Chapter 4

Evaluating the effects of Subclinical hypothyroidism on the female reproductive hormones and to find out the prevalence of hyperprolactinemia, and to study the correlation of Subclinical hypothyroidism with altered reproductive hormonal profile in Gujarat infertile female population

4.1 Introduction

Female infertility may be attributed to hormonal imbalances that need to be corrected. Thyroid disorders are the most common hormonal anomalies that interfere with reproductive physiology and thus affect fertility in women. Clinical and experimental evidences have suggested a physiological relevance between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-ovary (HPO) axis acting together as integrated system in regulation of many physiological processes including reproduction (Doufas A.G. & Mastorakos G., 2000). Thus, the thyroid hormones, along with the various hormones of HPT-HPO axis which mainly includes prolactin (PRL), follicle stimulating hormone (FSH) and luteinizing hormone (LH) coordinate and regulate reproduction (Goswami *et al.,* 2009). Disturbances in thyroid homeostasis result into either of the two clinical manifestations namely, hyperthyroidism and hypothyroidism, and of these two, hypothyroidism is more prevalent, especially in the females of reproductive age group.

Clinically hypothyroidism is a condition with a decrease in the thyroid hormones (T_3 and T_4) and increased levels of thyroid stimulating hormone (TSH) in the circulation. Hypothyroidism exerts significant effects on the reproductive system. Studies have shown an increase in the TSH levels to consecutively overlap with the PRL, FSH and LH secretions (Burrow *et al.,* 1986; Gharib *et al.,* 2005; Roberts *et al.,* 2004). The hypothyroid condition is found to be the cause of various reproductive abnormalities including menorrhagia, polymenorrhoea, oligomenorrhoea, anovulatory cycle as well as infertility. Oligomenorrhoea is reported as the most common menstrual cycle abnormality in hypothyroidism. An increase in TSH levels in hypothyroidism causes an increase in PRL secretion, a condition known as hyperprolactinemia which in turn results in ovulatory dysfunction (Padubiri *et al.,* 2015). An inter-related pathway controls the secretion of TSH and PRL. PRL is released by anterior pituitary gland and plays an important role in maintaining female fertility. Hyperprolactinemia causes decreased negative feedback on the Hypothalamus-Pituitary axis and thus increases the secretion and release of thyrotropin releasing hormone (TRH) which acts on thyrotropes and lactotropes causing an elevated secretion of both TSH and PRL. Thyroid hormones act synergistically with FSH and LH on ovary to secrete and maintain the normal level of estrogen and progesterone in a menstrual cycle. Anovulatory cycles with decreased fecundity and as result infertility have been found to be associated with thyroid dysfunction (Poppe *et al.,* 2003; Poppe *et al.,* 2007). Hyperprolactinemia inhibits follicular estradiol production and gonadotropin cyclicity leading to anovulation (Kalsum *et al.,* 2002).

Hyperprolactinemic condition inhibits FSH and Gonadotropin Releasing Hormone (GnRH) which activate ovulation. Hyperprolactinemia inhibit secretion of FSH causing hypo-estrogenism with ovarian dysfunction, menstrual abnormalities, suppression of ovulation and trouble getting pregnant and hence causing infertility. It reduces central FSH, LH levels. It decreases granulosa cells and estradiol levels resulting in short luteal phase and finally amenorrhea. TRH and estrogen, positive modulators of PRL, cause an increase in its secretion and action whereas dopamine is a negative modulator of PRL secretion (Gurmanpreet *et al.,* 2014). Delayed LH response to LH-releasing hormone has been reported in hypothyroid females with hyperprolactinemia (Distiller *et al.,* 1975; Larsen *et al.,* 1998; Valenti *et al.,* 1984; Honbo *et al.,* 1978). Studies show that 40 to 70% of hypothyroid infertile females have menstrual disturbances along with hyperprolactinemia and subclinical hypothyroidism (Serri *et al.,* 2003; Bohnet *et al.,* 1981). The data in the literature reports the effects of overt/clinical hypothyroidism on the pathway hormones of female reproductive system but not much data is available in the field for subclinical hypothyroidism (SCH) which can also have an impact on ovulation and menstrual cycles and other reproductive complications precipitating to infertility in females. A number of international and a few national studies have documented abnormal PRL, FSH and LH patterns in hypothyroidism but the estimation and correlation of hypothyroidism, hyperprolactinemia along with serum gonadotropins; LH and FSH, in combination has not been done in primary infertile females with subclinical hypothyroidism in Western India. Hence the study was conducted with the aim of evaluating the effects of SCH on the hormones of reproductive system and to find out the prevalence of hyperprolactinemia in Gujarat infertile female population and to study the correlation of SCH with altered reproductive hormonal profile.

4.2 Material and Methods

4.2.1 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) set up as per guidelines of the Indian Council of Medical Research (ICMR) and with the 1964 Helsinki declaration.

4.2.2 Study Population

The present retrospective study is a matched, case-control study. The study population consists of a total 110 healthy parous control and 270 IF-SCH females with primary infertility as case subjects, (which was screened and reported in the first objective of the present study. Details of The study population is as mentioned in chapter 2 [2.2.2]).

4.2.3 Blood collection and sample preparation

A volume of 1 ml blood samples aliquot was taken from the total 5 ml of the blood sample taken during the objective-1 study parameters from 110 control and 270 IF-SCH (Infertile females with subclinical hypothyroidism) subjects. The blood samples were collected by venous puncture from overnight fasting individuals on the 3^{rd} to 5^{th} day of the menstrual cycle, and serum was separated by centrifugation at 4000 g for 10 minutes at 22^0Cand was collected in Eppendorfand stored until the estimation of PRL and gonadotropins.

4.2.4 Estimation of PRL and Reproductive hormones

Estimation of PRL and gonadotropins- LH and FSH were done for the proposed objective to evaluate the effect and correlation of SCH on the female reproductive system in a total 110 control and 270 IF-SCH females which were screened out in the objective 1. The control and study subjects were subjected to serum PRL, LH and FSH level estimation 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. The test principle of estimation of PRL linked an enzyme immunoassay competition method with a final fluorescent detection (ELFA), while the principle of estimation of FSH and LH combine a one step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). Results were calculated by the instrument in relation to the calibration curve stored in memory of the device. These tests were carried out in the pathology laboratory [N Dalal Pathology Laboratory of "Ghanshyam clinic", Vadodara]. The normal/ reference range of hormones were as per the values provided in the kits used for the assays in the laboratory.

4.2.5 Sampling method

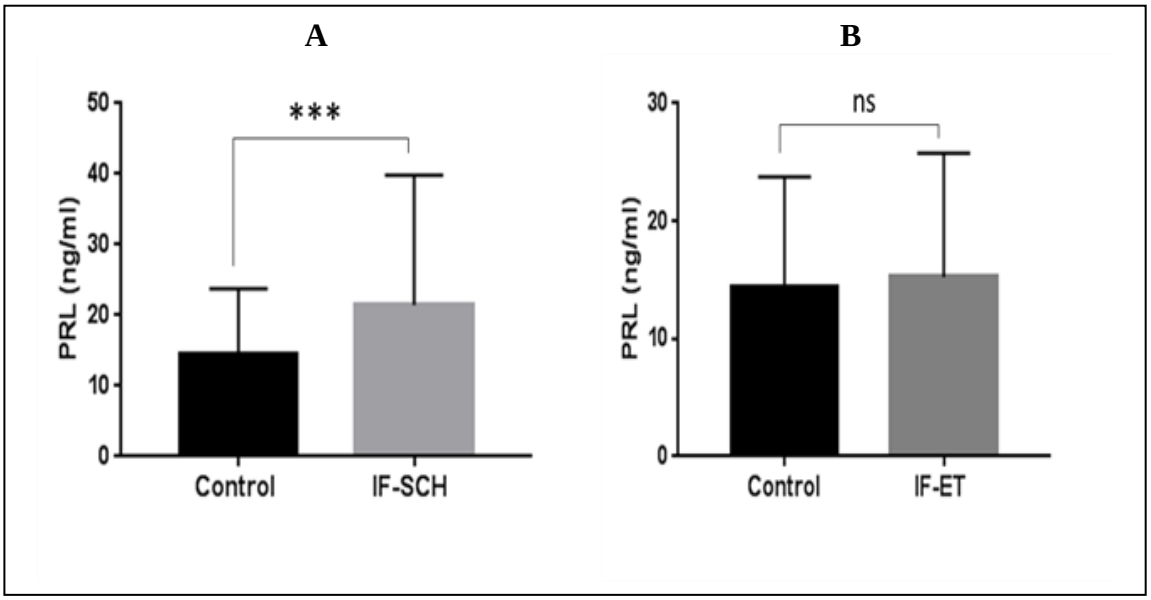
The sampling method for selecting the participants was purposive (also called convenience method) sampling method as this provides the best information by the members of the selected community.

4.3 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 PRL, LH and between age and PRL, LH, FSH as well as between LH and FSH. 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.

4.4 Results

Analysis of PRL, LH and FSH levels in the 110 control and 270 IF-SCH subjects revealed that IF-SCH subjects had significantly high (p=0.0002; Fig. 4.1A) PRL levels (mean ± SEM: 21.43 ± 1.119 ng/ml, Table 4.1.) compared to the Control females (mean ± SEM: 14.38 ± 0.896 ng/ml). The euthyroid infertile female (IF-ET) subjects were also assessed for the study parameter and the result was compared with the Control and IF-SCH subjects. On comparing the PRL levels in IF-ET females there was no significant difference (p=0.513; mean ± SEM: 15.23 ± 0.922 ng/ml, Fig. 4.1B, Table 4.2), as compared to Control subjects (mean ± SEM: 14.38 ± 0.895 ng/ml). We obtained significantly high PRL levels in IF-SCH (p=0.0004, mean ± SEM: 21.43 ± 1.119, Fig. 4.1C, Table 4.2), when compared with IF-ET females (mean ± SEM: 15.23 ± 0.922 ng/ml). Thus, our studies show the highest PRL levels in IF-SCH subjects, Fig. 4.1D; among the Control, IF-ET and IF-SCH subjects in the present study, Table 4.2.



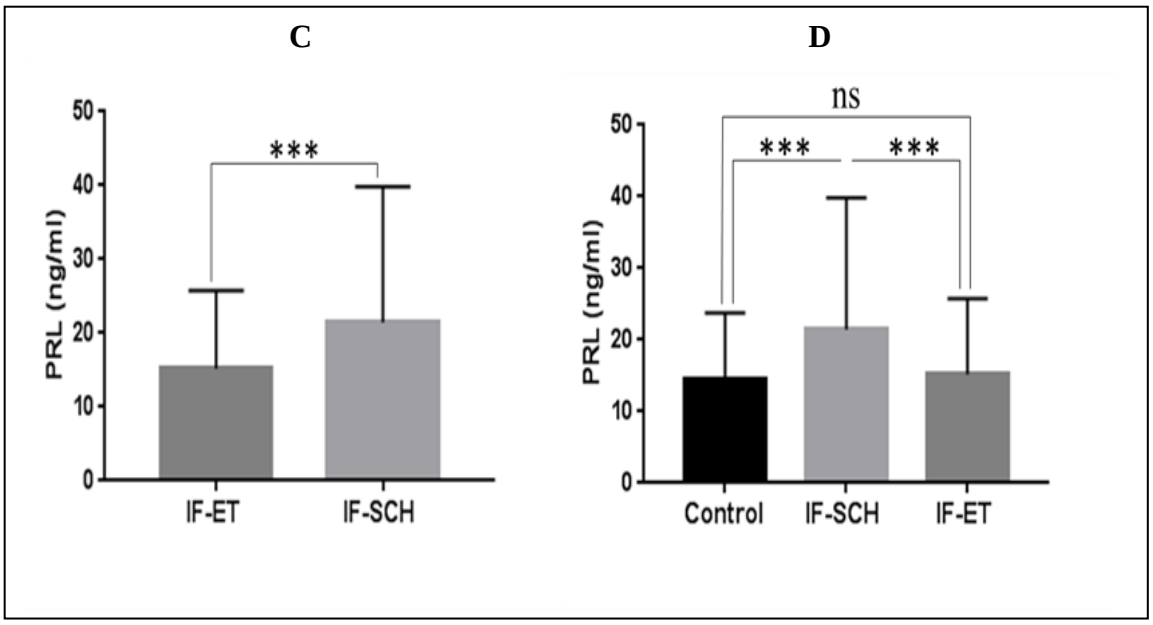


Figure 4.1 PRL levels in Control, IF-SCH and IF-ET subjects: A. PRL levels in Control and IF-SCH group (p=0.0001). **B.** PRL levels in Control and IF-ET group. **C.** PRL levels in IF-ET and IF-SCH group (p=0.0001). **D.** Comparative levels of PRL levels in Control, IF-ET and IF-SCH group(p=0.0001).

The study reports a significantly low (p=0.0006; Fig. 4.2A) LH level (mean ± SEM: 8.305 ± 0.570 m IU/ml; Table 4.1) in IF-SCH females as compared to controls (mean ± SEM: 11.29 ± 0.6448 m IU/ml). FSH level were also reported to be significantly low (p=0.0226; Fig. 4.2B) in IF-SCH subjects (mean ± SEM: 9.355 ± 0.536 m IU/ml; Table 4.1) as compared to controls (mean ± SEM: 11.42 ± 0.718m IU/ml). This need to be taken in to an account that, though there is a significant decrease in gonadotropin levels (both LH and FSH) in IF-SCH subjects as compared to Control group, the decreased levels of LH and FSH were still within the normal reference range provided for both the hormones respectively.

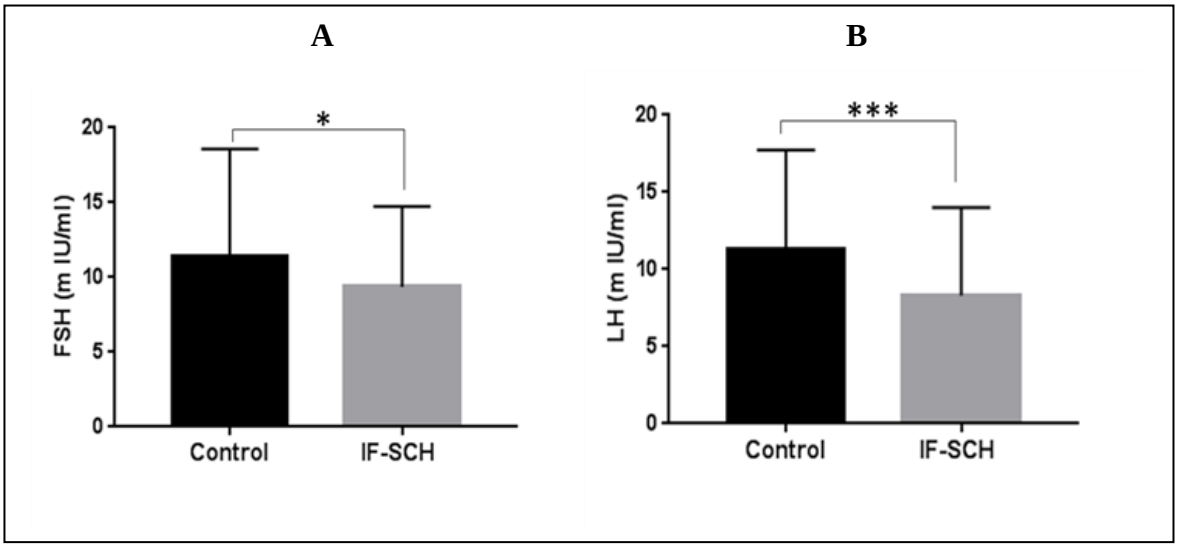


Figure 4.2 Gonadotropin levels in Control and IF-SCH subjects A. LH levels **B.** FSH levels

Table 4.1 PRL and Gonadotropins levels in Control and IF-SCH subjects

	PRL (ng/ml)	**LH (m IU/ml)**	**FSH (m IU/ml)**
Normal value	0.5– 20	4 – 20	2–30
Control	14.38 ± 0.895 ,n=110	11.29 ± 0.644, n=100	11.42 ± 0.718, n=100
IF-SCH	21.43 ± 1.119, n=270	8.305 ± 0.570, n=100	9.355 ± 0.536, n=100
p value (p value summary)	0.0002 (***)	0.0006 (***)	0.0226 (*)

Data represent mean ± SEM values, IF-SCH; Infertile females with subclinical hypothyroidism, LH; Leutinizing hormone, FSH; Follical stimulating hormone, *p<0.05, ***p=0.001.

The present study reports a significantly high prevalence rate 37% of hyperprolactinemia (Fig. 4.3C) in IF-SCH subjects (Table 4.2), while Control and IF-ET subjects reported prevalence of 19% (Fig. 4.3.A, Table 4.2) and 18% (Fig. 4.3B, Table 4.2) respectively. The prevalence rate was significantly high (p=<0.0001, Table 4.2) in IF-SCH subjects as compared to control subjects. No significant difference was found in the prevalence rate of hyperprolactinemia between control and IF-ET subjects (Table 4.2).

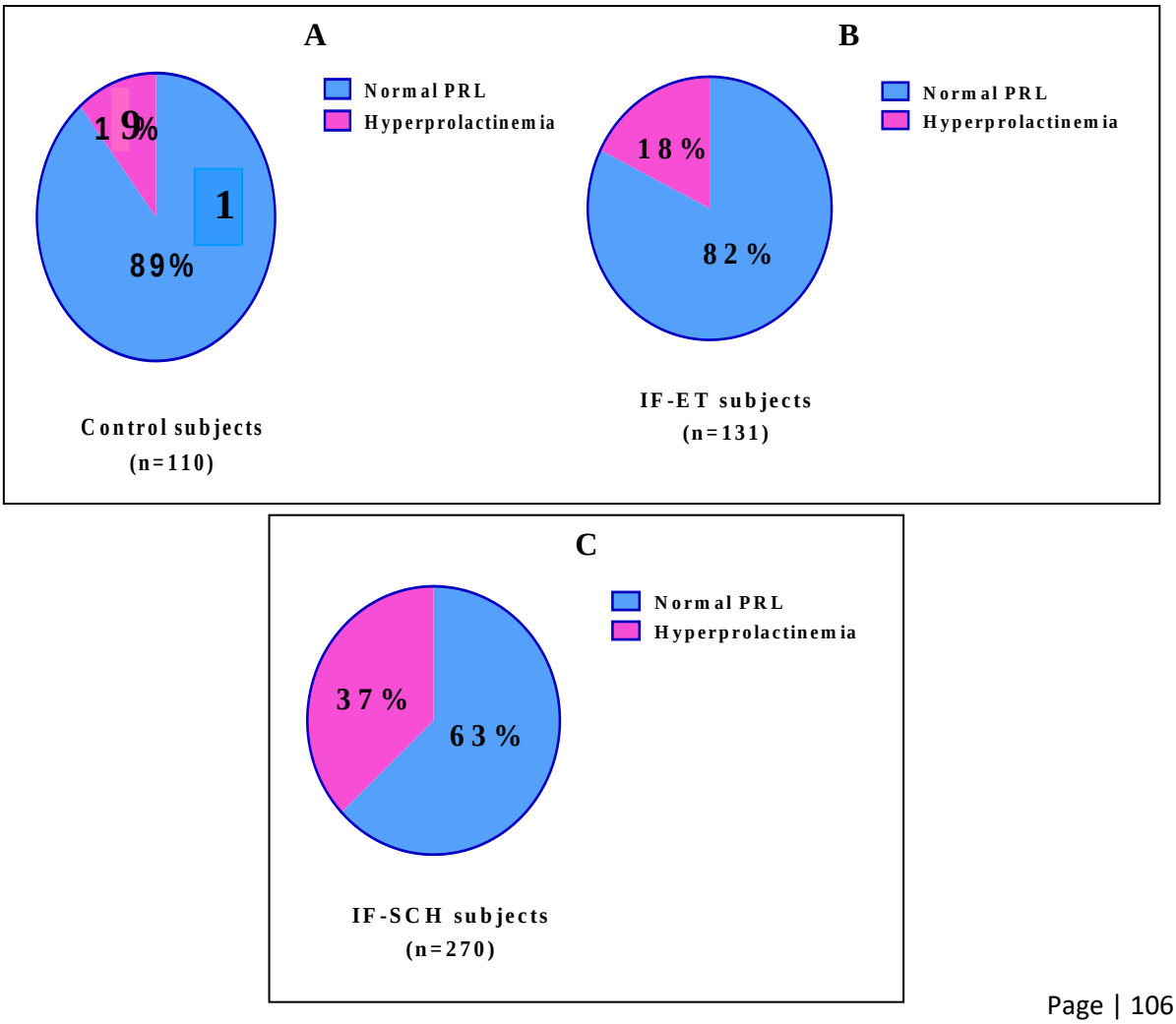


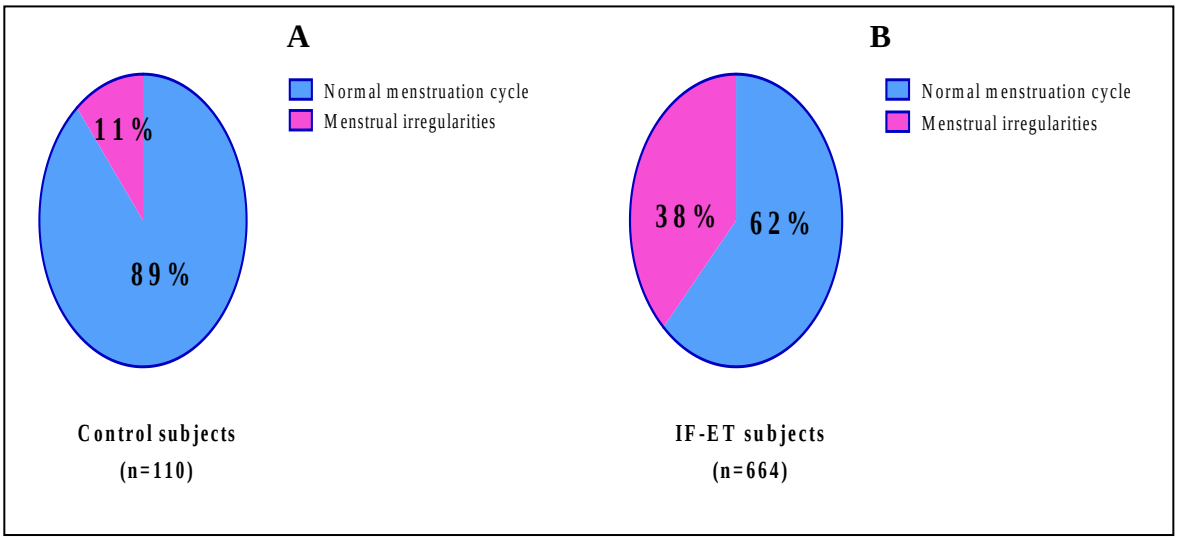
Figure 4.3 Prevalence of hyperprolactinemia in the study population: A. Prevalence of hyperprolactinemia in Control subjects. **B.** Prevalence of hyperprolactinemia in IF-ET. **C.** Prevalence of hyperprolactinemia in IF-SCH subjects.

Table 4.2 Comparative PRL levels and prevalence of Hyperprolactinemia in Control, IF-ET and IF-SCH subjects.

	Control subjects	**IF-ET subjects**	**IF-SCH subjects**	**P value (p value summary)**	
PRL (ng/ml)	14.38 ± 0.895, (n=110)	15.23 ± 0.922 (n=131)	21.43 ± 1.119, (n=270)	**Control Vs IF-ET**	**IF-ET Vs IF-SCH**
				0.513 (ns)	0.0004 (****)
Hyperprolactinemia (% Prevalence)	19 (n=100)	18 (n=130)	37 (n=270)	>0.9999 (ns)	<0.0001 (****)

Data presented as Mean ± SEM values, PRL; Prolactin, IF-ET; Euthyroid infertile females, IF-SCH; Infertile females with subclinical hypothyroidism

The details of the menstrual cycle of each control and study subjects were obtained by the questionnaire. We found a total 159 out of 270 IF-SCH females having menstrual irregularities, with prevalence rate of 59% (Fig. 4.4C, Table 4.3) as compared 12 out of 110 controls with 11% prevalence rate (Fig. 4.4A, Table 4.3), while 252 out of 664 with 38% presented with menstrual irregularities in IF-ET female subjects (Fig. 4.4B, Table 4.3). Further to find out the most prevailing type of menstrual irregularity the subjects were divided into five groups depending upon their menstrual cycle status as mentioned in the Table 4.4.



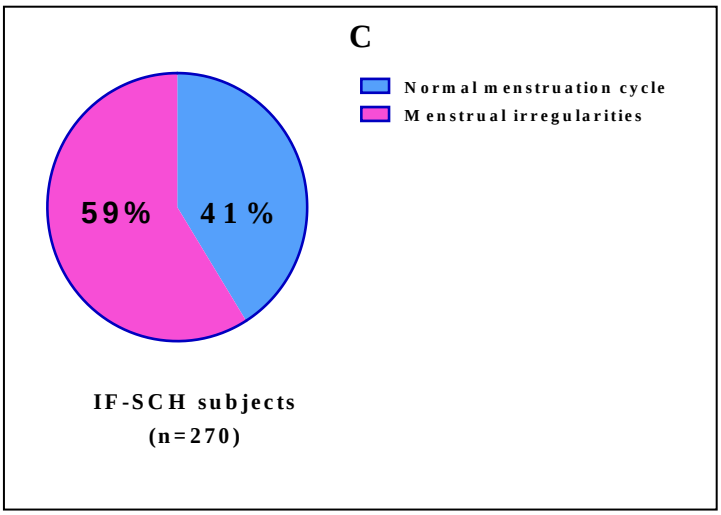


Figure 4.4 Prevalence of Menstrual irregularities in Control, IF-ET and IF-SCH subjects: A. Prevalence of menstrual irregularities in Control subjects. **B.** Prevalence of menstrual irregularities in IF-ET subjects. **C.** Prevalence of menstrual irregularities in IF-SCH subjects.

Table 4.3 Distribution of menstrual irregularities in the controls and IF-SCH subjects

	Total number N	**Normal menstrual Cycles N (%)**	**Menstrual Irregularities N (%)**
Control subjects	110	98 (89)	12 (11)
IF-SCH subjects	270	111(41)	159 (59)
IF-ET subjects	664	412 (62)	252 (38)

IF-ET; Euthyroid infertile subjects, IF-SCH; Infertile subjects with subclinical hypothyroidism, n; total numbers of the subjects, %; percentage.

The study reports 85 out of 270 with 32% of IF-SCH females with irregular menstrual cycles; Fig. 4.5C, Table 4.4 as against the control subjects in which 8 out of 110 resulting in 7% of irregularities in their menstrual cycles; Fig. 4.5A, Table 4.4) and IF-ET subjects 179 out of 664 with 27%; Fig. 4.5B, Table 4.4. Menorrhagia was 2% in (2 out of 110 control) and 19 from 270 giving the result 7% in IF-SCH females, and in IF-ET 40 out of 664 resulting into 6%. Oligomenorrhea was the most prevailing type of menstrual disorder (42 out of 270) with 15% in IF-SCH subject as compared to controls with 2% in 2 subjects out of 110 females and 20 out of 664 with 3% in IF-ET females diagnosed with the same; Fig. 4.5A, B and C, Table 4.4. The control group had no subjects with polymenorrhea as compared to 13 out of 270 IF-SCH females with 5% of subjects having polymenorrhea, Table 4.4.

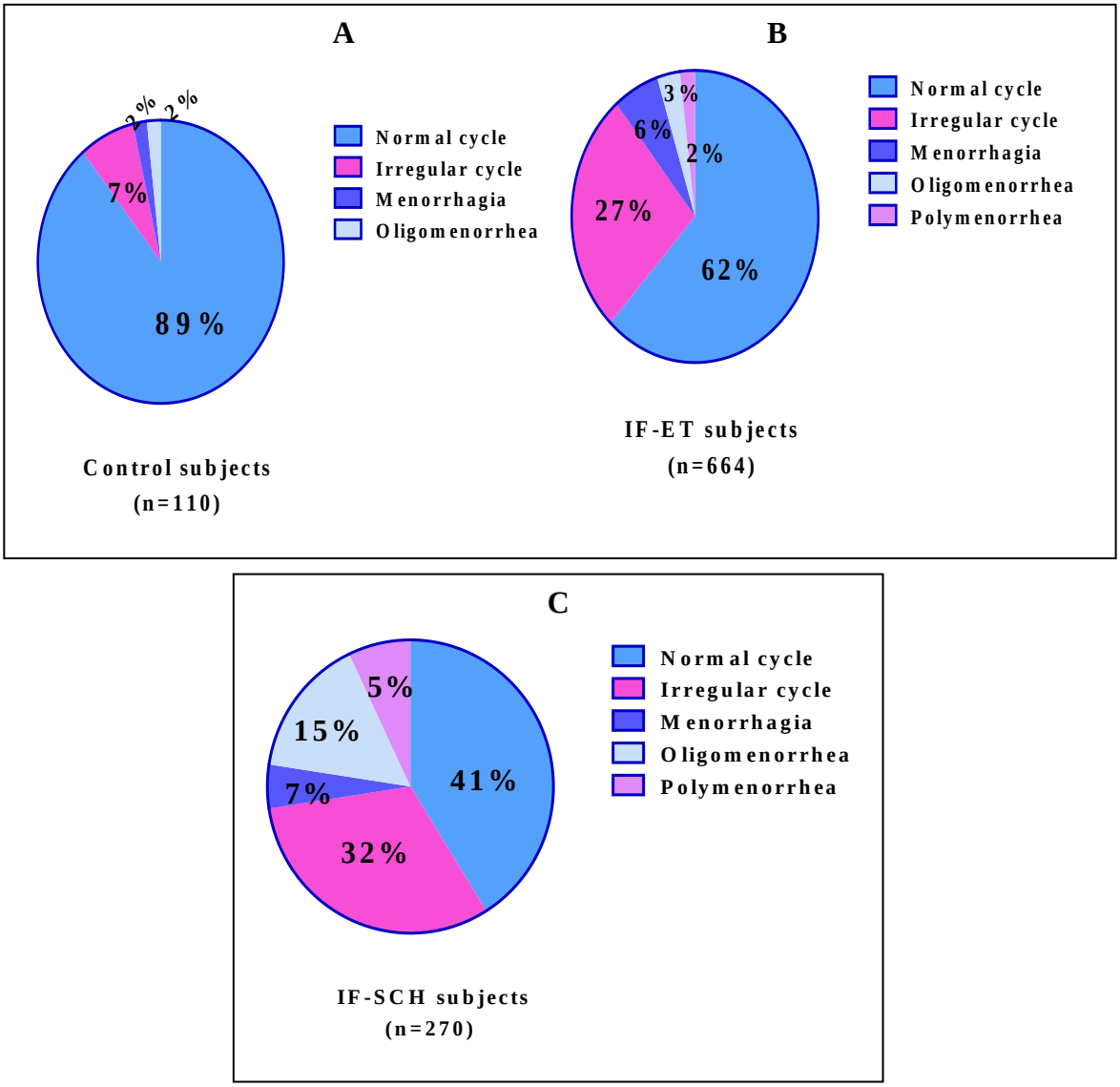


Figure 4.5 Menstrual status Control, IF-ET and IF-SCH subjects: A. Menstrual status in Control subjects. **B.** Menstrual status in IF-ET subjects. **C.** Menstrual status in IF-SCH subjects.

Table 4.4 Menstrual status of the IF-SCH subjects

Menstrual status	Control subjects N (%)	IF-ET subjects N (%)	IF-SCH subjects N (%)
Normal	98 (89)	412 (62)	111 (41)
Irregular	8 (7)	179 (27)	85 (32)
Menorrhagia	2 (2)	40 (6)	19 (7)
Oligomenorrhea	2 (2)	20 (3)	42 (15)
Polymenorrhea	0 (0)	13 (2)	13 (5)
Total	110 (100)	664 (100)	270 (100)

IF-ET; Euthyroid infertile subjects, IF-SCH; Infertile subjects with subclinical hypothyroidism, n; total numbers of the subjects, %; percentage.

A correlation study was carried out to find out and correlate the effect of an increased TSH levels with increased PRL and decreased gonadotropins (LH and FSH). PRL and LH and FSH levels were also compared and studied to find out the correlation with demographic characteristics (age and BMI) in subclinical hypothyroid primary infertile females (IF-SCH) and Control subjects. At first PRL and age, PRL and BMI, PRL and LH as well as PRL and FSH correlation study was carried out in control and IF-SCH females. The study reports a significant positive correlation (r=0.146, *p*=0.041, Fig. 4.6C, Table 4.5) between PRL and TSH in IF-SCH subjects, while the Control (Fig. 4.6A, Table 4.5) and IF-ET (Fig. 4.6B, Table 4.5) group did not report any correlation for the same.

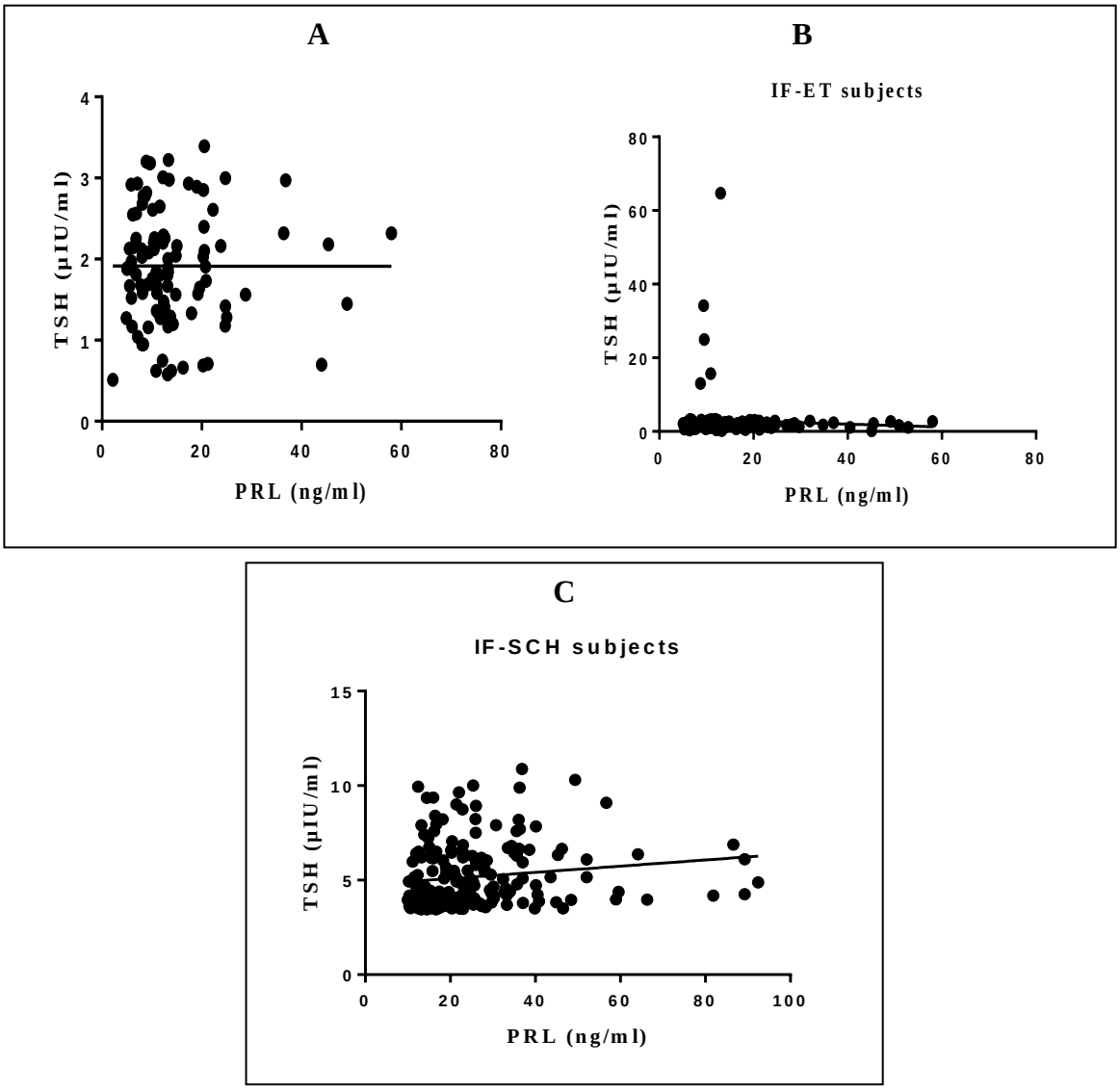


Figure 4.6 PRL and TSH correlation patterns: A. Correlation pattern in Control subjects. **B.** Correlation pattern in IF-ET subjects. **C.** Correlation pattern in IF-SCH subjects (r=0.146, *p*=0.041).

The correlation study between PRL and fT_3 revealed a significant negative correlation (r= -0.125, *p*= 0.040, Fig. 4.7B, Table 4.5) in IF-SCH subjects, a non significant negative correlation was also observed in Control subjects (r= -0.054, *p*=0.5786, Fig. 4.7A, Table 4.5).

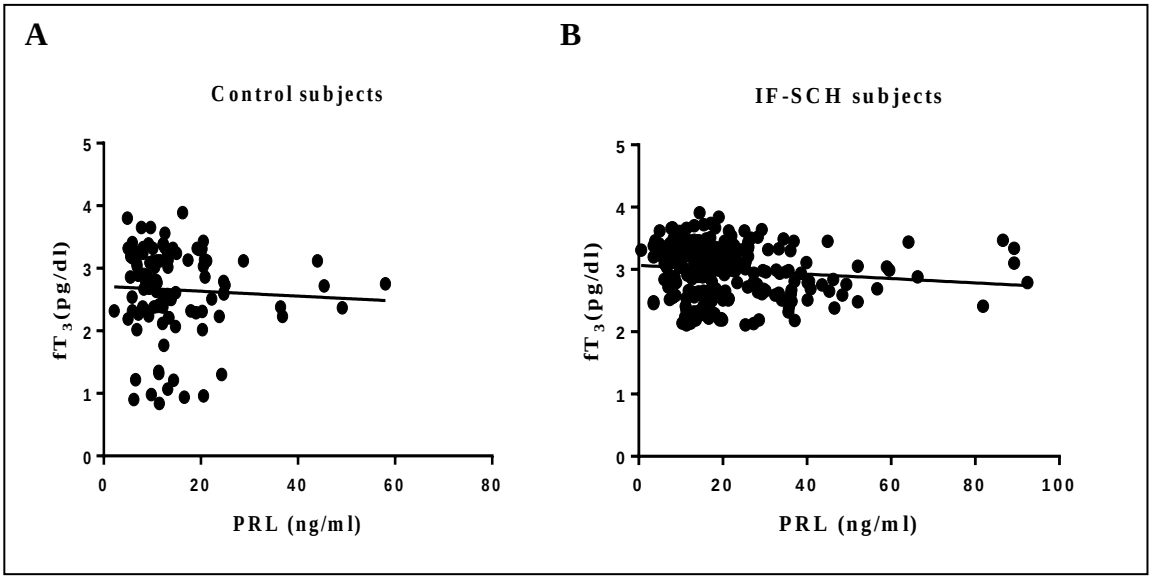


Figure 4.7 PRL and fT_3 correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing a non significant negative correlation (r= -0.054, *p*=0.5786) between PRL and fT_3levels. **B. Correlation pattern in IF-SCH subjects- IF-ET subjects** (n=270) were also showing a statistically significant negative correlation (r= -0.125, *p*= 0.040) between PRL and fT_3levels.

The correlation study between PRL and fT_4 revealed a significant negative correlation both in IF-SCH (r=-0.132, *p*=0.030, Fig. 4.8B, Table 4.5) in Control subjects (r=-0.212, *p*=0.026, Fig. 4.8A, Table 4.5).

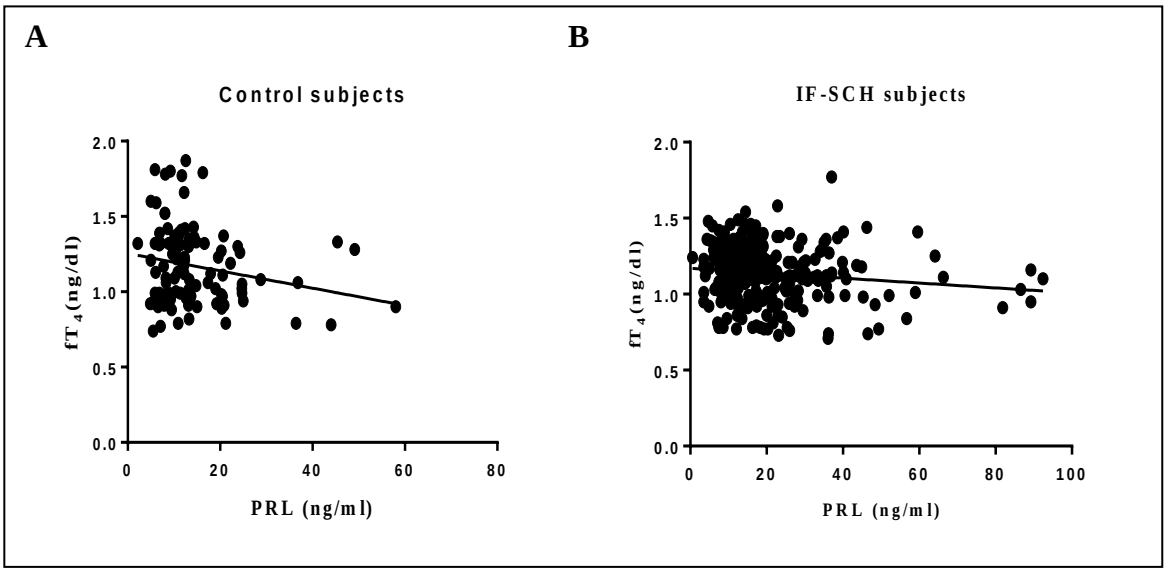


Figure 4.8 PRL and fT_4 correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing a significant negative correlation (r=-0.212, *p*=0.026) between PRL and fT_4levels. **B.** Correlation pattern in IF-SCH subjects- IF-SCH

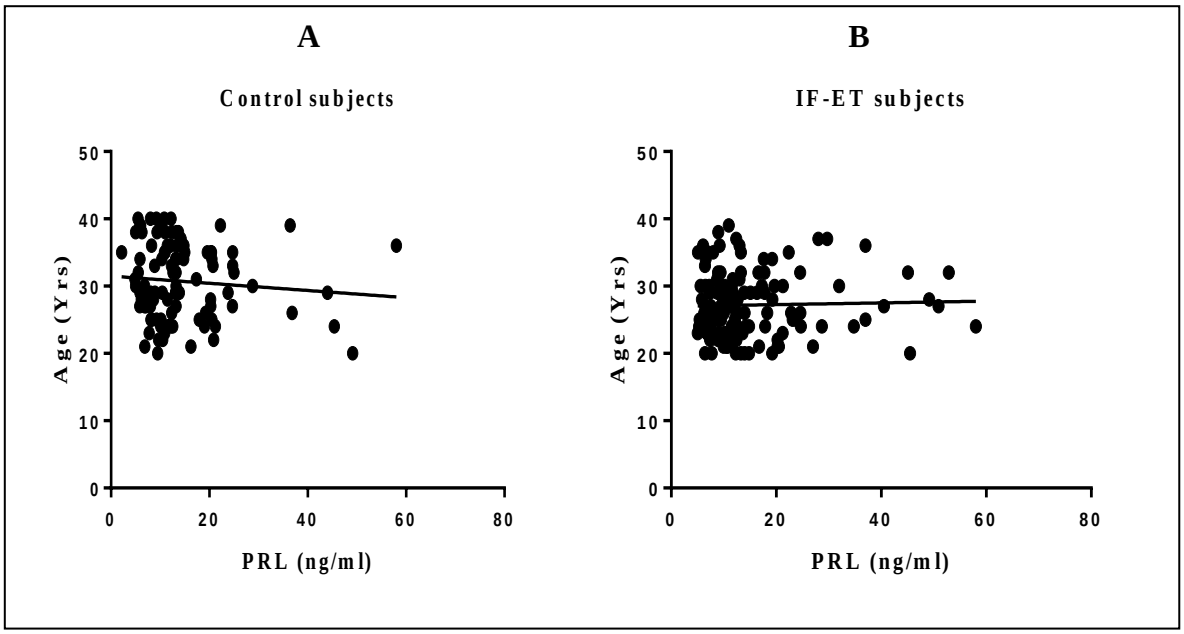
subjects (n=270) were also showing a statistically significant negative correlation (r=-0.132, *p*=0.030) between PRL and fT_3levels.

Table 4.5 Correlation between the PRL and TSH, PRL and fT_3, PRL and fT_4

	PRL with TSH			PRL with fT_3		PRL with fT_4	
No. of XY pairs	**Control**	**IF-ET**	**IF-SCH**	**Control**	**IF-SCH**	**Control**	**IF-SCH**
	100	131	200	100	270	100	270
Pearson r	-0.001	-0.062	0.146	-0.054	-0.125	-0.212	-0.132
95% Confidence	-0.198 to 0.195	-0.231 to 0.110	0.006 to 0.278	-0.238 to 0.135	-0.241 to -0.006	-0.384 to -0.026	-0.248 to -0.013
p value	0.992	0.479	0.041	0.5786	0.040	0.026	0.030
Significance of correlation	Ns	ns	*	ns	*	*	*

Data presented as Mean ± SEM values, PRL; Prolactin, fT_3; free triiodothyronin, fT_4;free thyroxine , IF-ET; Euthyroid infertile females, IF-SCH; Infertile females with subclinical hypothyroidism, * $p<0.05$

PRL and age correlation study revealed a significant positive correlation (r=0.124, *p*=0.044, Fig. 4.9C, Table 4.6.) in IF-SCH subjects and no correlation was observed in Control (Fig. 4.9A, Table 4.6.) or IF-ET (Fig. 4.9B, Table 4.6.) subjects. Further, PRL and BMI correlation study also resulted in a significant positive correlation (r=0.136, *p*=0.026, Fig. 4.10B, Table 4.6) in IF-SCH group and no correlation was found in Control females (Fig. 4.10A, Table 4.6).



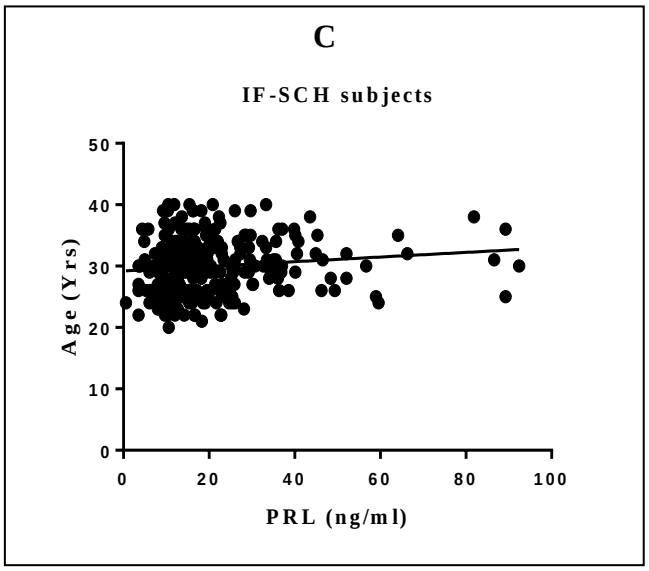


Figure 4.9 PRL and Age correlation in Control, IF-ET and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation between PRL levels and increased age. **B.** Correlation pattern in IF-ET subjects- IF-ET subjects (n=131) were also showing insignificant negative correlation between PRL levels and increased age. **C.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=265) were showing a statistically significant positive correlation (r=0.124, *p*=0.044) between PRL levels and increased age.

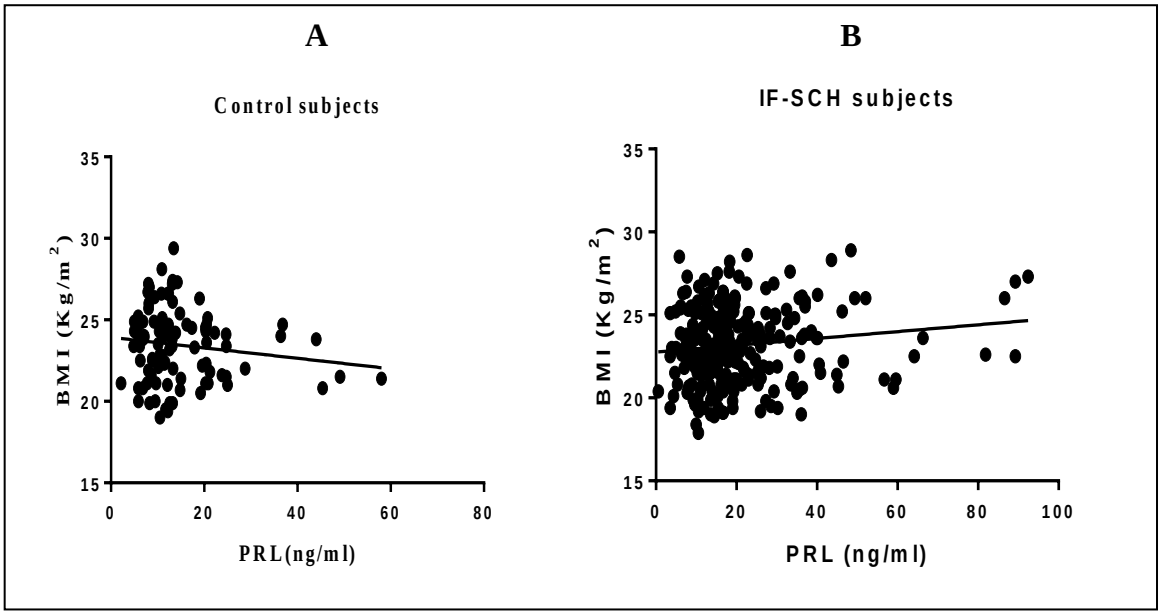


Figure 4.10 PRL and BMI correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation between PRL levels and high BMI. **B.** Correlation pattern in IF-SCH subjects- IF-ET subjects (n=269) were also showing a statistically significant positive correlation (r=0.136, *p*=0.026) between PRL levels and high BMI.

Table 4.6 Correlation between PRL levels and increased Age, PRL levels and high BMI

	PRL with Age			PRL with BMI	
No. of XY pairs	**Control**	**IF-ET**	**IF-SCH**	**Control**	**IF-SCH**
	100	131	265	100	269
Pearson r	-0.092	0.029	0.124	-0.155	0.136
95% Confidence	-0.283 to 0.107	-0.143 to 0.199	0.004 to 0.241	-0.341 to 0.043	0.0167 to 0.252
p value	0.364	0.744	0.044	0.124	0.026
Significance of correlation	NS	NS	*	NS	*

PRL; Prolactin, IF-ET; Euthyroid infertile females, IF-SCH; Infertile females with subclinical hypothyroidism, BMI; Body mass index, * p<0.05, ns; not significant.

Further to find out the effect of SCH on LH level correlation study between LH and TSH was carried out in Control and IF-SCH group. A negative correlation (r= -0.270, p=0.011, Fig. 4.11B, Table 4.7) was observed in IF-SCH subjects while no correlation was found with respect to the Control subjects (Fig. 4.11A, Table 4.7).

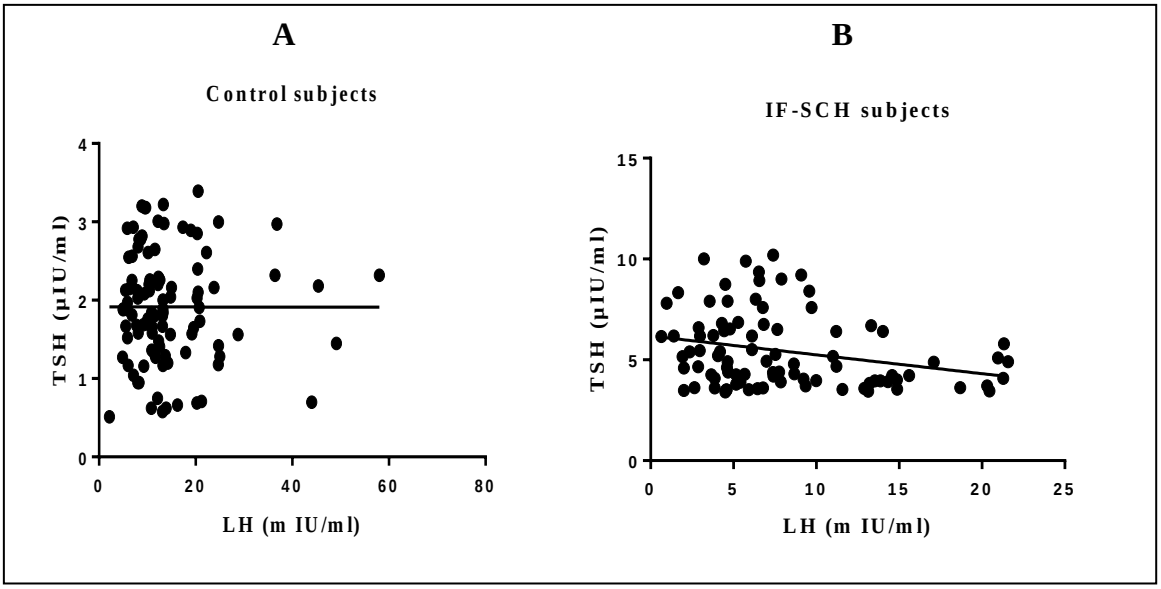


Figure 4.11 LH and TSH correlation in Control subjects and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation between LH and TSH levels. **B.** Correlation pattern in IF-SCH subjects. IF-SCH subjects (n=89) were showing a statistically significant (r=-0.270, p=0.011) negative correlation between LH and TSH levels.

On analyzing the correlation between LH levels and increased age we found insignificant negative correlation in both Control (r= -0.084, p=0.409, Fig. 4.12A, Table 4.7) and IF-SCH (r= -0.145, p=0.176, Fig. 4.12B, Table 4.7) subjects.

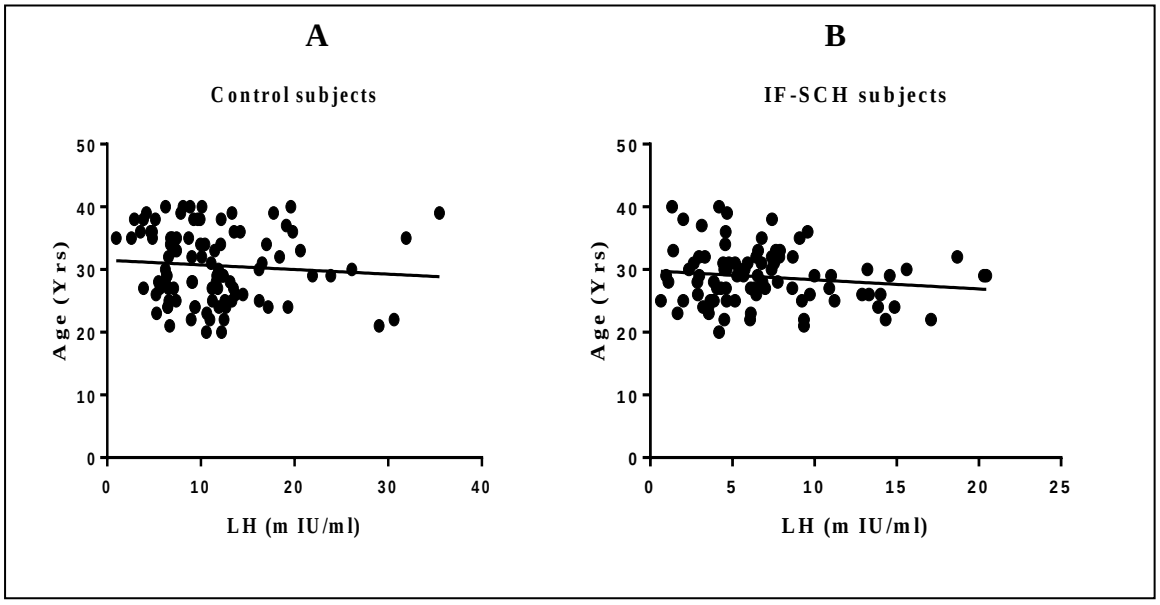


Figure 4.12 LH and Age correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation (r= -0.084, *p*=0.409) between LH levels and increased age. **B.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=89) were showed insignificant negative correlation (r= -0.145, *p*=0.176) between LH levels and increased age.

To understand the relation between BMI and serum LH levels, BMI and LH correlation was done and our study reports a negative correlation (r= -0.221, *p*= -0.221, Fig. 4.13B, Table 4.7) in IF-SCH group while there was no correlation in control subjects (r= -0.078, *p*= 0.441, Fig. 4.13A, Table 4.7).

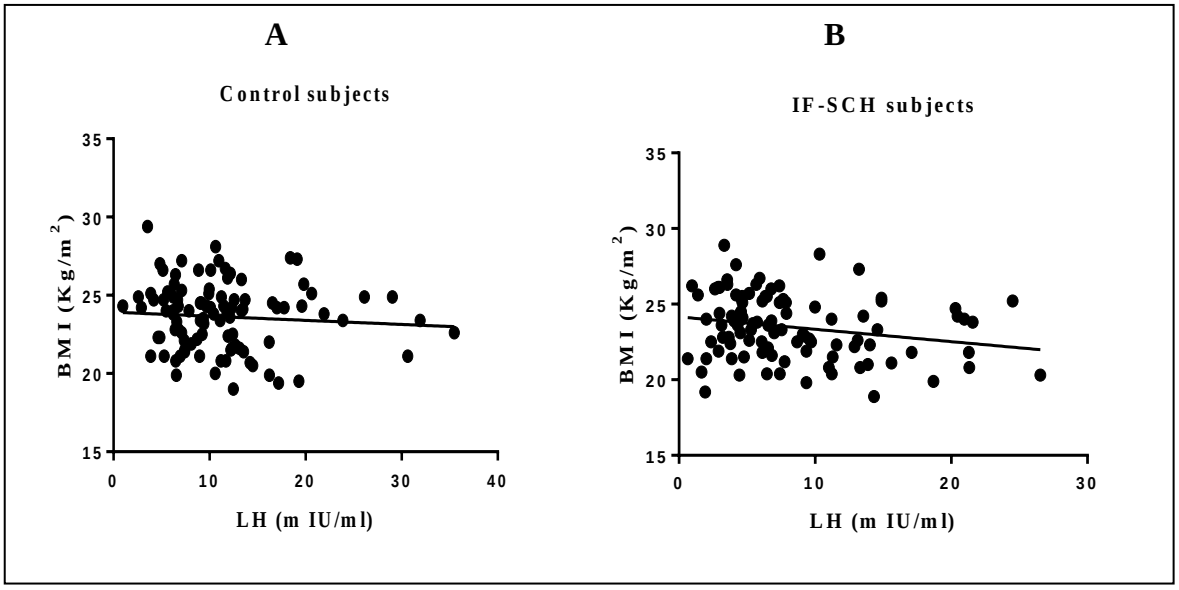


Figure 4.13 LH and BMI correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation (r= -0.078, *p*= 0.441) between LH levels and increased BMI. **B.** Correlation pattern in IF-SCH subjects- IF-SCH

subjects (n=100) group showed significant negative correlation (r= -0.221, *p*= -0.221) between LH levels and increased BMI.

Table 4.7 Correlation between the LH and TSH, LH and Age, LH and BMI

	LH with TSH		**LH with Age**		**LH with BMI**	
No. of XY pairs	**Control**	**IF-SCH**	**Control**	**IF-SCH**	**Control**	**IF-SCH**
	100	89	100	89	100	100
Pearson r	-0.001	-0.270	-0.084	-0.145	-0.078	-0.221
95% Confidence	-0.198 to 0.195	-0.453 to -0.065	-0.275 to 0.115	-0.343 to 0.066	-0.270 to 0.121	-0.400 to -0.026
p value	0.992	0.011	0.409	0.176	0.441	0.027
Significance of correlation	Ns	*	ns	ns	ns	*

LH; Leutinizing hormone, FSH; Follicle stimulating hormone, IF-SCH; Infertile females with subclinical hypothyroidism, BMI; Body mass index, * $p<0.05$, ns; not significant.

FSH and TSH correlation study reported decreasing FSH levels with increase in TSH levels resulting in a negative correlation (r= -0.267, *p*=0.016, Fig. 4.14.B, Table 4.8.) in IF-SCH subjects but not in Control females (r=.0.024, *p*= 0.813, Fig. 4.14.A, Table 4.8.). A negative correlation between FSH and age was observed in both Control (r= -0.221, *p*= 0.031, Fig. 4.15.A, Table 4.8.) and IF-SCH (r= -0.243, *p*=0.030, Fig. 4.15.B, Table 4.8.) females. FSH and BMI correlation study revealed a positive correlation both Control (r= 0.227, *p*=0.023, Fig. 4.16.A, Table 4.8.) and IF-SCH (r= 0.25, *p*=0.012, Fig. 4.16B, Table 4.8.) females. Further a positive correlation was observed between FSH and LH in IF-SCH (r= 0.203, *p*= 0.043, Fig. 4.17.A, Table 4.8).

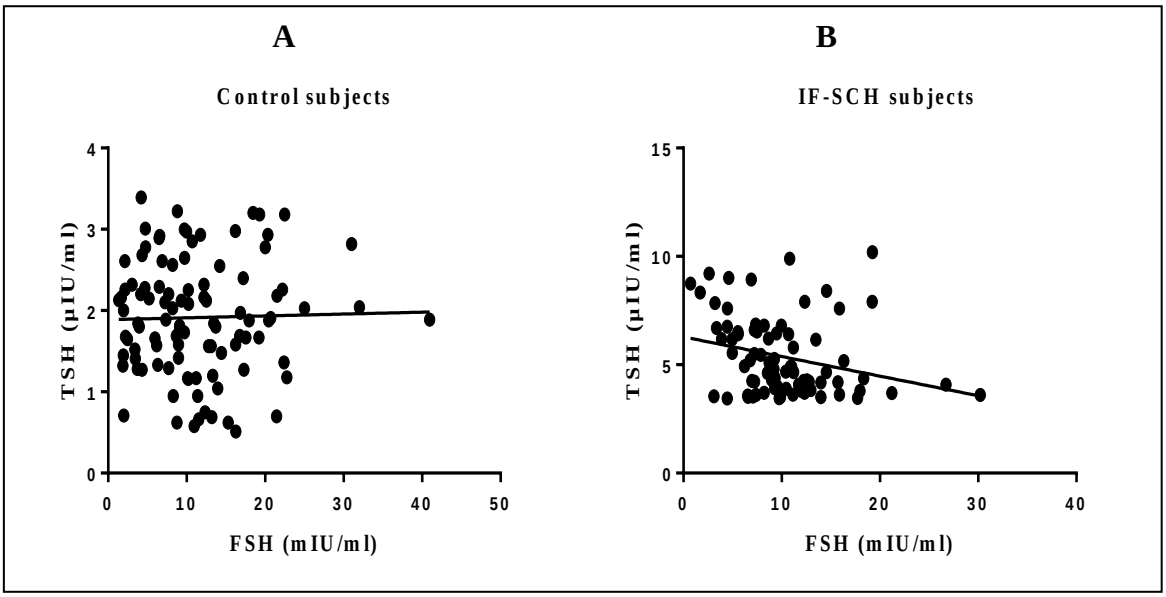


Figure 4.14 FSH and TSH correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation (r=.0.024, *p*= 0.813) between FSH and TSH levels. **B.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=100) group showed significant (r=-0.267, *p*=0.016) negative correlation (r= -0.267, *p*=0.016) between FSH and TSH levels.

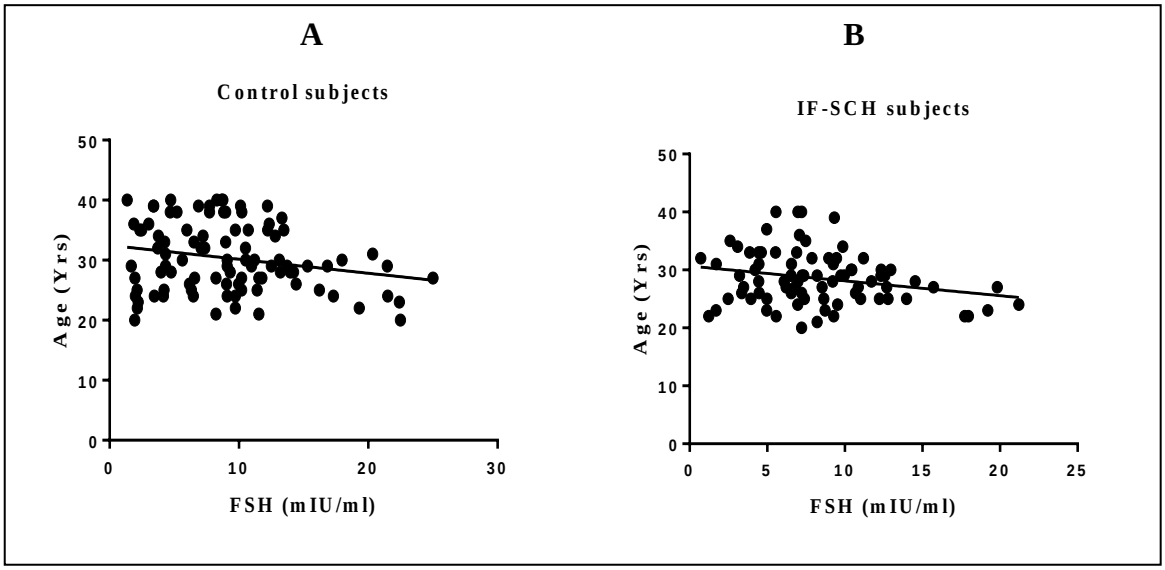


Figure 4.15 FSH and Age correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=95) were showing insignificant negative correlation (r= -0.221, *p*= 0.031) between FSH levels and increased age. **B.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=80) group showed significant negative correlation (r= -0.243, *p*=0.030) between FSH levels and increased age.

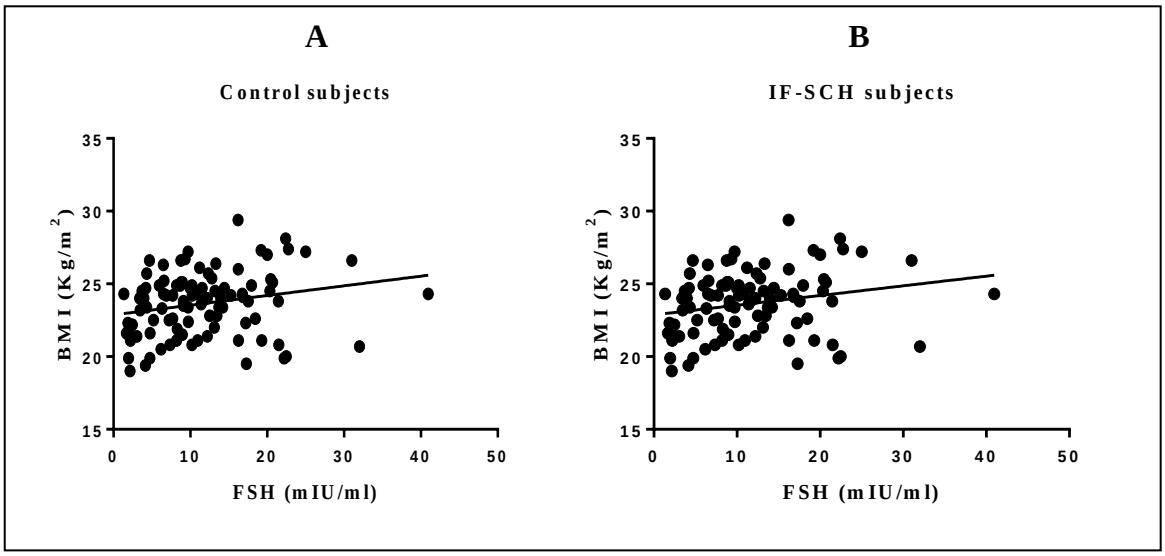


Figure 4.16 FSH and BMI correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing significant positive correlation (r= 0.227, *p*=0.023) between FSH levels and increased BMI. **B.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=100) group showed significant positive correlation (r= 0.25, *p*=0.012)between FSH levels and increased BMI.

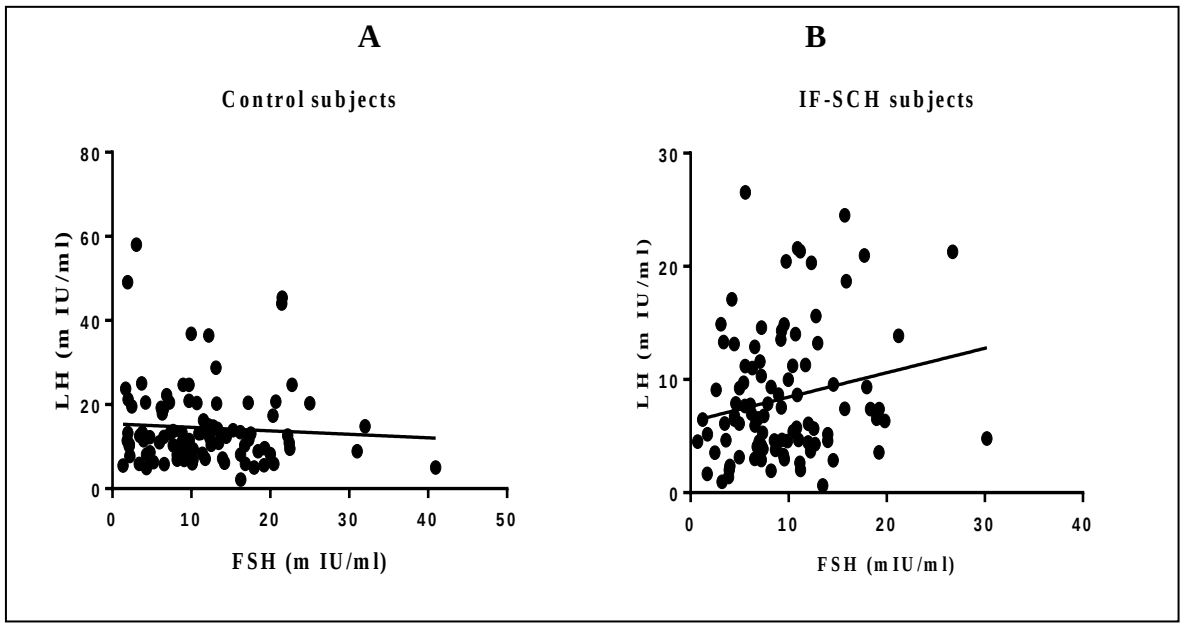


Figure 4.17 LH and FSH correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant negative correlation between FSH and LH levels. **B.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=100) group showed significant positive correlation (r= 0.203, *p*= 0.043) between FSH and LH levels.

Table4.8Correlation between FSH and TSH, FSH and Age, FSH and BMI, FSH and LH

	FSH with TSH		**FSH with Age**		**FSH with BMI**		**FSH with LH**	
No. of XY pairs	**Control**	**IF-SCH**	**Control**	**IF-SCH**	**Control**	**IF-SCH**	**Control**	**IF-SCH**
	100	81	95	80	100	100	100	100
Pearson r	0.024	-0.267	-0.221	-0.243	0.227	0.251	-0.063	0.203
95% Confidence	-0.173 to 0.219	-0.459 to -0.052	-0.405 to -0.021	-0.439 to -0.024	0.032 to 0.405	0.0572 to 0.426	-0.256 to 0.135	0.007 to 0.384
p value	0.813	0.016	0.031	0.030	0.023	0.012	0.535	0.043
Significance of correlation	NS	*	*	*	*	*	NS	*

LH; Leutinizing hormone, FSH; Follicle stimulating hormone, IF-SCH; Infertile females with subclinical hypothyroidism, BMI; Body mass index, * $p<0.05$, NS; not significant.

A correlation study between PRL and LH was also carried out to find out the relation between PRL and gonadotropin (LH). We found a significant negative correlation in IF-SCH subjects (r=-0.231, p=0.021, Fig 4.18B, Table 4.9), while in the Control group (r= 0.091, p=0.370, Fig. 4.18A, Table 4.9) no correlation was reported. The PRL-FSH correlation revealed a significantly very high negative correlation in IF-SCH female subjects (r=-0.281, p=0.005, Fig. 4.19B, Table 4.9) as compared to Control subjects (r=-0.235, p=0.018, Fig. 4.19A, Table 4.9).

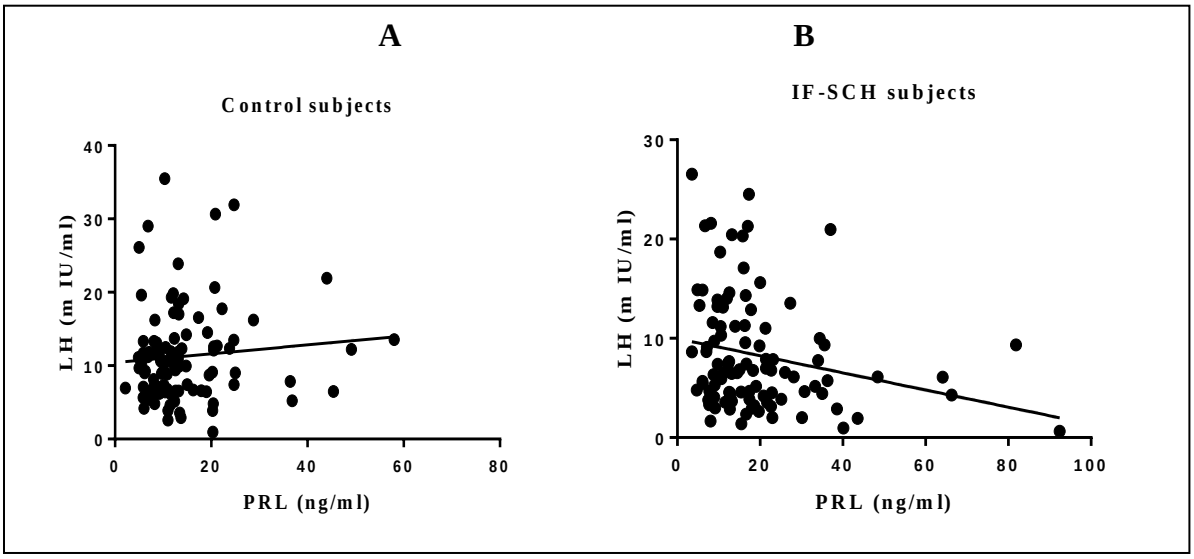


Figure 4.18 PRL and LH correlation in Control subjects and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing insignificant positive correlation (r= 0.091, p=0.370) between PRL and LH levels. **B .**Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=100) showed significant group showed negative correlation (r=-0.231, p=0.021) between PRL and LH levels.

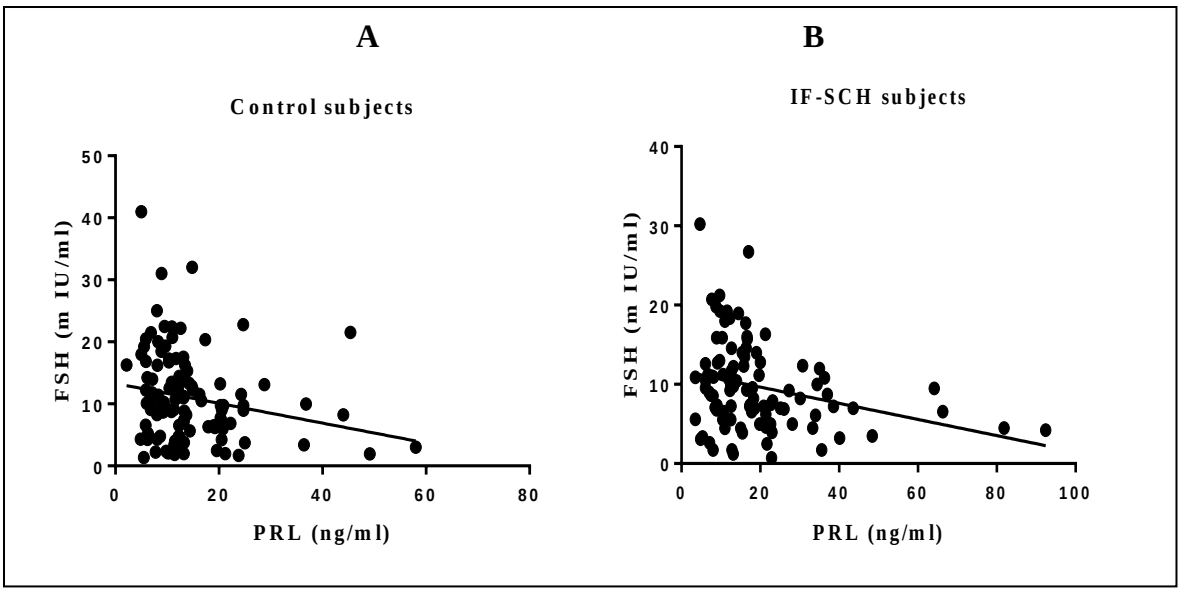


Figure 4.19 PRL and FSH correlation in Control and IF-SCH subjects: A. Correlation pattern in Control subjects- Control subjects (n=100) were showing significant (r=-0.235, *p*=0.018) negative correlation between PRL and FSH levels. **B.** Correlation pattern in IF-SCH subjects- IF-SCH subjects (n=100) showed significant (r=-0.281, *p*=0.005) group showed significant negative correlation between PRL and FSH levels.

Table4.9 Correlation between PRL and LH, FSH

	PRL with LH		PRL with FSH	
No. of XY pairs	**Control**	**IF-SCH**	**Control**	**IF-SCH**
	100	100	100	100
Pearson r	0.091	-0.231	-0.235	-0.281
95% Confidence	-0.108 to 0.282	-0.409 to -0.036	-0.413 to -0.041	-0.453 to -0.089
p value	0.370	0.021	0.018	0.005
Significance of correlation	Ns	*	*	**

PRL; Prolactin, LH; Leutinizing hormone, FSH; Follicle stimulating hormone, IF-SCH; Infertile females with subclinical hypothyroidism, , ns; not significant, * $p<0.05$

4.6 Discussion

In the present study we report increased PRL and decreased LH and FSH levels in primarily infertile females with subclinical hypothyroidism. PRL levels were significantly high precipitating to hyperprolactinemia at 37% prevalence. Secondly, we report a high prevalence of menstrual irregularities which is found to be 89% with oligomenorrhea in 15%, followed by menorrhegia in 7% of subclinical hypothyroid infertile females. Further the study reports a positive correlation between increased subclinical hypothyroidism and hyperprolactinemia, and also between hyperprolactinemia and increased age and BMI. A positive correlation is also reported between the levels of LH and FSH in infertile females. While a negative correlation was reported between hyperprolactinemia and LH, FSH, fT_3, fT_4 levels in infertile females suffering from subclinical hypothyroidism as compared to healthy parous control females.

Thyroid dysfunction in the form of Hypothyroidism is a common thyroid disorder which is known to adversely affect the female infertility. A close relationship exists between the HPT and HPO axis (Doufas A.G. & Mastorakos G., 2000). Hormonal disorders of female reproductive system are comprised in dysfunction of hypo-thalamic-pituitary ovarian axis. Fertility in females is the result of coordination of a range of hormone such as TSH, PRL, LH and FSH. This hormonal cascade required to be at the optimal level for reproduction to take place and thus alterations in these hormonal levels results in infertility among women of childbearing age (Prasad *et al.,* 2015).

Hypothyroidism associated hyperprolactinemia is the most common and prevalent hypothalamic pituitary axis disorder in women of reproductive age (M.Bals-Pratsch *et al.,*

1997; Choudhary *et al.,* 1995; Doufas *et al.,* 2000). Hypothyroidism in the absence of hyperprolactinemia can also result in infertility as thyroid hormones are required for the production of both estradiol and progesterone.

Various studies have reported about the effect of overt/clinical hypothyroidism and associated hyperprolactinemia as well as altered gonadotropin levels on the reproductive system and thus disturbing female fertility, but studies reporting the effect of subclinical hypothyroidism on prolactin (PRL) levels and associated gonadotropins; LH and FSH levels leading to female infertility for the Indian population is very scarce especially for Western India. The current understanding of the effect of increased thyrotropin releasing hormone levels in subclinical hypothyroidism (SCH) and an associated increase PRL levels which is termed as hyperprolactinemia which causes a decrease in LH and FSH levels resulting in female infertility (Fupare *et al.,* 2015; Chowdhury *et al.,* 2019; Goel *et al.,* 2015) requires retrospective studies with larger sample size. Further the available data on this topic is inadequate with largely varied results. This study therefore has been carried out to understand the alteration in reproductive hormone profile and its cause. The intent was to estimate serum PRL, LH and FSH levels and determine its correlation with subclinical hypothyroidism in primary infertile females with subclinical hypothyroidism (IF-SCH) so as to draw a causal relationship (if it exists) to female infertility in Gujarat population.

We found statistically significant PRL levels in IF-SCH females. The PRL levels in infertile euthyroid (IF-ET: Infertile females with normal TSH and fT3and fT4 levels) group did not show significant increase suggesting that the increase in PRL levels causing hyperprolactinemia is due to subclinical hypothyroidism in IF-SCH females. The prevalence of hyperprolactinemia in our study was 37% in IF-SCH subjects which can be attributed to subclinical hypothyroidism. Data in the literature have reported about 40 to 70% of hypothyroid infertile females with hyperprolactinemia and subclinical hypothyroidism (Goldsmith *et al.,* 1952; Serri *et al.,* 2003). Raber et al reported about 0%–40% are hyperprolactinemia in his study (Raber *et al.,* 2003). Affia Tasneem et al has reported a higher prevalence of hyperprolactinemia in females suffering from the thyroid disorders (Tasneem *et al.,* 2011), Turankar et al. (2013) has also reported the prevalence of hyperprolactinemia in overt as well as subclinical hypothyroid female subjects. Meire et al. (2003) reported about 18.5% of females having hyperprolactinemia in their study. Similar results were obtained in a study by Bahar et al. (2011) Sirohi et al. (2018) reporting 18 % of hyperprolactinemia prevalence in SCH females in their two different independent studies

Kumkum et al. (2006) has also reported Hyperprolactinemia in hypothyroid infertile women. Increased TSH along with hyperprolactinemia was mentioned in a study by Goswami et al. (2009). Though in the normal range, increase in serum TSH and PRL levels, were reported in several studies (Saxena *et al.,* 2016, Lal *et al.,* 2016., Sharma *et al.,* 2013., Hivre *et al.,* 2013). Verma et al. (2012) reported 18.3% hyperprolactinemia in her study in north Indian population, while another study reported 41% of prevalence in south Indian population. Pratinidhi *et al.* (2018) also confirms the high prevalence 48.2% of hyperprolactinemia in hypothyroid females. As per the study by Fupare et al. (2015) a greater percentage of infertile women with hypothyroidism exhibit hyperprolactinemia. Mehra D et al. (2018) also reported 46.15 % hypothyroid females have hyperprolactinemia. Thus overall, the prevalence of hyperthyroidism associated with hyperprolactinemia is reported to be ranging from 39% to as high as 57%; but research on the prevalence of hyperprolactinemia in subclinical hypothyroidism is inadequate and with varying results. The present study intended to focus on this neglected area and we report a significantly high prevalence of hyperprolactinemia (37%) in subclinical hypothyroidic infertile female population of Gujarat region.

Hypothyroidism can be the cause of the various menstrual abnormalities such as menorrhagia, polymenorrhoea, oligomenorrhoea, anovulatory cycle and thus causing infertility. Menstrual irregularities occur before the onset of overt hypothyroidism, which is the subclinical hypothyroid condition. Menstrual cycle is regulated by thyroid hormones, PRL, LH and FSH. Hyperprolactinemia inhibits secretion of FSH and LH thus alters the process of ovulation resulting in female infertility (Goswami *et al.,* 2009; Scott *et al.,* 1989; Mohan *et al.,* 2010; Sharma *et al.,* 2012; Abdelsalam KE and Ibrahim W., 2015). Worldwide Oligomenorrhea is found to be a common type of menstrual disorder among the women of reproductive age. To study the role, of subclinical hypothyroidism on female reproduction, the present study evaluated the menstrual irregularities in IF-SCH and control females. We report 89% of IF-SCH females with menstrual irregularities. The most prevalent menstrual abnormality in our study population was oligomenorrhea which was 15%, followed by menorrhegia in 7% in infertile females suffering from subclinical hypothyroidism. Data in the literature state about the prevalence of menstrual disturbances in hypothyroidism with varying magnitude. In a study by Acharya et al 57.5% patients had menstrual irregularities and 28.2% had oligomenorrhoea followed by 17.39% menorrhagia, while 21.73 % were infertile in subclinical hypothyroidic group (Acharya *et al.,* 2011). Armada-Dias et al. (2001) also reported oligomenorrhea followed by menorrhagia as the most prevailing menstrual

dysfunction. Krassas et al. (2000) in their study concluded that hyperprolactinemia resulting from primary hypothyroidism causes ovulatory dysfunction which is on account of insufficient corpus luteal progesterone secretion. Hyperprolactinemia also causes oligomenorrhoea or amenorrhoea as reported in the studies. Kumkum et al. (2006) and Kundu et al. (2021) have reported amenorrhoea in hyperprolactinemic patients who all had hypothyroidism and infertility. Turankar et al. (2013) also reported the alteration of menstrual cycle. Goswami et al. (2009) reported 61.2% infertile females with menstrual abnormalities and confirmed the association of hypothyroidism and hyperprolactinemia with amenorrhoea. Poppe et al. (2007) concluded that hypothyroidism is associated with a broad spectrum of reproductive disorders ranging from abnormal sexual development to menstrual irregularities and infertility. Kundu et al. (2021) reported 48% of the infertile women had menstrual disturbances. In other studies, Mehra *et al*. (2018) reported 57% and Sharma *et al*. (2013) reported 56% females presenting with menstrual abnormalities. All these studies confirm our finding that menstrual abnormalities are found in hypothyroid and subclinical hypothyroid infertile females with hyperprolactinemia.

Thyroid hormones act synergistically with FSH and LH on ovary to secrete and maintain the normal level of estrogen and progesterone, important for a regular menstrual cycle (Poppe et al. 2003; Poppe *et al.,* 2007). Similarly, high level of prolactin hormone can inhibit follicular estradiol production and gonadotropin cyclicity leading to anovulation (Poppe *et al.,* 2007). Hyperprolactinemia inhibits the secretion and action of LH and FSH at growing follicles in the ovary and thus affects positive feedback on gonadotropins leading to follicular immaturity and consequently infertility with anovulation (Kalsum *et al.,* 2002). Hyperprolactinemia causes delay in LH response leading to abnormal follicular development and anovulation. Hypothyroidism also alters the peripheral metabolism of estrogen by decreasing sex hormone binding globulin production. This is another pathway resulting in abnormal feedback at pituitary level impairing fertility (Poppe *et al.,* 2007; Emokpae *et al.,* 2011). FSH stimulates follicle development in the ovaries and is often used as a gauge of ovarian function. Elevated FSH level indicate poor follicle development and consequently, anovulatory cycles. Reduced levels of FSH may indicate hyperprolactinemia. LH triggers the release of the ovum from the ovary. Elevated LH levels can indicate ovarian dysfunction. Reduced levels of LH may indicate hyperprolactinemia and indicates a lack of secretions by the pituitary gland in general (Berinder *et al.,* 2007).

The data on alteration in gonadotropin levels due to subclinical hypothyroidism is scarce in literature, hence the study aimed to find out the effect of SCH on levels of gonadotropins and its correlation to SCH and to attribute a causal link to LH as FSH precipitating factors of female infertility in infertile women of reproductive age. The present study demonstrates that there is a significantly low level of LH in IF-SCH females as compared to controls group. Further FSH levels were also reported to be significantly highly low in IF-SCH having subclinical hypothyroidism and associated hyperprolactinemia, oligomenorrhea and menorrhegia. In the present study we report hypogonadism (low serum LH and FSH levels) in Acharya et al. (2011). K Mohan et al. (2010) showed increased PRL along with a decreased in LH and FSH levels in women with primary infertility. Bohnet et al. (1976), Matsuzaki et al. (1994) and Fupare et al. (2015) also reported decreased FSH and LH and higher levels of PRL in primary infertile women. As reported by Goswami et al. (2009) hyperprolactinemia in hypothyroid women causes amenorrhoea which is because of low LH and FSH levels. A study done by Digban et al. (2018) revealed that 30% (and Progesterone) infertile women had low FSH, LH. In the study by Suwal et al. (2018) 46.6% infertile women had with increase PRL, LH and FSH levels and and 43.3% women presented with infertility. Olooto et al.(2012) showed a decrease in LH and FSH in 56.2% and 51.0% of women with oligomenorrhea respectively.

A correlation study was carried out to find out and correlate the effect of an increased TSH levels with increased PRL and decreased gonadotropins (LH and FSH). PRL and LH and FSH levels were also compared and studied to find out the correlation with demographic characteristics (age and BMI) in subclinical hypothyroid primary infertile females (IF-SCH), infertile euthyroid (IF-ET) and Control subjects. The study reports a significant positive correlation between PRL and TSH in IF-SCH subjects, while the Control and IF-ET group did not report any correlation suggesting a positive correlation of subclinical hypothyroidism with hyperprolactinemia in infertile females. Our study is in accordance with studies reporting a positive correlation of PRL with TSH. (Bassey et., 2015; Hekimsoy *et al.,* 2010; Al Nahi *et al.,* 2014; Goel P *et al.,* 2015). We further report a significant negative correlation between hyperprolactinemia and fT_3 as well as fT_4 in primary infertile IF-SCH subjects as well as in Control group. Some other studies have reported similar findings (Pratinidhi *et al.,* 2018; Fupare *et al.,* 2015; Hekimsoy *et al.,* 2010). Hyperprolactinemia was also found to be positively correlated with the age in IF-SCH subjects but not in Control and euthyroid

infertile subjects. Further, PRL and BMI correlation study also showed a significant positive correlation in IF-SCH group but not in Control females.

To correlate SCH with gonadotropins (LH and FSH), the correlation study was carried out in Control and IF-SCH subjects. The correlation study between LH and TSH revealed a significant negative correlation in IF-SCH subjects. FSH and TSH correlation study also reported decreasing FSH levels with increase in TSH levels resulting in a negative correlation of SCH with FSH levels in IF-SCH subjects. Our finding supports the data of various studies showing a negative correlation of hypothyroidism/subclinical hypothyroidism with LH and FSH levels. Fupare et al. (2015) in their study showed that levels of both LH & FSH are negatively correlated with TSH and PRL levels. Chaudhary et al. (1995) has also shown a significantly negative correlation between elevated TSH levels in subclinical hypothyroidism infertile subjects with LH and FSH. Further a positive correlation was observed between FSH and LH in IF-SCH, confirming decreased levels of gonadotropins in subclinical hypothyroidism. Similarly Fupare et al. (2015) reported LH and FSH showing a strong positive correlation.

A correlation study between elevated PRL levels (hyperprolactinemia) and LH, FSH was also carried out to find out the relation between PRL and decreased gonadotropin levels LH and FSH (hypogonadism). We found a significant negative correlation in IF-SCH subjects. The PRL-FSH correlation revealed a significant negative correlation in Control subjects, and a significantly very high negative correlation in IF-SCH female subjects. Therefore, we infer that hyperprolactinemia and subclinical hypothyroidism play a key role in consequence of female infertility. Similar results were also obtained by a study conducted by Fupare et al. (2015) and other similar findings (Sadler *et al.,* 2004; Veena *et al.,* 2008).

The correlation between LH and age in the present study shows a non-significant negative correlation both in Control and IF-SCH subjects. Studies have shown that women with increased BMI have fertility problems (WHO 2000/Infertility; Catalano *et al.,* 2007; Gesink *et al.,* 2007; Verma *et al.,* 1982). Correlation studies between BMI values and serum LH levels, showed a negative correlation in IF-SCH group females. A negative correlation between FSH and age was observed in both Control and IF-SCH females. FSH and BMI correlation study revealed a positive correlation in both Control and IF-SCH females. Thus the present study reveals that infertility increases with increasing BMI which in turn increases the insulin levels. The net result of these changes is low estrogen levels which do not allow the ovaries to release eggs making a woman infertile.

4.7 Conclusions

Alterations in hormones of HPT-HPO axis interferes with reproductive ability. Hence it is very essential to keep them in complete balance while trying to conceive. FSH, LH, PRL and thyroid hormones are essential for the development and function of reproductive system and each of them need to be investigated in cases of infertility. While overt hypothyroidism is acknowledged and treated, subclinical hypothyroidism mainly remains neglected due to asymptomatic nature. Overt hypothyroidism associated hyperprolactinemia and consequent decrease in reproductive hormones finally causing fertility complications in women has been discussed adequately in several studies; but research on the prevalence, extent and correlation of hyperprolactinemia with subclinical hypothyroidism are few and with contradicting results. The present study reports a significantly high prevalence of hyperprolactinemia with subclinical hypothyroidism along with decreased LH and FSH levels subsequently causing menstrual abnormalities and consequently infertility especially in females with higher age and BMI of Gujarat region.

In the present study, we report elevated PRL levels and a decrease in LH and FSH levels in primarily infertile females suffering from subclinical hypothyroidism. Further the present study reports a high prevalence of menstrual irregularities with oligomenorrhea and menorrhea as the common menstrual disorders in subclinical hypothyroid infertile females. A positive correlation was observed between increased PRL and TSH, age and BMI. LH and FSH also show a positive correlation. While a negative correlation was reported between PRL and LH, FSH, fT_3, fT_4. BMI and age show a negative correlation with low LH and FSH levels in infertile females having subclinical hypothyroidism.

Thus, low LH and FSH along with disturbed menstrual cycles and oligomenorrhea as predominant menstrual disorder can be considered as the outcome of SCH in infertile females having higher age and high BMI; all of these precipitating into female infertility. Reproductive hormone profile evaluation should be considered as an early infertility work up for the primary infertile females with SCH and corrective measures to be taken if there is an anomaly. Thus, infertility work up of a woman requires a multidimensional diagnostic approach with hormonal assay including thyroid hormones especially TSH and prolactin levels, regardless of their menstrual cycle pattern at the time of first consultation. All SCH patients should asses the levels of PRL, LH and FSH. We advocate that subclinical hypothyroidism should not be ignored and hormonal assay should be one of the diagnostic tools in the management of primary infertility among the women of childbearing age. More

studies are needed to give a clear vision on the roles of hormones in causing female infertility.

Conclusively, we recommend that all primary infertile females must be screened for subclinical hypothyroidism as a preliminary diagnostic approach to identify the subsequent alteration in reproductive hormonal profile precipitating to infertility. This would help to minimize the untargeted efforts and be less stressful for the desiring would be happy mothers.

4.8 References

Abdelsalam KE, Ibrahim W. Relationship between TSH, T4, T3 and Prolactin in overweight and lean Sudanese PCOS Patients. International Journal of Biomedical Research. 2015;6(2):108-12.

Acharya N, Acharya S, Shukla S, Inamdar SA, Khatri M, Mahajan SN. Gonadotropin levels in hypothyroid women of reproductive age group. The Journal of Obstetrics and Gynecology of India. 2011 Oct;61(5):550-3.

Al-Nahi AS, Ajeena EH, Al-Khafagi SH. Correlation of Prolactin and Thyroid Hormones levels with Menstrual Patterns in Infertile Women. International Journal of Scientific & Engineering Research. 2014;5(12):1368.

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 Sep;34(9):1209-15.

Bahar A, Akha O, Kashi Z, Vesgari Z. Hyperprolactinemia in association with subclinical hypothyroidism. Caspian journal of internal medicine. 2011;2(2):229.

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

Bassey IE, Udoh AE, Essien OE, Isong IK, Gali RM, Archibong EE. Thyroid hormones and prolactin levels in infertile women in southern Nigeria. Journal of clinical and diagnostic research: JCDR. 2015 Mar;9(3):OC13.

Berinder K, Hulting AL, Granath F, Hirschberg AL, Akre O. Parity, pregnancy and neonatal outcomes in women treated for hyperprolactinaemia compared with a control group. Clinical endocrinology. 2007 Sep;67(3):393-7.

Bohnet HG, Dahlen HG, Wuttke W, Schneider HP. Hyperprolactinemic anovulatory syndrome. The Journal of Clinical Endocrinology & Metabolism. 1976 Jan 1;42(1):132-43.

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

Burrow GN. The thyroid gland and reproduction. In: Yen SSC, Jaffe RB, eds. Reproductive endocrinology. Philadelphia: WB Saunders, 1986:424–40.

Catalano PM. Management of obesity in pregnancy. Obstetrics & gynecology. 2007 Feb 1;109(2):419-33.

Choudhury SD, Goswami A. Hyperprolactinemia and reproductive disorders--a profile from north east. The Journal of the Association of Physicians of India. 1995 Sep 1;43(9):617-8.

Chowdhury SG, Sarkar AK. Comparative study of Serum PRL, LH, FSH in overt and subclinical hypothyroid females in reproductive age group. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2019;18(1):65-8.

Digban KA, Adu ME, Jemikalajah JD, Adama S. Hormonal profile of some infertile women in bida Nigeria. Libyan Journal of Medical Sciences. 2018 Jan 1;2(1):26.

DISTILLER LA, SAGEL J, MORLEY JE, OXENHAM E. Assessment of pituitary gonadotropin reserve using luteinizing hormone-releasing hormone (LRH) in states of altered thyroid function. The Journal of Clinical Endocrinology & Metabolism. 1975 Mar 1;40(3):512-5.

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

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

Fupare S, Gadhiya BM, Jambhulkar RK, Tale A. Correlation of thyroid hormones with FSH, LH and Prolactin in infertility in the reproductive age group women. Age. 2015;23(2.48):216-22.

Gesink Law DC, Maclehose RF, Longnecker MP. Obesity and time to pregnancy. Human reproduction. 2007 Feb 1;22(2):414-20.

Gharib H, Tuttle RM, Baskin HJ, Fish LH, Singer PA, McDermott MT. Subclinical thyroid dysfunction: a joint statement on management from the American Association of Clinical Endocrinologists, the American Thyroid Association, and the Endocrine Society. The journal of clinical endocrinology & metabolism. 2005 Jan 1;90(1):581-5.

Goel P, KahKaSha SN, GuPta BK, Goel K. Evaluation of serum prolactin level in patients of subclinical and overt hypothyroidism. Journal of clinical and diagnostic research: JCDR. 2015 Jan;9(1):BC15.

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

Goswami B, Patel S, Chatterjee M, Koner BC, Saxena A. Correlation of prolactin and thyroid hormone concentration with menstrual patterns in infertile women. Journal of reproduction & infertility. 2009 Oct;10(3):207.

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

Hekimsoy Z, Kafesçiler S, Güçlü F, Özmen B. The prevalence of hyperprolactinaemia in overt and subclinical hypothyroidism. Endocrine journal. 2010:1009160482-.

Hivre MD, Bhale DV, Mahat RK, Bujurge AA. Study of serum TSH and prolactin levels in patients of female infertility. Int J Rec Tre Sci Tech. 2013;9:144-5.

Honbo KS, Van Herle AJ, Kellett KA. Serum prolactin levels in untreated primary hypothyroldism. The American journal of medicine. 1978 May 1;64(5):782-7.

K Mohan MS. Follicle Stimulating Hormone, Luteinizing Hormone and Prolactin Levels in Infertile Women in North Chennai, Tamilnadu. J Bio sci Res 2010;Vol. 1(1):6.

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

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

Kumkum A, Jasmine K, Shweta G, PAL AN. Hyperprolactinema and its coorelation with hypothyroidism in infertile women. J ObstetGynaecol India,2006;56;68-71.

Kundu S, Rao SS, Singh K, Rao R. Study of thyroid profile and prolactin levels in female infertility patients: An institutional analysis. Journal of the Scientific Society. 2021 Jan 1;48(1):13.

Lal RZ, Biyani S, Lodha R. Correlation of thyroid hormones with FSH, LH and Prolactin in infertility in the Reproductive Age Group women. IAIM. 2016;3(5):146-50.

Matsuzaki T, Azuma K, Irahara M, Yasui T, Aono T. Mechanism of anovulation in hyperprolactinemic amenorrhea determined by pulsatile gonadotropin-releasing hormone injection combined with human chorionic gonadotropin. Fertility and sterility. 1994 Dec 1;62(6):1143-9.

Mehra D, Gupta HP, Singh S, Gupta U, Chandra A. Evaluation of thyroid and prolactin levels and its correlation in patients with Infertility. Evaluation. 2018 Dec;4(12).

Meier C, Christ-Crain M, Guglielmetti M, Huber P, Staub JJ, Müller B. Prolactin dysregulation in women with subclinical hypothyroidism: effect of levothyroxine replacement therapy. Thyroid. 2003 Oct 1;13(10):979-85.

Olooto WE, Adeleye AO, Amballi AA, Mosuro AO. Pattern of reproductive hormones (follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, and prolactin) levels in infertile women in Sagamu South Western Nigeria. Der Pharmacia Lettre. 2012;4(2):549-53.

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

Poppe K, Velkeniers B, Glinoer D. Thyroid disease and female reproduction. Clin Endocrinol (Oxf). 2007;66(3):309-21. Review.

Poppe K, Velkeniers B. Thyroid disorders in infertile women. Ann Endocrinol (Paris). 2003;64(1):45-50.

PR L. Davies TF, Hay ID. The thyroid gland. William's textbook of endocrinology. Philadelphia: WB Saunders. 1998:389-515.

Prasad B, Parmar D, Sharma NC. A Study on Serum FSH, LH and prolactin levels among infertile women. Int J Med Res Health Sci 2015;4:876-8.

Pratinidhi SA, Zope RD, Alka S. Correlation of prolactin levels with thyroid hormone levels in thyroid disorders, infertility and menstrual disorders. International Journal of Clinical and Biomedical Research. 2018 Apr 15:12-5.

Raber W, Gessl A, Nowotny P, Vierhapper H. Hyperprolactinaemia in hypothyroidism: clinical significance and impact of TSH normalization. Clinical endocrinology. 2003 Feb;58(2):185-91.

Roberts CG, Ladenson PW. Hypothyroidism. Lancet. Hypothyroidism. Lancet. 2004;363(9411).

Sadler T.W. Longmans medical embryology, 9th ed, Lippincott Williams Wilkins, Philadelphia, pp 3- 49, 2004.

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

Scott MG, Ladenson JH, Green ED, Gast MJ. Hormonal evaluation of female infertility and reproductive disorders. Clinical chemistry. 1989 Apr 1;35(4):620-9.

Serri O, Chik CL, Ur E, Ezzat S. Diagnosis and management of hyperprolactinemia. Cmaj. 2003 Sep 16;169(6):575-81.

Sharma N, Baliarsingh S, Kaushik GG. Biochemical association of hyperprolactinemia with hypothyroidism in infertile women. Clinical laboratory. 2012 Jan 1;58(7-8):805-10.

Sharma P, Suvama P, Nitin T. Female infertility and its correlation with serum prolactin and TSH concentration-an unmatched case control study. J Pharm Biomed Sci. 2013;30(30):902-7.

Sirohi T, Singh H. Estimation of serum prolactin levels and determination of prevalence of hyperprolactinemia in newly diagnosed cases of subclinical hypothyroidism. Journal of Family Medicine and Primary Care. 2018 Nov;7(6):1279.

Suwal R, Maharjan BR, Nepal AK, Lamsal M, and Baral N, "Prolactin and Reproductive Hormone Status in Women with Oligomenorrhea and Infertility Problem," International Research Journal of Pharmacy and Medical Sciences (IRJPMS), Volume 1, Issue 4, pp. 52-54, 2018.

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

Turankar S, Sonone K, Turankar A. Hyperprolactinaemia and its comparision with hypothyroidism in primary infertile women. Journal of clinical and diagnostic research: JCDR. 2013 May;7(5):794.

Valenti G, Ceda GP, Denti L, Tarditi E, Speroni G. Gonadotropin secretion in hyperthyroidism and hypothyroidism. Ricerca in clinica e in laboratorio. 1984 Jan;14(1):53-63.

Veena BS, Upadhya S, Adiga SK, Pratap KN. Evaluation of oxidative stress, antioxidants and prolactin in infertile women. Indian journal of clinical biochemistry. 2008 Apr;23(2):186-90.

Verma BL, Kumar A, Srivastava RN. Measurement of body-build based on weight and height: and index for adults in an Indian population. Indian Journal of Public Health. 1982 Jul 1;26(3):133-43.

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)