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Longitudinal Changes in Thyroid Hormones During Conception Cycles and Early Pregnancy

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Abstract

Objective: The aim of this study was to characterize changes in free triiodothyronine (fT3), free thyroxine (fT4) and thyroid-stimulating hormone (TSH) during the follicular and luteal phase and during subsequent early pregnancy in individual women.

Method: TPOAb negative women with a viable pregnancy (n=49) had fT3, fT4 and TSH measured longitudinally in serum samples at baseline/non-pregnant (gestation week 0), ovulation (gestation week 2), mid-luteal phase (gestation week 3) and twice weekly from gestation weeks 4 to 6.5. Patient groups received in their conception cycle either no medication (n=13), low ovarian stimulation, (n=17) or controlled ovarian hyperstimulation (COH) for IVF treatment (n=19).

Results: Women receiving COH had a transient drop in TSH at the time of ovulation followed by a peak at mid-luteal (p=0.024). Levels of fT3 and fT4 at each gestation week were not significantly different between the treatment groups, whereas TSH levels were significantly higher at all gestation weeks (p=0.036) in the COH group compared to the natural and low stimulation groups. There were significant changes in thyroid function once pregnancy was established (gestation week 4) through to gestation week 6.5, with a gradual decrease in serum fT3 (r=-0.104, p=0.030) and TSH (r=-0.123 p=0.031), whilst fT4 levels remained constant. 3 women (6.1%) had TSH levels >4.0 mU/L during their pregnancy although these were isolated measurements.

Conclusion: Thyroid hormones in individual women did not remain constant but showed discrete changes. TSH was significantly lower at time of ovulation in women who received high doses of ovarian stimulation medication for IVF, and was higher throughout pregnancy than for the other groups. Serum fT3 and TSH decreased significantly during early pregnancy irrespective of medication given in the conception cycle.

Keywords: Thyroid-stimulating hormone; Free triiodothyronine; Free thyroxine; Pregnancy

Introduction

Maternal thyroid hormones play a critical role in fetal development, particularly in early pregnancy, since the fetal thyroid gland does not become functional until the second trimester. TSH levels are known to decrease during the first trimester, correlated with an increase in human chorionic gonadotropin (hCG) [1]. The decrease in TSH is most likely due to a weak stimulatory effect of hCG on TSH receptors on the thyroid. The most commonly accepted reason for TSH reducing slightly in the first trimester is that there is homology between the alpha subunits of both TSH and hCG [2]. As such, hCG can act on TSH receptors at the thyroid and stimulate them, thereby increasing T4 and T3. This feed back to the hypothalamus, decreasing TRH, TSH and, in turn, decreasing T3 and T4 as part of the natural negative feedback loop. TSH is therefore lowest when hCG is at its highest because hCG rises until 8-10 weeks of pregnancy and then plateaus and starts falling when TSH starts recovering. Such changes during pregnancy have resulted in the need for pregnancy-stage specific reference ranges to prevent the misdiagnosis of disease [3-5].

Thyroid autoimmunity is characterized by the presence of antithyroid antibodies. Of particular interest is the marker antithyroid peroxidase which is responsible for thyroid hormone synthesis [6]. Approximately 10-20% of euthyroid women (having TSH within a defined normal range) are positive for thyroid peroxidase antibody (TPOAb) [7,8], of which 16% will develop a TSH that exceeds 4.0 mIU/L by the end of the third trimester. About 50% of TPOAb positive women will develop postpartum thyroiditis, which is a consequence of the immunological changes that occur during pregnancy [9]. There is evidence that women who test positive for TPOAb during pregnancy tend to have higher TSH levels, which may indicate a slight impairment of thyroid function [10-13]. Numerous studies have reported that TPOAb positive women have an increased risk of miscarriage [14-19] as well as an increased risk of preterm birth [20]. Therefore, TPOAb is a strong risk factor for thyroid dysfunction both during and after pregnancy [21].

The clinical classification of thyroid dysfunction has been debated for over a decade. Only recently has a general consensus been reached that the upper limit for TSH in pregnancy is lower than in non-pregnant adults. A generally accepted upper reference interval for TSH in the first trimester of pregnancy is an upper limit of 2.5 mIU/L [9]. However, universally accepted reference ranges for thyroid function tests during pregnancy are still needed. Until then, laboratories need to adapt their own assay-dependent, gestational age specific and population specific reference ranges for thyroid testing in pregnant women [7].

Adequate treatment of thyroid conditions in pregnancy, particularly during early gestation greatly minimises pregnancy risks and complications, resulting in significantly improved outcomes and highlighting the need for correct diagnosis. Despite this need for detection of thyroid dysfunction in pregnancy, most studies that report on changes in thyroid function tests in pregnancy are cross-sectional in design, using blood obtained from pathology laboratories undertaking first trimester screening [3] or retrospective cohorts where the small changes in individual women were not the focus [22]. Whilst providing practical guidance on the interpretation of clinical screening tests and pregnancy outcomes in relation to thyroid status [23], large cohorts do not describe the changes that may occur during early pregnancy (especially prior to gestation week 12), nor are there established reference intervals at this time during pregnancy. The focus of our longitudinal study was to document changes in the concentration of thyroid hormones, namely fT3, fT4 and TSH during the follicular and luteal phase and during subsequent early pregnancy in individual women. We collected blood in 49 women in both the conception cycle and then the subsequent period of pregnancy monitoring until the time of a fetal heart on ultrasound scan at 6.5 weeks gestation.

Materials and Methods

Patients

Ethical approval to undertake this research project was given by the Joondalup Health Campus Human Research Ethics Committee (Ethics Approval Number 1414) and the Edith Cowan University Human Research Ethics Committee (Ethics Approval Number 12077). Women who attended Fertility North for fertility treatment were recruited after evidence of a positive pregnancy blood test 14 days after ovulation, namely if the serum hCG concentration was >25 IU/mL measured on a Centaur XP automated analyser (Siemens Healthcare Pty. Ltd., Bayswater, Victoria 3153, Australia). The 49 recruited pregnant women conceived either in an unstimulated monitoring 'natural' cycle (n=13), after low dose FSH stimulation with either intercourse (n=10) or intra-uterine insemination (n=7) or a high dose FSH controlled ovarian stimulation cycle (n=19) for subsequent *in-vitro* fertilisation (IVF) treatment. All women were non-smokers, iodine replete (reside in Western Australia), BMI <35 and TPOAb negative. One woman was excluded from the study that tested positive for TPOAb.

Sample collection and processing

All dates were according to the gestational age relative to Day 1 of the cycle in which conception occurred. Blood samples were collected from each woman at the following times:

a) Conception cycle. Blood was available for 49 women at baseline/non-pregnant (day 2-3), week 2 (around the time of ovulation) and week 3 (mid-luteal phase).

b) Pregnancy monitoring. Blood samples were collected in all 49 women from week 4, at the time of the serum positive pregnancy test, twice weekly until fetal heartbeat was seen by ultrasound (approximately week 6.5).

The blood samples were stored frozen at -20°C until assayed for thyroid hormones.

Thyroid assays and quality control

The precision of the Siemen's thyroid hormone reagents (fT3, fT4 and TSH) were analysed using both commercial QC material (Bio-rad Lyphochek Immunoassay Plus Control 1, 2 and 3, Irvine, CA) and pooled patient serum. The within-assay coefficient of variation, determined by analyzing 20 replicates of 3 serum pools for fT3 (4.5-5.5 pmol/L), fT4 (15.0-17.5 pmol/L) and TSH (1.8-2.6 pmol/L), was ≤ 3.1%, ≤ 5.2 and ≤ 2.8 respectively. The concentrations of each pooled sample were close to the average levels of thyroid hormones typically found in each patient. Quality control material (three levels) provided by the manufacturer were also included in each assay. The blood samples from each woman were thawed at room temperature and analyzed together on the same day in one batch to eliminate between-assay variability for each woman.

Statistical analysis

Analyses were performed using IBM SPSS Statistics Version 23. The relationships between each of the thyroid hormones during pregnancy were analyzed using bivariate correlations (2-tailed, Spearman) to determine the correlation coefficient (r) and p-value (significance). General linear models and repeated measures ANOVA were used to measure the relationships between thyroid hormones, gestation week and hCG. Differences were statistically significant when $p<0.05$. Concentrations for TSH were log-transformed and the geometric mean and standard deviation were obtained from the anti-log values.

Results

fT3 levels before and during early pregnancy

The fT3 concentrations between each of the three treatment groups were not significantly different (p=0.065) before or during early pregnancy (gestation weeks 2 to 6.5), with mean concentrations of 4.65 ± 0.12 pmol/L in the natural group, 4.52 ± 0.15 pmol/L in the low ovarian stimulation group and 4.64 ± 0.09 pmol/L in the controlled ovarian hyperstimulation group. There were no significant differences observed between gestation weeks 2-6.5 for all patients (p=0.343), although there was a gradual decreasing trend seen in early pregnancy (gestation weeks 4-6.5) (r=-0.104, p=0.030).

ft4 levels before and during early pregnancy

The fT4 concentrations between each of the three treatment groups were not significantly different (p=0.336) before or during early pregnancy (gestation weeks 2 to 6.5), with mean concentrations of 15.0 ± 0.2 pmol/L in the natural group, 14.4 ± 0.2 pmol/L in the low ovarian stimulation group and 15.0 ± 0.2 pmol/L in the controlled ovarian hyperstimulation group. There were no significant differences observed for all patients during their conception cycle or during early pregnancy (gestation weeks 2-6.5) (p=0.123).

TSH levels before and during early pregnancy

TSH levels were significantly higher between gestation weeks 0-6.5 in the controlled ovarian hyperstimulation group (p=0.036), with a mean of 1.84 ± 0.06 mIU/L compared to only 1.27 ± 0.05 mIU/L in the natural group and 1.30 ± 0.06 mIU/L in the low stimulation group. Pairwise comparisons of TSH concentrations within the controlled hyperstimulation group revealed a significant difference (p=0.024) between the time of ovulation (gestation week 2), with a mean concentration of 1.49 mIU/L, compared to a peak level of 1.97 mIU/L at mid-luteal phase (gestation week 3). There was also a trend of lower TSH levels at time of ovulation within the natural and low stimulation groups, however non-significant (p=0.106 and p=0.052 respectively). When combining all patient groups together, there was a strong significant difference between gestation weeks 2 and 3 (p=0.001) and gestation weeks 2 and 4 (p=0.008). Once pregnancy was established (gestation week 4), the mean TSH concentration decreased significantly during early pregnancy (up to gestation week 6.5) (r=-0.123 p=0.031). 19 patients (38.8%) in this study at one or more points during their pregnancy had a TSH measurement >2.5 mU/L and of these patients, 3 (6.1%) also had TSH >4.0 mU/L, although these were isolated measurements.

Relationship between thyroid hormones and hCG

fT3, fT4 and TSH were all positively correlated with each other (p<0.005). However, all significant correlations were very weak (r<0.3) and unlikely to be a direct consequence of an interaction between the hormones, with very weak effect sizes - the strongest being between fT3 and fT4 with only about 8.4% (r2). fT3 and TSH levels were negatively correlated with levels of hCG (Table 1) during early pregnancy (p=0.005 and p=0.001 respectively).

Gestation Week	Mean (mU/mL)	Sem	95% CI	
			Lower	Upper
4	251.9	30.1	191.6	312.2
4.5	1384.1	182.2	1019.2	1749
5	5187.5	565.4	4054.8	6320.2
5.5	13852.5	1167.7	11513.3	16191.6
6	29686.4	2183.5	25312.4	34060.4
6.5	53844.4	3525.2	46782.6	60906.2

Table 1: Concentrations of QhCG during early pregnancy.

Thyroid distribution in conception cycles and early pregnancy

Serum samples from all women in the study that had viable pregnancies (n=49) and tested negative for TPOAb (<28 U/ml) were used to calculate levels for TSH (median and 95% CI) and fT3 and fT4 (both mean ± 1sd) between gestation weeks 2-6.5 for each of the treatment groups (Tables 2-4). Where available, non-pregnant levels were calculated from both screening bloods (day 2 of the menstrual cycle taken prior to the conception cycle), or at gestation week 0 (day 2) of the conception cycle.

Sample type	n	TSH (mIU/L)	fT3 (pmol/L)	fT4 (pmol/L)
Adult reference range	229	0.55-4.78	3.50-6.50	11.5-22.7
Non-pregnant	13	1.43 (0.56-2.87)	4.71 ± 0.35	15.7 ± 1.8
2 weeks gestation	13	1.01 (0.39-2.55)	4.61 ± 0.44	14.2 ± 2.2
3 weeks gestation	13	1.24 (0.67-2.58)	4.50 ± 0.41	15.2 ± 1.6
4 weeks gestation	13	1.10 (0.65-3.63)	4.62 ± 0.47	15.2 ± 1.3
4.5 weeks gestation	13	1.17 (0.53-2.09)	4.63 ± 0.37	15.5 ± 1.2
5 weeks gestation	13	1.09 (0.69-2.12)	4.52 ± 0.41	15.4 ± 1.5
5.5 weeks gestation	13	0.98 (0.53-2.66)	4.65 ± 0.32	15.4 ± 1.1
6 weeks gestation	12	0.95 (0.50-2.28)	4.55 ± 0.27	15.1 ± 1.3
6.5 weeks gestation	10	1.02 (0.33-2.01)	4.53 ± 0.36	15.9 ± 2.0

Table 2: Serum concentrations of TSH (median and 95% CI), fT3 and fT4 (both mean ± 1sd) in pregnant women conceiving in a natural menstrual cycle. The adult reference range was provided by the assay manufacturer. TSH ranges obtained from screening bloods (usually day 2 of the menstrual cycle) in the few months prior to conception cycle, whilst fT3 and fT4 ranges obtained from baseline (gestation week 0) measurements.

Sample type	n	TSH (mIU/L)	fT3 (pmol/L)	fT4 (pmol/L)
Adult reference range	229	0.55-4.78	3.50-6.50	11.5-22.7
Non-pregnant	15	1.04 (0.47-2.39)	4.44 ± 0.69	14.4 ± 2.4
2 weeks gestation	15	0.95 (0.40-2.48)	4.71 ± 0.46	12.7 ± 2.6
3 weeks gestation	17	1.29 (0.53-3.12)	4.72 ± 0.42	14.4 ± 2.7
4 weeks gestation	17	1.59 (0.48-2.54)	4.66 ± 0.42	14.2 ± 2.3
4.5 weeks gestation	17	1.25 (0.52-2.40)	4.42 ± 0.44	14.0 ± 2.5
5 weeks gestation	17	1.31 (0.51-2.52)	4.57 ± 0.41	13.6 ± 2.2
5.5 weeks gestation	17	1.04 (0.48-2.43)	4.47 ± 0.39	14.0 ± 2.3
6 weeks gestation	16	1.01 (0.39-2.57)	4.39 ± 0.35	14.4 ± 2.2
6.5 weeks gestation	9	0.89 (0.54-2.69)	4.41 ± 0.41	15.2 ± 1.4

Table 3: Serum concentrations of TSH (median and 95% CI), fT3 and fT4 (both mean ± 1sd) in pregnant women conceiving in a cycle with mild ovarian stimulation. The adult reference range was provided by the assay manufacturer. TSH ranges obtained from screening bloods (usually day 2 of the menstrual cycle) in the few months prior to conception cycle, whilst fT3 and fT4 ranges obtained from baseline (gestation week 0) measurements.

Sample type	n	TSH (mIU/L)	fT3 (pmol/L)	fT4 (pmol/L)
Adult reference range	229	0.55-4.78	3.50-6.50	11.5-22.7
Non-pregnant	19	1.76 (0.62-3.56)	Not available	Not available

2 weeks gestation	19	1.49 (0.49-1.71)*	4.76 ± 0.47	14.9 ± 2.7
3 weeks gestation	19	1.97 (0.66-3.84)*	4.74 ± 0.41	15.5 ± 2.0
4 weeks gestation	19	1.81 (0.96-3.61)	4.78 ± 0.50	14.9 ± 1.8
4.5 weeks gestation	19	1.75 (0.78-3.82)	4.70 ± 0.56	15.0 ± 2.4
5 weeks gestation	19	1.94 (0.61-3.36)	4.66 ± 0.49	15.0 ± 2.5
5.5 weeks gestation	19	1.77 (0.63-3.02)	4.67 ± 0.46	15.0 ± 2.4
6 weeks gestation	19	1.66 (0.68-3.31)	4.73 ± 0.39	14.3 ± 2.2
6.5 weeks gestation	18	1.49 (0.41-2.97)	4.51 ± 0.40	14.8 ± 2.3

Table 4: Serum concentrations of TSH (median and 95% CI), fT3 and fT4 (both mean ± 1sd) in pregnant women conceiving in an IVF cycle with controlled ovarian hyperstimulation, and bled at different times in their conception cycle and during early pregnancy. *denotes values are significantly different ($p<0.05$). The adult reference range was provided by the assay manufacturer. TSH ranges obtained from screening bloods (usually day 2 of the menstrual cycle) in the few months prior to conception cycle.

Discussion

Maternal thyroid changes during pregnancy

During the first trimester, maternal changes in thyroid hormones are well known and the rise in hCG results in very mild and transient increases in fT4 and fT3, which via a negative feedback, leads to a decrease in TSH [24,25]. An increase in free T4 has been noted toward the end of the first trimester by some authors [26], but not by all studies [27], and it is important to note that measurement of thyroid hormones in pregnancy is complex. Factors such as rising Thyroid Binding Globulin (TBG) and falling albumin levels can change binding dynamics [28] and there are unknown effects on direct free thyroid hormone assays. Furthermore, it is known that levels of fT3 are largely determined by peripheral de-iodination of fT4 [29,30]. Two main types of deiodinase are found-Type I in the liver, kidney and thyroid and this enzyme determines measured serum levels of fT3. Type II deiodinase is found in the pituitary and may be differently regulated from Type I. This differential regulation of the two deiodinases can explain apparent "discrepancies" in fT4, fT3 and TSH levels. These discrepant values are common in clinical practice and can occur in acute illness such as 'sick euthyroid syndrome' [31,32] or in starvation [33], uraemia [34] or diabetes [35].

Despite the active thyroid changes that occur in the first trimester and the importance to fetal development, most published studies during this time are cross-sectional and do not look at any time points prior to gestation week 8. The current study focused on gestational weeks 4 to 6.5, with blood sampling twice weekly in individual women in order to establish longitudinal distribution of thyroid levels in early pregnancy.

In this study, there were no significant changes documented in fT4 levels during the time period, with mean concentrations from gestation week 4 to 6.5 similar to the non-pregnant concentration at week 0. We did note however, gradual small but significant decreases in fT3 from gestation weeks 4 to 6.5. As expected, TSH concentration dropped significantly with gestational age, from gestation week 4 onwards. This strongly correlated with the rapid rise in hCG during this time, which is consistent with the trend in the first trimester of pregnancy [25]. The relationships between each of the hormones fT3, fT4 and TSH during gestation were demonstrated to be statistically significant. There were no significant differences between non-pregnant (gestation week 0) and pregnant (gestation week 4) fT3, fT4 or TSH levels.

The lack of change in fT4 is not surprising as many authors note that changes in thyroid hormones are minimal because the compensatory decrease in TSH occurs very rapidly to re-establish normal free thyroid hormones. The issues of the type of measurement of fT4 in pregnancy also need to be considered and to more exactly document changes to free T4, complex and expensive methods such as ultrafiltration or dialysis have been proposed. However, these are not practical for clinical practice.

The reduction in fT3 initially appeared surprising. However, when considering the changes that occur in activity of the Type I deiodinase in states of starvation and in diabetes these changes are not dissimilar to those that occur in early pregnancy. Early pregnancy is a state where fasting rapidly results in low glucose due to the high levels of insulin and activity of lipolytic hormones. In contrast, after a meal pregnant women are more likely to be hyperglycaemic (similar to diabetic women) as they have higher levels of insulin but concurrent insulin resistance. A reduction in Type I deiodinase activity may result in reduced fT3 levels. However, in these situations, Type II deiodinase which controls pituitary T3 levels, is not affected. As such, normal/high levels of fT4 in the pituitary are converted normally to fT3 and this results in reduction of TSH from the hypothalamic –pituitary axis. It is also interesting that fT3 levels decreased in early pregnancy, a time when women often experience nausea and/or vomiting which puts the body in a state similar to starvation, resulting in decreased peripheral conversion of T4 to T3.

This study showed that all thyroid markers remained positively correlated with each other, although a very weak effect size. There is no literature that examines the interaction between fT3, fT4 and TSH during this early gestation period, so it is difficult to establish what are considered expected changes in these levels. It is known that serum fT3 and fT4 levels increase slightly at 10-12 weeks when hCG is at its highest, but remain within normal limits [36], whilst TSH concentrations decrease, fT3 and fT4 levels also decrease later in pregnancy when hCG starts to decline. Furthermore, the changes in thyroid hormones that take place at different time points during gestation are a result of complex effects that may be seen only momentarily. Hence the gradual decrease in fT3 that was observed during gestation weeks 4 to 6.5 is not likely to have any effect on the pregnancy itself, especially when levels are within expected 'normal' limits.

Thyroid function during conception cycles

This present study observed a significant difference in TSH levels between time of ovulation (gestation week 2) and time of conception (gestation week 3). Another study has observed a weak association between TSH and estradiol levels (borderline significance) which occurs at time of ovulation in the menstrual cycle [37]. In addition, this present study observed significantly higher TSH levels in women who were undertaking IVF treatment. There have been a few authors that have investigated the relationship between thyroid function and controlled ovarian hyperstimulation (COH) in IVF cycles and show that TSH increases significantly with a rise in oestrogen [38,39] and elevated TSH is linked to decreased fertilisation rates in those women

undertaking IVF treatment [37]. The reasons as to why TSH is higher in this treatment group is uncertain, especially since these patients are undertaking fertility treatment due to a number of various underlying conditions (for example endometriosis, PCOS, male factor). A large proportion of the IVF patients in this study have had difficulties conceiving in the past and have also suffered recurrent miscarriage, which is consistent with the study on higher TSH and fertilisation rates [37]. A more recent study investigating IVF patients and TSH levels following treatment reported that TSH levels peaked 1 week after the hCG trigger (mid-luteal) [40] which is supported by the results of this present study that showed TSH was significantly higher at the same time point.

There were no significant differences observed in fT3 or fT4 levels during ovulation or luteal phase in any of the treatment groups. fT4 was slightly higher (non-significant) during the mid-luteal phase (gestation week 3) in the IVF treatment group when TSH was also at its highest. These results are inconsistent with a previous study that reported a significant decrease in fT4 after ovarian stimulation (the same time point) [38]. However, the literature has shown that the relationship between fT4 and TSH is complex and non-linear [41] and this present longitudinal study has further contributed to our understanding of thyroid function at various time points during the menstrual cycle and very early pregnancy.

TSH and TPOAb in Pregnancy

There have been numerous studies that have investigated the role of thyroid hormones in maintaining early pregnancy or in recurrent miscarriage [42-49] and the literature suggests there is an increased association with miscarriage and abnormal levels of thyroid hormones. A relationship between elevated TSH serum levels and risk of miscarriage has been reported previously [50], however rates of subclinical hypothyroidism during pregnancy are very low, between 2-3% [51]. Other studies in women undergoing fertility treatment cycles have reported that elevated TSH levels (above 2.5 mlU/L) do not adversely affect clinical outcomes [22,23]. The results of this present study showed a higher than average rate of hypothyroidism (6.1%) (defined as TSH levels >4.0 mU/L), however the 3 patients with elevated TSH all had successful pregnancies. The mean TSH concentrations measured for all woman in this study were within normal ranges during early pregnancy.

Thyroid autoantibody measurement is a common marker of autoimmune thyroid disease, and women who test positive should be excluded from thyroid hormone studies, as these women may have higher TSH levels [10-13] and a greater risk of miscarriage [52]. More importantly, inclusion of these women when calculating thyroid reference intervals could result in falsely high values. This study excluded 1 woman (2.0%) from the analysis as she tested positive for TPOAb. The prevalence of 2.0% (1/50) TPOAb recorded in this study is lower than another study that reported 5.4% of women undertaking ART tested positive for TPOAb [53], although this was in a much larger cohort (n=688). The TPOAb positive woman excluded from this study had undergone an OI (low ovarian stimulation) cycle. This woman had thyroid levels within the 'normal' ranges determined in this study and was a viable pregnancy (single fetal heartbeat detected). Interestingly, there is a known elevated risk of miscarriage with the presence of TPOAb and this woman had a previously failed cycle and also had PCOS, which is associated with an increased prevalence of autoimmune thyroiditis [54,55]. This woman's medical history was an indicator of the association between reproductive disorders and thyroid dysfunction.

Thyroid reference ranges in early pregnancy

A woman's thyroid status during pregnancy is difficult to establish without gestational age-specific reference ranges. In addition to maternal thyroid changes during gestation [26], there are significant differences in reference intervals between immunoassays [56-58], highlighting the need for further research to establish reliable common limits to correctly diagnose thyroid dysfunction in pregnancy.

There is a current paucity of literature that examines thyroid reference ranges prior to gestation week 9, and most research is performed even later in the first trimester. Although the number of patients in this present study were not large enough to provide reference ranges, the data obtained is invaluable, documenting important thyroid distribution data and characterising changes that occur during early pregnancy between gestation weeks 4 to 6.5.

TSH concentrations were log transformed to normalise the data before determining appropriate concentration ranges. The median for each gestation week in the natural and low stimulation groups fell below 1.6 mU/L, with the high stimulation group having a median of above 1.66 mU/L at each gestation week except week 2 (ovulation) and week 6.5 (both 1.49 mU/L). The normal first trimester upper range recommended by the Guidelines of the American Thyroid Association is 2.5 mU/L [9]. 38.8% of patients in this study at one point during their pregnancy had at least one TSH measurement >2.5 mU/L and 6.1% of patients had at least one measurement >4.0 mU/L. Elevated TSH levels did not affect pregnancy outcome as the patients included in this study all had viable pregnancies. Levels that are considered "normal" during early pregnancy are constantly debated. The cut-off point of 2.5 mU/L for TSH, particularly for women undertaking ART, was crossed at one or more time points by a high percentage of women in this study.

Mean thyroid hormones levels in this study did not differ between non-pregnant (gestation week 0) and early pregnancy (gestation weeks 4 to 6.5). However, it is known that first trimester thyroid function is different to pre-pregnancy, in particular a decrease in TSH, which is the same trend observed in the present study. Median levels of TSH in this study (1.37 mU/L) were higher than published levels later in the first trimester (0.89 mU/L) [58] and (0.77 mU/L) [59] indicating that levels of TSH would likely continue to decrease in later first trimester weeks and therefore would end up being significantly different to non-pregnant levels. fT4 levels between gestation weeks 4 to 6.5 reported in this study for the natural treatment group (14.96 pmol/L) were similar to levels reported by another study later in the first trimester (gestation weeks 9 to 13) (15.1 pmol/L) [59]. To the best of our knowledge, fT3 data during early pregnancy has not been well documented and so the gradual decrease that was observed in this study between gestation weeks 4 to 6.5 is a novel finding.

In summary, this study has provided important thyroid distribution data for a critical time in pregnancy, when the growing fetus relies on maternal levels of thyroxine for neurocognitive development. We established that the average non-pregnant levels of all thyroid markers (gestation week 0) did not predict the levels during early pregnancy (from gestation week 4 to 6.5), however there were significant differences noted between multiple time points during conception cycles for TSH levels. It may be helpful for clinicians to be aware that thyroid levels may change week by week, despite all upper limits still

falling within the "normal" range during the first trimester. It would be useful to confirm the levels found in this study with a larger cohort and establish references ranges. Despite the known adverse effects of thyroid disease during pregnancy, the prevalence of the disease is low and further research is needed on pregnancy outcomes and treatment of women with thyroid disease.

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