Chapter 2

Aim and Hypothesis

2.1 Aim of the study

The present study aims to find out the involvement of a hitherto unnoticed cause of thyroid dysfunction, namely, subclinical hypothyroidism and its consequence to female infertility. Subclinical hypothyroidism mostly remains unaddressed due to its asymptomatic nature. The study is designed to screen infertile females of Gujarat for the prevalence of infertility as a subsequence of subclinical hypothyroidism and to look for an etiological effect of autoimmunity, environmental pollutants as well as genetic factors. Further the the study aims to look for correlation in alterations in levels of reproductive hormones, oxidative stress and lipid profile with female infertility, either independent or as a cumulative effect, which in turn can be considered as important check points in treatment of infertility and hence could then be used to treat infertility with greater success and less side effects without targeting the reproductive system. In conclusion the present study aims to contribute to our understanding of the etiology of this multi factorial disorder, which can further be used as a diagnostic approach for the management of primary infertility in females.

2.2 Hypothesis of the study

Of the many nature's gifts to humans, nothing is more precious than parenthood. The human body works in a much-synchronized manner fulfilling this multi factorial task of maintaining fertility. Anything that can cause a break in this harmony results in Infertility. Female infertility remains a global health problem and is very common in women of reproductive age (Gangul*yet al.,* 2010; SRS, 2013; Kaneda *et al.*; 2020; United Nations World Fertility prospects, 2008; niti.gov.in.Total fertility rate 2000-2016; Purkayastha*et al.,* 2021). The cause of female infertility can be difficult to diagnose. Numerous apparent medical conditions can contribute to infertility, while sometimes the causes of infertility could be due to other underlying medical conditions which may not be obvious and hence difficult to diagnose. Since thyroid hormones exert effect on fertility at various steps, evaluation of thyroid functions in treatment of infertility as well as in treating relevant pathologies become important. While there are studies on overt conditions some of these abnormalities are due to "subclinical" conditions which also must be addressed (Krassas *et al.,* 2000; Poppe *et al.,* 2007; Jefferys *et al.,* 2015).

Infertility could be due to alterations in the hypothalamus–pituitary–thyroid (HPT) axis. Clinical and experimental studies have suggested a close inter relationship between the Hypothalamic-Pituitary-Thyroid (HPT) axis and the Hypothalamic-Pituitary-Ovarian (HPO)

axis. In normal/ euthyroid thyroid condition feedback mechanism regulates and maintains the normal thyroid hormone levels in the circulation, figure 2.1.

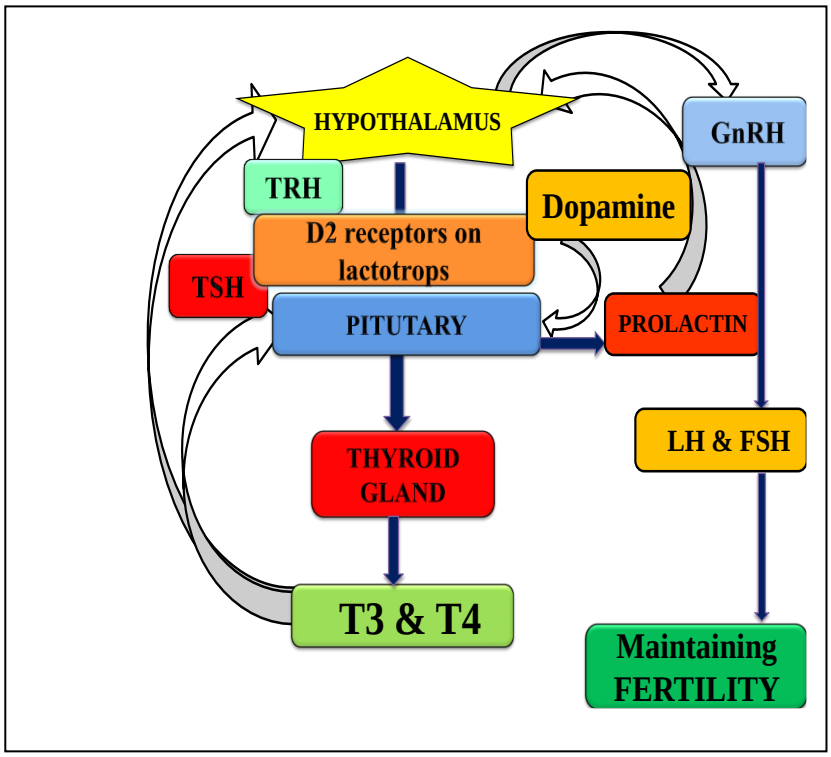

Figure 2.1Cross talk between HPT & HPO axis in Euthyroid condition: Feedback mechanism maintains the thyroid hormone levels in the circulation in normal thyroidal condition via cross talk between HPT and HPO axis.

Decreased levels of thyroid hormones due to thyroid destruction or by any of the etiological factors results in hypothyroidism. The etiological factors which can cause hypothyroidism are many. Of these the three prominent factors are:

1. Immunological factor: Autoimmune Thyroid Disease-AITD (Presece and association of Anti-TPO Abs), (Alexander et al., 2017).
2. Environmental factor: Endocrine disruptors-EDCs (Presence and association of Polychlorinated Biphenyls-PCBs), (Diamanti*et al.*, 2009).
3. Genetic factor: Single Nucleotide Polymorphisms –SNPs (Presence and association of *PDE8B* polymorphisms), (Arnaud-Lopez *et al.*, 2008).

Hypothyroidism via feedback loop causes an increase in TRH which stimulates excess prolactin secretion. Increased TSH also causes increased prolactin levels and this is due to increased production of TRH. Increased TRH causes decrease in dopamine due to which prolactin levels increase leading to hyperprolactinemia. Hypothyroidism followed by hyperprolactinemia, may cause ovulatory dysfunction, luteal phase defects, and even oligomenorrhea and amenorrhea. Such condition can cause infertility, figure 2.2.

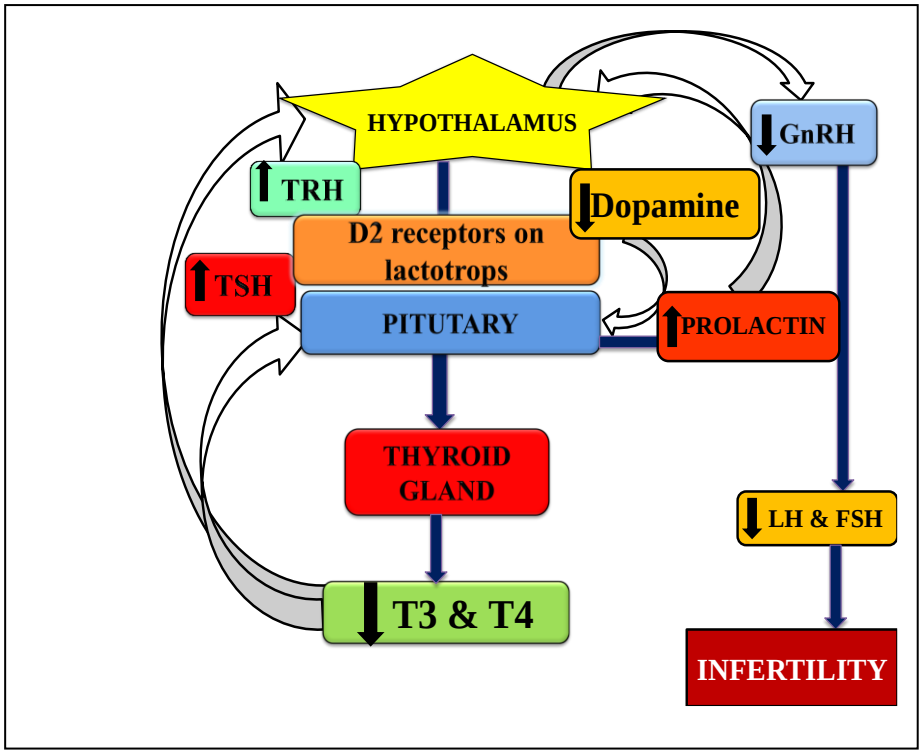

Figure 2.2Cross talk between HPT & HPO axis in Clinical/Overt Hypothyroidism: Decreased thyroid hormone levels in the circulation increases TSH and prolactin levels and decreases the levels of LH and FSH thus causing infertility in overt/ clinical hypothyroidism.

The role of overt/ clinical hypothyroidism in causing infertility in reproductive age females is well documented. But literature studies discussing the prevalence and the role of Sub Clinical Hypothyroidism (SCH) in etiology of female infertility are scarce and almost nil for the Gujarat population. SCH acts as a silent perpetrator of infertility and goes unnoticed owing to asymptomatic condition. In SCH there is an increase in TSH level but the level of thyroid hormones do not decrease and are within the normal range unlike clinical hypothyroidism where thyroid hormones level goes down. Increased TSH in SCH causes an increase in TRH and prolactin levels and thus decreases LH and FSH levels. SCH is associated with short luteal phase and insufficient progesterone secretion. Luteal phase defect (LPD) occurs when the luteal phase is shorter, progesterone levels during the luteal phase are below normal, and it interferes with the implantation of embryos resulting in infertility and thus hinders female fertility, figure 2.3.

Etiological factor such as “Autoimmune thyroid disease” (AITD) is found to be very high in infertile subclinical hypothyroid females. Another factor, “Endocrine Disrupting Chemicals” (EDCs) disturbing the thyroid system are termed as Thyroid disruptors (TDs). TDs such as “Polychlorinated biphenyls” (PCBs) may interfere with thyroid homeostasis and may cause SCH. “Single nucleotide polymorphisms” (SNPs) of several genes could also be an

etiological factor resulting in SCH. One of the candidate genes responsible for variation in TSH levels is *PDE8B* (PhoshpoDiEstrase8B gene). Genetic variations due to SNPs are frequent in PDE8B gene and many SNPs have been identified to cause higher TSH levels and a positive correlation between this and various diseases have been established. However, such a link with SCH and infertility is lacking both for world and Indian populations with no data for western part of the country.

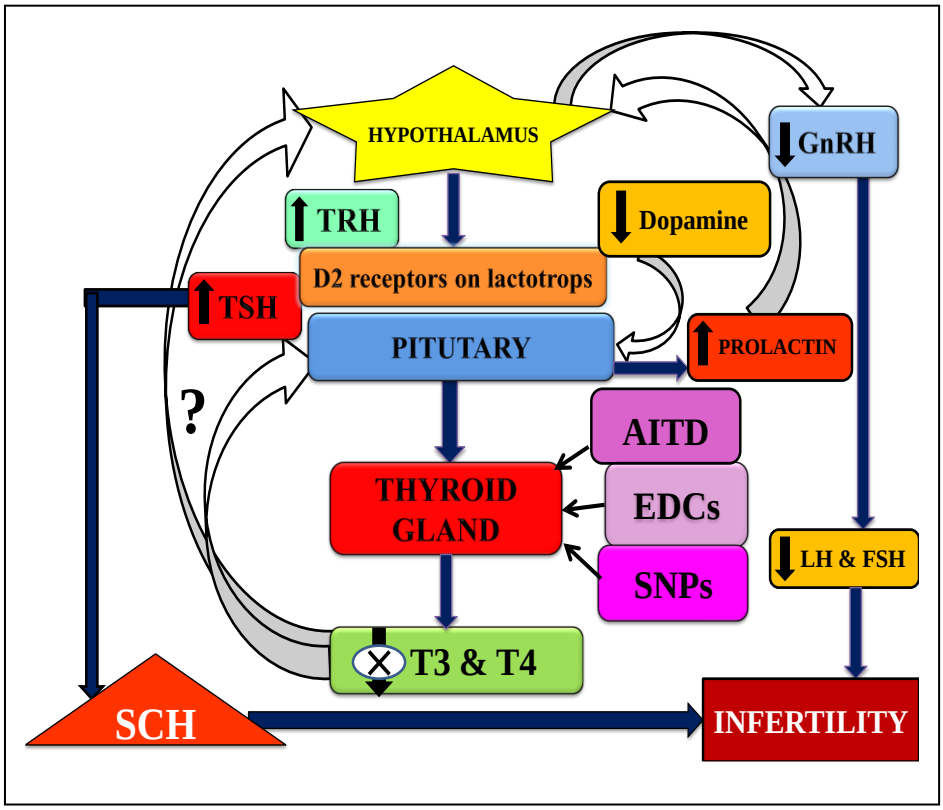

Figure 2.3 Cross talk between HPT & HPO axis in Subclinical Hypothyroidism: In subclinical hypothyroidism there is an increase in the TSH levels but the level of thyroid hormones is within the normal range. Etiological factors such as AITD, EDCs and SNPs cause destruction in thyroid gland. Increased TSH causes increased TRH and prolactin levels as a result LH and FSH level decreases resulting into infertility.

As depicted in the figure 2.4, as a preliminary investigation, the study hypothesizes the independent or cumulative involvement of three etiological factors causing SCH which are AITD (presence of anti TPO-Antibodies), EDCs (presence of Polychlorinated Biphenyls-PCBs) and SNPs (*PDE8B* polymorphisms). The study hypothesis states that all these three factors either independently or as a cumulative factors are affecting the thyroid gland homeostasis leading to subclinical hypothyroidism (SCH), which in turn via a cross talk between HPT and HPO axis results into female infertility due to reproductive hormones imbalance, increased oxidative stress as well as altered lipid profile.

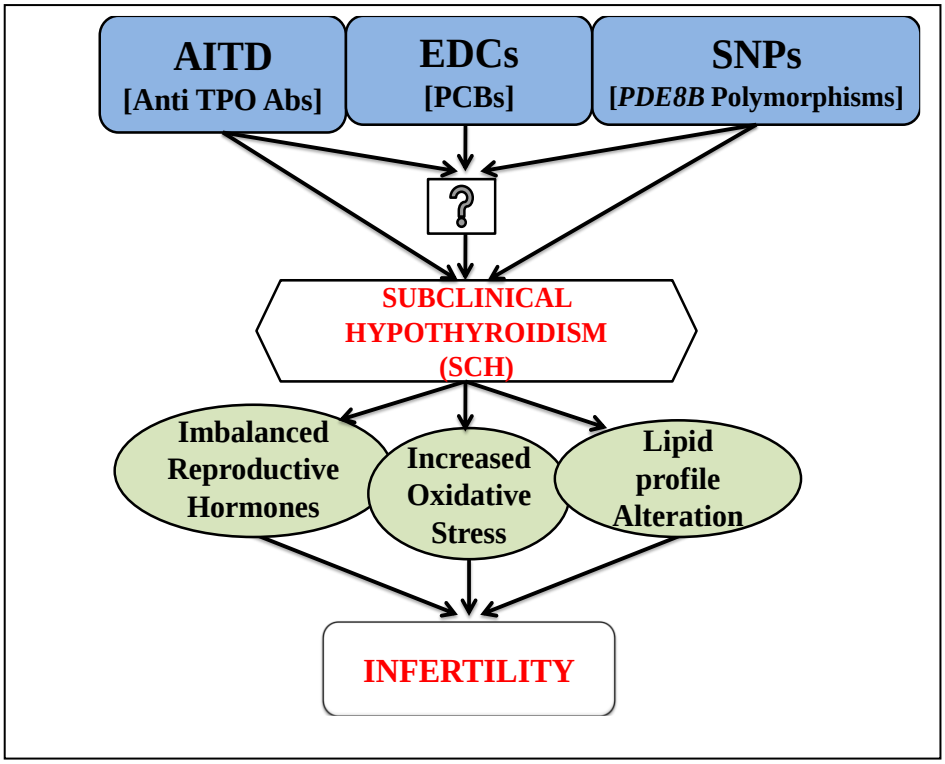

Figure 2.4 Hypothesis of the study: The most important etiological factors AITD, EDCs and SNPs either independently or as a cumulative effect causes subclinical hypothyroidism which subsequently disturbs the levels of reproductive hormones, increases the oxidative stress and alters the lipid profile resulting into infertility in females.

Though the potential consequences of SCH can lead to infertility standardized treatment to correct this are uncommon and well-designed clinical trials addressing various unnoticed issues regarding thyroid dysfunction and fertility are still needed. Treating thyroid dysfunction can reverse several abnormalities that impede reproduction and thus improve fertility. Early detection prevents the conversion of subclinical hypothyroidism to overt hypothyroidism by treating with hormones and with careful follow-up. This could then be used to treat infertility with greater success and less side effects without disturbing the reproductive system.

2.3 References

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Objectives

1. **Screening of infertile female population of Gujarat (Vadodara and its suburbs) for the prevalence of Subclinical Hypothyroidism (SCH) related infertility. And to find out the prevalence of Autoimmune thyroid disease (AITD) and to study the correlation of SCH with AITD in primarily infertile female population from Gujarat.**

A. To estimate the prevalence of Subclinical Hypothyroidism (SCH) in control females and infertile females to emphasis on its screening importance in females with infertility.

- Thyroid Function Test [TFT]:

(i) Thyroid Stimulating hormone (TSH) estimation.

(ii) Free tri-iodothyronine (fT_3) estimation.

(iii) Free thyroxine (fT_4) estimation.

B. To find out the prevalence and involvement of AITD in control females and infertile females with subclinical hypothyroidism.

(i) Repeat TSH estimation in SCH subjects.

(ii) Repeat fT_3 estimation in SCH subjects.

(iii) Anti Thyroperoxidase (TPO)-Antibody estimation.

2. **Evaluating the effects of SCH on the hormones of reproductive system and to find out the prevalence of hyperprolactinemia in Gujarat infertile female population, and to study the correlation of SCH with altered female reproductive hormonal profile.**

- To estimate the female reproductive hormone levels in control females and infertile females with subclinical hypothyroidism.

(i) Estimation of Prolactine (PRL) levels.

(ii) Estimation of Leutinising Hormone (LH) levels.

(iii) Estimation of Follical Stimulating Hormone (FSH) levels.

3. **To estimate and explore the effect of SCH on Oxidative stress levels and lipid profile, and to find out the correlation of SCH with the oxidative stress levels along with alterations in the lipid profile in infertile female population from Gujarat region.**

A. Oxidative stress levels estimation in control females and infertile females with subclinical hypothyroidism.

(i) Estimation of Malondialdehyde-MDA (Lipid Peroxidation) levels.

(ii) Change in the activity of Catalase (CAT) enzyme.

(iii) Change in the activity of Superoxide dismutase (SOD) enzyme.

B. Estimation of lipid profile alterations in control females and infertile females with subclinical hypothyroidism.

(i) Estimation of Total Cholesterol (TC).

(ii) Estimation of Triglycerides (TG).

(iii) Estimation of Low Density Lipoprotein (LDL) cholesterol.

(iv) Estimation of High Density Lipoprotein (HDL) cholesterol.

4. Estimating the levels of Polychlorinated Biphenyls (PCBs) and evaluating the correlation of PCBs with the cause of SCH in Gujarat infertile female population.

- To estimate PCBs levels in blood samples of control females and infertile females with subclinical hypothyroidism following the extraction and detection by GC-MS method.

5. To evaluate the prevalence and association of *PDE8B* polymorphisms with Subclinical hypothyroidism and female infertility, And to study the possible genotype-phenotype correlation with the cause of SCH and infertility in Gujarat infertile female population.

(i) Genotyping of *PDE8B* rs4704397 polymorphism in control females and infertile females with subclinical hypothyroidism.

(ii) Genotyping of *PDE8B* rs6885099 polymorphism in control females and infertile females with subclinical hypothyroidism.