These data might reflect an abnormally low (fT_4) value for patients who present a mildly increased serumTSH (Abalovich *et al.*, 2002; Andersen *et al.*, 2002; Baloch *et al*., 2003). Some authors have proposed restricting the upper normality limit of serum TSH to 2·5 mU/l. Today, however, there is no agreement among endocrinologists about the most appropriate (physiologically relevant) upper limit of normality for serum TSH (Brabant *et al.*, 2006). Studies investigating the association between SCH and infertility are mostly based on the previous upper serum TSH levels. In the study by Bohnet *et al.* (1981) SCH was considered as an infertility factor by itself. Bals-Pratsch *et al.* (1997) did not observe corpus luteum insufficiency in infertile women with SCH. Gerhard *et al.* (1991) reported a positive correlation between basal TSH, LH and testosterone concentrations in the early follicular phase and women with an elevated serum TSH had a lower pregnancy rate than those women with a normally stimulated serum TSH. In the study by Shalev *et al.* (1994) the prevalence of SCH was reported in infertile women with ovulatory dysfunction. Grassi *et al.* (2001) found prevalence of ovulatory dysfunction and autoimmune thyroid disease in infertile females concluding that mean duration of infertility was significantly longer in the patients with SCH and autoimmunity. Arojoki *et al.* (2000) found SCH prevalence in 4% women presenting with primary infertility. The prevalence of increased serum TSH was highest in the group with ovulatory dysfunction. In a case–control study, Poppe *et al.* (2002) reported a high prevalence of SCH in women with primary infertile. Considering the largest cohorts published, the prevalence of SCH in infertile women ranged from 2% to 4% (Poppe *et al*., 2008) and most cases with SCH were associated with ovulatory dysfunction. Raber *et al.* (2003) in his study on 283 infertile females reported that 34% had SCH, and more frequent abortions were also observed in the women with SCH.

1.6.1.1.4 Prevalence of SCH with female infertility in India

In India, it has been estimated that about 42 million people suffer from thyroid diseases (Unnikrishnan *et al*., 2011; Purkayasthaa*et al*., 2021). Universal salt iodization program in

India changed the thyroid status of India. India is in the transition phase from iodine deficiency to iodine sufficiency, and this is expected to change the thyroid status of the population (Unnikrishnan *et al.*, 2011). Thyroid disorders were the most common endocrine abnormalities, seen in 21.6% of the infertile population in a study by Deshpande *et al.* (2019). Varma *et al.* (2012) found the prevalence of hypothyroidism in infertile women to be 23.9% with 62.7% infertile female suffering from subclinical and 37.3% were with clinical hypothyroidism. In a study by Abdul *et al.* (2015) it was found that 62.5% of hypothyroid infertile women were with subclinical & 37.5% were with clinical hypothyroidism. Prevalence of subclinical hypothyroidism with 50.5% was more common than overt hypothyroidism in a studydone by Priya *et al.* (2015). In the study by Malarasai *et al.* (2016) the major thyroid dysfunction was found to be hypothyroidism with 30% in the infertile women and 6%, out of which 22% of the cases were found to have subclinical hypothyroidism. Prasad *et al.* (2019) reported thatin patients with menstrual disorders, 40% had thyroid dysfunction, with 20% having subclinical hypothyroidism, 14% with overt hypothyroidism. Anti-TPO antibodies were present in 28% of patients with menstrual disorders. Sheela *et al.* (2017) reported subclinical hypothyroidism 10% higher than the national average in south Indian population. Though data on the prevalence of SCH in infertile females are scarce, in conclusion studies in different parts of India report overall high prevalence of SCH in infertile females, but no studies were found to be reported for the western part particularly for the Gujarat region with data on the prevalence of SCH in infertile females. The present study mainly aims to study the role of thyroid in female infertility with the focus on subclinical hypothyroidism leading to female infertility in the western population of India.

1.7 Autoimmune Thyroid Disease (AITD) and female infertility

Autoimmune thyroid disease (AITD) is one of the most common causes for the thyroid destruction resulting into overt and subclinical hypothyroidism (Hollwell *et al.*, 2002). The prevalence of AITD is 5–10 times higher in women than in men and might be explained by genetic factors, the effects of estrogens and possibly chromosome X abnormalities (Prummel *et al*., 2004; Weetman *et a..*, 2004; Tomer *et al.*, 2014). The prevalence of thyroid autoimmunity ranges from 5 to 20%, but it may reach up to 25% in infertile women (Poppe *et al*., 2003; Artini *et al.*, 2013; Unuane *et al.*, 2013, Carp *et al*., 2012). Thyroid dysfunction is more frequent in women who have thyroid autoimmunity (Van den Boogaard *et al.*, 2011). The importance of AITD is twofold. First, it is the most common autoimmune disorder in

women, affecting 5–10% in the childbearing period; second, it is the most frequent cause of thyroid dysfunction. Numerous studies have investigated the prevalence of AITD in women with infertility concluding significantly increased incidence of AITD in women with infertility compared fertile subjects. In a case-controlled study by Poppe *et al.*, (2003) comparing 438 women of infertile couples a risk of AITD with infertility was reported. Gerhard *et al.* (1991) showed that 44% of women with AITD had endometriosis, compared with only 9% in women without AITD. In a study by Janssen *et al.* (2004) a strong association was shown between AITD and women with polycystic ovarian syndrome (PCOS) as the cause of infertility. The prevalence of AITD was 26·9% compared to 8·3% in women without PCOS (Gerhard *et al.*, 1991; Poppe *et al*., 2002; Russev *et al.*, 1996; Kutteh *et al.*, 1999; Kaider *et al.*, 1999; Reimand *et al.*, 2001; Janssen *et al.*, 2004). Although AITD represents an organ-specific autoimmune disorder, associations between AITD and non-organ-specific autoimmune disease states have also been described, suggesting a shared immune-genetic background. The underlying pathogenic mechanisms associating AITD and infertility remain largely speculative, as neither animal models nor *in vitro* data on this issue are available.

The 2017 American Thyroid Association (ATA) pregnancy guidelines, based upon the data to date and a number of recent studies has confirmed adverse effects of subclinical hypothyroidism and thyroid peroxidase antibody (TPO-Abs) positivity on obstetrical outcomes (Vanderpump *et al.*, 1995; Bjoro *et al*., 2000). Recent studies since the 2017 guidelines have provided important insights into the complex interactions of thyroid diseases with fertility. Higher TSH and/or anti-TPO antibodies are associated with infertility and decreased ovarian reserve in women and such women treatment for subclinical hypothyroidism improves pregnancy outcomes. AITD may be present without the thyroid disease and it has been found that Anti-TPO abs are associated with increased risk of miscarriage in euthyroid infertile women. It is well established that a proportion of people with AITD have normal serum TSH. As increased rates of sub fertility are seen in euthyroid women with AITD it is the management of this group that has created the greatest debate among clinicians. (Kaider *et al.*, 1999; Kutteh *et al.*, 1999). There is a strong correlation between thyroid immunityand infertility and miscarriage. Thyroid vehicle antibodies exert their impact in a TSH structured but also in a TSH unbiased manner. Hence it is suggested that autoimmune thyroiditis should be diagnosed and treated in infertile patients. Autoimmune conditions implicated in sub fertility and reproductive health includes anti phosphor lipid antibodies, diabetes mellitus and systemic lupus erythematosus (Prummel *et*

al., 2005). There has been very old theory over the importance of thyroid auto antibodies in the sub fertility setting. The prevalence of thyroid autoimmunity has been found to be consistently increased in the sub fertile population compared with fertile controls. It has been suggested that thyroid auto antibodies are an early sign of lymphocytic infiltration and therefore a predictor of thyroid disease (Scofield *et al*., 2004).

Increased TSH levels due to decreased levels of thyroid hormones or due to thyroid destruction by any of the etiological factor such as Autoimmune thyroid disease (AITD) causes hypothyroidism. There are many etiological factors which are known to alter the thyroid gland metabolism and thus disrupt the thyroid hormones homeostasis and causes hypothyroidism. AITD is found to be very high in infertile subclinical hypothyroid females and the study hypothesizes the independent or cumulative involvement AITD (presence of anti TPO-Antibodies), and thus affecting the thyroid gland homeostasis leading to subclinical hypothyroidism (SCH), which in turn results into female infertility in subclinical hypothyroid females.

1.8 SCH and alteration in female reproductive system

The thyroid hormones affect function of almost all the organs in the body. It plays a major role in the ‘‘milieu interior’’ of the body by bringing about changes in other organ systems, like sympathetic system, reproductive system and endocrine glands. It is well known that in both sexes thyroid hormones influence sexual development andreproductive function; clinical and experimental evidences suggest that the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-ovary axis are physiologically related and act together as unified system in a number of pathological conditions (Duofas *et al.*, 2000; Afifa *et al*., 2011). Ovulation in females is coordinated and regulated by functionally intact hypothalamic-pituitary-ovarian axis by various hormones including follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and thyroid hormones, figure 1.11.

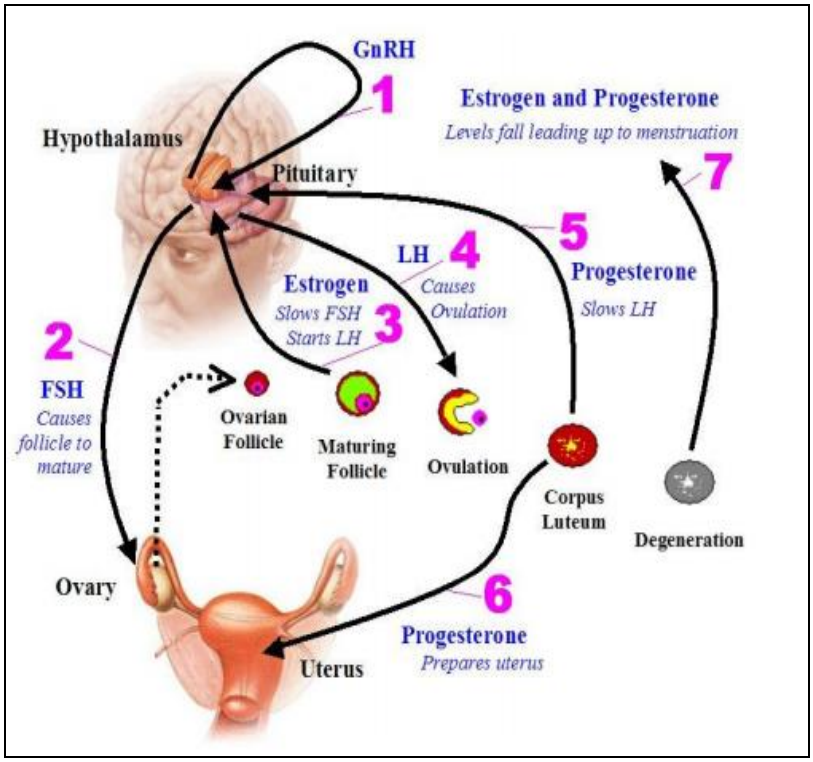

Figure 1.11 Hypothalamus Pituitary Ovarian-HPO Axis: Coordination and regulation of ovulation in female by hypothalamic-pituitary-ovarian axis by various hormones, which are 1.GnRH2.FSH3.Estrogen 4.LH5.Progesterone. GnRH, Gonadotropin releasing hormone; FSH, Follicle stimulating hormone; LH, Luetinizing hormone (Guyton & Hall, 11th Edition, 2006).

Reproductive system is a precious boon given to living organisms through which life can continue. It is the way through which genetic components are preserved and can be inherited to the offspring. The female reproductive system consists of four main internal organs: Ovary, Fallopian tube, Uterus and Vagina. The normal reproductive years of the female are characterized by rhythmic cyclical changes in the rates of secretion of hormones and corresponding physical changes in the ovaries and other sexual organs. The duration of the cycle averages 28 days but can vary from 20 to 45 days. Abnormal cycle length is frequently associated with decreased fertility. Only a single ovum is normally released from the ovaries each month, so that normally only a single fetus will begin to grow at a time in the uterine endometrium which is prepared in advance for implantation of the fertilized ovum at the required time of the month. The ovarian changes that occur during the sexual cycle depend completely on the gonadotropic hormones FSH and LH, secreted by the anterior pituitary gland. The hormones of the female reproductive system include (i). Gonadotropin releasing hormone (GnRH), is a hypothalamic hormone (ii). The anterior pituitary sex hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH); both of which are secreted in response to the release of GnRH from the hypothalamus and (iii). The ovarian hormones estrogen and progesterone are secreted by the ovaries in response to the two female sex hormones from the anterior pituitary gland. In the absence of these hormones, the ovaries remain inactive, which is the case throughout childhood, when almost no pituitary

gonadotropic hormones are secreted. At age 9 to 12 years, the pituitary begins to secrete progressively more FSH and LH, which leads to onset of normal monthly sexual cycles beginning between the ages of 11 and 15 years, called pubertal changes. The regulation of reproductive hormones is depicted in the figure 1.12. Ovarian and uterine cycles are controlled by hormones FSH and LH. FSH initiates the follicular growth and secretion of estrogens by the growing follicles. While LH stimulates the growth and development of the ovarian follicles and the secretion of estrogens which in turn results in ovulation, formation of corpus luteum and production of estrogens, progesterone, relaxin and inhibin by corpus-luteum. Overall, estrogen causes ovulation and progesterone causes implantation.

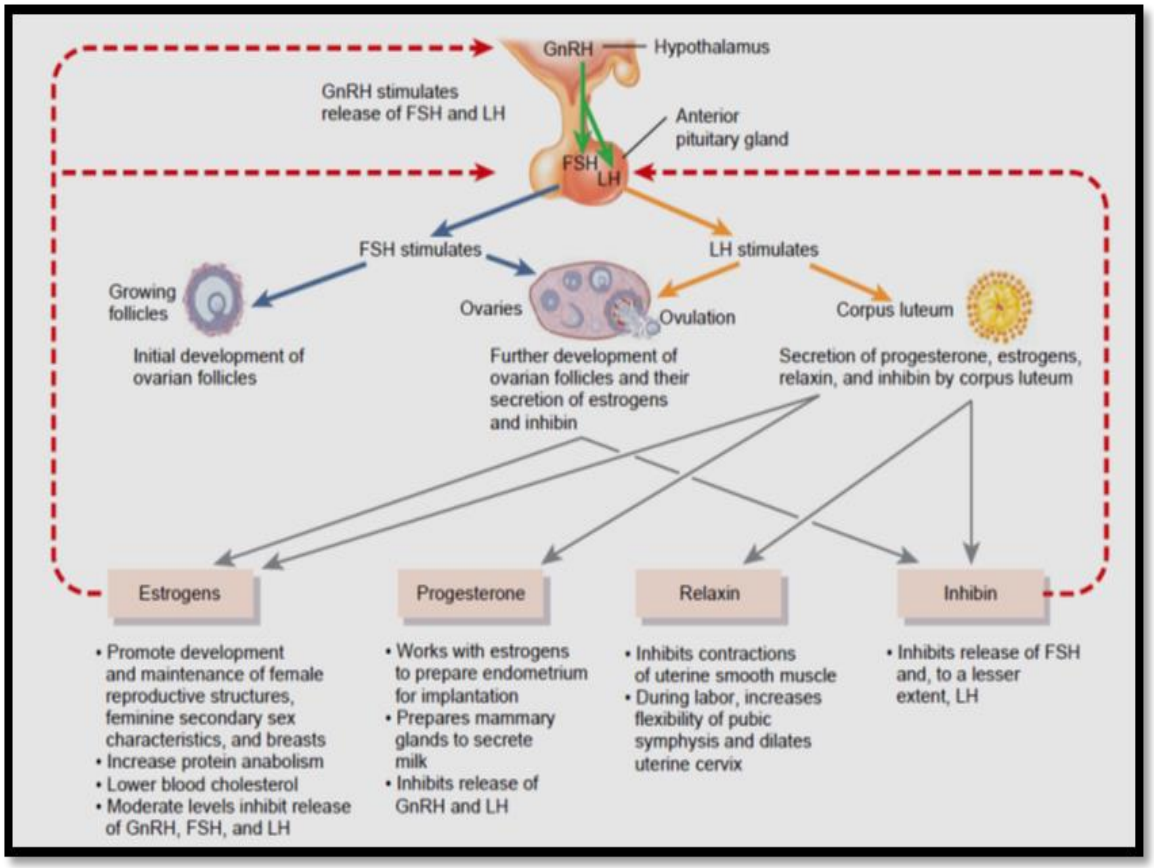

Figure 1.12 Regulation of reproductive hormones: The regulation and functions of female reproductive hormones GnRH; FSH; LH; Estrogen; Progesterone; Relaxin and Inhibin. GnRH, Gonadotropin Releasing Hormone; FSH, Follicle Stimulating Hormone; LH, Luetinizing Hormone (Guyton & Hall, 11thEdition, 2006).

The regulation occurs through a negative feedback mechanism. The levels of hormones at different stages are shown in the figure 1.13. FSH causes maturation of follicles but as the level of estrogen increases, due to maturation of follicle, LH also increases resulting in ovulation. Once the ovulation is over the corpus luteum which produces the hormone progesterone is left behind.

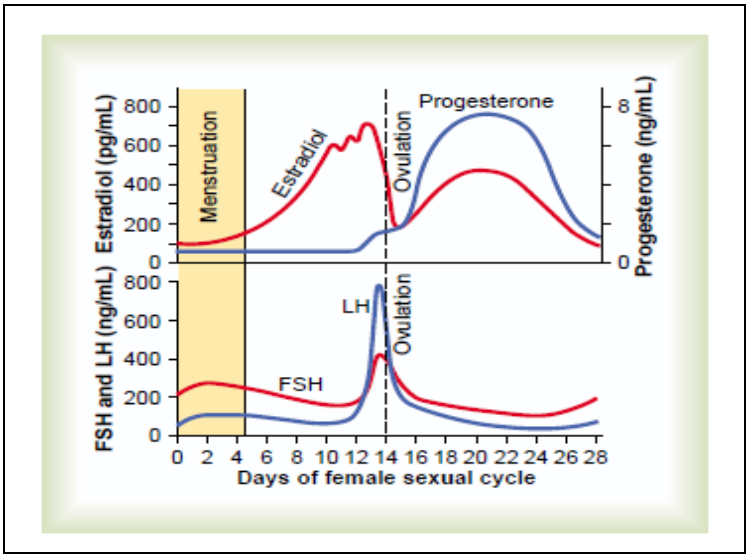

Figure 1.13 Levels of reproductive hormones during the female sexual cycle: FSH causes maturation of follicles and as the level of estrogen increases, due to maturation of follicle, LH also increases resulting in ovulation on the 14th day of the female sexual cycle. FSH, Follicle Stimulating Hormone; LH, Luetinizing Hormone (Guyton & Hall, 11th Edition; 2006).

Prolactin (PRL) also called as Luteotropic hormone is released by anterior pituitary and plays an important role in fertility and conception. PRL acts by inhibiting FSH and GnRH. High PRL levels inhibit secretion of FSH causing hypo-estrogenism with ovarian dysfunction, menstrual abnormalities, suppression of ovulation and infertility. The pituitary gland may cause excess production of PRL known as hyperprolactinemia, which reduces estrogen production and may cause infertility. Hyperprolactinemia is the presence of abnormally-high PRL levels in the blood. PRL is primarily associated with breast development during pregnancy and induces lactation. However, prolactin also binds to specific receptors in the gonads, lymphoid cells, and liver. Hyperprolactinaemia may occur primarily as a result of normal physiological changes during pregnancy, breastfeeding, mental stress, hypothyroidism, or sleep. Pathologically, it may be due to diseases affecting the hypothalamus and pituitary gland or secondary to disease of other organs such as the liver, kidneys, ovaries and thyroid. Women with hyperprolactinemia are hypothyroid and have a decreased negative feedback on hypothalamo-pituitary-thyroid axis. This results in increased secretion of TRH which acts on thyrotropes andlactotropes causing a significant rise in the levels of both TSH and PRL while reducing central FSH, LH levels resulting in decrease in granulosa cells and estradiol levels resulting in short luteal phase and finally amenorrhea.The release of PRL is suppressed by dopamine in the brain and any reduction in dopamine will raise PRL and hinder the secretion and release of FSH and LH which result in menstrual dysfunction and disrupt ovulation. Hence it is very essential to get all the hormones in balance to increase the chances of becoming pregnant. Hyperprolactinemia has been identified as a possible cause of infertility in women. High PRL levels can interfere with ovulation causing hormone imbalance, irregular menstruation, anovulation which reduces the

chance of becoming pregnant and cause low progesterone levels also. Hypothyroidism leads to increase levels of thyroid releasing hormone (TRH), TRH in turn stimulates secretion of TSH and PRL, and PRL inhibits gonadotrophins which decreases levels of serum FSH and serum LH.

Numerous studies have shown abnormal PRL, FSH and LH patterns in hypothyroidism. Thyroid hormones act synergistically with FSH and LH on ovary to secrete and maintain the normal level of estrogen and progesterone in a menstrual cycle. Anovulatory cycles with decreased fecundity and consequently infertility have been found to be associated with thyroid dysfunction (Poppe *et al*., 2003; Poppe *et al.*, 2007). Similarly, high level of PRL hormone can inhibit follicular estradiol production and gonadotropin cyclicity leading to anovulation (Kalsum *et al.*, 2002). It has been seen that estrogen and thyrotropin releasing hormone (TRH) are positive modulators of PRL leading to its increased secretion and action whereas dopamine is a negative modulator of PRL secretion (Gurmanpreet *et al*., 2014; Saxena *et al.*, 2016). High level of thyroid stimulating hormone, TSH is found to be associated with increased prolactin secretion that can lead to ovulatory dysfunction (Padubiri *et al*., 2015). Thyroid hormones help to maintain the normal serum level of progesterone and estradiol essential for normal reproductive function. Therefore, isolated thyroid dysfunction can also cause infertility. Thyroid hormones secreted by thyroid gland as well as prolactin hormone secreted by anterior pituitary have a major implication on the fertility of a female.

Thyroid hormones along with the FSH and LH have synergistic action at granulosa cell and thus have a stimulatory effect on its growth as well as on the secretion of steroid hormones from ovary that are responsible for normal reproductive function (Poppe *et al*., 2007). Increased serum PRL level has also been implicated to affect fertility potential by suppressing hypothalamic-pituitary-gonadal axis and GnRH pulsatility. Hperprolactinemia interferes with the secretion and action of gonadotropins at growing follicles in the ovary thus impairing gonadal steroid secretion which further affects positive feedback on gonadotropins leading to follicular immaturity and consequently infertility with anovulation (Kalsum *et al.*, 2002). PRL level in serum is regulated by both hypothalamus and pituitary. Dopamine, a neurotransmitter and progesterone inhibits its secretion and synthesis respectively under normal physiological condition. In case of hypothyroidism, low serum level of thyroxine (T4) causes decreased negative feedback on the hypothalamo-pitutary axis leading to increased TRH secretion which further stimulates thyrotrophs and lactotrophs secretion from the pituitary, thereby increasing the level of both TSH and PRL(Emokpae *et al.*, 2011). Increased TRH production in the cases of hypothyroidism promotes hyperprolactinemia

which in turn affects pulsatile secretion of GnRH. This leads to delay in LH response leading to abnormal follicular development and anovulation. Hypothyroidism also alters the peripheral metabolism of estrogen by decreasing sex hormone binding globulin production. This may be another pathway by which it may have resulted in abnormal feedback at pituitary level impairing the fertility (Poppe *et al*., 2007). The data on subclinical hypothyroidism causing imbalance in the reproductive hormones leading to infertility in females is inadequate and nil for the western population, the present study thus aims to find out the alterations in reproductive hormone levels due to SCH on female fertility in Gujarat population.

1.9 SCH, oxidative stress (OS) and female infertility

Clinical relevance of the association between thyroid and reproductive health is known for long but its patho-physiological mechanisms are poorly understood. Oxidative stress (OS) is the most accepted initiator of many diseases including infertility (Halliwell *et al*., 1994). Among free radicals the most well studied are reactive oxygen species (ROS) (Kang *et al*., 2003). Studies have shown that both hyperthyroidism and hypothyroidism are associated with enhanced oxidative stress involving enzymatic and non-enzymatic antioxidants (Resch *et al*., 2002). Thyroid hormones are among the most important humoral factors involved in setting the basal metabolic rate. Variations in the levels of thyroid hormones can be one of the main physiological modulators of in vivo cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increase in reactive oxygen species induced by deficiency of thyroid hormones can lead to oxidative stress condition. Under physiological conditions (Mancini *et al.*, 2012), ROS generation is controlled by a large number of anti free radical systems which act as protective mechanisms, such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase as well as non-enzymatic antioxidants, among which the most important are vitamins C and E, carotenoids, and glutathione.

Hypothyroidism-associated oxidative stress is the consequence of both increased production of free radicals and reduced capacity of the anti oxidative defense. Hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria leads to accelerated production of free radicals (i.e., super-oxide anion, hydrogen peroxide, and hydroxyl radical as well as lipid peroxides), which consequently leads to oxidative stress (OS). Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress (Pasupathi *et al.*, 2008). There is a growing evidence of possible role of highly reactive products of oxygen, in

infertility. Imbalance between these oxidants and antioxidants is responsible for tissue injury and affects fertility. OS may induce pregnancy complications; while intake of antioxidant nutrients (multivitamins) may have a beneficial role in maintaining an intact female fertility system (Ruder *et al.*, 2009). Thyroid hormones are associated with the oxidative and antioxidative status of the organism. Data on the effect on oxidative status in response to hypothyroidism are few (Dumitriu *et al.*, 1988; Konukoglu *et al.*, 2002; Torun *et al*., 2009). Thyroid dysfunctions increase LPO reactions and ROS as documented by some studies (Venditti *et al.*, 2006; Messarah *et al*., 2007). Lipid peroxidation (LPO) is reported to be high in hyperlipidaemia, which is very common in hypothyroidism (Nanda *et al.*, 2008; Konukoglu *et al.*, 2002). Santi *et al.* (2010) observed an increased activity of CAT in the SCH group and an association between lipid parameters and CAT or SOD activities. Duarte *et al.* (2010) demonstrated that CAT was significantly higher in subjects with hypercholesterolemia. Santi *et al.* (2010) showed hypercholesterolemia has a stronger influence on the development of oxidative stress in overt hypothyroid (OH) patients with increased levels of thiobarbituric acid reactive substances (TBARS), SOD, CAT and Vitamin E (Santi *et al.*, 2010). Elevated MDA levels were also shown in subclinical hypothyroidism (Torun *et al.*, 2009), while Baskol *et al.* (2007) showed patients with primary hypothyroidism had elevated malondialdehyde (MDA) levels while superoxide dismutase (SOD) was not different from controls. Mancini *et al.* (2010) showed low Total Antioxidant Capacity (TAC) levels in hypothyroid patients (Mancini *et al.*, 2010a). The data on OS contributing to female infertility as a consequence of SCH is not documented for western part of India, thus the present study aims to find out the association of SCH and OS in infertile females of Gujarat region.

1.10 SCH, alteration in lipid profile and female infertility

A correlation of lipid profile alteration among hypothyroid patients is universally accepted. Thyroid hormones have significant effect on the synthesis, mobilization and metabolism of lipids. Hypothyroidism greatly increases the plasma concentrations of cholesterol, phospholipids, and triglycerides and excessively causes deposition of fat in the liver. Patho-genetic mechanism of increased low-density lipoprotein (LDL) in hypothyroidism as proposed by Duntas LH (2005) is depicted in figure 1.14. Hypothyroidism reduces the expression of LDL mRNA and the number of LDL receptors and the binding of LDL to LDL receptor, leading to increased half-life of LDL, reduced degradation of LDL in the

fibroblasts, enhanced residence time in serum, and susceptibility to oxidation. The relationship between SCH and lipid profile is yet to be clearly understood.

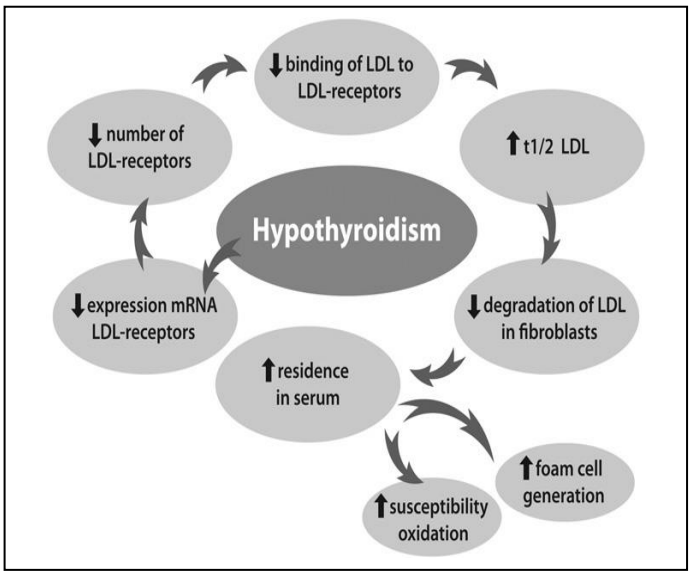

Figure 1.14 Mechanism for increased LDL in hypothyroidism: Hypothyroidism reduces the expression of LDL mRNA and the number of LDL receptors and the binding of LDL to LDL receptor, leading to increased half-life of LDL, reduced degradation of LDL in the fibroblasts, enhanced residence time in serum, and susceptibility to oxidation (Duntas *et al.*, 2002).

Increased levels of total cholesterol (TC), lowdensitylipoprotein cholesterol (LDL-C), and apolipoprotein (apo) B have been reported in the patients with clinical as well as subclinical hypothyroidism (Wiseman *et al.*, 1993; Maya *et al.*, 2002). Hypercholesterolemia is commonly associated with hypothyroidism due up regulation of LDL-receptor expression by thyroid hormones (Huesca-G C*et al.*, 2002). Studies have reported elevated TC and/or LDL-C in SCH as compared with controls (Santi *et al.*, 2012; Efstathiadou *et al.*, 2001; Yildirimkaya *et al.*, 1996; S. Miura *et al.*, 1994). Thyroid hormones may stimulate hydroxyl methylglutaryl coenzyme A (HMG CoA) and induce an increased synthesis of cholesterol. The LDL-C receptor gene contains a thyroid hormone responsive element (TRE) that increases of LDL-C receptor synthesis. Thyroid hormones and their function are low in target tissue in SCH, which influences lipid profiles by this mechanism (Turhan *et al.*, 2008; Lu *et al.*, 2011). Adriana *et al.* reported a positive correlation between TSH and total cholesterol and LDL fraction as well as thyroid hormones (T3 and FT4) showing correlation with triglyceride levels (Santi *et al.*, 2012). Some studies have shown that TSH was also associated with deleterious changes in serum lipids i.e. HDLC, LDL-C, and the ratio of LDL-C to HDL-C(Taddei *et al.*, 2003; Lee *et al.*, 2004; Iqbal *et al.*, 2006). Many evidences suggest that dyslipidemia is a major determinant in the progression of infertility (Broughton *et al.*, 2017; Pugh *et al.*, 2017). Hypercholesterolemia has a stronger influence on the development of oxidative stress in overt hypothyroid (OH) patients whereas patients with SCH had altered lipid profiles, increased lipid peroxidation, and induction of enzymatic defense. Oxidative stress biomarkers are seemed to be associated with secondary hypercholesterolemia to

hypothyroidism, whereas hypothyroidism per se does not cause oxidative stress in SCH patients. On the other hand, high-plasma lipids can be considered as an oxidation substrate for the oxidative stress (Santi *et al.*, 2010). Studies also suggest that an increased oxidative stress in both hypothyroid and SCH states can be explained by both the insufficient increase in the antioxidant status and the altered lipid metabolism (Torun *et al.*, 2009). During clinical and SCH the lipid profile alteration is seen but, relationship between SCH and lipid profile is still controversial. Lipid profile alterations are reported for hypothyroid infertile female subjects, but adequate data is unavailable for SCH and alterations in the lipid profile subsequently resulting into the female infertility. Hence the study aims to estimate and explore the effect of SCH on lipid profile and secondly to find out the correlation of SCH with alterations in the lipid profile in infertile female population from Gujarat region.

1.11 Polychlorinated Biphenyls (PCBs) as cause of SCH leading to female infertility

The substantial burden of the deposition and accumulation of a large number of industrial chemicals in the environment during the last few decades have now reached to a considerably high proportion worldwide. In India too, due rapid urbanization and industrialization there is upshot of various serious health consequences. The disruption of thyroid homeostasis by environmental chemicals known as thyroid disruptors (TDs) is one amongst the most prevailing etiological factors causing thyroid disorders with the data reporting hypothyroidism meddling female infertility. Poly Chlorinated Biphenyls (PCBs) are well known example amongst TDs. PCBs are industrial chemicals which interfere with thyroid homeostasis at multiple levels, figure 1.15 (Boas *et al.*, 2006). PCBs are one of the numerous "Persistant Organic Pollutants" (POPs), the productions of which were banned in 1970s, but still these contaminants are characteristically detected in the surrounding environment (Breivik *et al.*, 2002) as well as in human tissues (Fisher *et al.*, 1999). PCBs are resistant to molecular degradation and 1.5 metric tons have accumulated on earth's surface. In the year 2001, more than 100 countries all around the world have signed "The Stockholm Convention", a commitment to restrict or discontinue use of POPs and PCBs are one among the total 12 chemicals in the list of these chemicals (Patrick *et al.*, 2009, Annex *et al.*, 2008 and Kim *et al.*, 2013).

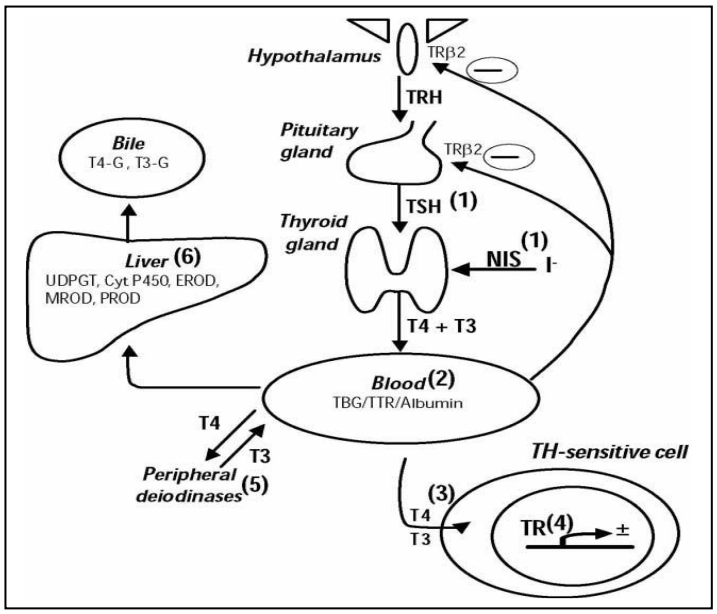

Figure 1.15 Possible mechanisms of action of PCBs on the hypothalamic–pituitary–thyroid axis: (1).Synthesis of THs, interference with NIS, TPO or TSH receptor (2). Transport proteins (3).Cellular uptake mechanisms (4).The TH receptor (5). Iodothyronine deiodinases (6). Metabolism of THs in the liver. (Boas *et al.*, 2006)

Humans get exposed to PCBs through contaminated water, sea foodcaught in contaminated waters, dairy products, fats & oils, contaminated air around hazardous waste sites at work, accidental fires or spills, improper disposal, old fluorescent lighting, capacitors, hydraulic fluid, adhesives, fire retardants, pesticides, inks, carbonless reproducing papers, old electrical appliances and devices such as TV's, refrigerators and microwaves, etc to which we come in contact routinely in our day to day life (Patrick *et al.*, 2009; Diamanti *et al.*, 2009). A number of studies on human populations have shown PCBs levels in the environment altering thyroid homeostasis and hypothyroidism with increased TSH levels and presence of PCBs in their blood and body tissues (Hagmar *et al.*, 2003; Hagmar *et al.*,2001; Persky*et al.*, 2001;Hsu *et al.*, 2005). Chemicals used as pesticides, plasticizers, antimicrobials, and flame retardants apart from being beneficial, also effect human health by disrupting hormonal balance and result in developmental and reproductive abnormalities (Casals-casas *et al.*, 2011). Various studies demonstrated a positive correlation between PCB exposure and TSH and overall studies pin point towards subtle, but significant, effects of low-dose PCB exposure on human thyroid function (Persky *et al.*, 2001; Osius *et al.*, 1999; Schell *et al.*, 2004; Tasker *et al.*, 2005; Bloom *et al.*, 2003; Ribas-Fito *et al.*, 2003). Most epidemiological studies on general population in deed showed negative associations between PCBs and thyroidhormones like T3 or T4, and positive associations with TSH (Takser *et al.*, 2005; Chevrier *et al.*, 2008; Alvarez *et al.*, 2009; Kim *et al.*, 2013). Effects of PCB exposure apart from thyroid homeostasis have also been studied for various reproductive destructions (Reddy *et al.*, 2006; Rier *et al.*, 1996; Rozati *et al.*, 2009; Gore *et al.*, 2015; Yang *et al.*, 2011; Chavrier *et al.*, 2013; Yao *et al.*, 2017). However, studies evaluating the correlation of female infertility with PCBs levels are

very few and inconsistent (Chavrier *et al*., 2013; Cohn *et al*., 2011; Han *et al*., 2016; Buck *et al.*, 2009).

There is growing evidence of worldwide contamination by PCBs and other similar environmental chemicals which have banned long back to the same extent as that of the chemicals which have not been phased out. As PCBs have long biologic half-life and easy accumulation in the food chain as well as the continuous production of structurally similar compounds their exposure remains widespread even though PCBs are no longer manufactured. In midst of paucity on toxicity and exposure data for the number of chemicals to which people are exposed at national level and specifically for western India, this study is designed to assess the safe level of exposure to hazardous man-made chemicals that remain persistent in the body for longer periods and casing infertility via disturbing the endocrine system. Hence present study aims for a quantitative analysis of PCBs levels in primarily infertile female population of Gujarat and correlate the etiological effect of PCBs on subclinical hypothyroidism in consequence female infertility.

1.12 SCH, Single Nucleotide Polymorphisms (SNPs) and female infertility

The sharing of 99.5% genomic sequence level identity of humans implies that the phenotypic diversity arises on account of the remaining 0.5% difference as well as epigenetic modifications. Genetic variation occurs within and among populations, leading to polymorphisms that could be associated with genetic trait or also a phenotype in the presence of an environmental stimulus (Brookes *et al*., 1999; Rebbeck *et al.*, 2004; Hirschhorn *et al.*, 2005). Normal TSH levels in serum are finely regulated in humans. Nevertheless, serum thyroid parameters show substantial inter-individual variability (Practice Committee of the American Society for Reproductive Medicine, 2015), in which genetic variations are provedas the major factors in several populations. It has been shown that altered TSH levels are related to genetic factors in up to 65% of the cases (Bernadette*et al.*, 2013; Panikar *et al.*, 2011; Malinowski *et al.*, 2014). A number of genes have been identified that are associated with altered thyroid function (Arnaud-Lopez*et al.*, 2008; Panicker*et al.*, 2008; Peeters*et al.*, 2003), the most studied among them is Phosphodiesterase8B gene (*PDE8B*) which has been reported as a modulator of TSH levels. *PDE8B* encodes a cyclic adenosine mono phosphate (cAMP) specific phosphodiesterase (PDE) enzyme (Medici *et al.*, 2015). Figure 1.16 depicts the intracellular signaling pathways following activation of this cyclase. The cAMP and cGMP synthesized act as second messengers in the cellular responses. Phosphodiesterases *(PDE)* in turn inactivate cAMP and cGMP (Bender *et al.*, 2006).

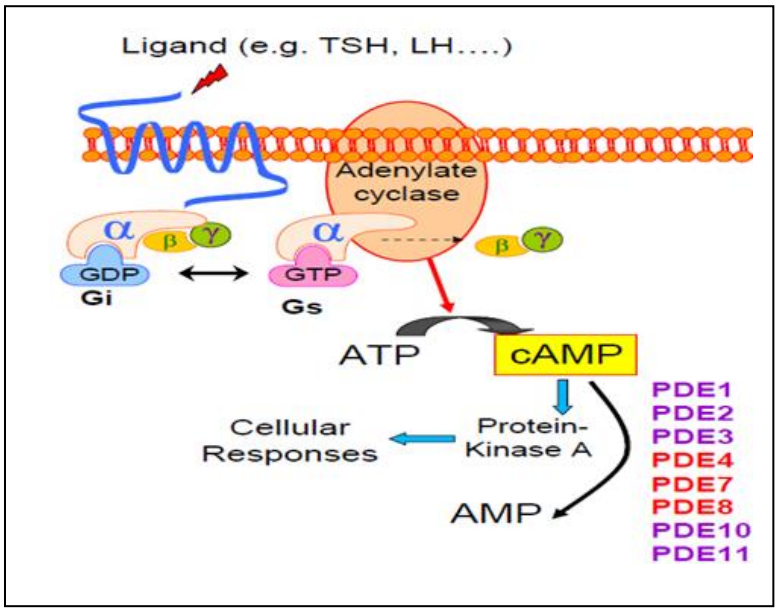

Figure 1.16 Intracellular Signaling pathways following activation of cyclase (Bender *et al.*, 2006).

It is reported that *PDE8B* is undetectable in the pituitary (Persani *et al.*, 2001), and thus Mariotti *et al.* (2010) proposed that *PDE8B*influences serum TSH levels through its effect on TSH dependent thyroid hormone synthesis and secretion and it could act primarily in the thyroid by inactivating cAMP produced after TSH stimulation, figure 1.17. Indeed, of the 5 major isoforms of *PDE8B*, the major isoform *PDE8B1* and minor isoforms *PDE8B2* and *PDE8B3* are abundantly expressed in the thyroid. *PDE8B* could therefore influence serum TSH levels through its effect on TSH dependent thyroid hormone synthesis and secretion. Several single nucleotide polymorphisms (SNPs) for *PDE8B* havebeen demonstrated to be associated with increased levels of serum TSH. Of these SNPs, rs4704397 showed strongest association, while rs6885099 polymorphism has also been shown toincrease TSH levels, but to a lesser extent (Arnaud-Lopez *et al.*, 2008). The relevance of human reproductionto PDE has been well-documented (Hayashi *et al.*, 2002; Soderling *et al.*, 1998; Gamanuma *et al.*, 2003, Horvath *et al.*, 2008).

PDE8B rs4704397 polymorphism has been reported for subclinical hypothyroidism in pregnancy (Shields *et al.*, 2009; Yang *et al.*, 2015) and for recurrent miscarriage (Granfors *et al.*, 2012). But there is no report on the role of PDE8B polymorphisms in subclinical hypothyroidism in female infertility. Overt or clinical hypothyroidism is symptomatic and has an adverse effect on the reproductive health contributing to infertility (Weiss *et al.*, 2014; Saran *et al.*, 2016). However, subclinical hypothyroidism (SCH) is silent and asymptomatic hence it is often undiagnosed. SCH occurs due to multiple factors (Biondi *et al.*, 2008). Among these factors, the present study focuses on the SNPs (genetic factor) as one of the etiological factors for SCH and subsequently for the infertility (Mansuri *et al.*, 2020).

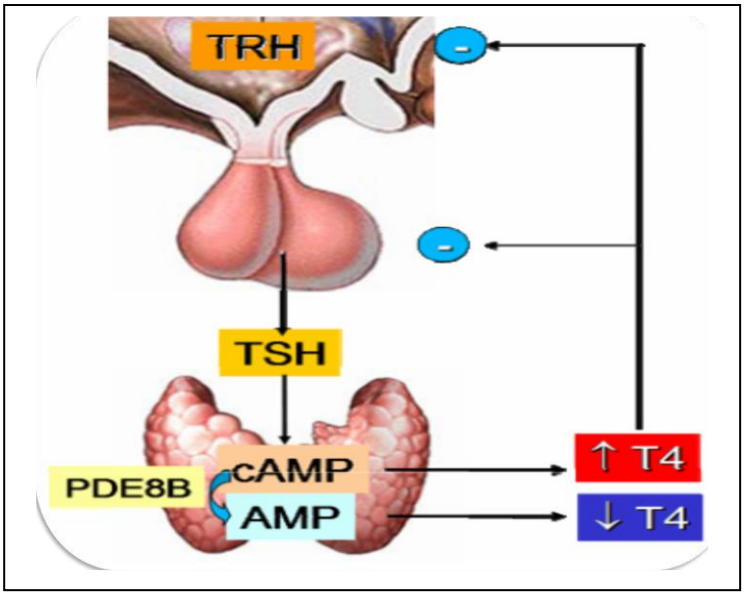

Figure 1.17 Hypothetical mechanisms linking *PDE8B* to serum TSH levels (Mariotti *et al.*, 2010).

We aim to find out the prevalence rate of *PDE8B* polymorphism and explore association of *PDE8B* rs4704397and rs6885099 polymorphisms with subclinical hypothyroidism and infertility and to correlate the *PDE8B* polymorphism with the cause of subclinical hypothyroidism and consequently infertility in primarily infertile females of Gujarat.

1.13 References

Abalovich M, Gutierrez S, Alcaraz G, Maccallini G, Garcia A, Levalle O. Overt and subclinical hypothyroidism complicating pregnancy. Thyroid. 2002 Jan 1;12(1):63-8.

Abdul R, Seema M. Effect of clinical/sub-clinical hypothyroidism on fertility in infertility case and the response of treatment for hypothyroidism on fertility in cases of infertility. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2015;14(2):5-8.

Adamson PC, Krupp K, Freeman AH, Klausner JD, Reingold AL, Madhivanan P. Prevalence & correlates of primary infertility among young women in Mysore, India. The Indian journal of medical research. 2011 Oct;134(4):440.

Agarwal A, Singh A, Hamada A, Kesari K. Cell phones and male infertility: a review of recent innovations in technology and consequences. International braz j urol. 2011 Aug;37(4):432-54.

Allow AK, Sadek SM, Maryam B. distribution of infertility factors among infertile couples in Yemen. J Clin Dev Biol. 2016;1(3):1-4.

Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, Goni F, Basterrechea M, Sunyer J. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environmental science & technology. 2009 Oct 15; 43(20):7909-15.

Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T4 and T3 in normal subjects: a clue to the understanding of subclinical thyroid disease. The Journal of Clinical Endocrinology & Metabolism. 2002 Mar 1;87(3):1068-72.

Annex C. of the Stockholm Convention on Persistent Organic Pollutants. United Nations Environmental Programme, Geneva. 2008 Aug 27;29.

Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, Sestu N, Maschio A, Busonero F, Albai G, Dei M, Lai S, Mulas A, Crisponi L, Tanaka T, Bandinelli S, Guralnik JM, Loi A, Balaci L, Sole G, Prinzis A, Mariotti S, Shuldiner AR, Cao A, Schlessinger D, Uda M, Abecasis GR, Nagaraja R, Sanna S, Naitza S 2008. Phosphodiesterase8B gene variants are associated with serum TSH levelsand thyroid function. Am J Hum Genet 82:1270–1280.

Arojoki M, Jokimaa V, Juuti A, Koskinen P, Irjala K, Anttila L. Hypothyroidism among infertile women in Finland. Gynecological endocrinology. 2000 Jan 1;14(2):127-31.

Artini PG, Uccelli A, Papini F, Simi G, Di Berardino OM, Ruggiero M, Cela V. Infertility and pregnancy loss in euthyroid women with thyroid autoimmunity. Gynecological Endocrinology. 2013 Jan 1;29(1):36-41.

Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid: official journal of the American Thyroid Association. 2003 Jan;13(1):3-126.

Bals-Pratsch M, De Geyter C, Müller T, Frieling U, Lerchl A, Pirke KM, Hanker JP, Becker-Carus C, Nieschlag E. Episodic variations of prolactin, thyroid-stimulating hormone, luteinizing hormone, melatonin and cortisol in infertile women with subclinical hypothyroidism. Human reproduction (Oxford, England). 1997 May 1;12(5):896-904.

Barbieri RL. The initial fertility consultation: recommendations concerning cigarette smoking, body mass index, and alcohol and caffeine consumption. American journal of obstetrics and gynecology. 2001 Nov 1;185(5):1168-73.

Baskol GÜ, Atmaca H, Tanrıverdi F, Baskol ME, Kocer D, Bayram FA. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment. Experimental and Clinical Endocrinology & Diabetes. 2007 Sep;115(08):522-6.

Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacological reviews. 2006 Sep 1;58(3):488-520.

Benksim A, Elkhoudri N, Addi RA, Baali A, Cherkaoui M. Difference between primary and secondary infertility in Morocco: frequencies and associated factors. International journal of fertility & sterility. 2018 Jul;12(2):142.

Bernadette Biondi. The Normal TSH reference range: what haschanged in the last decade? J Clin Endocrinol Metab. 2013; 98(9): 3584-3587.

Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008; 29(1): 76-131.

Bjoro T, Holmen J, Krüger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). European journal of endocrinology. 2000 Nov 1;143(5):639-47.

Bloom MS, Weiner JM, Vena JE, Beehler GP. Exploring associations between serum levels of select organochlorines and thyroxine in a sample of New York state sportsmen:: the New York State Angler Cohort Study. Environmental research. 2003 Sep 1;93(1):52-66.

Bohnet HG, Fiedler K, Leidenberger FA. Subclinical hypothyroidism and infertility. The Lancet. 1981 Dec 5;318(8258):1278.

Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Human reproduction. 2007 Jun 1;22(6):1506-12.

Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs I, Weetman AP, Wiersinga WM. Is there a need to redefine the upper normal limit of TSH?. European journal of endocrinology. 2006 May 1;154(5):633-7.

Breivik K, Sweetman A, Pacyna JM, Jones KC. Towards a global historical emission inventory for selected PCB congeners—a mass balance approach: 2. Emissions. Science of the Total Environment. 2002 May 6;290(1-3):199-224.

Brookes, A. J. The essence of SNPs. Gene. 1999.234, 177–186. doi: 10.1016/S0378-1119(99)00219-X

Broughton DE, Moley KH. Obesity and female infertility: potential mediators of obesity's impact. Fertility and sterility. 2017 Apr 1;107(4):840-7.

Buck Louis GM, Dmochowski J, Lynch C, Kostyniak P, McGuinness BM, Vena JE. Polychlorinated biphenyl serum concentrations, lifestyle and time-to-pregnancy. Human Reproduction. 2009 Feb 1;24(2):451-8.

Carp HJ, Selmi C, Shoenfeld Y. The autoimmune bases of infertility and pregnancy loss. Journal of autoimmunity. 2012 May 1;38(2-3):J266-74.

Casals-Casas C, Desvergne B. Endocrine disruptors: from endocrine to metabolic disruption. Annual review of physiology. 2011 Mar 17;73:135-62.

Chandra A, Copen CE, Stephen EH. Infertility and impaired fecundity in the United States, 1982-2010: data from the National Survey of Family Growth. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2013.

Chevrier C, Warembourg C, Gaudreau E, Monfort C, Le Blanc A, Guldner L, Cordier S. Organochlorine pesticides, polychlorinated biphenyls, seafood consumption, and time-to-pregnancy. Epidemiology. 2013 Mar 1:251-60.

Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. American journal of epidemiology. 2008 Aug 1; 168(3):298-310.;

Chowdhury SH, Cozma AI, Chowdhury JH. Infertility. Essentials for the Canadian Medical Licensing Exam: Review and Prep for MCCQE Part I.

Cohn BA, Cirillo PM, Sholtz RI, Ferrara A, Park JS, Schwingl PJ. Polychlorinated biphenyl (PCB) exposure in mothers and time to pregnancy in daughters. Reproductive Toxicology. 2011 Apr 1;31(3):290-6..

Collins G, Thakore S, Goldfarb JM. IVF outcomes in young patients with unexplained infertility: an analysis of 273,779 cycles from the 2011-2013 society for assisted reproductive technology clinic outcome reporting system registry. Fertility and Sterility. 2016 Sep 1;106(3):e171.

Cyriac DD, Justine B, NoÃ PJ. Who is responsible for couple infertility: A clinic-based survey in Ouagadougou (Burkina Faso). International Journal of Nursing and Midwifery. 2017 Feb 28;9(2):10-6.

Datta J, Palmer MJ, Tanton C, Gibson LJ, Jones KG, Macdowall W, Glasier A, Sonnenberg P, Field N, Mercer CH, Johnson AM. Prevalence of infertility and help seeking among 15 000 women and men. Human reproduction. 2016 Sep 1;31(9):2108-18.

Deshpande PS, Gupta AS. Causes and prevalence of factors causing infertility in a public health facility. Journal of human reproductive sciences. 2019 Oct;12(4):287.

Deyhoul N, Mohamaddoost T, Hosseini M. Infertility-related risk factors: a systematic review. Int J Womens Health Reprod Sci. 2017 Jan 1;5(1):24-9.

Dhaibar HA, Patadia H, Mansuri T, Shah R, Khatri L, Makwana H, Master S, Robin P. Hexachlorobenzene, a pollutant in hypothyroidism and reproductive aberrations: a perceptive transgenerational study. Environmental Science and Pollution Research. 2021 Mar;28(9):11077-89.

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocrine reviews. 2009 Jun 1;30(4):293-342.

Direkvand-Moghadam A, Sayehmiri K, Delpisheh A. The global trend of infertility: an original review and meta-analysis. International Journal of Epidemiologic Research. 2014;1(1):35-43.

Dosiou C. Thyroid and fertility: recent advances. Thyroid. 2020 Apr 1;30(4):479-86.

Doufas AG, Mastorakos G. The hypothalamic-pituitary-thyroid axis and the female reproductive system. Annals of the New York Academy of Sciences. 2000 Apr;900(1):65-76.

Duarte MM, Moresco RN, Duarte T, Santi A, Bagatini MD, Da Cruz IB, Schetinger MR, Loro VL. Oxidative stress in hypercholesterolemia and its association with Ala16Val superoxide dismutase gene polymorphism. Clinical biochemistry. 2010 Sep 1;43(13-14):1118-23.

Dumitriu L, Bartoc R, Ursu H, Purice M, Ionescu V. Significance of high levels of serum malonyl dialdehyde (MDA) and ceruloplasmin (CP) in hyper-and hypothyroidism. Endocrinologie. 1988 Jan 1;26(1):35-8.

Duntas LH. Thyroid disease and lipids.*Thyroid.* 2002; 12 (4):287-293.

Efstathiadou Z, Bitsis S, Milionis HJ, Kukuvitis A, Bairaktari ET, Elisaf MS, Tsatsoulis A. Lipid profile in subclinical hypothyroidism: is L-thyroxine substitution beneficial?. European Journal of Endocrinology. 2001 Dec 1;145(6):705-10.

Elhussein OG, Ahmed MA, Suliman SO, Adam I. Epidemiology of infertility and characteristics of infertile couples requesting assisted reproduction in a low-resource setting in Africa, Sudan. Fertility research and practice. 2019 Dec;5(1):1-5.

Elussein EA, Magid YM, Omer MM, Adam I. Clinical patterns and major causes of infertility among Sudanese couples. Tropical doctor. 2008 Oct;38(4):243-4.

Emokpae MA, Osadolor HB, Ohonsi AO. Sub-clinical hypothyroidism in infertile Nigerian women with hyperprolactinaemia. Nigerian Journal of Physiological Sciences. 2011;26(1).

European IVF-Monitoring Consortium (EIM), European Society of Human Reproduction and Embryology (ESHRE), Kupka MS, D'Hooghe T, Ferraretti AP, de Mouzon J, Erb K, Castilla JA, Calhaz-Jorge C, De Geyter C, Goossens V. Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE. Human reproduction. 2016 Feb 1;31(2):233-48.

Farhi J, Ben-Haroush A. Distribution of causes of infertility in patients attending primary fertility clinics in Israel. IMAJ-Israel Medical Association Journal. 2011 Jan 1;13(1):51.

Fisher BE. Most unwanted. Environmental health perspectives. 1999 Jan;107(1):A18-23.

Freundl G, Godehardt E, Kern PA, Frank-Herrmann P, Koubenec HJ, Gnoth C. Estimated maximum failure rates of cycle monitors using daily conception probabilities in the menstrual cycle. Human Reproduction. 2003 Dec 1;18(12):2628-33.

Fritz MA, Speroff L. Induction of ovulation. Gynecologic Endocrinology and Infertility, 8th Edition. Eds. Fritz M, Speroff L,. Wolters Kluwer Health/Lippincot Williams & Wilkins. 2011.

Gamanuma M, Yuasa K, Sasaki T, Sakurai N, Kotera J, Omori K. Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. Cell Signal. 2003; 15(6): 565-574.

Ganguly S, Unisa S. Trends of infertility and childlessness in India: Findings from NFHS data. Facts, views & vision in ObGyn. 2010;2(2):131.

Gelbaya TA, Potdar N, Jeve YB, Nardo LG. Definition and epidemiology of unexplained infertility. Obstetrical & gynecological survey. 2014 Feb 1;69(2):109-15.

Gerhard I, Becker T, Eggert-Kruse W, Klinga K, Runnebaum B. Thyroid and ovarian function in infertile women. Human reproduction. 1991 Mar 1;6(3):338-45.

Gillett M. Subclinical hypothyroidism: subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Clin Biochem Rev. 2004;25:191–4.

Gnoth C, Godehardt E, Frank-Herrmann P, *et al.*. Definition and prevalence of subfertility and infertility. Hum Reprod. 2005 May;20(5):1144-7. doi: 10.1093/humrep/deh870. PMID: 15802321

GOLDSMITH RE, STURGIS SH, JACOB L, STANBURY JB. The menstrual pattern in thyroid disease. The Journal of Clinical Endocrinology & Metabolism. 1952 Jul 1;12(7):846-55.

Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. Executive summary to EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocrine reviews. 2015 Dec 1; 36(6):593-602.

Granfors M, Karypidis H, Hosseini F, Skjöldebrand-Sparre L, Stavreus-Evers A, Bremme K, *et al.*. Phosphodiesterase 8B gene polymorphism in women with recurrent miscarriage: a retrospective case control study. BMC Med Genet. 2012; 13:121.

Grassi G, Balsamo A, Ansaldi C, Balbo A, Massobrio M, Benedetto C. Thyroid autoimmunity and infertility. Gynecological Endocrinology. 2001 Jan 1;15(5):389-96.
Grodstein F, Goldman MB, Cramer DW. Body mass index and ovulatory infertility. Epidemiology. 1994 Mar 1:247-50.

Gurmanpreet, Gill HS, Bedi GK, Kaur A. Correlation of TSH and Prolactin in patients with primary infertility. Scholars journal of applied medical sciences. 2014;2(4B):1267-1269.

Gurunath S, Pandian Z, Anderson RA, Bhattacharya S. Defining infertility--a systematic review of prevalence studies. Hum Reprod Update. 2011;17(5):575–88.

Guyton AC, Hall JE. Textbook of Medical physiology 11 th Edition. Elsevier sounders. China. 2006;17:1-201.

Habbul PP, Shaikh AK. Study of thyroid dysfunction in association with infertility. Int J Biochem Res Rev. 2016;11(2).

Hagmar L, Rylander L, Dyremark E, Klasson-Wehler E, Erfurth EM. Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. International archives of occupational and environmental health. 2001 Apr;74(3):184-8.

Hagmar L. Polychlorinated biphenyls and thyroid status in humans: a review. Thyroid. 2003 Nov 1;13(11):1021-8.

Halliwell, B. &Gutteridge, J.M.C. (1994). *Free radical in biology and medicine*. Clarendon Press. 2nd ed., Oxford, 1979.

Han L, Hsu WW, Todem D, Osuch J, Hungerink A, Karmaus W. In utero exposure to polychlorinated biphenyls is associated with decreased fecundability in daughters of Michigan female fisheaters: a cohort study. Environmental Health. 2016 Dec;15(1):1-3.

Hayashi M, Shimada Y, Nishimura Y, Hama T, Tanaka T. Genomic organization, chromosomal localization, and alternative splicing of the human phosphodiesterase 8B gene. BiochemBiophys Res Commun. 2002; 297(5): 1253-1258.

Hayashi M, Shimada Y, Nishimura Y, Hama T, Tanaka T. Genomic organization, chromosomal localization, and alternative splicing of the human phosphodiesterase 8B gene. BiochemBiophys Res Commun. 2002; 297(5): 1253-1258.

Healy DL, Trounson AO, Andersen AN. Female infertility: causes and treatment. The Lancet. 1994 Jun 18;343(8912):1539-44.

Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. Nature reviews genetics. 2005 Feb;6(2):95-108.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). The Journal of Clinical Endocrinology & Metabolism. 2002 Feb 1;87(2):489-99.

Horvath A, Giatzakis C, Tsang K, Greene E, Osorio P, Boikos S.A cAMP-specific phosphodiesterase (PDE8B) that is mutated in adrenal hyperplasia is expressed widely in human and mouse tissues: a novel PDE8B isoform in human adrenal cortex. Eur J Hum Genet. 2008; 16(10): 1245-1253

Hruska KS, Furth PA, Seifer DB, Sharara FI, Flaws JA. Environmental factors in infertility. Clinical obstetrics and gynecology. 2000 Dec 1;43(4):821-9.

Hsu PC, Lai TJ, Guo NW, Lambert GH, Leon Guo Y. Serum hormones in boys prenatally exposed to polychlorinated biphenyls and dibenzofurans. Journal of Toxicology and Environmental Health, Part A. 2005 Sep 1;68(17-18):1447-56.

Huesca-G C, Franco M, Monta LF, Mass F, Posadas-Romero C. Chronic hypothyroidism induces abnormal structure of high-density lipoproteins and impaired kinetics of apolipoprotein AI in the rat. Metabolism-Clinical and Experimental. 2002 Apr 1;51(4):443-50.

Inhorn MC. Global infertility and the globalization of new reproductive technologies: illustrations from Egypt. Social science & medicine. 2003 May 1;56(9):1837-51.

Iqbal A, Jorde R, Figenschau Y. Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: the Tromsø Study. Journal of internal medicine. 2006 Jul;260(1):53-61.

Janssen OE, Mehlmauer N, Hahn S, Offner AH, Gartner R. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. European journal of endocrinology. 2004 Mar 1;150(3):363-70.

Jefferys A, Vanderpump M, Yasmin E. Thyroid dysfunction and reproductive health. ObstetGynaecol. 2015;17(1):39–45.

Joshi JV, Bhandarkar SD, Chadha M, Balaiah D, Shah R. Menstrual irregularities and lactation failure may precede thyroid dysfunction or goitre. Journal of postgraduate medicine. 1993 Jul 1;39(3):137.

Kaider AS, Kaider BD, Janowicz PB, Roussev RG. Immunodiagnostic evaluation in women with reproductive failure. American Journal of Reproductive Immunology. 1999 Dec;42(6):335-46.

Kalsum A, Jalali S. Role of hyperprolactenemia in infertility. Pakistan J Med Res. 2002;41(3):18.

Kaneda T, Greenbaum C, Kline K. 2020 World Population Data Sheet. Population Reference Bureau. [Online]. Available: https://bit. ly/3tJgT51. 2020.

Kang, D. & Hamasaki, N. (2003). Mitochondrial oxidative stress and mitochondrial DNA. *Clinical Chemistry and Laboratory Medicine* , 41, pp. 1281-1288.

Katole A, Saoji AV. Prevalence of primary infertility and its associated risk factors in urban population of central India: A community-based cross-sectional study. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2019 Oct;44(4):337.

Kim S, Park J, Kim HJ, Lee JJ, Choi G, Choi S, Kim S, Kim SY, Moon HB, Kim S, Choi K. Association between several persistent organic pollutants and thyroid hormone levels in serum among the pregnant women of Korea. Environment international. 2013 Sep 1;59:442-8.

Konukoglu D, Ercan M, Hatemi H. Plasma viscosity in female patients with hypothyroidism: effects of oxidative stress and cholesterol. Clinical hemorheology and microcirculation. 2002 Jan 1;27(2):107-13.

Konukoglu D, Ercan M, Hatemi H. Plasma viscosity in female patients with hypothyroidism: effects of oxidative stress and cholesterol. Clinical hemorheology and microcirculation. 2002 Jan 1;27(2):107-13.

Krassas GE, Pontikides N, Kaltsas TH, Papadopoulou PH, Paunkovic J, Paunkovic N, H. Duntas L. Disturbances of menstruation in hypothyroidism. Clinical endocrinology. 1999 May;50(5):655-9.

Krassas GE. Thyroid disease and female reproduction. Fertility and sterility. 2000 Dec 1;74(6):1063-70.

Krassas GE. Thyroid disease and female reproduction. Fertility and sterility. 2000 Dec 1;74(6):1063-70.

Kumar D. Prevalence of female infertility and its socioeconomic factors in tribal communities of Central India. Rural and Remote Health. 2007 May 1;7(2):1-5.

Kutteh WH, Yetman DL, Carr AC, Beck LA, Scott Jr RT. Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction. Fertility and sterility. 1999 May 1;71(5):843-8.

Lee WY, Suh JY, Rhee EJ, Park JS, Sung KC, Kim SW. Plasma CRP, apolipoprotein A-1, apolipoprotein B and Lp (a) levels according to thyroid function status. Archives of medical research. 2004 Nov 1;35(6):540-5.

Lincoln SR, Ke RW, Kutteh WH. Screening for hypothyroidism in infertile women. The Journal of reproductive medicine. 1999 May 1;44(5):455-7.

Lu L, Wang B, Shan Z, Jiang F, Teng X, Chen Y, Lai Y, Wang J, Xue H, Wang S, Li C. The correlation between thyrotropin and dyslipidemia in a population-based study. Journal of Korean medical science. 2011 Feb 1;26(2):243-9.

Macaluso M, Wright-Schnapp TJ, Chandra A, Johnson R, Satterwhite CL, Pulver A, Berman SM, Wang RY, Farr SL, Pollack LA. A public health focus on infertility prevention, detection, and management. Fertility and sterility. 2010 Jan 1;93(1):16-e1.

Malaiarasi N, Santhanalakshmi L. The association of thyroid dysfunctions with infertility in females. Int J Adv Res. 2016; 4 (7): 1017-1024.

Malinowski JR, Denny JC, Bielinski SJ, Basford MA, Bradford Y, Peissig PL, *et al.*. Genetic variants associated with serum thyroid stimulating hormone (TSH) levels in European Americans and African Americans from the eMERGE Network. PLoS One. 2014; 9(12): e111301.

Mancini A, Giacchi E, Raimondo S, Di Segni C, Silvestrini A, Meucci E. Hypothyroidism, oxidative stress and reproduction. Hypothyroidism—Influences and Treatments. 2012 Feb 8:117-34.

Mansuri T, Jadeja Sh.D, Singh M, Begum R, Robin P. Phosphodiesterase 8B polymorphism rs4704397 is associated with infertility in subclinicalhypo-thyroid females: a case-control study. Int J Fertil Steril. 2020; 14(2): 122-12 9. doi: 10.22074/ijfs.2020.6015.

Maraka S, Ospina NM, O'Keeffe DT, Espinosa De Ycaza AE, Gionfriddo MR, Erwin PJ, Coddington III CC, Stan MN, Murad MH, Montori VM. Subclinical hypothyroidism in pregnancy: a systematic review and meta-analysis. Thyroid. 2016 Apr 1;26(4):580-90.

Mariotti S, Naitza S, Cao A. Phosphodiesterase 8B (PDE8B) gene variants and TSH levels.Hot Thyroidol. 03/10:1-10.

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS medicine. 2012 Dec 18;9(12):e1001356.

Masoumi SZ, Parsa P, Darvish N, Mokhtari S, Yavangi M, Roshanaei G. An epidemiologic survey on the causes of infertility in patients referred to infertility center in Fatemieh Hospital in Hamadan. Iranian journal of reproductive medicine. 2015 Aug;13(8):513.

Mathews TJ. Delayed childbearing: more women are having their first child later in life. US Department of H, ealth and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2009.

Medici M, Visser WE, Visser TJ, Peeters RP. Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand? Endocr Rev. 2015; 36(2): 214-244.

Miura S, Iitaka M, Yoshimura H, Kitahama S, Fukasawa N, Kawakami Y, Sakurai S, Urabe M, Sakatsume Y, ITO K, Ishii J. Disturbed lipid metabolism in patients with subclinical hypothyroidism: effect of L-thyroxine therapy. Internal Medicine. 1994;33(7):413-7.

Mokhtar S, Hassan HA, Mahdy N, Elkhwsky F, Shehata G. Risk factors for primary and secondary female infertility in Alexandria: a hospital-based case-control study. J Med Res Inst. 2006;27:251-61.

Mya MM, Aronow WS. Subclinical hypothyroidism is associated with coronary artery disease in older persons. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2002 Oct 1;57(10):M658-9.

Nanda N, Bobby Z, Hamide A. Oxidative stress and protein glycation in primary hypothyroidism. Male/female difference. Clinical and experimental medicine. 2008 Jun;8(2):101-8.

Naz MS, Dovom MR, Tehrani FR. The menstrual disturbances in endocrine disorders: a narrative review. International Journal of Endocrinology and Metabolism. 2020 Oct;18(4).

niti.gov.in Total fertility rate 2000-2016.

Olu Pearce T. She will not be listened to in public: Perceptions among the Yoruba of infertility and childlessness in women. Reproductive Health Matters. 1999 Jan 1;7(13):69-79.

Osius N, Karmaus W, Kruse H, Witten J. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environmental health perspectives. 1999 Oct; 107(10):843-9.

Padubiri VG, Daftary SN; Physiology. In Shaw's textbook of Gynaecology, 16th edition, Elsevier, New Delhi, 2015:37-48.

Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JR, Weedon MN, Singleton A, Hernandez D, Evans J, Durant C, Ferrucci L, Melzer D, Saravanan P, Visser TJ, Ceresini G,

HattersleyAT,Vaidya B, Dayan CM, Frayling TM 2008 A common variation in deiodinase1 gene DIO1 is associated with the relative levels of free thyroxine andtriiodothyronine. J Clin Endocrinol Metab 93:3075–3081.

Panicker V. Genetics of thyroid function and disease. Clinical Biochem Rev. 2011; 32(4): 165-175.

Pasupathi P, Latha R. Free radical activity and antioxidant defense mechanisms in patients with hypothyroidism. Thyroid Sci. 2008;3(12):1-6.

Patrick L. Thyroid Disruption: Mechanisms and Clinical Implications in Human Health. Alternative Medicine Review. 2009 Dec 1;14(4).

Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, Visser TJ 2003 Polymorphisms in thyroid hormonepathway genes are associated with plasma TSH and iodothyroninelevels in healthy subjects. J Clin Endocrinol Metab 88:2880–2888.

Persani L, Borgato S, Lania A, Filopanti M, Mantovani G, Conti M, Spada A. Relevant cAMP-specific phosphodiesterase isoforms in human pituitary: effect of Gsα mutations. The Journal of Clinical Endocrinology & Metabolism. 2001 Aug 1;86(8):3795-800.

Persky V, Turyk M, Anderson HA, Hanrahan LP, Falk C, Steenport DN, Chatterton Jr R, Freels S, Great Lakes Consortium. The effects of PCB exposure and fish consumption on endogenous hormones. Environmental health perspectives. 2001 Dec;109(12):1275-83.

Poppe K, Glinoer D, Tournaye H, Devroey P, Velkeniers B. Impact of the ovarian hyperstimulation syndrome on thyroid function. Thyroid. 2008 Jul 1;18(7):801-2.

Poppe K, Glinoer D, Van Steirteghem A, Tournaye H, Devroey P, Schiettecatte J, Velkeniers B. Thyroid dysfunction and autoimmunity in infertile women. Thyroid. 2002 Nov 1;12(11):997-1001.

Poppe K, Glinoer D. Thyroid autoimmunity and hypothyroidism before and during pregnancy. Human reproduction update. 2003 Mar 1;9(2):149-61.

Poppe K, Velkeniers B, Glinoer D. The role of thyroid autoimmunity in fertility and pregnancy. Nature clinical practice Endocrinology & metabolism. 2008 Jul;4(7):394-405.

Poppe K, Velkeniers B, Glinoer D. Thyroid disease and female reproduction. Clinical endocrinology. 2007 Mar;66(3):309-21.

Poppe K, Velkeniers B. Female infertility and the thyroid. Best practice & research Clinical endocrinology & metabolism. 2004 Jun 1;18(2):153-65.

Poppe K, Velkeniers B. Thyroid disorders in infertile women. InAnnales d'endocrinologie 2003 Feb 1 (Vol. 64, No. 1, pp. 45-50).

Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertility and sterility. 2013 Jan 1;99(1):63.

Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertility and sterility. 2015 Jun 1;103(6):e44-50.

Practice Committee of the American Society for Reproductive Medicine. Subclinical hypothyroidism in the infertile female population: a guideline. FertilSteril. 2015; 104(3): 545-553.

Prasad A, Singh M. Role Of Thyroid Dysfunction In Patients With Menstrual Disorders Volume 18, Issue 8 Ser. 8 (August. 2019), PP 18-21.

PRB. World population data sheet. Population Reference Bureau. With a special focus on human needs and sustainable resources. 2016.

Priya DM, Akhtar N, Ahmad J. Prevalence of hypothyroidism in infertile women and evaluation of response of treatment for hypothyroidism on infertility. Indian journal of endocrinology and metabolism. 2015 Jul;19(4):504.

Prummel MF, Strieder T, Wiersinga WM. The environment and autoimmune thyroid diseases. European Journal of Endocrinology. 2004 May 1;150(5):605-18.

Prummel MF, Wiersinga WM. Thyroid peroxidase autoantibodies in euthyroid subjects. Best practice & research Clinical endocrinology & metabolism. 2005 Mar 1;19(1):1-5.

Pugh SJ, Schisterman EF, Browne RW, Lynch AM, Mumford SL, Perkins NJ, Silver R, Sjaarda L, Stanford JB, Wactawski-Wende J, Wilcox B. Preconception maternal lipoprotein levels in relation to fecundability. Human Reproduction. 2017 May 1;32(5):1055-63.

Purkayastha N, Sharma H. Prevalence and potential determinants of primary infertility in India: Evidence from Indian demographic health survey. Clinical Epidemiology and Global Health. 2021 Jan 1;9:162-70.

Purkey HE, Palaninathan SK, Kent KC, Smith C, Safe SH, Sacchettini JC, Kelly JW. Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: rationalizing rodent PCB toxicity. Chemistry & biology. 2004 Dec 1;11(12):1719-28.

Raber W, Nowotny P, Vytiska-Binstorfer E, Vierhapper H. Thyroxine treatment modified in infertile women according to thyroxine-releasing hormone testing: 5 year follow-up of 283 women referred after exclusion of absolute causes of infertility. Human reproduction. 2003 Apr 1;18(4):707-14.

Rebbeck TR, Spitz M, Wu X. Assessing the function of genetic variants in candidate gene association studies. Nature Reviews Genetics. 2004 Aug;5(8):589-97.

Reddy B.S., Rozati R., Reddy S., Kodampur S., Reddy P., Reddy R.: "High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study". *Fertil. Steril.*, 2006, *85,* 775.

Reimand K, Talja I, Metsküla K, Kadastik Ü, Matt K, Uibo R. Autoantibody studies of female patients with reproductive failure. Journal of Reproductive Immunology. 2001 Aug 1;51(2):167-76.

Resch, U., Helsel, G., Tatzber, F. &Sinzinger, H.. Antioxidant status in thyroid dysfunction. *Clinical Chemistry and Laboratory Medicine*. 2002, 40, pp. 1132-1134.

Ribas-Fitó N, Sala M, Cardo E, Mazón C, De Muga ME, Verdú A, Marco E, Grimalt JO, Sunyer J. Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns. Occupational and environmental medicine. 2003 Apr 1; 60(4):301-3.

Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. Fundamental and applied toxicology. 1993 Nov 1;21(4):433-41.

Ross DS, Cooper D, Mulder J. Subclinical hypothyroidism in nonpregnant adults. Waltham, MA: UpTo-Date. 2020.

Ross DS, Lockwood CJ, Cooper DS, Mulder JE. Overview of thyroid disease in pregnancy. Wellesley, MA: UpToDate. 2006.

Roussev RG, Kaider BD, Price DE, Coulam CB. Laboratory evaluation of women experiencing reproductive failure. American journal of reproductive immunology. 1996 Apr;35(4):415-20.

Rozati R, Bakshi HA, Baludu S, Sharma RS. Impact of high plasma concentrations of dioxin and polychlorinated biphenyls (PCBS) in South Indian women with endometriosis. Medical Journal of Islamic World Academy of Sciences. 2009; 17(1):37-44.

Ruder, E.H. Hartman, T.J. & Goldman, M.B. Impact of oxidative stress on female fertility. *Current Opinions in Obstetrics & Gynecology*. 2009, 21 (3), pp. 219-22.
Sample Registration Survey (SRS), conducted by the Registrar General of India, the country's official source of birth and death data, 2013.

Sample Registration Survey (SRS), The Registrar General of India, the country's official source of birth and death data; 2013.

Santi A, Duarte MM, de Menezes CC, Loro VL. Association of lipids with oxidative stress biomarkers in subclinical hypothyroidism. International Journal of Endocrinology. 2012 Oct;2012.

Santi A, Duarte MM, Moresco RN, Menezes C, Bagatini MD, Schetinger MR, Loro VL. Association between thyroid hormones, lipids and oxidative stress biomarkers in overt hypothyroidism. Clinical Chemistry and Laboratory Medicine. 2010 Nov 1;48(11):1635-9.

Saran S, Gupta BS, Philip R, Singh KS, Bende SA, Agroiya P, *et al.*. Effect of hypothyroidism on female reproductive hormones.Indian J Endocrinol Metab. 2016; 20(1): 108-113.

Saxena S, Gupta R, Agarwal L, Srivastava PC, Mallick AK. Correlation of serum thyroid stimulating hormone and prolactin in female infertility–a case control study. Indian Journal of Obstetrics and Gynecology Research. 2016;3(4):388-92.

Schell LM, Gallo MV, DeCaprio AP, Hubicki L, Denham M, Ravenscroft J, Akwesasne Task Force on the Environment. Thyroid function in relation to burden of PCBs, p, p′-DDE, HCB, mirex and lead among Akwesasne Mohawk youth: a preliminary study. Environmental toxicology and pharmacology. 2004 Nov 1; 18(2):91-9.

Scofield RH. Autoantibodies as predictors of disease. The Lancet. 2004 May 8;363(9420):1544-6.

Shah K, Batzer F. Infertility in the developing world: The combined role for feminists and disability rights proponents. IJFAB: International Journal of Feminist Approaches to Bioethics. 2010 Sep;3(2):109-25.

Shalev E, Eliyahu S, Ziv M, Ben-Ami M. Routine thyroid function tests in infertile women: are they necessary?. American journal of obstetrics and gynecology. 1994 Nov 1;171(5):1191-2.

Shamila S, Sasikala SL. Primary report on the risk factors affecting female infertility in South Indian districts of Tamil Nadu and Kerala. Age (years). 2011;20(25):25-30.

Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. European urology. 2016 Oct 1;70(4):635-45.

Sharma S, Khinchi MP, Sharma N, Agrawal D, Gupta MK. Female infertility: an overview. International Journal of Pharmaceutical Sciences and Research. 2011 Jan 1;2(1):1.

Sheela WG, P V, Mohanambal CM. Thyroid Disorder and Menstrual Disorders - A tertiary care hospital based cross sectional study in Silk city Kancheepuram, South India. Indian Journal of Obstetrics and Gynecology Research. 2017;4(2).

Shields BM, Freathy RM, Knight BA, Hill A, Weedon MN, Frayling TM, *et al.*. Phosphodiesterase 8B gene polymorphism is associated with subclinical hypothyroidism in pregnancy. J Clin Endocrinol Metab. 2009; 94(11): 4608-4612.

Smith S, Pfeifer SM, Collins JA. Diagnosis and management of female infertility. Jama. 2003 Oct 1;290(13):1767-70.

Soderling SH, Bayuga SJ, Beavo JA. Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. ProcNatlAcad Sci USA. 1998; 95(15): 8991-8996.

Sohrabvand F, Jafari M, Shariat M, Haghollahi F, Lotfi M. Frequency and epidemiologic aspects of male infertility. Acta Medica. Iranica. 2015;53(4):231-235.

Soltani M, Shairi MR, Roshan R, Rahimi CR. The impact of emotionally focused therapy on emotional distress in infertile couples. International journal of fertility & sterility. 2014 Jan;7(4):337.

Sudha G, Reddy KS. Causes of female infertility: a cross-sectional study. International Journal of Latest Research in Science and Technology. 2013;2(6):119-123.

Sun H, Gong TT, Jiang YT, Zhang S, Zhao YH, Wu QJ. Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990–2017: results from a global burden of disease study, 2017. Aging (Albany NY). 2019 Dec 15;11(23):10952.

Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Jama. 2004 Jan 14;291(2):228-38.

Tabong PT, Adongo PB. Infertility and childlessness: a qualitative study of the experiences of infertile couples in Northern Ghana. BMC pregnancy and childbirth. 2013 Dec;13(1):1-0.

Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, Salvetti A, Ferrannini E, Monzani F. Impaired endothelium-dependent vasodilatation in subclinical hypothyroidism: beneficial effect of levothyroxine therapy. The Journal of Clinical Endocrinology & Metabolism. 2003 Aug 1;88(8):3731-7.

Takser L, Mergler D, Baldwin M, De Grosbois S, Smargiassi A, Lafond J. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environmental health perspectives. 2005 Aug;113(8):1039-45.

Talwar PP, Go OP, Murali IN. Prevalence of infertility in different population groups in India and its determinants. Statistics and demography. New Delhi: National Institute of Health & Family Welfare & Indian Council of Medical Research. 1986.

Tasneem A, Fatima I, Ali A, Mehmood N, Amin MK. The incidence of hyperprolactinaemia and associated hypothyroidism: local experience from Lahore. Pak J Nuclear Med. 2011;1:49-55.

Tomer Y. Mechanisms of autoimmune thyroid diseases: from genetics to epigenetics. Annual Review of Pathology: Mechanisms of Disease. 2014 Jan 24;9:147-56.

Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. Clinical endocrinology. 2009 Mar;70(3):469-74.

Turhan S, Sezer S, Erden G, Guctekin A, Ucar F, Ginis Z, Ozturk O, Bingol S. Plasma homocysteine concentrations and serum lipid profile as atherosclerotic risk factors in subclinical hypothyroidism. Annals of Saudi medicine. 2008 Mar;28(2):96-101.

Unisa S. Childlessness in Andhra Pradesh, India: treatment-seeking and consequences. Reproductive health matters. 1999 Jan 1;7(13):54-64.

United Nations World Fertility Prospects, 2008.

Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. Indian journal of endocrinology and metabolism. 2011 Jul;15(Suppl2):S78.

Unuane D, Tournaye H, Velkeniers B, Poppe K. Endocrine disorders & female infertility. Best Practice & Research Clinical Endocrinology & Metabolism. 2011 Dec 1;25(6):861-73.

Unuane D, Tournaye H, Velkeniers B, Poppe K. Endocrine disorders & female infertility. Best Practice & Research Clinical Endocrinology & Metabolism. 2011 Dec 1;25(6):861-73.

Unuane D, Velkeniers B, Anckaert E, Schiettecatte J, Tournaye H, Haentjens P, Poppe K. Thyroglobulin autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment?. Thyroid. 2013 Aug 1;23(8):1022-8.

Unuane D, Velkeniers B. Impact of thyroid disease on fertility and assisted conception. Best Practice & Research Clinical Endocrinology & Metabolism. 2020 Jul 1;34(4):101378.

van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JA, Goddijn M, Bisschop PH. Significance of (sub) clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Human reproduction update. 2011 Sep 1;17(5):605-19.

Vanderpump MP, Tunbrldge WM, French JA, Appleton D, Bates D, Clark F, Evans JG, Hasan DM, Rodgers H, Tunbridge F, Young ET. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clinical endocrinology. 1995 Jul;43(1):55-68.

Venditti P, Meo SD. Thyroid hormone-induced oxidative stress. Cellular and Molecular Life Sciences CMLS. 2006 Feb;63(4):414-34.

Verma I, Sood R, Juneja S, Kaur S. Prevalence of hypothyroidism in infertile women and evaluation of response of treatment for hypothyroidism on infertility. International journal of applied and basic medical research. 2012 Jan;2(1):17.

Wang C, Crapo LM. The epidemiology of thyroid disease and implications for screening. Endocrinology and Metabolism Clinics. 1997 Mar 1;26(1):189-218.

Weetman AP. Cellular immune responses in autoimmune thyroid disease. Clinical endocrinology. 2004 Oct;61(4):405-13.

Weiss RV, Clapauch R. Female infertility of endocrine origin. Arquivos Brasileiros de Endocrinologia & Metabologia. 2014;58:144-52.

WHO/Infertility/2016.

Wiseman SA, Powell JT, Humphries SE, Press MA. The magnitude of the hypercholesterolemia of hypothyroidism is associated with variation in the low density lipoprotein receptor gene. The Journal of Clinical Endocrinology & Metabolism. 1993 Jul 1;77(1):108-12.

World Health Organization, World Health Organization. Infecundity, infertility, and childlessness in developing countries. DHS comparative reports. 2004;9.

World Health Organization. Recent advances in medically assisted conception: report of a WHO scientific group [meeting held in Geneva from 2 to 6 April 1990]. World Health Organization; 1992.

World Health Organization. Reproductive health indicators for global monitoring: report of the second interagency meeting, WHO Geneva, 17-19 July 2000. World Health Organization; 2001.

World Health Organization. Reproductive health indicators forglobal monitoring: Report of the second interagency meeting, 2001. Geneva: World Health Organization; 2001. p.23.

World Health Organization. Reproductive health indicators: guidelines for their generation, interpretation and analysis for global monitoring. World Health Organization; 2006.

Yang CY, Huang TS, Lin KC, Kuo P, Tsai PC, Guo YL. Menstrual effects among women exposed to polychlorinated biphenyls and dibenzofurans. Environmental research. 2011 Feb 1;111(2):288-94

Yang S, Tao J, Zhang J, Fan J, Qian W, Shu K. Genetic association study of phosphodiesterase 8B gene with subclinical hypothyroidism in pregnant women. Endocrine Res. 2015; 40(4): 199-203.

Yao M, Hu T, Wang Y, Du Y, Hu C, Wu R. Polychlorinated biphenyls and its potential role in endometriosis. Environmental Pollution. 2017 Oct 1;229:837-45.

YILDIRIMKAYA M, ÖZATA M, YILMAZ K, KILINÇ C, GÜNDOGAN MA, KUTLUAY T. Lipoprotein (a) Concentration in Subclinical Hyothyroidism Before and After Levo-Thyroxine Therapy. Endocrine journal. 1996;43(6):731-6.

Zargar AH, Wani AI, Masoodi SR, Laway BA, Salahuddin M. Epidemiologic and etiologic aspects of primary infertility in the Kashmir region of India. Fertility and sterility. 1997 Oct 1;68(4):637-43.

Zegers-Hochschild F, Adamson GD, Mouzon J, Ishihara O, Mansour R, Nygren K. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology. 2009; 92: 1520–24.

Zhou Z, Zheng D, Wu H, Li R, Xu S, Kang Y, Cao Y, Chen X, Zhu Y, Xu S, Chen ZJ. Epidemiology of infertility in China: a population-based study. BJOG: An International Journal of Obstetrics & Gynaecology. 2018 Mar;125(4):432-41.