Chapter 3

Screening of infertile female population for the prevalence of Subclinical Hypothyroidism, and to study the prevalence, involvement and the correlation of Autoimmune thyroid disease with Subclinical hypothyridism in primarily infertile female population from Gujarat

3.1 Introduction

Infertility is a serious health concern affecting 15% of couples of reproductive ages worldwide in developing countries (Gurunath *et al.*, 2011; Mascarenhas *et al.*, 2012; Direkvand *et al.*, 2014) and female infertility has been recognized as a global public health issue by the WHO (WHO/Infertility/2016). World Total Fertility Rate (TFR) has declined drastically from 4.9 in the year 1950 to 2.6 in 2010 showing almost 50% decrease (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, as reported by the "Population Reference Bureau's 2020 World Population Data Sheet" (Kaneda *et al.*; 2020). Further 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 (United Nations World Fertility prospects, 2008). 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; Purkayastha *et al.*, 2021). According to the data of surveys of all the four rounds of National Family Health Survey (NFHS), NFHS I-IV women in the reproductive age group, the prevalence of infertility has shown a remarkable increase during the most recent decade in India with western region showing a high increase in the primary infertility rate (Ganguly *et al.*, 2010). 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 (SRS, 2013).

Female infertility apart from being a psychological burden is also considered as a social stigma in Indian society. The challenges of infertility can put down a woman feeling discouraged, especially if there is no evident reason for her being infertile. Data in literature on the prevalence of infertility in women with chronic endocrine disorders are however scarce and observational studies in this area should clarify this issue. Some of these abnormalities are "subclinical" which are now must be focused. When reproductive considerations are examined, one possible reason could be thyroid dysfunction. Thyroid hormones have a direct effect on all aspects of female reproduction hence disruption in

thyroid function can lead to infertility (Krassas *et al*., 2000; Poppe *et al*., 2007; Jefferys *et al*., 2015)**.** Thyroid dysfunction can be classified as hypothyroidism (low thyroid hormone levels) and hyperthyroidism (high thyroid hormone levels). As compared to hyperthyroidism, hypothyroidism is reported to be more common in infertile females thus being a hypothyroid may be linked to infertility. Hypothyroidism can be further classified as overt/ clinical hypothyroidism (elevated TSH and low thyroid hormone levels) and subclinical hypothyroidism (SCH) (elevated TSH and normal thyroid hormone levels). Overt hypothyroidism is a clinical state with very low levels of thyroid hormones in the circulation where obvious and apparent symptoms of hypothyroidism can be seen. Studies have reported the association of overt hypothyroidism with infertility, miscarriage, and adverse pregnancy outcomes in female populations. Subclinical hypothyroidism (SCH) on the other hand is a milder form of hypothyroidism and is defined as an elevated TSH concentration in conjunction with normal free thyroxine (fT_4) levels (Alexander *et al*., 2017; Ross *et al*., 2006; Ross *et al*., 2020).

Subclinical hypothyroidism is a condition in which a slightly raised thyroid stimulating hormone (TSH) signal is representing an early, mild thyroid failure. Subclinical hypothyroidism state suggests possible dysfunction of the thyroid which may increase the risk of infertility and problems with pregnancy. SCH is a common diagnosis among women of reproductive age and as such it can affect women planning conception and pregnant women (Surks *et al*., 2004). SCH acts as a silent perpetrator of infertility as by and large goes unnoticed owing to asymptomatic condition. The role of overt/ clinical hypothyroidism in causing infertility in reproductive age females is well documented since long. But literature studies discussing the prevalence and the role of SCH in etiology of female infertility are very scares. TSH is considered as a sensitive indicator of the thyroid status and thus of SCH. In euthyroid condition which indicates normal thyroid hormones levels, serum TSH level ranges from 0.3–4.0 μIU/mL and is finely regulated within an individual. 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; Fatourechi *et al*., 2003). Considering the largest cohorts published, SCH or mild thyroid failure has a prevalence of approximately 2–4% in infertile women (Poppe *et al*., 2008).

Numerous recent studies report the prevalence ranging from 11-27% in different populations all over the world indicating a considerable burden of SCH as a silent precipitator of female infertility (Orouji Jokar *et al*., 2018; Feldthusen *et al*., 2015; Dosiou *et al*., 2020). In India, it

has been estimated that about 42 million people suffer from thyroid diseases, and hypothyroidism is the most common endocrine abnormality, seen in infertile population. The prevalence of SCH is reported to be high in various populations. In India too prevalence of SCH is high as 15-25% as reported for the population of various parts of the country but not for the western region of India (Unnikrishnan *et al*., 2011; Purkayasthaa *et al*., 2021).

Autoimmune thyroid disease (AITD) is one of the most common etiological factors amongst the causes for the thyroid destruction resulting into overt and subclinical hypothyroidism (Hollwell *et al.,* 2002). The prevalence of AITD is higher in women than in men with the prevalence of 5 to 20% and thyroid dysfunction is more frequent in women who have thyroid autoimmunity (Poppe *et* al., 2006; Prummel *et al*., 2004; Unuane *et al.*, 2013; Van den Boogaard *et al.*, 2011). Well known forms of thyroid dysfunction are Hashimoto's Thyroiditis (HT) and Grave's Disease (GD). Hashimoto's Thyroiditis (hypothyroidism) and Grave's disease (hyperthyroidism) are organ specific autoimmune diseases. Hashimoto's Thyroiditis is characterized by the presence of auto antibodies in the serum against the thyroglobulin (anti TG -Ab) or Thyroperoxidase (anti TPO-Ab) while Grave's disease is characterized by the presence of Thyrotropin receptor auto antibodies in the serum of the patient (Kuharić et al., 2017; He et al., 2016). Elevated levels of thyroid auto antibodies, such TPO- Ab and TG-Ab induce chronic inflammation in the thyroid gland, which leads to the loss of functional tissue. Thyroid autoimmunity has been found to be related to subclinical hypothyroidism (SCH). The presence of TPO-Ab, has been associated with miscarriage, preterm birth, and post-partum thyroid disease. The American Society for Reproductive Medicine states the routine measurement of TSH and anti TPO-abs in infertile women when TSH levels are ≥2.5 mIU/L (Sen *et al*., 2014; Glinoer *et al.*, 2011; PCASRM, 2015). Studies have investigated the prevalence of AITD in different populations concluding significantly increased incidence of AITD in women with infertility (Poppe *et al.*, 2003; Poppe *et al*., 2002; Janssen *et al.*, 2004; Poppe K *et al*., 2004)). 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 Ab) positivity on obstetrical outcomes and pregnancy complications, including infertility. Thus TPO-Ab status must be considered in decision-making (Alexander *et al*., 2017; Vanderpump *et al.*, 1995; Bjoro *et al*., 2000). Screening and treatment in women of infertile couples, as proposed by Poppe K *et al*. (2004) and modified by Unuane *et al.* (2011) in a review, are summarized in figure 2.1 in algorithmic format.

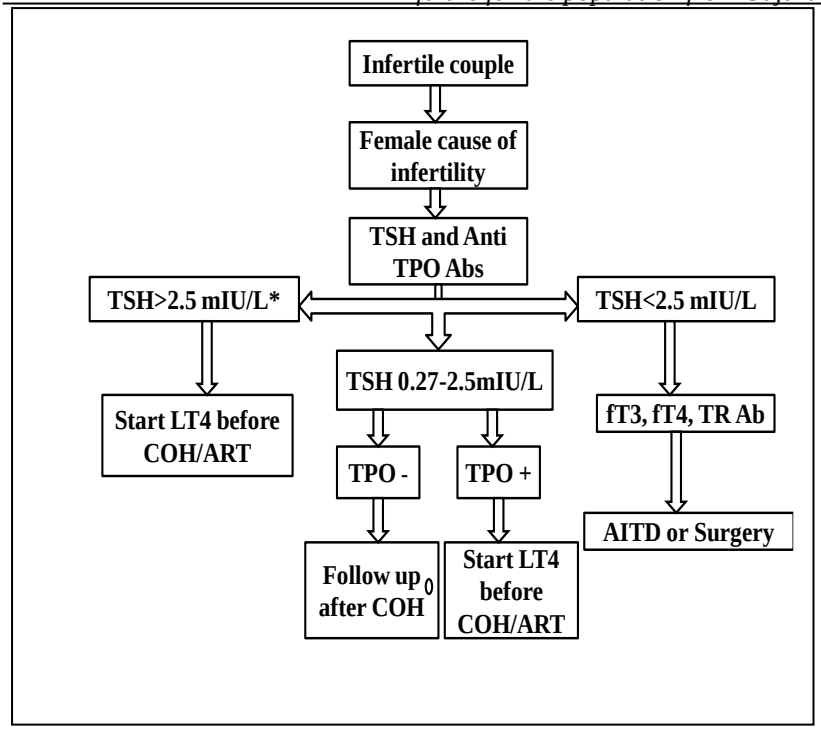

Figure 3.1 The workflow to identify infertile women with thyroid problems: The workflow to identify infertile women with thyroid problem. *, consider measuring anti-Tg antibodies; °, consider treatment when altered function after Controlled Ovarian Hyperstimulation –COH; ART, assisted reproductive technology (proposed by Poppe K *et al*., 2004 modified by Unuane *et al.*, 2011).

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. Since thyroid functions exert effect over fertility with various mechanisms, evaluation of thyroid functions during both pregnancy and treatment of infertility as well as in treating relevant pathologies become important. Treating "subclinical" thyroid dysfunction can reverse menstrual abnormalities and thus improve fertility. The study focuses on the involvement of autoimmune factor in causing the subclinical hypothyroidism leading to female infertility. The study is designed to screen the infertile female population for the prevalence of SCH, looking for AITD and establishing a correlation with SCH with infertility and AITD, which in turn can be consider as an important step in treatment of infertility. To take in to an account, very few studies are reported which discuss about the prevalence as well as role of SCH in female infertility for Indian population and there is a scarcity of data that reports the prevalence rate of SCH related female infertility in the western India especially for the Gujarat population. Hence the main aim of this primary study is to find out the prevalence of subclinical hypothyroidism which is a need of an hour due to the rapid increase of female infertility cases during last few decades and as SCH usually goes undiagnosed due to its asymptomatic nature and mostly remains unnoticed as a major cause of female infertility. We therefore aimed (i) to estimate the prevalence of SCH to emphasis on its screening importance in females with infertility in infertile females from Gujarat population and (ii) to find out the prevalence and involvement

of AITD (anti TPO-Abs), (iii) to find out the correlation between TSH and different demographic factors, (iv) to find out the correlation between TSH and fT_3, fT_4 and (v) to find out the correlation between TSH and AITD in infertile control and infertile female population.

3.2 Material and Methods

3.2.1 Ethical consideration

It was ensured that the study design complies with the ethical standards of the Institutional Ethical Committee for Human Research (IECHR), Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (FS/IECHR/BC/PR/1) and with the 1964 Helinki declaration.

3.2.2 Study Population

The present retrospective study is a matched, case-control study and includes female subjects only. The study population consists of a total 1000 infertile females with primary infertility as case subjects and 135 healthy females as controls. Control females were selected from general public by arranging health-checkup camps through newspaper advertisements as well by the means of field work. Infertile female patients were taken from the Ghanshyam Clinic (of Dr. Mahesh Pandya, Vadodara), which is a very well known and highly recognized infertility treatment center which receives a large number of female infertility cases from all over Gujarat. Rational of the study was explained in details to controls as well as patients and the females who show willingness to participate were enrolled as study population. A detailed self-administered questionnaire (please refer the attachments) was filled up after thorough counseling and a written consent (please refer the attachments) was obtained from each and every participant. Controls inclusion criteria are healthy fertile females who have at least once delivered healthy full term babies and are must be non pregnant at the time of enrollment for the present study. They should neither have any history of reproductive and/or thyroid problems nor on any medication. Infertile females at their first visit to the clinic and the patients who didn't have gone to any other infertility center before were selected only to avoid any interruption from the previous medical treatment. The study mainly focused on the finding of prevalence of primary infertility, the patients coming for the treatment of secondary infertility were excluded from the study population. Patient inclusion criteria were primary infertility diagnosis and duration of more than one year of unprotected intercourse without pregnancy. The exclusion criteria were male factor infertility, any tubal anomaly, congenital or urogenital tract anomaly and history of thyroid disease/ medication/ surgery,

PCOS (Polycystic Overian Syndrom) or any other disorders or diseases. Infertile subjects taking antidepressants**,** antipsychotics and females with liver and kidney diseases were also excluded. Because females are the most fertile in the age of 20-40 years; the subjects which are within this age group were selected for the study. General information such as contact details, health characteristics, menopausal status, smoking and/ or tobacco addiction, medical history of family, residential details, alcohol consumption and dietary habits (particularly as related to preference) were investigated by the questionnaire. To find out the geographical difference in the reference range of thyroid hormones, the study protocol was discussed with local endocrinologists and gynaecologists of private as well as government hospitals and with their kind suggestions the reference range of TSH, fT_3 and fT_4 for euthyroid, overt hypothyroid, SCH and hyperthyroid were decided which was further confirmed with the literature.

3.2.3 Blood Collection and Serum Separation

To screen out SCH in the study population 0.5 ml overnight fasting blood samples were collected by venous puncture in a total 135 control females and 1000 infertile females and serum was separated for the estimation of serum TSH levels. The control subjects having serum TSH levels in the normal/ euthyroid range (0.35-3.5 µIU /ml) and infertile females with TSH levels above normal range (3.5-10 µIU/ml) were only included and requested for the further blood collection. To avoid repeated blood collection separately for each different objective, the blood samples of the study subjects were collected in only at once in a single sitting for all the proposed objectives of the present study. A total volume of 5 ml blood samples was collected in this screened out study subjects by venous puncture from overnight fasting individuals. The control and study subjects were requested to come during the 3-5th day of their menstruation cycle for the reproductive hormonal estimation in the second objective. From this 5 ml blood sample, 1 ml sample was taken for the estimation of different study parameters of this objective1 and serum was separated by centrifugation at 4000 g for 10 minutes at 22^0C and serum was collected in eppendorf for thyroid function test (TFT). The remaining 4 ml blood samples were further distributed and processed accordingly for the respective objective parameters. Owing to the nonspecific nature of the overt hypothyroidism related symptoms (e.g., fatigue), the diagnosis of SCH is based on laboratory testing. First only serum TSH and fT4 levels were estimated in all the 1000 infertile and 135 control subjects for the screening of SCH. On obtaining the levels of TSH and fT4 levels, the subjects were further classified into normal (euthyroid-ET), above normal (subclinical

hypothyroid-SCH), high (overt/ clinical hypothyroid-OHT) or low (hyperthyroid-HYPT) depending on reference range of TSH and thyroid hormone levels, table 3.1.

Table 3.1 Reference (normal) range of TSH, fT_3 and fT4 levels followed for the selecting the subjects

Likely Diagnosis	**TSH Levels (µIU/ml)**	**fT_3 (pg/ml)**	**fT4 (ng/dl)**
Euthyroid (ET)	0.35-3.5	2.3-4.2	0.9-1.76
Subclinical hypothyroidism (SCH)	3.5-10(High)	Normal	Normal
Overt/ Clinical Hypothyroid (OHT)	> 10	Low	Low
Hyperthyroid (HYPT)	< 0.35	High	High

TSH, Thyroid Stimulating Hormone; fT_3, free triiodothyronine; fT4, free tyroxine

Patients with TSH value between 3.5 to 10µl/ml were considered as subclinical hypothyroid (270 infertile female subjects were turned out to be SCH out of total 1000) and selected as the study population. While on the other hand euthyroid (112 control female subjects were tested euthyroid out of total 135) controls were included in the study. The euthyroid control subjects and Subclinical hypothyroid (SCH) infertile subjects were the final study population of the present study and were further estimated by the thyroid function test (TFT) for further evaluation.

3.2.4 Thyroid function test-TFT (Hormonal Estimation)

A total of 270 infertile and 110 controls were further subjected to TFT which includes the test for the serum TSH, free T3 (fT3) and fT4 by enzyme-linked fluorescence immunoassay (ELFA) on mini VIDAS® immuno-analyzer (BioMérieux India Pvt. Ltd., India). "VIDAS is an automated quantitative test for use on the VIDAS instrument for the quantitative measurement of serum using the ELFA technique". Solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents are ready to use and all of the assay steps were carried out automatically. The test principle of estimation of TSH, fT_3 and fT_4 linked an enzyme immunoassay competition method with a final fluorescent detection (ELFA). Results were calculated by the instrument in relation to the calibration curve stored in memory of device, and then printed the results. These tests were carried out in the pathology laboratory (N Dalal Pathology Laboratory, Vadodara) of the Ghanshyam clinic.

3.2.5 Strategies for Screening and Management of IF-SCH

Infertile females having TSH values between 3.5 and 10 µIU/ml with normal fT4 were considered as infertile subclinical hypothyroid female and named as IF-SCH females. Fertile females having TSH values within the normal/ euthyroid range (i.e. 0.35-3.5 µIU/ml) and fT4 levels within the normal range were included as controls in the present study. IF-SCH females/case group are defined as the infertile females who have subclinical hypothyroidism with no other clinical menifestations. In addition, they should not be under any type of medication, including thyroid disorder. And the control group includes fertile, perous, healthy euthyroid females with no medical history of thyroid or any other disorder. Control group does not include any subclinical hypothyroid female. The figure 3.2 shows the strategies for screening and management of the IF-SCH subjects followed for the present study.

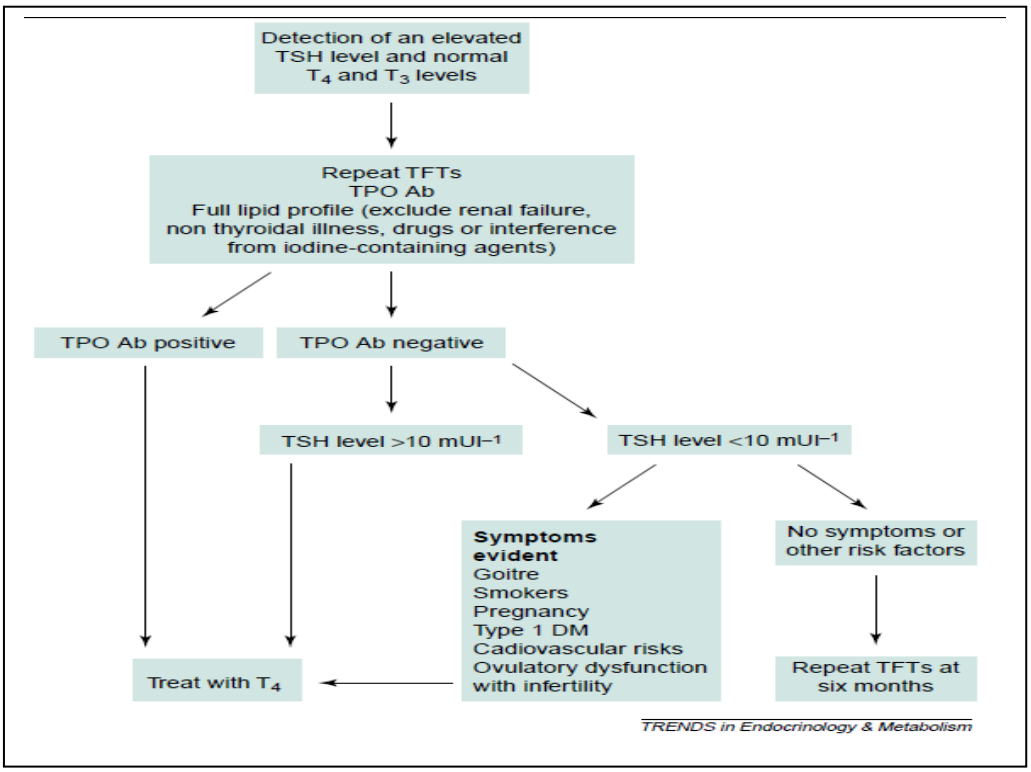

Figure 3.2 Strategies for Screening and Management of IF-SCH

3.2.6 Confounding variables

The confounding variables such as age, body mass index (BMI), smoking and hemoglobin (Hb) levels were tested in control and IF-SCH females. The age was represented compared in years and the female body mass index (BMI) was measured according to the following equation: dividing the weight in kilograms by the height in squared meters (kg/m2) (Flegal *et al*., 2005), figure 3.2. Hemoglobin estimation was done by Drabkin's method (Cook JD *et al*., 1985). Drabkin's reagent (ferricyanide) converts the haemoglobin to cyanmethemoglobin (CMG) and absorbance of CMG is proportional to the haemoglobin concentration. The

protocol was followed as per method. The optical density was measured at 540nm against distilled water.

Table 3.2The parameter of Body mass index (European Society of Human Reproduction and Embryology, 2009)

BMI	**kg/m^2**
Underweight	≤ 18.5
Normal	18.5-24.9
Overweight	25-29.9
Obesity	≥ 30

BMI, Body Mass Index

3.2.7 Anti-TPO Antibody test

Estimation of Anti TPO antibodies was done by kit (Dr. Fenning BioMed GmbH; Germany) based Enzyme Linked Immunosorbant Assay (ELISA) method. Anti TPO antibodies were determined from serum of patients. The serum was diluted 1:101 before use. Recombinant human TPO antigen is coated on to microtiter well plate. During the test performance the anti TPO antibodies of standard as well as samples binds to the immobilized recombinant TPO specifically. These antibodies are further detected using anti-h-IgG peroxidise conjugate. The calibration of the calibrators for the quantative detection is based on WHO reference serum ―1st International Reference Preparation MRC 66/387‖ for TPO –Antibodies. The estimation was done at 450 nm within 30 after completion of assay. TPO values smaller than 70 IU/ml was judged as negative (Cut off = 70 IU/ml).

3.2.8 Sampling method

The sampling method for selecting the participants was purposive (also called convenience method) sampling method as this provides the best information by the members of the selected community.

3.3 Statistical analysis

All the statistical analysis was done by using Prism 5 software (GraphPad Software Inc.; 2007). The tests done were Non-parametric unpaired t-test, Fishers exact test for retrospective data and One-way ANOVA test whichever is applicable. The correlation studies were done by using Pearson correlation coefficients to find out the correlation between TSH and fT_3, fT_4 and Anti-TPO Abs. Pearson's correlation coefficient was calculated to determine the relationship. A two–tailed, at minimum 95% confidence intervals and a p-value <0.05 was considered statistically significant.

3.4 Results

To find out the prevalence rate of SCH in the selected study population, screening of SCH in 135 control and 1000 infertile female volunteers were carried out by estimating TSH levels, as this is the primary and only laboratory test to screen for subclinical hypothyroidism. Out of total 135 controls females enrolled for the present study 110 (82 %) females were euthyroid, 8 (6%) were overt/ clinical hypothyroid (CT-OHT), 7 (5%) females (CT- SCH) were subclinical hypothyroid and 10 (7%) were tested as hyperthyroid (CT-HYPT), figure 3.3A and table 3.2. Whereas out of 1000 infertile females who were participated in the study in which 664 females (66.4%) were euthyroid (IF-ET), 20 (2%) were overt/ clinical hypothyroid (IF-OHT), 270 (27 %) infertile females (IF-SCH) were tested as SCH and 46 (4.6 %) females were showing hyperthyroid (IF-HYPT) status, figure 3.3B,table 3.3. As this primary study mainly aims to find out the prevalence rate of SCH in infertile population of western India, the present study reports a significantly high prevalence rate which is 27 % in infertile females as against 5% in control females. 270 SCH infertile females were then nominated as IF-SCH females (the case subjects) and were selected as the final study population along with 112 euthyroid control females. All the further estimations were done in these 270 IF-SCH and 112 control females for the present study.

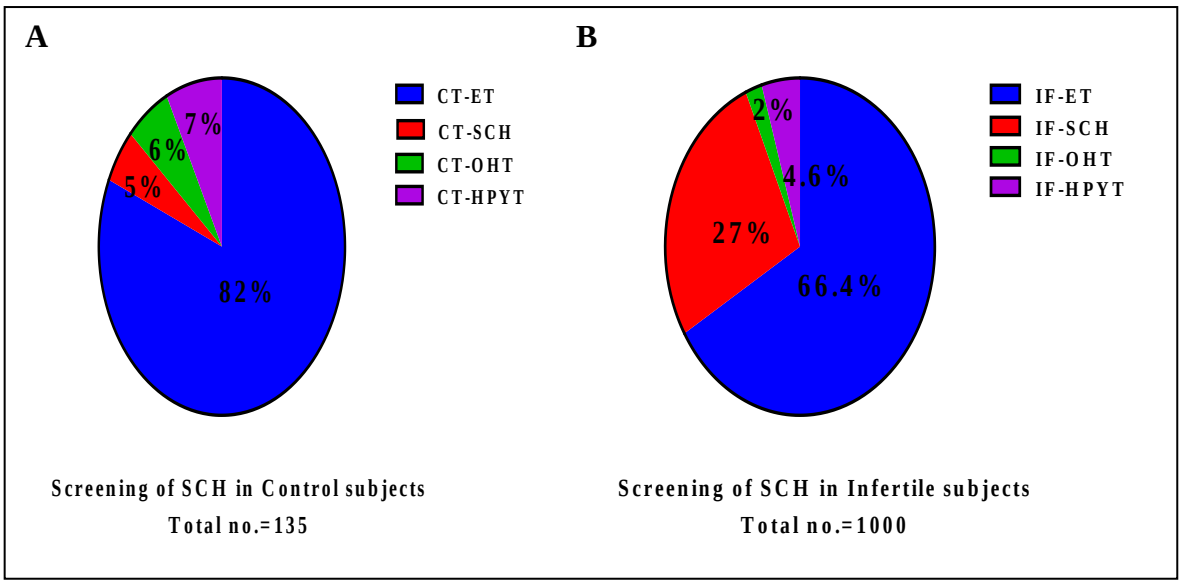

Figure 3.3 Screening of SCH: A. Control females. B. Infertile females. Infertile female subjects show significantly very high prevalence of subclinical hypothyroidism. CT-ET, Control Euthyroid; CT-SCH, Control Subclinical Hypothyroid; CT-OHT Overt / clinical Hypothyroid; CT-HYPT, Control Hyperthyroid; IF-ET, Infertile Euthyroid ; IF-SCH Subclinical Hypothyroid; IF-OHT, Overt / clinical Hypothyroid IF-HYPT, Infertile Hyperthyroid.

Table 3.3 Screening of SCH in study population

Subjects	**Control females** **N (%) (Type)**	**Infertile females** **N (%) (Type)**
Total No.	135 (CT)	1000 (IF)
Euthyroid (ET)	110 (82) (CT-ET)	664 (66.4) (IF-ET)
Subclinical Hypothyroid (SCH)	7 (5) (CT-SCH)	270 (27) (IF-SCH)
Overt / clinical Hypothyroid (OHT)	8 (6) (CT-OHT)	20 (2) (IF-OHT)
Hyperthyroid (HYPT)	10 (7) (CT-HYPT)	46 (4.6) (IF-HYPT)

The demographic details of all the subjects were checked to find out the effects of cofounding variables which includes the age, Body Mass Index (BMI), the levels of Hemoglobin (Hb) and smoking habits in Control and IF-SCH subjects. The age of IF-SCH females were significantly higher with p value= 0.0007 (mean ± SEM: 31.58 ± 0.266) as compared to controls (mean ± SEM: 29.85 ± 0.447), figure 3.4A; table 3.3. Figure 3.4B; table 2.3 show that BMI of the case subjects was also higher with p value = 0.0245 (mean ± SEM: 23.63 ± 0.130) as compared to Control subjects (mean ± SEM: 23.09 ± 0.196). We do not found any difference between IF-SCH subjects (p= 0.394; mean ± SEM: 11.69 ± 0.167) and controls (mean ± SEM: 11.56 ± 0.080) with respect to Hemoglobin (Hb) levels, table 3.4.

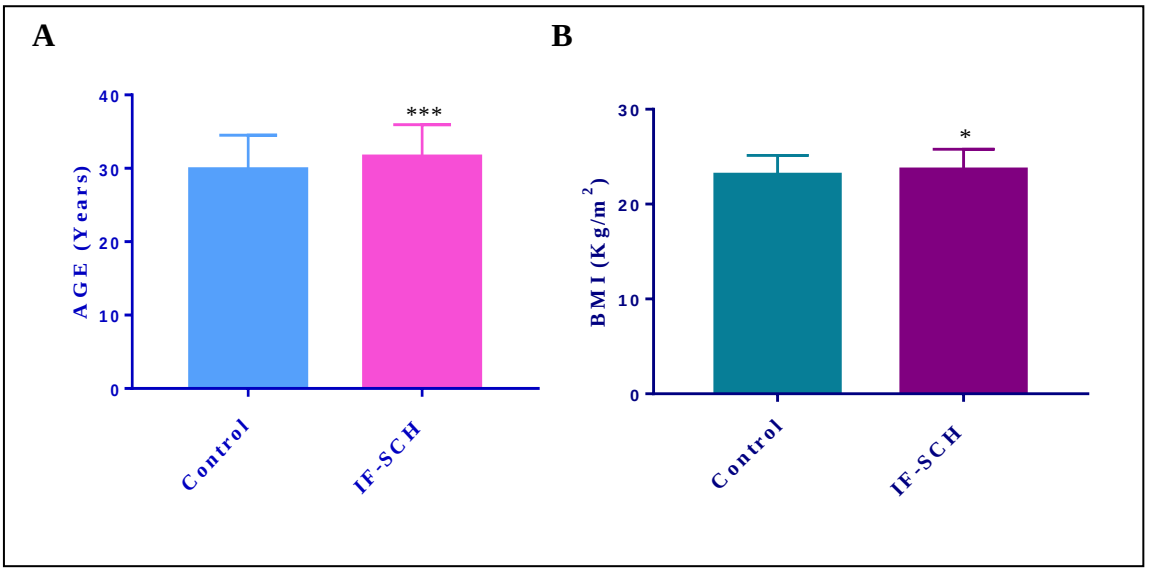

Figure 3.4 Demographic factors (cofounding variables) in Control and IF-SCH females: A. Age. The age of the IF-SCH subjects were significantly high (p=0.001) as compared to the control subjects. **B.** Body Mass Index, BMI**.** The BMI of the IF-SCH subjects were significantly high (p<0.05) as compared to the control group.

The present study reports no difference in age at menarche between the IF-SCH group and the Control group. Smoking habits, alcohol or tobacco addiction in any of the subject of the study population, table 3.3. The residential details with respect to the urban and rural did not report any significant difference in IF-SCH subjects as compared to the Control subjects, while the IF-SCH subjects were having 13% residents near the industrial areas which is significantly high as against only 4% in the control group, table 3.3. When annual family income was compared the control group reported 25% subjects from high income class, 57% from middle class and 18% were falling in low income class, whereas in the IF-SCH group 30% subjects belonged to high income class, 62% from middle class and 8% were from low income class indicating no significant difference in higher and middle class while the IF-SCH subjects where showing significant difference as compared to the control subjects, table 3.3. No difference in literacy levels was reported between the groups. The IF-SCH group reported significantly high number of working women as compared to the control subjects, table 3.4.

Table 3.4 Demographic details (cofounding variables)

Study subjects	**Control subjects**	**IF-SCH subjects**	**P value (p value summary)**
Total Nos.	110	270	-
Age (years)	29.85 ± 0.45	31.58 ± 0.27	0.0007 (***)
BMI (kg/m$^{2)}$	23.09 ± 0.20	23.63 ± 0.13	0.0245 (*)
Hb (gm/dl)	11.69 ± 0.17	11.56 ± 0.08	Ns
Age atmenarche(Yrs)	13.1 ± 0.12	13.3 ± 0.17	Ns
Smokers (%)	0	0	-
Alcohol addiction	0	0	-
Tobacco addiction	0	0	-
Urban resident	76%	70%	-
Rural resident	20%	17%	-
Industrial resident	4%	13%	S
High income class	25%	30%	-
Middle income class	57%	62%	-
Low income class	18%	8%	S
Literate/ Illiterate	90%/ 10%	92%/ 8%	Ns
Working/ Non working	13%/ 87%	25%/ 75%	S

The IF-SCH group subjects were divided into two subgroups group A and B to on the basis of the TSH levels to find out the more prevailing sub range for the subclinical hypothyroidism in the selected population. The data revealed that the group A subjects with the TSH levels 3.5-7 µIU/ml was representing significantly very higher prevalence with 221 out of total 270 subjects at 82% as compared the group B with 49 out of 270 IF-SCH subjects at 18% with TSH levels 7.1-10 µIU/ml, figure 3.5; table 3.6.

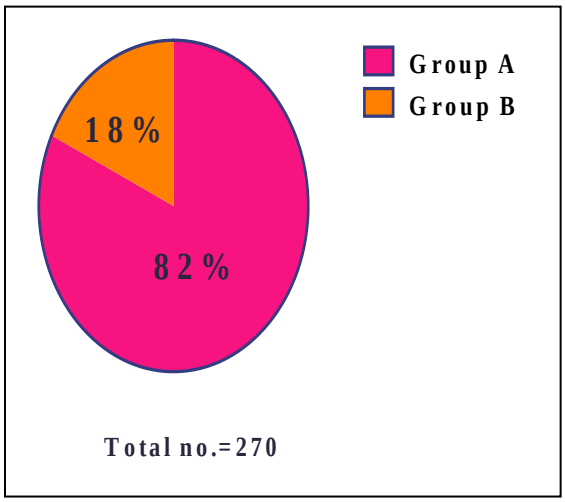

Figure 3.5 IF-SCH subjects groups: The IF-SCH subjects in group A were having significantly higher prevalence as compared to group B.

Table 3.5 IF-SCH subjects groups

Sr. no.	TSH levels (µIU/ml)	Prevalence n (%)
Group –A	3.5-7	221(82)
Group-B	7.1-10	49 (18)

The IF-SCH subjects were further divided into four age groups to compare the prevalence of infertility between different age groups. The group I, II, III and IV were consisting of the IF-SCH females of 20-25, 26-30, 31-35 and 36-40 years of age respectively. We found that the group III with IF-SCH females of 31-35 years of age group with the highest infertility prevalence with 39% followed by group II with 31% and group IV at 21%. The IF-SCH females with the age group of 20-25 years of age with 9% were reported the lowest infertility prevalence amongst the all four groups, figure 3.6; table 3.6.

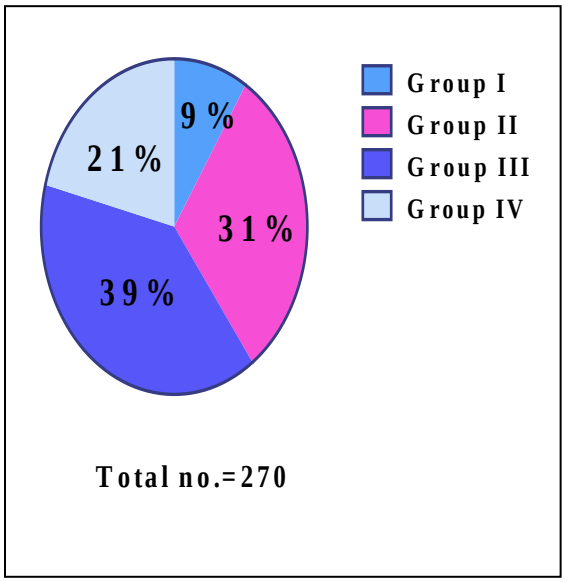

Figure 3.6 Prevalence of infertility in different age groups of IF-SCH subjects: The prevalence of infertility was significantly high in the age group of 31-35 Yrs.

Table 3.6 Prevalence of infertility in different age groups of IF-SCH subjects

Group No.	Age group (Yrs)	Infertility prevalence N (%)
Group-I	20-25	24 (9)
Group-II	26-30	84 (31)
Group-III	31-35	106 (39)
Group-IV	36-40	56 (21)

Further to find out the duration of infertility years of IF-SCH subjects the group was divided into four subgroups, group 1, 2, 3 and 4. The data revealed that the infertility duration was highest in group 2 with 5-7 yrs of duration showing 42% followed by group 3 having 8-10 yrs at 26%, group 1 at 20% and group 4 at 12% showing 11-13 yrs of infertility duration, figure 3.7; table 3.7.

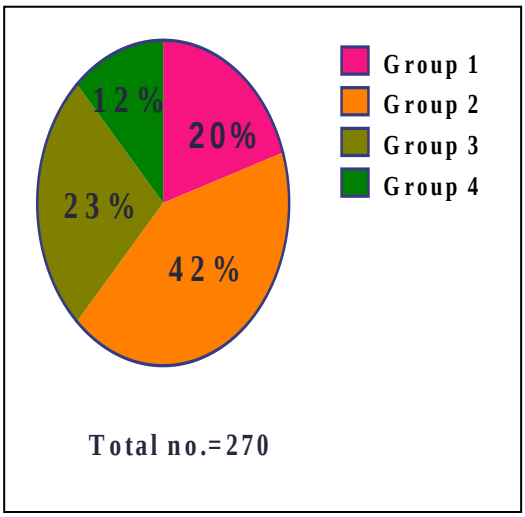

Figure 3.7 Duration of infertility in years of IF-SCH subjects: The group 2 with 5-7 yrs of infertility duration was showing the highest

Table 3.7 Duration of infertility in years of IF-SCH subjects

Sr no.	Duration of infertility(Yrs)	Total cases (%)
Group1	2-4	55 (20)
Group2	5-7	112 (42)
Group3	8-10	70 (26)
Group4	11-13	33 (12)

The correlation study analysis was done to find out the correlation of TSH with various cofounders. The correlation study of TSH with age of the control (Person r = -0.033 and p=0.734) and IF-SCH subjects (Pearson r = -0.068 and p= 0.268) showed no significant correlation, figure 3.8; table 3.8.

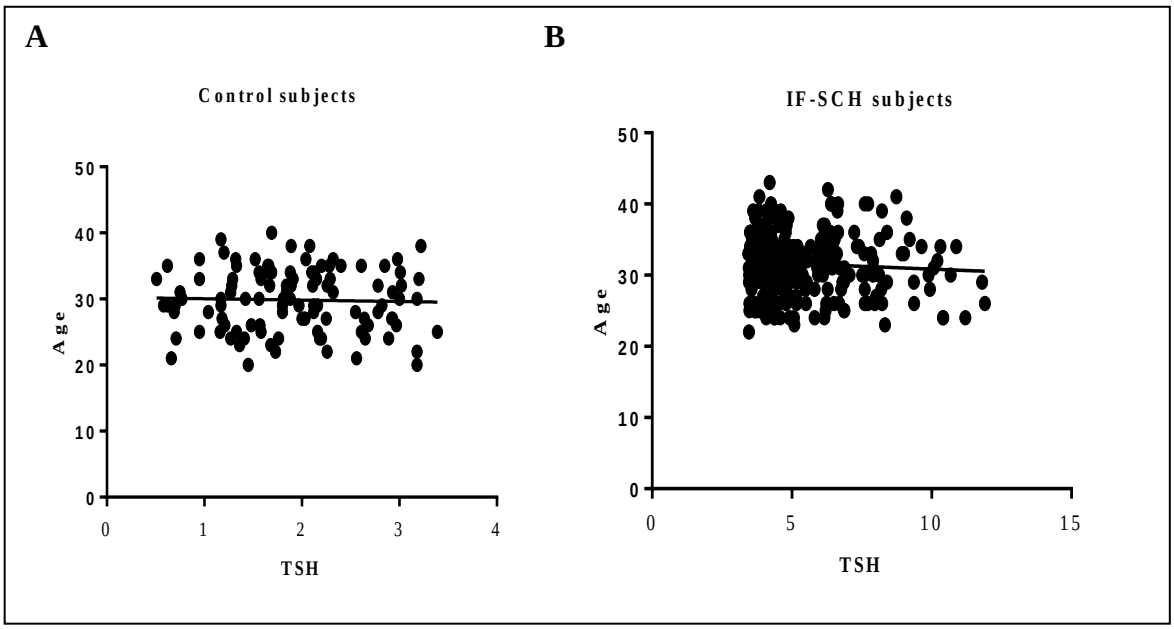

Figure 3.8 Pattern of correlation between TSH and Age in Control and IF-SCH subjects: The control (Person r = -0.033, p=0.734) and IF-SCH subjects (Pearson r = -0.068, p= 0.268) reported no correlation between TSH and age levels.

Table 3.8 Correlation between TSH and Age

	TSH with Age	
	Control subjects	**IF-SCH subjects**
No. of XY pairs	110	270
Pearson r	-0.03	-0.07
95% Confidence	-0.22 to 0.16	-0.19 to 0.05
P value	0.73	0.27
Significance of correlation	Ns	Ns

Also TSH and BMI correlation analysis for control (Pearson r = 0.007 and p= 0.734) and IF-SCH subjects (Pearson r = -0.068 and p= 0.268) showed no significant correlation, figure 3.9; table 3.8.

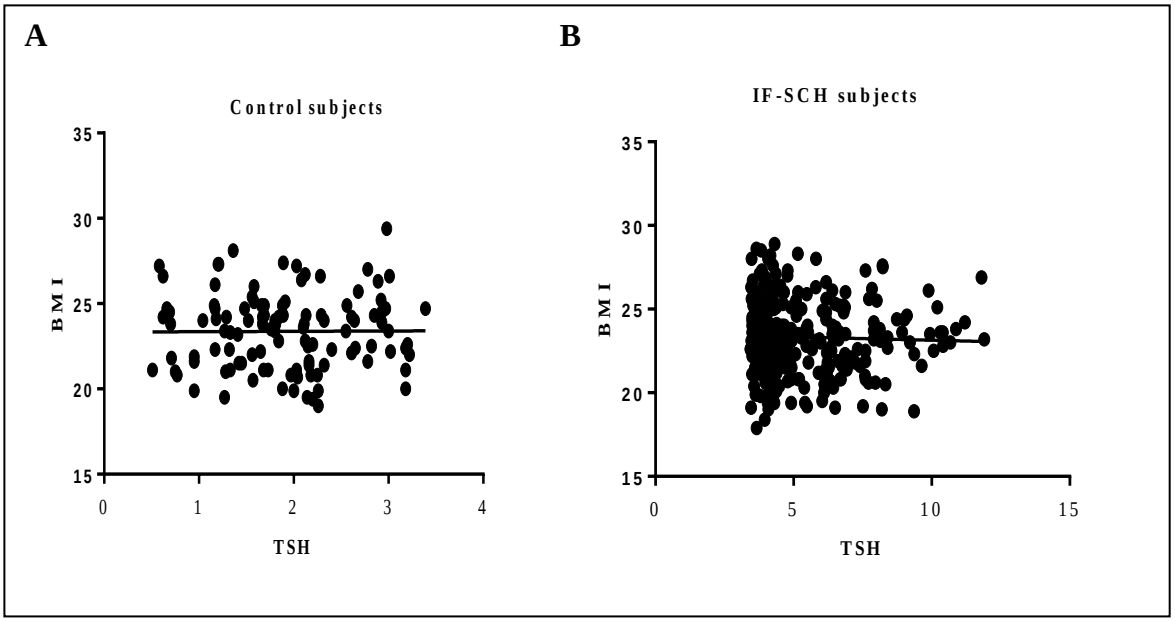

Figure 3.9 Pattern of correlation between TSH and BMI in Control and IF-SCH subjects: The control (Pearson r = 0.007, p= 0.734) and as IF-SCH subjects (Pearson r = -0.068, p= 0.268) reported no correlation between TSH and BMI levels.

Table 3.9 Correlation between TSH with BMI

	TSH with BMI	
	Control subjects	**IF-SCH subjects**
No. of XY pairs	110	270
Pearson r	0.001	-0.03
95% Confidence	-0.18 to 0.19	-0.15 to 0.09
P value	0.95	0.59
Significance of correlation	Ns	Ns

TSH and Hb correlation study in control (Pearson r=-0.006 and p=0.799) and IF-SCH subjects (Pearson r= 0.041 and p= 0.504) also could not report any significant correlation, figure 3.10; table 3.9.

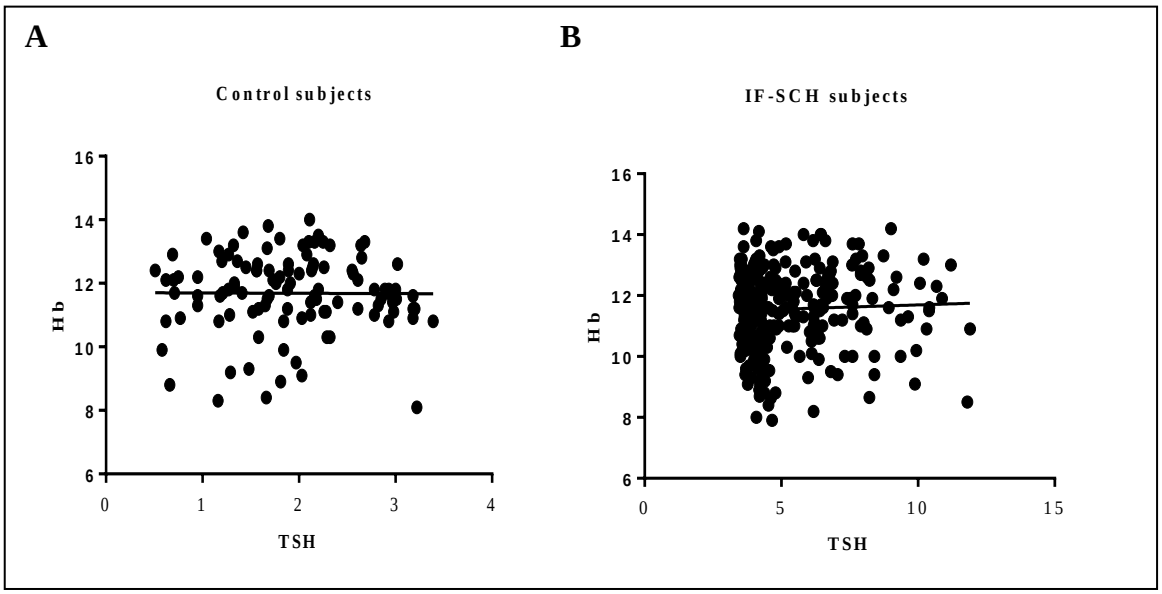

Figure 3.10 Pattern of correlation between TSH and Hb in Control and IF-SCH subjects: The control (Pearson r=-0.006, p=0.799) and as IF-SCH subjects (Pearson r= 0.041, p= 0.504) reported no correlation between TSH and Hb levels.

Table 3.10 Correlation between TSH and Hb

	TSH with Hb	
	Control subjects	**IF-SCH subjects**
No. of XY pairs	110	270
Pearson r	-0.006	0.041
95% Confidence	-0.193 to 0.182	-0.079 to 0.159
P value	0.799	0.504
Significance of correlation	Ns	Ns

Analysis of TSH, fT3 and fT4 levels in the studied subjects revealed that IF-SCH subjects had significantly very high ($p<0.0001$; figure 3.11) TSH levels (mean ± SEM: 5.364 ± 0.115μIU/ml; table 3.10) compared to the control females (mean ± SEM: 1.908 ± 0.068 μIU/ml) and they had no significant difference in fT_3 levels (p= 0.5532, mean ± SEM: 2.7± 0.025 pg/ml; fig.3.11) compared to the controls (mean ± SEM: 2.8 ± 0.065 pg/ml). There was no significant difference in fT_4 levels (p=0.2227, mean ± SEM: 1.22 ± 0.0249; figure 3.11, table 3.10) between IF-SCH and control subjects (mean ± SEM: 1.195±0.0318 ng/dl). The prevalence rate of Anti-TPO Antibodies was also reported to be significantly high at 20% in IF-SCH as against 9% control subjects, figure 3.12; table 3.10.

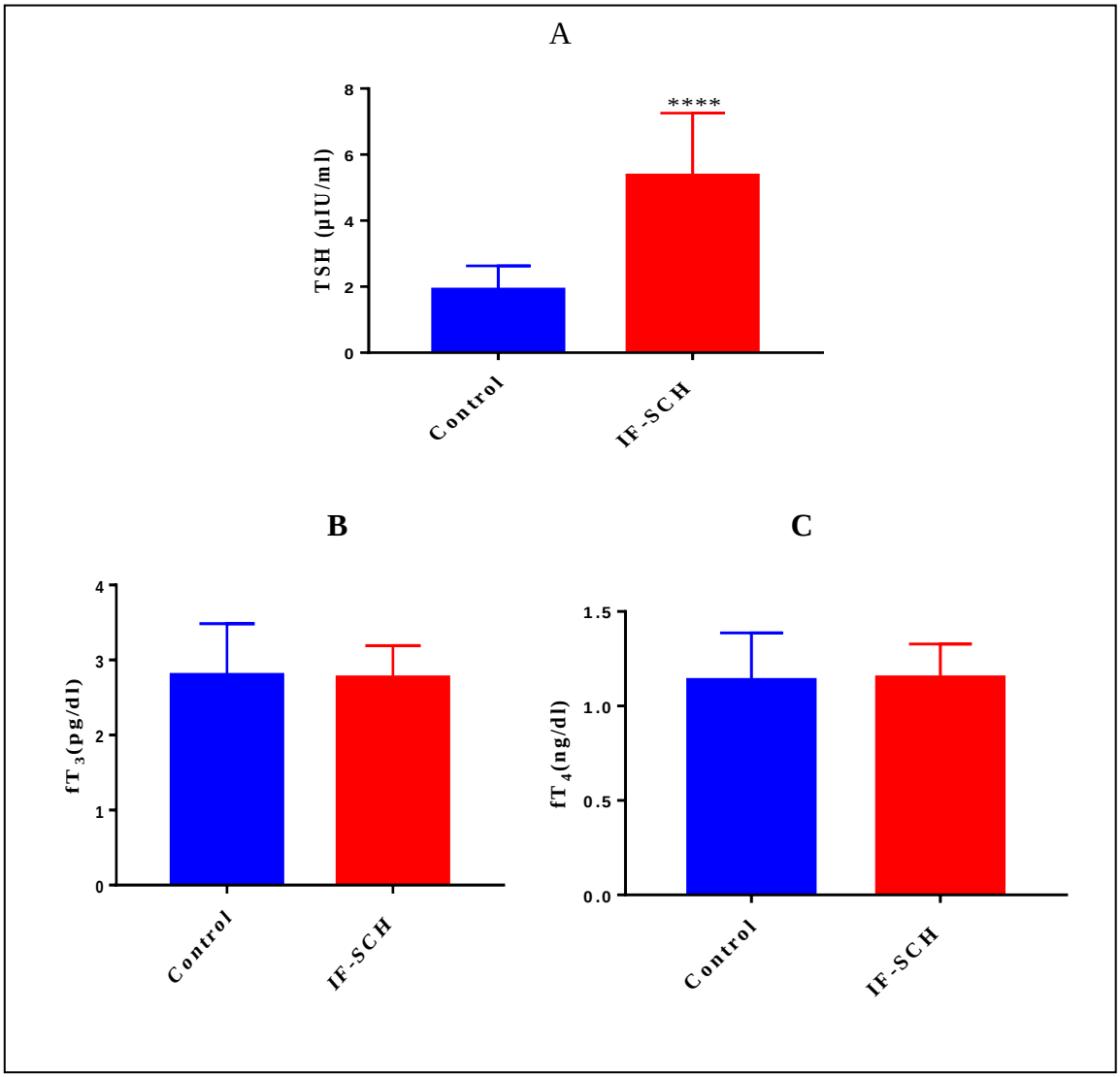

Figure 3.11 TSH levels in the study population: A. TSH levels in Control and IF-SCH subjects. The IF-SCH group subjects were showing significantly very high ($p<0.0001$) TSH levels as compared to the control group subjects. **B. fT_3 levels in Control and IF-SCH subjects:** The Control and the IF-SCH group reported no significant difference in fT_3 levels. **C. fT_4 levels in Control and IF-SCH subjects.** No significant difference was reported in fT_4 between the Control and IF-SCH subjects.

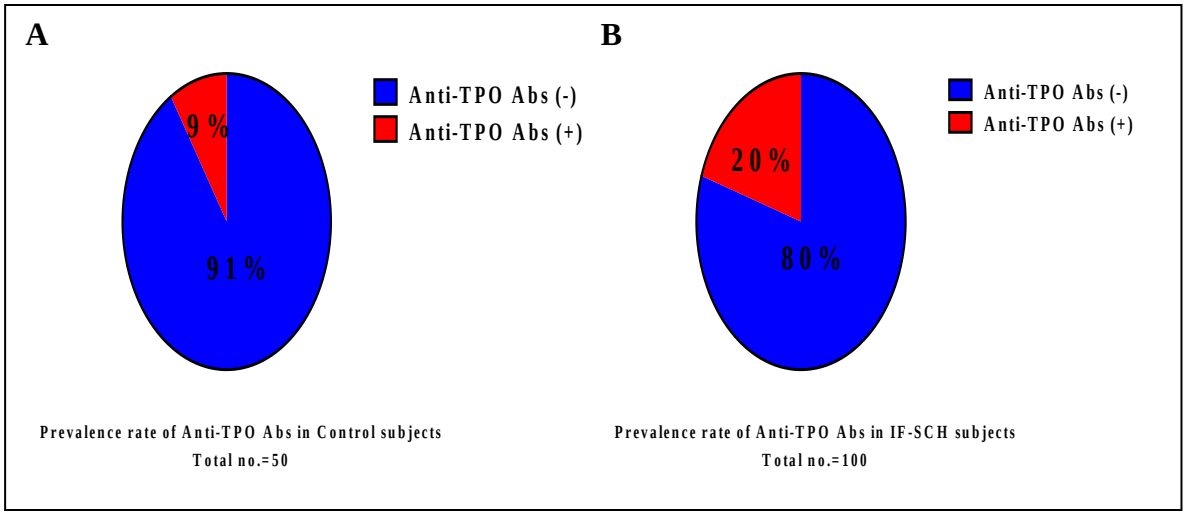

Figure 3.12 Prevalence rate of AITD (Anti-TPO antibodies): A. Control, B. IF-SCH subjects: The IF-SCH group reported the higher prevalence of AITD with 20% females having presence positive anti TPO antibodies as compared to the Control subjects at 9%.

Table 3.11 TSH, fT_3 and fT_4 levels and Anti-TPO prevalence in the study population

	Reference Value	**Control subjects**	**IF-SCH subjects**	**p value (p value summary)**
TSH (µIU/ml)	0.35-4.00	1.908 ± 0.0680 (n=100)	5.364 ± 0.115 (n=270)	<0.0001 (****)
fT_3(pg/dl)	0.9-.1.76	2.8 ± 0.065 (n=100)	2.7± 0.025 (n=270)	0.553 (ns)
fT_4(ng/dl)	2.3-4.2	1.14 ± 0.024 (n=100)	1.15 ± 0.010 (n=270)	0.545 (ns)
Anti-TPO Abs prevalence	0-9 IU/ml	9 % (n=50)	20 % (n=100)	0.0432 (*)

Data presented as Mean ± SEM

On comparing TSH levels in controls (mean ± SEM; 1.908 ± 0.068) and IF-ET (mean ± SEM; 1.888 ± 0.041) subjects we found no significant difference (p=0.8483) figure 3.13A; table 3.11. While the levels of TSH in IF-ET subjects (mean ± SEM; 1.888 ± 0.041) and IF-SCH (p=<0.0001, mean ± SEM; 5.364 ± 0.115) showed significant difference as shown in figure 3.13B; table 3.11. The IF-OHT subjects were having very high TSH levels (p= <0.0001; mean ± SEM; 31.53 ± 4.345) as compared to IF-SCH (mean ± SEM; 5.364 ± 0.115) figure 3.13C; table 3.11. Figure 2.13D depicts the comparative TSH levels of the Control, IF-SCH and IF-ET group subjects which reported a significantly very high TSH levels in IF-SCH subjects as compared to the Control and IF-ET subjects.

The prevalence rate analysis of Anti-TPO Abs in the study population revealed 9%, 11% and 20% rate in control, IF-ET and IF-SCH subjects respectively figure 3.14, table 3.11. The difference in percentage of IF-ET and control was not significant (p= 0.814) and the trend was also same for the IF-ET and IF-SCH though nearly significant with p=0.117, The IF-OHT subjects reported with the highest prevalence of anti TPO-Abs; figure 3.14, table 3.11

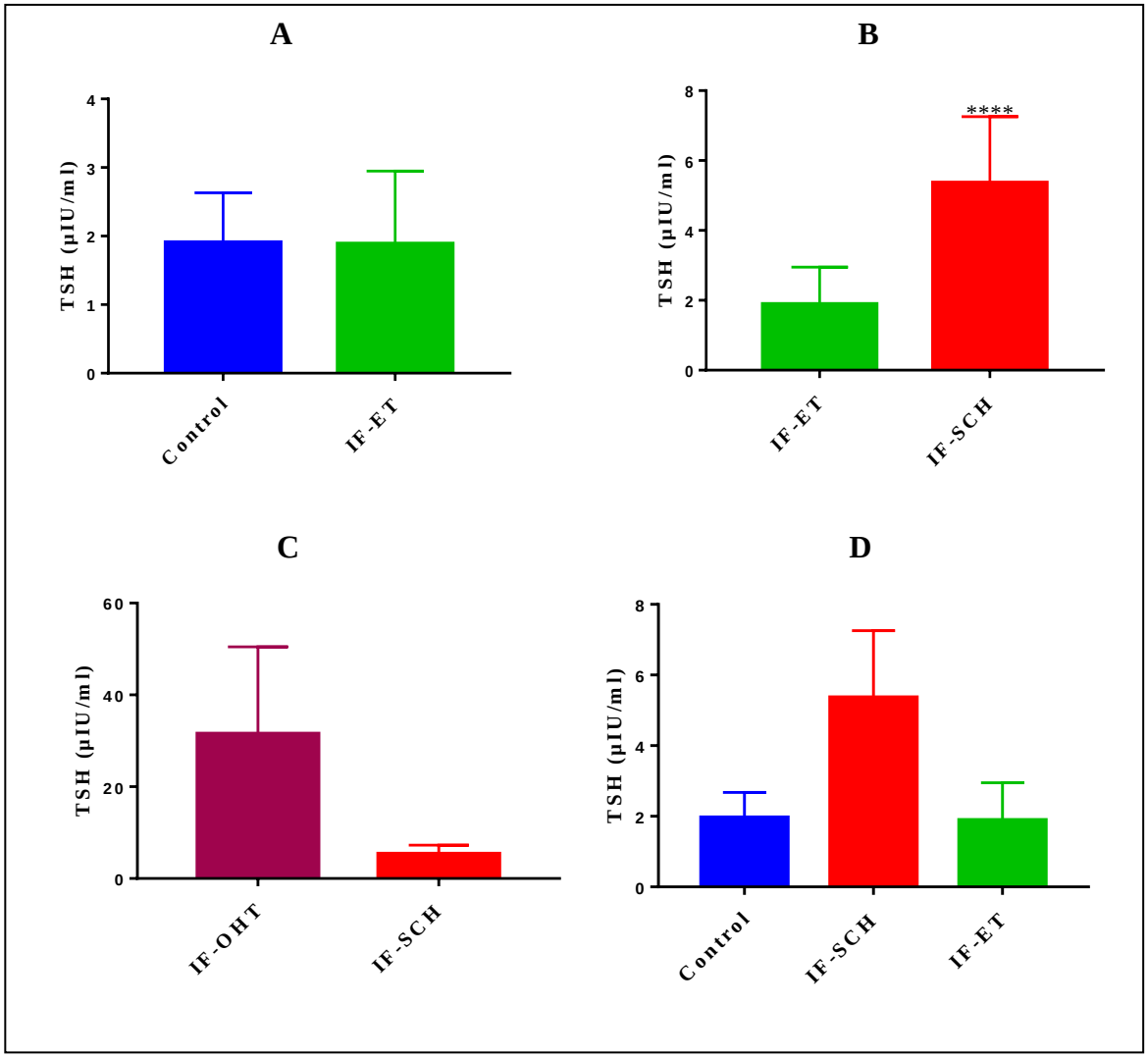

Figure 3.13 TSH levels: A. TSH levels in Control and IF-ET subjects. No significant difference in TSH levels between the groups. **B. TSH levels in IF-ET and IF-SCH subjects.** The TSH levels were significantly high ($p<0.0001$) in IF-SCH subjects as compared to the IF-ET subjects. **C.TSH levels in IF-SCH and IF-OHT subjects.** The IF-OHT group subjects reported significantly very high TSH levels as compared to the IF-SCH subjects. **D.TSH levels in Control, IF-SCH and IF-ET.** The IF-SCH subjects were reported significantly very high TSH levels as compared to the Control and IF-ET subjects.

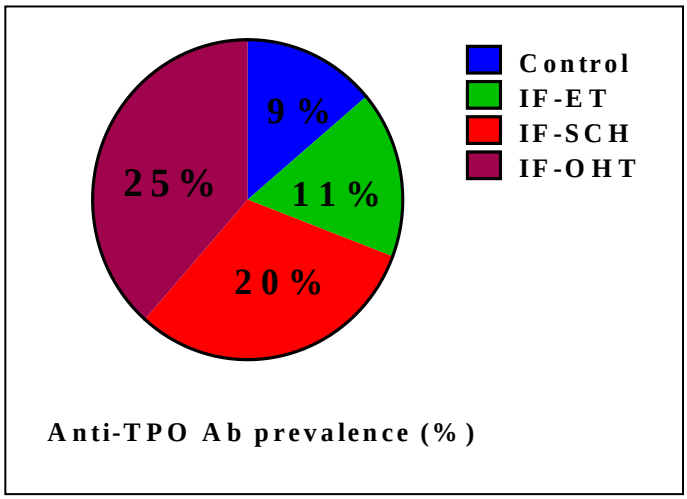

Figure3.14 Anti-TPO Abs prevalence in Control, IF-SCH and IF-ET subjects: Amongst the Control, IF-ET and IF-SCH group, the IF-SCH subjects group was having the highest Anti-TPO Abs prevalence.

Table 3.12 TSH levels and Anti- TPO Abs prevalence

Study subjects	TSH (µIU/ml)	Anti-TPO Ab prevalence	P value (p value summary)
Control subjects	1.91 ± 0.07 (n=100)	9 % (n=50)	-
IF-ET subjects	1.89 ± 0.04 (n=664)	11% (n=100)	-
IF-SCH subjects	5.36 ± 0.12 (n=270)	20% (n=200)	-
IF-OHT subjects	31.53 ± 4.35 (n=20)	-	-
Control vs IF-ET subjects	-	-	0.8483 (ns)
IF-ET vs IF-SCH subjects	-	-	<0.0001 (****)
IF-OHT vs IF-SCH subjects	-	-	<0.0001 (****)

Data presented as Mean ± SEM

Correlation analysis of TSH with fT_3, fT_4 and Anti-TPO Abs was carried out to find out the correlation of TSH with thyroid hormones and anti-TPO Abs. The study revealed that there is no significant correlation of TSH with fT_3 in control (Pearson r =0.116 and p=0.229) as well as in IF-SCH subjects (Pearson r=0.036 and p= 0.561), figure 3.15; table 3.12.

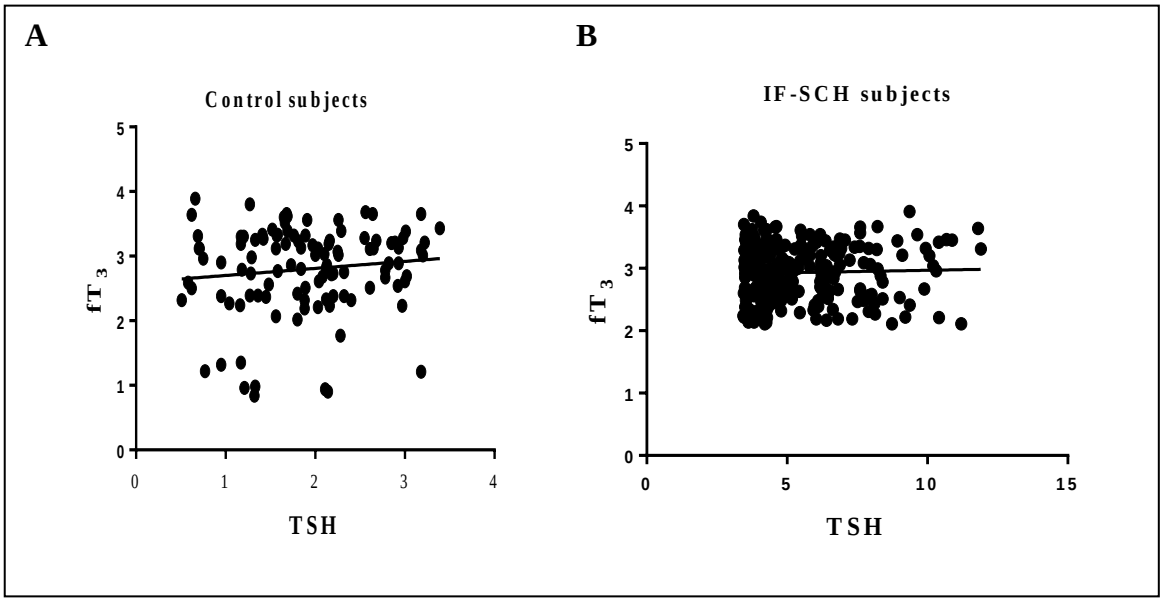

Figure 3.15 Pattern of Correlation between TSH and fT_3 levels: A. Control subjects (Pearson r =0.116, p=0.229) **B. IF-SCH subjects** Pearson (r=0.036, p= 0.561)**.** No significant correlation was found between the TSH and fT_3 levels.

TSH and fT_4 correlation analysis in control (Pearson r= -0.053 and p=0.582) and IF-SCH subjects (Pearson r=0.0168 and p= 0.784) as shown in figure 2.16; table 2.12, resulted in no significant correlation.

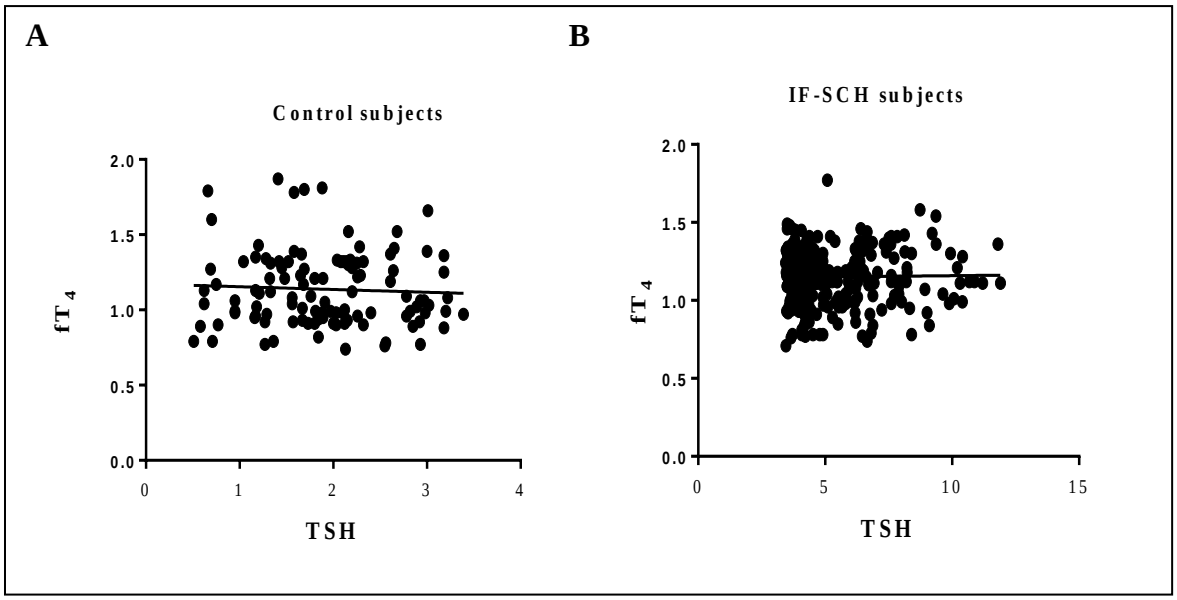

Figure 3.16 Pattern of Correlation between TSH and fT_4 levels: A. Control subjects (Pearson r= -0.053 and p=0.582)**. B. IF-SCH subjects** (Pearson r=0.0168 and p= 0.784)**.** No significant correlation was found between TSH and fT_4 levels.

On analyzing the correlation of Anti-TPO Abs with TSH no significant correlation between Control (Pearson r= -0.215 and 0.579), IF-SCH subjects (Pearson r= 0.012 and p=0.931) and IF-ET (Pearson r= -0.171and p=0.616) subjects, figure 3.17A and C, table 3.13.

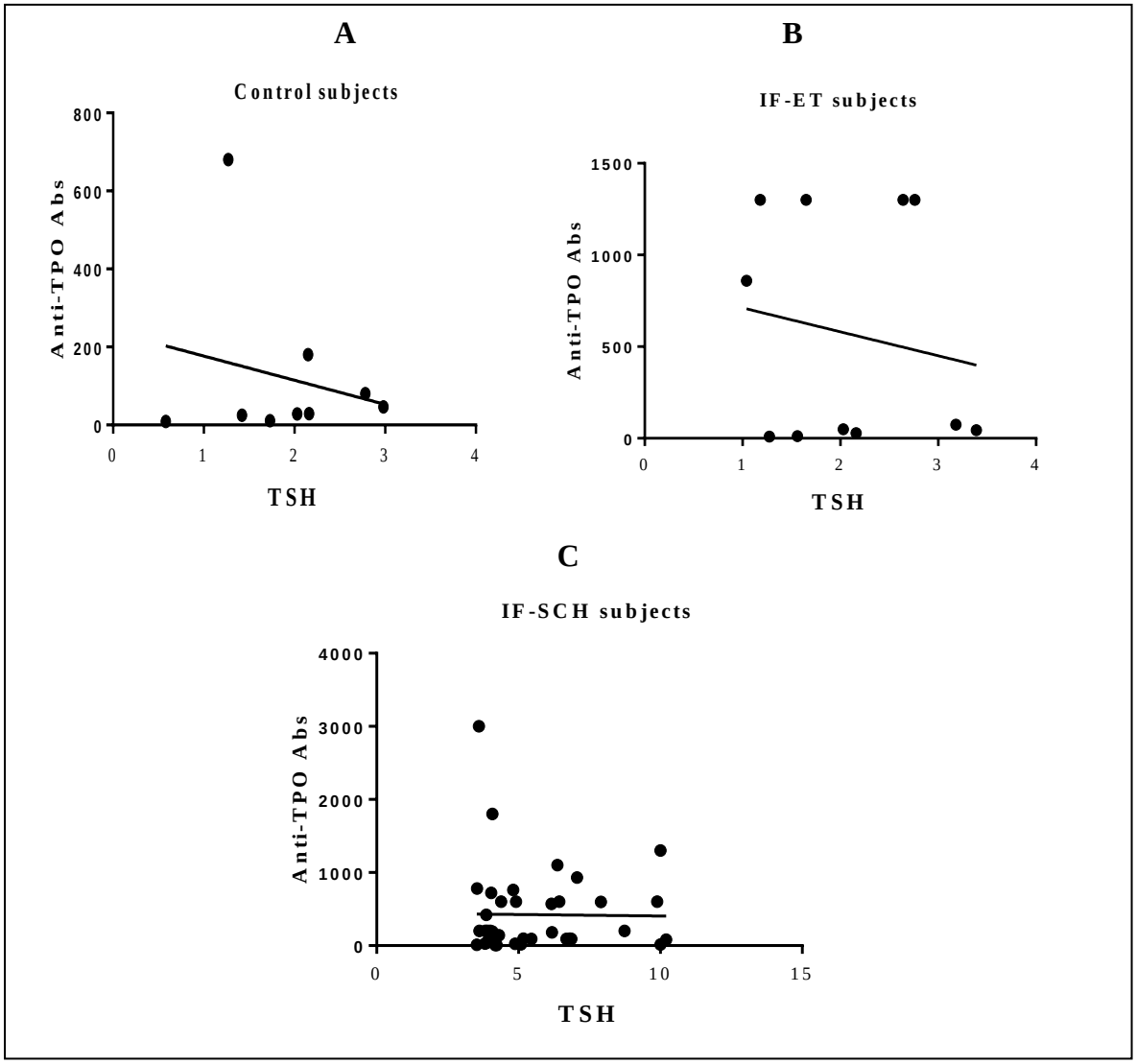

Figure 3.17 Pattern of Correlation between TSH levels and positive Anti-TPO Abs: A. Control subjects. B. IF-ET subjects. C. IF-SCH subjects. No significant correlation between TSH levels and Anti-TPO Abs was reported for Control (Pearson r= -0.215, 0.579), IF-ET (Pearson r= -0.171, p=0.616)and IF-SCH subjects (Pearson r= 0.012, p=0.931).

Table 3.13 Correlation of TSH with fT_3 and fT_4 in the study population

	TSH with fT_3		**TSH with fT_4**	
	Control Subjects	**IF-SCH Subjects**	**Control Subjects**	**IF-SCH Subjects**
No. of XY pairs	110	270	110	270
Pearson r	0.116	0.036	-0.053	0.0168
95% confidence	-0.073 to 0.296	-0.084 to 0.154	-0.238 to 0.136	-0.103 to 0.136
P value	0.229	0.561	0.582	0.784
Significance of correlation	Ns	Ns	Ns	Ns

Table 3.14 Correlation between TSH and anti-TPO Abs

	TSH with Anti-TPO Abs		
	Control subjects	**IF- ET subjects**	**IF- SCH Subjects**
No. of XY pairs	9	11	40
Pearson r	-0.215	-0.171	-0.0142
95% confidence	-0.769 to 0.524	-0.699 to 0.478	-0.324 to 0.299
P value	0.579	0.616	0.931
Significance of correlation	Ns	Ns	Ns

3.5 Discussion

The present study mainly aims to find out the prevalence of thyroid disorders in infertile female population and reports the higher prevalence of hypothyroidism with 29% as compared to hyperthyroidism with 5% in Control females. And further amongst the infertile hypothyroid women the prevalence of subclinical hypothyroidism was significantly very high with 27 % as compared to overt/ clinical hypothyroidism with 2% in infertile female population of western India. Subclinical hypothyroid infertile female subjects had significantly very high Thyroid Stimulating Hormone (TSH) levels with no difference in thyroid hormones fT_3 and fT_4 levels as compared to the healthy control female subjects. As high as 82% of the subclinical hypothyroid subjects were falling within the sub-range of TSH levels 3.5-7 µIU/ml and rest 18% infertile females had TSH levels 7.1-10 µIU/ml. We found no significant difference in TSH levels in controls and euthyroid infertile females. While the levels of TSH in euthyroid infertile females and infertile females with subclinical hypothyroidism, IF-SCH subjects reported significantly very high TSH levels in the females with subclinical hypothyroidism. The infertile subjects with overt/ clinical hypothyroidism reported very high TSH levels as compared infertile females of euthyroid and subclinical hypothyroid group. Correlation analysis revealed no significant correlation of TSH levels with the levels of thyroid hormones fT_3 and fT_4 in the study subjects. The prevalence of Anti-TPO Antibodies was also reported to be significantly high with 20% in IF-SCH females and 25% in infertile females with overt/ clinical hypothyroidism, while TSH level with Anti-TPO Abs positivity correlation analysis was not found to be significant. The study on demographic details and effects of cofounding variables revealed that the ages and BMI of IF-SCH females with were significantly higher, but no difference in the age at menarche, Hemoglobin (Hb) levels as compared to the control females. No infertile female subject with smoking habits, alcohol or tobacco addiction was reported. The residential details with respect to the urban

and rural did not report any significant difference, while the IF-SCH subjects were having 13% residents near the industrial areas, which is significantly high. When annual family income was compared, no significant difference in higher and middle class while the IF-SCH females were showing significant less numbers in low income class and high numbers in high and middle class groups. No difference in literacy levels was reported between the groups. The subclinical hypothyroid infertile group reported significantly high number of working women as compared to the control subjects. On comparing the prevalence of infertility between different age groups, we found that the IF-SCH females of 31-35 years of age group with the highest infertility prevalence with 39% followed by the age group of 25-30 yrs with 31%. The data on the duration of infertility years revealed that the infertility duration was highest in the group with 5-7 yrs of duration showing 42% followed by 8-10 yrs at 26%, the 2-4 yrs at 20% and at 12% showing 11-13 yrs of infertility duration. Further, the correlation study analysis was done to find out the correlation of TSH levels with various confounders and the data reported that the TSH levels with age, BMI and Hb levels as well as with all the other confounders reported no correlation with TSH levels.

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 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). World TFR was estimated to be near to the replacement level of fertility in the year 2020 (Kaneda *et al.*; 2020) and is expected to further fall by the year 2050 for many countries including India (United Nations World Fertility prospects, 2008; niti.gov.in.Total fertility rate 2000-2016; Purkayastha *et a..*, 2021). The prevalence of female infertility has shown a remarkable increase during the most recent decade in India with western region showing a high increase in the primary infertility rate (Ganguly *et al.*, 2010; SRS, 2013).

In developing countries, one among four couples suffers from infertility and in these couples, thyroid diseases are among one of the key perpetrators. 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 HPO axis and dysfunctions of the thyroid may lead to infertility (Poppe et al., 2007). Infertility could also be due to alterations in the HPT axis. The most common endocrine disorder that causes infertility in reproductive age women is hypothyroidism. Clinical and experimental studies have suggested a close inter relationship between the HPT axis and the HPO axis. In normal/ euthyroid thyroid condition feedback mechanism regulates and maintains the normal thyroid hormone levels in the circulation. 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. 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 may hinder female fertility. SCH is found to be more prevalent than that of overt hypothyroidism affecting women in reproductive age (Krassas et al., 2000; Poppe et al., 2008). Primary health caregivers most often pick up overt hypothyroidism easily; however, SCH with its subtle symptoms most often goes unnoticed. SCH is now being challenged and studies have discussed the importance of screening for SCH. 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 reported that 34% had SCH with frequent abortions observed in the infertile females with subclinical hypothyroidism. In a case–controlled study, Poppe *et al.* (2002) reported a high prevalence of SCH in women with primary infertility. 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. In some of the older studies a study by Bohnet *et al.* (1981) SCH was considered as an infertility factor by itself and 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 decreased rate of pregnancy. In the study by Shalev *et al.* (1994) the prevalence of SCH was reported in infertile women with ovulatory dysfunction. Nevertheless the prevalence of SCH amongst infertile females is common; there is a scarcity on available data. Prevalence of subclinical hypothyroid in developed world is 4-15% (Armada et al., 2001). There are a few studies reporting the prevalence of hypothyroidism, ranging from 15-25% in Indian population in different parts of the country. 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 and the country 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). Among all cities, Kolkata recorded the highest prevalence (21.67%) while others showed comparable rates ranging from 8.88% (Hyderabad) to 11.07% (Delhi). 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 (50.5%) was more common than overt hypothyroidism in a study done 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 that in 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. The present study reports the higher prevalence of hypothyroidism with 29% as compared to hyperthyroidism with 5% in primarily infertile females. And further amongst the infertile hypothyroid women the prevalence of subclinical hypothyroidism was significantly very high with 27 % as compared

to overt/ clinical hypothyroidism with 2% and further 82% of the females reported TSH levels in the sub-range of SCH with 3.5-7 µIU/ml in subclinical hypothyroid infertile female population of Gujarat region.

SCH has been a challenging task 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 (Abalovich *et al.*, 2002; Andersen *et al.*, 2002; Baloch *et al.*, 2003). While some authors have proposed restricting the upper normality limit of serum TSH to 2·5 5µIU/ml, 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. It has been found that miscarriage rates are higher among women with SCH and a TSH level > 4 µIU/ml, but the association is less clear in women with TSH levels between 2.5 µIU/ml and 4 µIU/ml. Likewise, placental abruption, preterm delivery, and premature rupture of membranes are more common among women with SCH and TSH levels > 4 µIU/ml. Since there is disagreement regarding TSH limits, the available research on subclinical hypothyroidism and infertility is hard to evaluate. This is because TSH limits vary widely across studies and include only a small number of patients, making it improper to act on any reported conclusions. However, a study by Rao et al (2020), compared subjects with subclinical hypothyroidism and with normal thyroid function and reported that the median TSH level for the women with subclinical hypothyroidism was 5.13 µIU/ml, with 50% of the values falling between 3.56 and 6.70 µIU/ml.

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 on the prevalence of SCH in infertile females. The present study mainly focuses on the role of thyroid in female infertility with main focus on subclinical hypothyroidism subsequent to female infertility in the western population of India. To obtain a more precise TSH levels in the selected population, SCH was further divided into two sub-range groups the group “A” subjects with TSH levels above the upper limit of normal which is 3.5-7 µIU/ml and group “B” subjects were those with TSH levels between 7.1-10 µIU/ml. The present study reports a significantly high prevalence of SCH with TSH levels in the range of 3.5-10 µIU/ml in infertile females of Gujarat region. If we consider 2.5µIU/ml as the upper limit of TSH levels in infertile females of the present study; the prevalence rate of SCH will be than increased up to the double i.e. around 50%, but as 0.3-3.5 µIU/ml is the most accepted upper limit of TSH levels in the infertile females; the

present study has also taken this value as the reference range for finding out the prevalence which we report at the rate of 27% with mean TSH levels 5.36 µIU/ml and 82% of subjects falling in the sub-range of 3.5-7 µIU/ml in infertile females of the western part of India, which to our knowledge is the first study reporting the prevalence for the western part, specifically for the population of Gujarat region.

Infertility is a fall out of multiple factors hence proper evaluation of this disorder should involve a multipronged approach. The association of female infertility with various anthropometric parameters as well as different socioeconomic conditions is being documented since long. Amongst the various risk factors for female infertility the female age is the most important (Talwar *et al.*, 1997; Mokhtar *et al.*, 2006; Deshpande *et al.*, 2019). Fertility declines with age in both men and women, but the effects of age are much greater in women. Infertility in females has been associated with age and the aged women experience a considerable decline in the fertility rate with time span (Menken et al., 1986; Kumar et al., 2007). In their 30s, women are about half as fertile as they are in their early twenties and women's chance of conception declines significantly after age 35 (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. Apart from these, conditions 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; Rich-Edwards et al., 2002) and stress (Soltani *et al.*, 2014), are also 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). In the present study the during the demographic details and effects of various confounders associated with female infertility on TSH levels, we found that the ages of IF-SCH subjects were significantly higher as compared to the Control females. Infertile subjects with subclinical hypothyroidism were reporting a significant increased in ages with mean age of 31.6 yrs. Further on evaluating the difference in age at menarche, the data revealed no difference in the age at menarche between the Control and infertile subjects. The basal metabolic rate, BMI of IF-SCH was significantly

higher with mean BMI value of 23.63 kg/m^2. Hemoglobin (Hb) levels of IF-SCH show no difference as compared to the Control females. No Control or IF-SCH subject with smoking habits, alcohol or tobacco addiction was reported. Further with respect to the residential details IF-SCH females were more from near the industrial areas with 13% which is significantly high as compared to the Control females with 4%. When annual family income was compared, the IF-SCH females were showing significant less numbers in low income class and high numbers in high and middle class groups. No difference in literacy levels was reported between the groups. The IF-SCH group reported significantly high number of working women with 25% as compared to the Control subjects which reported 13%. On comparing the prevalence of infertility between different age groups, we found that the subclinical hypothyroid infertile females of 31-35 years of age group with the highest infertility prevalence with 39%. The data on the duration of infertility years revealed that the infertility duration was highest with 42%in the group with 5-7 yrs of duration of infertility.

SCH occurs due to multiple factors. Some of them include congenital agenesis, defect in synthesis due to iodine deficiency or anti-thyroid drugs, autoimmune thyroid diseases, post-surgery, hypo-pituitarism, TSH deficiency, history of thyroid dysfunction/thyroid surgery, family history of thyroid disease, goitre, thyroid auto antibodies, diabetes type I, history of miscarriage/preterm delivery, other autoimmune disorders, history of subfertility, history of therapeutic head or neck irradiation, age ≥30 years, recent exposure to iodinated radiological contrast agents, environmental pollutants, genetic factors, mutations and SNPs etc (Ross *et al.*, 2020; Biondi et al., 2008). Of these factors, the objective of the present study mainly focuses on the AITD. Autoimmune conditions implicated in infertility and reproductive health includes anti- phospholipid antibodies, diabetes mellitus and systemic lupus erythematosus (Prummel *et al.*, 2005). Clinical and experimental studies have suggested a close relationship between the HPT and HPO axis. Increased TSH levels due to decreased levels of thyroid hormones or due to thyroid destruction by and AITD can cause 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 SCH and subsequently to female infertility. In present study to evaluate possible correlation between the autoimmunity associated with increased TSH levels and infertility, anti TPO-Abs prevalence rate were evaluated. 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). AITD is one of the most common etiological factors amongst the causes for the thyroid destruction resulting into overt and subclinical hypothyroidism (Hollwell *et al.*, 2002). The prevalence of AITD is higher in women than in men (Prummel *et al*., 2004; Weetman *et al.*, 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). There is a strong correlation between thyroid immunity and infertility. Thyroid vehicle antibodies exert their impact in a TSH structured but also in a TSH unbiased manner. Hence it is suggested that auto immune thyroiditis should be diagnosed and treated in infertile patients (Vanderpump et al., 2005). 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). The importance of AITD is twofold. First, it is the most common autoimmune disorder in women and second, it is the most frequent cause of thyroid dysfunction. Antibodies against various thyroid antigens can be detected in AITD contributing to propagation of the autoimmune disease and can also have a functional role, directly modulating thyroid hormone synthesis causing hyper- or hypothyroidism, respectively. Hypothyroidism is the more prevalent variant and is linked to thyroid autoimmunity. The circulating thyroid antibodies and the hypothyroidism that often follows AITD have effects on many tissues. The endometrium and ovaries are not spared, and therefore this common morbidity might have an impact on fertility. Neither animal model nor *in vitro* studies are available discussing the pathogenic mechanism of the association of AITD and female infertility. While a single specific and direct pathophysiological mechanism through which autoimmune thyroid disease causes infertility has not been identified, there are multiple gynecological co-morbidities that might perpetuate infertility (Kuharić et al., 2017). 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; Roussev *et al.,* 1996; Kutteh *et al.*, 1999; Kaider *et al.*, 1999; Reimand *et al.*, 2001; Janssen *et al.*, 2004). 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 Ab) positivity on obstetrical outcomes (Vanderpump *et al.*, 1995; Bjoro *et al*., 2000). The prevalence of thyroid autoimmunity has been found to be consistently increased in the subfertile population compared with fertile controls. It has been suggested that thyroid auto antibodies are an early sign of lymphocytic in filtration and therefore a predictor of thyroid disease (Scofield *et al*., 2004). Prasad *et al.* (2019) reported that in 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. Thyroid autoimmunity has been found to be related to subclinical hypothyroidism (Johnson et al., 2006) and the presence of TPO-Abs has been associated with miscarriage, preterm birth, and post-partum thyroid disease (Thangaratinam et al., 2011; Sen et al., 2014). Thyroid peroxidase it is an enzyme that responsible of iodide oxidation and transports it to follicle cavity (Carrasco et al., 2005). Acquired hypothyroidism can result from many reasons like previous injury of thyroid or by exposure to environmental radiation and nearly 61 different mutations have been reported in TPO gene (Ris- Stalpersa et al., 2010). The mutation in TPO gene may prevent the thyroid peroxidase to binding with the heme or the unable of enzyme to binding with thyroglobulin (AL-Ramahi et al., 2011). Treatment of pregnant women with subclinical hypothyroidism and positive TPO-Ab is warranted with the long-term thyroxine therapy in most of the cases (Garber et al., 2012). The American Society for Reproductive Medicine states the routine measurement of TSH in infertile women attempting pregnancy, but not that of TPO-abs, unless TSH levels are ≥2.5 mIU/L (PCASRM, 2015). The 2017 American Thyroid Association (ATA) pregnancy guidelines confirm the adverse effects of subclinical hypothyroidism and thyroid peroxidase antibody positivity on obstetrical outcomes. The guidelines have provided important insights into the complex interactions of thyroid diseases with fertility, concluding that higher TSH and/or anti-TPO Abs are associated with infertility and decreased ovarian reserve in women and confirmed that treatment of subclinical hypothyroidism with TSH >4 improves miscarriage rates recommending levothyroxine treatment in TPO-Ab-positive women who wish to conceive. According to the 2017 guidelines, pregnancy complications, including infertility, were reported to be related to thyroid autoimmunity and thyroid function thus TPO-Ab status must be considered in decision-making if the TSH level is more than 2.5μIU/ml (Alexander et al., 2017). The mechanisms for the association of TPO-Ab with infertility are unclear with some suggesting

that presence of TPO-Ab is a marker of very early thyroid dysfunction, whereas others attributing the association to the presence of a state of autoimmunity adversely affecting fertility (Shields et al., 2013). TPO-Ab positivity is associated with increased risk of pregnancy complications, with 1.7–2.5-fold increased risk of premature delivery, spontaneous premature delivery, and very premature delivery, independent of TSH or thyroxine levels as reported by Vissenberg et al. (2015). Vanderpump et al (1995) reported that if TPO antibodies are present, 4.3% of patients with SCH progress to clinically overt hypothyroidism and if TPO antibodies are absent only 2.6 % patients reported with progression to overt hypothyroidism. 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). 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. Many studies also report about a proportion of people with AITD having normal serum TSH with increased rates of infertility in euthyroid women with AITD, and the management of this group has been a challenge for the clinicians. As AITD can also be present without thyroid dysfunction and therefore remain undiagnosed (Geva et al., 1997; Poppe et al., 2004). This association correlated in part with an increased oestrogen/ progesterone ratio characteristic for this syndrome. Studies confirmed that treatment of subclinical hypothyroidism with TSH >4 improves miscarriage rates, and that anti-TPO Abs are associated with increased risk of miscarriage in euthyroid infertile women (Alexander et al., 2017). The present study reports the prevalence of anti-TPO Abs in overt/ clinical hypothyroid subjects with 25% and with 11% prevalence in euthyroid infertile subjects and s significantly high prevalence with 20% in IF-SCH females as compared to control subjects in Gujarat population.

3.6 Conclusions

The present study reports the following findings in the Gujarat population:

1. Hypothyroidism is more common thyroid disorder than hyperthyroidism and Subclinical hypothyroidism type is the more prevailing hypothyroid disorder as compared to overt/ clinical hypothyroidism in primarily infertile women of reproductive age. Most of the subclinical hypothyroid infertile females were falling in

the zone where there is very less increase in the TSH level and majority of patients were asymptomatic, which was expected as patients with SCH lack frank symptoms of hypothyroidism. To our knowledge is the first study reporting the prevalence for the western part, specifically for the population of Gujarat region.

2. Ages of subclinical hypothyroid infertile females were higher with a considerable number were above 30 yrs. And females of 31-35 years of age group reported the highest infertility prevalence. The females in their twenties reported less prevalence may be due to a change in the thyroid status with increasing age as upcoming trend of late marriage and delayed age for the planning of the first child is now becoming very common. While the females in late thirties reporting less prevalence mainly due to give up tendency of the human nature. There may more percentage of females representing with infertility as females do not go for the treatment more openly due to fear of the society. The data on the duration of infertility years revealed that the infertility duration was highest in the group with 5-7 years this can be justified by the ignorance towards the disease at the early stage.
3. Body mass index, BMI of subclinical hypothyroid females was also reported to be higher indicating a change in the life style and diet as well as lack of exercise resulting in disturbances in the thyroid homeostasis leading to the disease of obesity.
4. No difference in the Hemoglobin (Hb) levels than normal in subclinical hypothyroid women confirming the no anemic status in the subjects.
5. No cases of subclinical hypothyroid infertile females with smoking habits, alcohol or tobacco addiction was reported in the data of the present study. But there are chances in actual scenario as in Indian culture women do not openly share their details as far as these habits are concerned.
6. A significant number of subclinical infertile females were documented residing near the industrial areas drawing the attention towards the hazards of rapid urbanization and industrialization to the thyroid regulation and female infertility.
7. A marked up gradation in the economic status of the country during the last few decades has also contributed for the disease. We report majority of the infertile females with subclinical hypothyroidism coming from the middle and high income group and less from the low income class. The economic status of the family changes the life style mainly causing sedentary life on one hand and on the other hand increase in the stress is also found to survive in the present world full of competition.

8. An increase in the nation's literacy level has also played a role in the nation's fertility rate. Higher education has provided awareness for the newer fertility management and infertility treatments. The study report no difference in literacy levels was reported between the fertile and infertile group.
9. Women empowerment and trend and need of the more women coming at the work places out the home are found to be interfering with maintaining female fertility. In working women there is a common trend of late marriage, late family planning and delayed age at the time of first birth, no time for health work ups, disturbed sleep, work stress, imbalanced and non timely food habits, exposure to occupational toxic and hazardous chemicals, less participation in the societal activities as well dual responsibilities of home and work place resulting into a compromised fertility status have been documented. We also report a high number of working women in subclinical hypothyroid infertile females.
10. Thyroid autoimmunity has been reported as important factor amongst the cause of thyroid destruction causing hypothyroidism. The present study reports the prevalence of anti-TPO Abs in subclinical hypothyroid infertile females. We also found the prevalence of anti TPO Abs in overt/ clinical hypothyroid subjects. The study reported the presence of anti-TPO Abs even in euthyroid subjects also, which can be justified by the presence of non thyroidal autoimmunity.

Causes of infertility are difficult to diagnose and as cases of female infertility are increasing rapidly 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 infertility in the back drop of thyroid dysfunction. Apart from the obvious and apparent clinical conditions abnormalities which are "subclinical" like subclinical hypothyroidism is now must be focused and observational studies in this area should simplify this issue. As SCH is largely asymptomatic, it goes undiagnosed, resulting in infertility. It is essential to include evaluation of thyroid related hormones and autoimmunity as a standard practice along with other tests to ascertain the causes of infertility should be performed as part of the work-up in women with infertility. Though the potential consequences of subclinical hypothyroidism 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. Since thyroid functions exert effect over fertility with various mechanisms, evaluation of thyroid functions during both pregnancy and treatment of infertility as well as in treating relevant pathologies become important. Treating

thyroid dysfunction can reverse menstrual abnormalities and thus improve fertility. Thus it's a need of an hour that thyroid function test should be routinely recommended for all reproductive age group women, as it helps in detection of hypothyroidism in the early stage, that can be treated medically with hormones and it also cost-effective and unnecessary treatment as well as surgery complications of pregnancy can be prevented and burden to the society is decreased on early diagnosis and management. Early detection prevents the conversion of subclinical hypothyroidism to overt hypothyroidism by treating with hormones with careful follow-up. On the other hand, medications given to alter the levels of reproductive hormones have serious repercussions on the health of females with long-term implications. Treatment of infertility is usually done by direct targeting the reproductive system, instead of looking for the involvement of other factors, such as AITD, as a cause of subclinical hypothyroidism and infertility. The present study establishes an association of anti- TPO antibody with infertility in infertile females with subclinical hypothyroidism. This autoimmune approach could be used to identify subclinical hypothyroid patients and treat infertility with greater success and fewer side-effects without disturbing the reproductive system.

Thus concluding, this primary retrospective study is pinpointing the health workers and researchers in the field towards a high prevalence of subclinical hypothyroidism in infertile females and advocating the etiology of thyroid autoimmunity with subclinical hypothyroidism subsequent to female infertility when reproductive considerations are examined. The study emphasizes on the concern of female age and planning for first child birth at an early reproductive age. The present also confirms the interference of the obesity, industrial localization and life style factor with subclinical hypothyroidism consequent to female fertility.

In conclusion the present study reports a high prevalence of subclinical hypothyroidism in infertile females with primary infertility and reiterates the importance of screening of subclinical hypothyroidism along with thyroid autoimmunity as a diagnostic tool in infertility management.

3.7 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.

Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. Thyroid. 2017 Mar 1;27(3):315-89.

AL-Ramahi,I.J.(2011). Molecular and Genetic Study in samples of Iraqi patients with Thyroid Disorders. *Gen. Eng and Biotech Inst Postgrad Stud* **.** *Iraq,* PhD thesis.

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.

Armada-Dias L, Carvalho JJ, Breitenbach MM, Franci CR, Moura EG. Is the infertility in hypothyroidism mainly due to ovarian or pituitary functional changes?. Brazilian Journal of Medical and Biological Research. 2001;34:1209-15.

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.

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.

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.

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.

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

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.

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.

Carrasco, N. (2005). Thyroid hormone synthesis - thyroid iodine transport. In The Thyroid, 9th Edition, L.E. Braverman and R.D. Utiger, eds. (*Philadelphia ,Pennsylvania, USA: Lippincott Williams & Wilkins*), 37-52

Cook JD, Dallman PR, Bothwell TH. Serum ferritin: measurement of iron status. A report of the International Nutritional Anemia Consultative Group (INACG). Kansas, USA: International Nutritional Anemia Consultative Group (INACG). 1985:35-54.

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.

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.

European Society of Human Reproduction and Embryology**,** 2009

Fatourechi V, Klee GG, Grebe SK, Bahn RS, Brennan MD, Hay ID, McIver B, Morris III JC. Effects of reducing the upper limit of normal TSH values. Jama. 2003 Dec 24;290(24):3195-6.

Feldthusen AD, Pedersen PL, Larsen J, Toft Kristensen T, Ellervik C, Kvetny J. Impaired fertility associated with subclinical hypothyroidism and thyroid autoimmunity: the Danish General Suburban Population Study. Journal of pregnancy. 2015 Oct;2015.

Flegal, K. M., Graubard, B. I., Williamson, D. F., and Gail, M. H. (2005). Excess deaths associated with underweight, Overweight, and Obesity. *J American Med Assoc* ; 293(15), Pp1861-1867).

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.

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

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of ClinicalEndocrinologists and the American Thyroid Association. Thyroid. 2012;22(12):1200–35.

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.

Geva, E., Lessing, J.B., Lerner-Geva, L., Azem, F., Yovel, I. & Amit,A. (1997) The presence of antithyroid antibodies in euthyroid patients with unexplained infertility and tubal obstruction. *American Journal of Reproductive Immunology*, **37**, 184–186.

Glinoer D. Miscarriage in women with positive anti-TPO antibodies: is thyroxine the answer? *J Clin Endocrinol Metab*. 2006;91(7):2500-2502.

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.

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

He H, Jing S, Gong F, Tan YQ, Lu GX, Lin G. Effect of thyroid autoimmunity per se on assisted reproduction treatment outcomes: A meta-analysis. *Taiwan J Obstet Gynecol*. 2016;55(2):159-165.

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.

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.

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. The Obstetrician & Gynaecologist. 2015 Jan;17(1):39-45.

Johnson N, Chatrani V, Taylor-Christmas A-K, et al. Population reference values and prevalence rates following universal screening for subclinical hypothyroidism during pregnancy of an Afro- Caribbean Cohort. *Eur Thyroid J*. 2014;3(4):234-239.

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.

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

Kuharić M, Rozić D, Karner I. Thyroid Autoimmunity and Infertility. SEEMEDJ 2017; 1(2); 1-10.

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.

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

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.

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.

Menken J, Trussell J. and Larsen U. Age and infertility. Science. 1986 Sep 26;233 (4771): 1389-

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.

Orouji Jokar T, Fourman LT, Lee H, Mentzinger K, Fazeli PK. Higher TSH levels within the normal range are associated with unexplained infertility. The Journal of Clinical Endocrinology & Metabolism. 2018 Feb;103(2):632-9.

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.

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

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.

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

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.

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.

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.

Rao M, Wang H, Zhao S, Liu J, Wen Y, Wu Z, Yang Z, Su C, Su Z, Wang K, Tang L. Subclinical hypothyroidism is associated with lower ovarian reserve in women aged 35 years or older. Thyroid. 2020 Jan 1;30(1):95-105.

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.

Rich-Edwards JW, Spiegelman D, Garland M,Hertzmark E, Hunter DJ, Colditz GA, Willett WC. And Manson JE. Physical activity, body mass index and ovulatiory disorder infertility. Epidemiology. 2002 Mar;13(2): 184-90.

Ris- Stalpersa,C,and Bikker.H. (2010). Genetics and phenomics of ypothyroidism and goiter due to TPO mutations: *Mol.Cell Endocrinol*; 322 . Pp:38–43

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.

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

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

Sen A, Kushnir VA, Barad DH, Gleicher N. Endocrine autoimmune diseases and female infertility. *Nat Rev Endocrinol*. 2014;10(1):37-50.

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.

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, Knight BA, Hill AV, Hattersley AT, Vaidya B. Five-year follow-up for women with subclinical hypothyroidism in pregnancy. J Clin Endocrinol Metab. 2013;98(12):E1941–5.

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.

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.

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.

Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. *BMJ*. 2011;342: d2616.

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

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, 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.

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.

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.

Vissenberg R, Manders VD, Mastenbroek S, Fliers E, Afink GB, Ris-Stalpers C, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum Reprod Update. 2015;21(3):378–87.

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

WHO/Infertility/2016.