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Reproductive Biology Insights

Impact of Controlled Ovarian Hyperstimulation on Thyroid Function

Kathrin Fleischer[1,2], Alex F. Muller[3], Femke P. Hohmann[1], Frank H. de Jong[4], Marinus J.C. Eijkemans[5], Bart C. Fauser[6] and Joop S.E. Laven[1]

[1]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, The Netherlands. [2]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, The Netherlands. [3]Department of Internal Medicine, Diaconessen Hospital Utrecht, The Netherlands. [4]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands. [5]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands. [6]Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, The Netherlands.

ABSTRACT

OBJECTIVE: To compare the effects of different protocols of controlled ovarian hyperstimulation on thyroid function with those of the natural menstrual cycle.

STUDY DESIGN: Prospective controlled study.

SETTING: University Medical Center.

PATIENTS: A total of 97 women without a history of endocrine disease undergoing intrauterine insemination either in a natural cycle, or with mild ovarian hyperstimulation, or in vitro fertilization (IVF).

MAIN OUTCOME MEASURES: estradiol (E_2), thyroxine binding globulin (TBG), free thyroxine (FT_4), total thyroxine (TT_4) and thyroid stimulating hormone (TSH) during the midluteal phase.

RESULTS: In the IVF group midluteal E_2, TBG, and TT_4 were significantly higher; midluteal FT_4 was significantly lower (mean difference: −1.46 pmol/L; $P < 0.001$) and midluteal TSH was significantly higher (mean difference: 0.52 mU/L; $P = 0.015$).

CONCLUSIONS: Ovarian hyperstimulation in IVF is associated with lower midluteal FT_4 and higher midluteal TSH levels compared to the natural cycle.

KEYWORDS: ovarian hyperstimulation, thyroid function, neurodevelopmental delay, Assisted Reproductive Technology (ART)

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CORRESPONDENCE: kathrin.fleischer@radboudumc.nl

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Introduction

In healthy pregnant women, the thyroid gland maintains euthyroidism, with only minor fluctuations in serum free thyroxine (FT_4) and thyroid stimulating hormone (TSH).[1,2] A high circulating chorionic gonadotropin level in the first trimester leads to interactions with the TSH receptors, prompting a temporary increase in FT_4 and the partial suppression of TSH.[1,2] Also, estradiol (E_2) promotes increased serum levels of T_4-binding globulin (TBG), accompanied by a decrease in serum FT_4, which is followed by a rise in TSH and total thyroxine (TT_4), resulting in a new equilibrium.[1,2] In women undergoing assisted reproductive technology (ART) procedures, however, controlled ovarian hyperstimulation (OH) leads to E_2 levels that are usually higher than in the first trimester of pregnancy.[2] In women with subclinical or overt hypothyroidism, or in euthyroid women with thyroid autoimmunity (TAI), there is an increased frequency of adverse pregnancy outcomes (ie, a higher risk of miscarriage, preterm delivery, and impaired

psychomotor development in the offspring).[3–10] Whether alterations of thyroid function during OH could influence ART and pregnancy outcomes is unclear. Several studies have evaluated thyroid parameters during OH,[11–14] or in the first month of pregnancy after OH.[15–17] However, the evidence concerning the effect of OH on thyroid function is contradictory,[18] probably due to the fact that alterations in thyroid function during OH are dependent on the duration and the amount of increment in E_2 levels. Our aim was to investigate the changes in thyroid function during OH. Therefore, we conducted a study to determine the effect of different regimes of OH on thyroid function during ART, and to compare these effects with the natural menstrual cycle.

Materials and Methods

Overall study design. We studied 97 women from infertile couples who presented at our center. The participants were prospectively included between January 2000 and February 2003 after their written informed consent was provided. The study was approved by the Institutional Review Board of the Erasmus University Medical Centre (Rotterdam, the Netherlands). We included 20 women who underwent intrauterine insemination (IUI) without ovarian stimulation in a natural cycle (IUI-NC), 27 women who underwent IUI with mild OH (IUI+MOH), and 50 women undergoing in vitro fertilization (IVF) who were participating in a randomized study[19] with three different stimulation protocols for assisted reproduction. The inclusion criteria for IUI-NC were: 1) age between 18 and 38 years; 2) cervical hostility; or 3) male factor infertility, according to Tygerberg's strict criteria.[20] The inclusion criterion for IUI+MOH was unexplained subfertility. The inclusion criteria in women undergoing IVF were: 1) age between 20 and 38 years; 2) body mass index between 19 kg/m^2 and 29 kg/m^2; 3) history of regular menstrual cycles ranging from 25–35 days; 4) no relevant systemic disease, severe endometriosis, or uterine or ovarian abnormalities; 5) no more than three previous IVF cycles; and 6) no previous IVF cycle with a poor response or OH syndrome.[19] The exclusion criterion for all participants in this study was history of a previous endocrine disease, as assessed from the patients' medical history. Women with known thyroid disease and/or on any thyroid medication were also excluded from the study.

E_2, TBG, FT_4, TT_4, and TSH were determined at cycle days 2–5 (baseline), at the day of (or the day before) human chorionic gonadotropin (hCG) injection, at the day of IUI/ovum pick-up (OPU) and 6–7 days after insemination/OPU (midluteal).

ART treatment. In IUI-NC, follicular growth was monitored by transvaginal ultrasound. In IUI+MOH, a daily dose of 75–150 IU of human menopausal gonadotropin (Metrodin HP®; Merck KGaA, Darmstadt, Germany) was administered subcutaneously (sc), starting on day 5 of the cycle, until a transvaginal scan showed at least one follicle with a diameter of 17–18 mm. The aim of MOH was to obtain two or three dominant follicles with a diameter of at least 18 mm. In both IUI-NC and IUI-MOH, a sc dose of 5.000 IU hCG (Profasi®, Merck KGaA) was administered when the leading follicle had reached an average diameter of 17–18 mm. IUI was performed approximately 36–40 hours later.

After assignment to IVF, patients were randomized to one of the three treatment protocols: 1) treatment with the gonadotropin-releasing hormone (GnRH) agonist, triptoreline (Decapeptyl®; Ferring B.V. Group, Hoofddorp, the Netherlands), 1 mg sc daily, starting 1 week before the expected menses; 2) treatment with recombinant follicle stimulating hormone (recFSH) 150 IU sc daily (GONAL-f®; Merck KGaA), beginning once downregulation was achieved; or 3) treatment with the same dose of recFSH, starting on cycle day 2 or 5, in combination with the GnRH antagonist, cetrorelix (Cetrotide®; Merck KGaA), at a dose of 0.25 mg sc daily, beginning when the largest follicle reached a diameter of 14 mm.[19] The aim of OH in IVF was to obtain three or more dominant follicles. Administration of both the GnRH agonist/antagonist was continued up to and including the day of hCG administration. When the patient had at least three follicles with a diameter ≥15 mm and the leading follicle had reached a diameter of ≥18 mm, administration of recFSH was discontinued, and ovulation was induced with 10.000 IU hCG sc (Pregnyl®; Schering-Plough Corporation, Kenilworth, NJ, USA). Approximately 35 hours after the hCG injection, transvaginal ultrasound-guided oocyte retrieval was performed. Subsequently, IVF with or without intracytoplasmic sperm injection was performed. Luteal support was given using the vaginal administration of 200 mg of progesterone (Progestan®; Schering-Plough Corporation) three times daily, starting on the day of oocyte retrieval until the pregnancy test.

Hormone assays. Serum from collected blood samples was coded and stored at –20°C until analysis. All samples were analyzed in a random order in one series. TSH levels were determined with the Immulite® 2000 analyzer (Siemens AG, Munich, Germany) and FT_4 with the Vitros® ECi analyzer (Ortho Clinical Diagnostics, Johnson & Johnson Medical BV, Tilburg, the Netherlands). Reference ranges were: TSH, 0.4–4.0 mU/L; and FT_4, 11–25 pmol/L. TT_4 was determined with an in-house radioimmunoassay (RIA) with a reference range of 64–132 nmol/L. TBG was determined by RIA (Brahms, Berlin, Germany). E_2 was determined by RIA (Siemens-DPC, Los Angeles, CA, USA). Inter- and intraassay coefficients of variation were, respectively: TSH <4.1% and <3.4%; FT_4 <5.4% and <2.7%; TT_4 <4.6% and <2.8%; E_2 <10.2% and <8.8%; and TBG <3.8% and <3.3%.

Statistical analysis. Statistical analysis was limited to subjects for whom both a baseline sample and at least one other sample during the study period were available. In total, 25 of 388 samples were missing (five at time point 2, two samples in IUI-NC and three samples in IUI-MOH; five at time point 3, two samples in IUI-NC and three samples in

IUI-MOH; and 15 at time point 4, four samples in IUI-NC and eleven samples in IUI-MOH). Among 78 of 97 women, sampling was complete at all time points. We excluded four women from the statistical analysis because there was no blood sample available from time point 1. The subjects undergoing IVF in the three different stimulation protocols were analyzed together in one group (IVF). Comparisons of the baseline data between the three groups were performed using the chi-square test for qualitative data and the Kruskal–Wallis test for continuous data. Results are presented as the median and range, unless otherwise indicated. A linear mixed model was used 1) to compare hormone levels at various time points during OH compared to baseline; and 2) to calculate the mean differences and 95% confidence intervals (CI) between baseline and the midluteal hormone levels, and to compare the differences between the three treatment groups. Correlations are expressed as Pearson's correlation coefficient. All data analyses were performed using SPSS version 18.0 (IBM Corporation, Armonk, NY, USA). Statistical significance was set at the $P < 0.05$ level.

Results

Baseline characteristics. The clinical and endocrine baseline characteristics of women enrolled in this study are shown in Table 1. The participants did not differ significantly with respect to their family history of thyroid disease, age, body mass index, and E_2, TBG, FT_4, and TT_4 levels at baseline. The three treatment groups differed significantly in terms of cause of infertility ($P = 0.004$) and TSH levels ($P = 0.001$). Of all patients, eight in the IVF group and none in the IUI groups underwent a fertility treatment in the previous cycle. After exclusion of these previously treated patients from the statistical analysis, similar results were obtained.

Differences in thyroid function between the time points by treatment group. Table 2 shows the mean values and 95% CI of the hormone levels by time point. Serum E_2 peaked in all groups on the day of hCG administration (visit 2); serum TBG peaked in the IUI+MOH and IVF group at the midluteal phase (visit 4); FT_4 significantly decreased in the IVF group at ovum pick-up (visit 3) and at the midluteal phase (visit 4); and TT_4 and TSH peaked in the IVF group at the midluteal phase (visit 4). Compared to baseline, TSH levels in the IUI-NC group were significantly lower upon hCG administration (visit 2) and at the midluteal phase (visit 4), whereas the TSH levels in the IVF group were significantly higher at the midluteal phase (visit 4).

Differences in thyroid function between the treatment groups. Figure 1 shows the mean hormone levels with standard error (SE) bars of the three treatment groups by time point, and Table 3 summarizes the differences of the midluteal hormone levels as they change from baseline.

Correlations. There was a significant correlation between midluteal TBG and E_2 ($r = 0.61$; $P < 0.001$; Fig. 2), between midluteal FT_4 and TBG ($r = -0.3$; $P < 0.005$; Fig. 3), and between midluteal E_2 and FT_4 ($r = -0.28$; $P = 0.01$; Fig. 4). Finally, there was no significant correlation between midluteal FT_4 and TSH ($r = 0.08$; $P = 0.47$; data not shown).

Discussion

In this prospective controlled study, we investigated the effects of different regimes of OH on thyroid function. We found higher midluteal E_2, TBG, and TT_4 serum levels and

Table 1. Patient characteristics.

	IUI-NC (n = 20)	IUI+MOH (n = 27)	IVF (n = 50)	*P*-VALUE
No. of patients with a family history of thyroid disease (%)	0	3 (11)	0	.09
Cause of infertility (%)				
Unexplained	0	27 (100)	15 (30)	.004
Male factor	20 (100)	0	19 (38)	
Tubal factor	0	0	7 (14)	
Combination (male and female factor)	0	0	4 (8)	
Other (endometriosis/ovulation disorder)	0	0	5 (10)	
Patient age (yr)[a]	31 (21–41)	32 (22–39)	33 (25–38)	.16
BMI (kg/m²)[a]	24 (19–33)	23 (20–39)	23 (20–29)	.74
Baseline E_2 (pmol/L)[a]	188 (10–824)	158 (43–469)	137 (7–389)	.11
Baseline TBG (mg/L)[a]	20 (11–26)	20 (15–24)	20 (9–30)	.11
Baseline FT_4 (pmol/L)[a]	16 (14–22)	16/13–24	14 (12–20)	.07
Baseline TT_4 (nmol/L)[a]	92 (76–127)	94 (73–118)	91 (7–129)	.08
Baseline TSH (mU/L)[a]	1.7 (0.8–4)	1.7 (0.1–4.4)	0.8 (0.1–4.4)	.001

Note: [a]Values are medians (range).

Table 2. Serum hormone levels by time points.

ART TREATMENT	VISIT	E_2 (pmol/L)	TBG (mg/L)	FT_4 (pmol/L)	TT_4 (nmol/L)	TSH (mU/L)
IUI-NC n = 20	1	185 (146–236)	19.6 (18.8–20.4)	16.6 (16.0–17.2)	95.6 (92.6–98.6)	1.92 (1.69–2.15)
	2	546 (424–704)**	18.5 (17.7–19.4)	16.4 (15.7–17.0)	92.4 (89.3–95.5)	1.54 (1.29–1.78)*
	3	352 (274–454)**	18.9 (18.1–19.8)	16.8 (16.2–17.5)	93.8 (90.7–96.9)	1.85 (1.60–2.09)
	4	428 (328–561)**	19.0 (18.1–20.0)	16.6 (15.9–17.3)	91.6 (88.3–95.0)	1.52 (1.26–1.78)*
IUI+MOH n = 27	1	164 (138–196)	19.3 (18.6–20.1)	16.4 (15.9–17.0)	94.1 (91.7–96.5)	1.72 (1.49–1.95)
	2	957 (794–1154)**	18.6 (17.8–19.4)	16.0 (15.4–16.5)	90.8 (88.3–93.4)	1.61 (1.37–1.86)
	3	616 (512–743)**	19.3 (18.5–20.1)	16.1 (15.6–16.7)	93.1 (90.5–95.7)	1.83 (1.59–2.08)
	4	556 (442–698)**	20.6 (19.6–21.6)*	15.8 (15.1–16.5)	93.2 (90.1–96.4)	1.73 (1.43–2.03)
IVF n = 50	1	127 (106–152)	19.7 (18.7–20.6)	14.8 (14.5–15.1)	93.8 (90.4–97.2)	.95 (0.81–1.09)
	2	2695 (2252–3224)**	19.1 (18.1–20.1)	14.4 (14.1–14.7)	90.1 (86.7–93.6)	.95 (0.82–1.09)
	3	1387 (1159–1659)**	20.7 (19.7–21.7)	14.2 (13.9–14.5)*	95.9 (92.5–99.3)	1.12 (0.98–1.26)
	4	1676 (1400–2005)**	24.1 (23.1–25.1)**	14.2 (13.9–14.5)**	106.4 (103–110)**	1.25 (1.11–1.39)**

Notes: Visit: 1=baseline; 2=hCG-administration; 3=intra uterine insemination/ovum pick-up; 4=midluteal.
Data are presented as means with 95% confidence intervals.
Tests of significance reflect pair wise comparisons with results obtained at visit 1.
*$P \leq .05$; **$P \leq .001$.

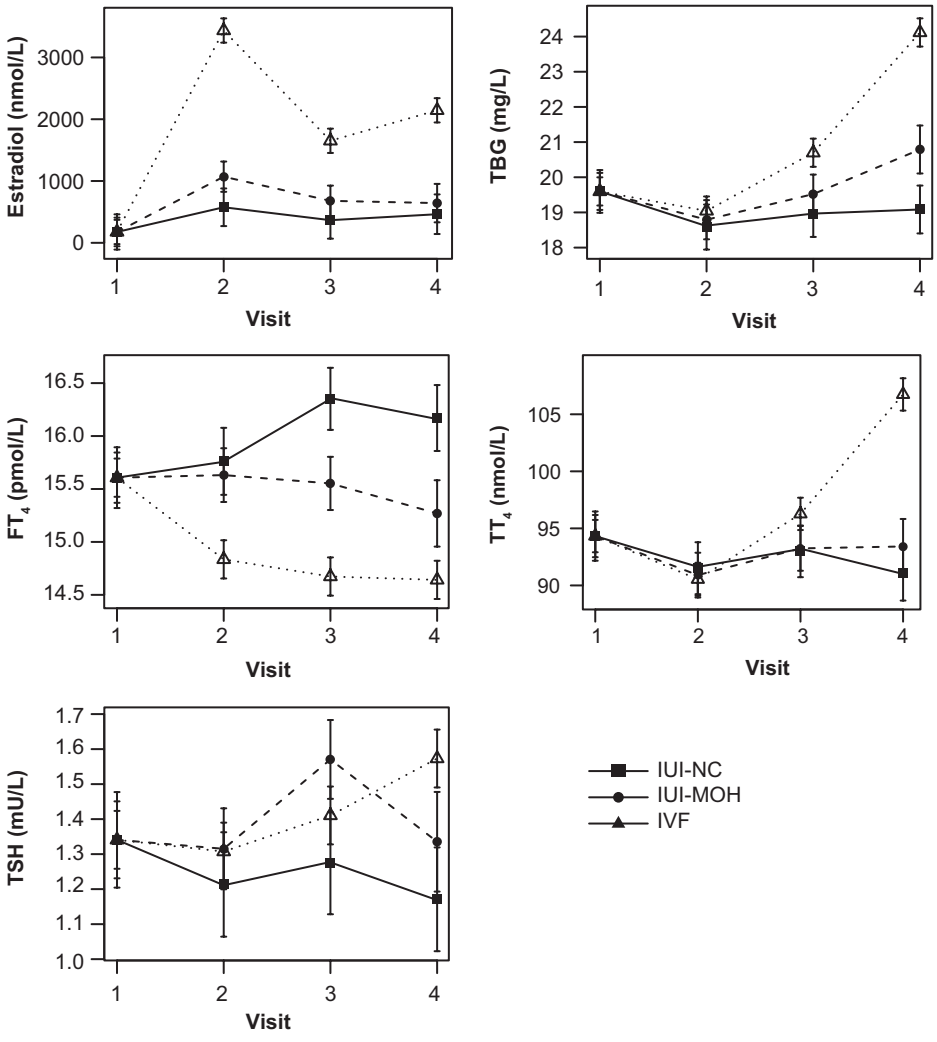

Figure 1. Hormone levels after adjustment for baseline values (mean ± SE) by treatment group and visit.
Notes: Visit 1=baseline, 2=hCG, 3=intra-uterine insemination/ovum pick-up, 4=midluteal.

Table 3. The differences of midluteal serum hormone levels between ART treatments as change from baseline.

ART TREATMENT		E_2 (pmol/L)		TBG (mg/L)		FT_4 (pmol/L)		TT_4 (nmol/L)		TSH (mU/L)	
		FD* (95% CI)	*P*	MD** (95% CI)	*P*	MD** (95% CI)	*P*	MD** (95% CI)	*P*	MD** (95% CI)	*P*
IVF versus	IUI-NC	1.47 (1.06–1.87)	.000	5.03 (2.82–7.23)	.000	−1.46 (0.63–2.29)	.001	15.9 (7.81–23.99)	.000	0.52 (0.1–0.93)	.015
	IUI+MOH	3.16 (2.12–4.76)	.000	3.36 (1.16–5.57)	.003	−0.71 (−0.11–1.53)	.08	13.24 (5.16–21.32)	.002	0.14 (−0.27–0.55)	NS
IUI+MOH versus	IUI-NC	1.37 (0.84–2.23)	.004	1.67 (−1.01–4.35)	NS	−0.75 (−1.72–0.22)	NS	2.66 (−7.17–12.5)	NS	0.38 (−0.09–0.86)	NS

Notes: *FD (fold difference) between ART treatments in change from baseline; data were back-transformed from logarithmic values because of lack of normal distribution; **MD (mean difference) between ART treatments in change from baseline.
Abbreviation: NS, not significant.

Figure 2. Correlation between midluteal TBG (linear scale) and E_2 levels (log scale).

Figure 3. Correlation between midluteal FT4 and TBG levels.

Figure 4. Correlation between midluteal E_2 (log scale) and FT4 (linear scale) levels.

lower FT_4 serum levels after OH compared to the natural cycle. Furthermore, we found higher midluteal TSH serum levels in IVF women compared to those with a natural cycle. Women undergoing OH for IVF showed the highest E_2 levels from the moment of hCG administration, and the lowest FT_4 levels during the midluteal phase. These lower FT_4 serum levels are likely to be a result of the administered FSH, resulting in elevated E_2 and consequently leading to increased TBG serum levels. Our study confirms the association between elevated E_2 and TBG serum levels, and the reduction in FT_4 levels during OH.[11] Our results also indicate that OH during IVF may induce suboptimal thyroid hormone levels around the time of implantation. We think that the regulation of thyroid hormone levels during OH and in early pregnancy after IVF needs further study, since any condition resulting in a rise of TSH or a decreased FT_4 could potentially be harmful for pregnancy outcomes.

None of the participants suffered from clinical or subclinical hypothyroidism and, during the study, TSH and FT_4 levels were within the normal range. However, at baseline, women undergoing IVF had markedly lower FT_4 and TSH values than did women undergoing IUI-NC or IUI+MOH. Therefore, one could speculate that women undergoing IVF, with a longer history of infertility were principally different from those in the two other groups. An explanation could be that their pituitary–thyroid axis might work at a lower set point, contributing to their infertility.

Several studies have reported on thyroid function during OH in IVF patients.[11–14] Muller et al[11] reported in a study of 65 women a significant decrease in FT_4 and an increase in E_2, TBG, and TSH at stimulation at day 14 when compared to baseline. In a prospective cohort study of 57 euthyroid or hypothyroid-treated women, Gracia et al[12] showed a rapid increase in E_2 (which peaks upon hCG administration), an increase in TSH that peaks 1 week later and, simultaneously, a significant increase in FT_4 and TT_4. The authors also recognized elevated TBG levels that remained high during the first trimester.[12] Monteleone et al[13] observed in 31 euthyroid women no significant changes in FT_4 and TSH levels during OH, or at the day of the pregnancy test as compared to baseline. Kim et al[14] randomized 64 women with subclinical hypothyroidism to levothyroxine treatment or no treatment during IVF. Although the authors described differences in thyroid parameters between the treatment and control groups during OH, they did not report the changes of TSH and FT_4 when compared to baseline.[14] Poppe et al[15–17] examined, in three studies, thyroid function in the first month of pregnancy after IVF, and they found an increase of FT_4 and TSH levels compared to prestimulation levels. The results in a number of these studies are concordant with ours and some are not. However, all studies were limited to patients during or after OH in IVF without the inclusion of an unexposed control group.

Maternal thyroid hormone levels in the first trimester of pregnancy are related to the developmental indices of the child. Pop et al[6] reported that the developmental index of a child is related to the mother's first trimester FT_4 level, irrespective of whether or not TSH and thyroid peroxidase antibodies (TPO-Ab) were elevated. Women with a first-trimester FT_4 value equal to or below the tenth percentile had a relative risk of 5.8 for a child with a developmental index, which was more than one standard deviation below the mean at 10 months of age. None of these women were suffering from clinical or subclinical hypothyroidism.[6] Another study by the same group[7] consisted of a 2-year follow-up of children born to mothers who had hypothyroxinemia without a recorded status of TPO-Ab at 12 weeks of gestation. Here, too, a significantly delayed mental and motor development of the offspring was found at 1 year (a 10-point lower intelligence score and an 8-point lower motor score than normal controls) and 2 years of age (an 8-point lower intelligence score and a 10-point lower motor score than normal controls).[7] Li et al[21] investigated thyroid function in women at 16–20 weeks of gestation retrospectively, and evaluated the intelligence and motor score of their children at 25–30 months of age in order to examine the individual effects of subclinical hypothyroidism, hypothyroxinemia, and elevated TPO-Ab titers on neuropsychological development. Children of women with subclinical hypothyroidism, hypothyroxinemia, and elevated TPO-Ab titers had mean intelligence scores 8.88, 9.30, and 10.56 points lower than those of the controls and mean motor scores were 9.98, 7.57, and 9.03 points lower than those of the controls.[21] Finally, a large population-based cohort study[22] on maternal hypothyroxinemia at 13 weeks' gestation, as well as children's verbal and nonverbal cognitive development at 18 months and 30 months, which included 3,659 children and their mothers, reported that mild and severe maternal hypothyroxinemia was associated with a significantly higher risk of expressive language delay across all ages. Severe maternal hypothyroxinemia also predicted a significantly higher risk of nonverbal cognitive delay.[22]

Within an individual, thyroid hormone concentrations are maintained within relatively narrow limits. Therefore, test results that fall considerably outside the normal range for the individual being tested may appear normal when using conventional population-based reference intervals, and a test result within the laboratory's reference limits, is not necessarily normal for that individual.[23] This emphasizes that FT_4 serum levels in the lower reference range, as found in our study before and after OH, could be of importance for individual women during a subsequent pregnancy. The mother herself may synthesize and secrete just enough T_4 and T_3 to meet her own needs, but the amount of FT_4 reaching the embryo or the fetus might not be sufficient for its normal neurodevelopment.[6,7]

The physical, neurological, and developmental health of children born after ART has been examined in many epidemiological studies, with contradictory results. Most small studies reported no effect,[24–31] whereas the larger studies have found an increased risk of developmental delay.[32–35] A recent

systematic review and meta-analysis has also addressed the question of developmental delay in children born after ART. The authors conclude that the risk of serious developmental delay following ART appears to be small, but that larger studies conducted on samples of low- and high-risk children conceived by ART are needed.[36] In another recent systematic review,[37] it was concluded that many existing studies of the neurodevelopmental effects of ART were difficult to interpret due to methodological shortcomings. Furthermore, the included studies did not allow for a conclusion to be drawn about the risk of minor neurodevelopmental disorders, because their detection requires more specific tests at ages above 2 years.[37]

The limitations of our study are that we did not monitor the TAI state and the thyroid function during the first trimester in our population. Therefore, one could argue that the observations from our study only represent a transient phenomenon with only marginal or no clinical significance. On the other hand, if women who are receiving OH treatment tend to have lower serum FT_4, already during the first days of pregnancy, increased levels of E_2 could further contribute to a disturbance of their thyroid function, and a clinically significant effect might be observed, for example, in women with preexisting hypothyroxinemia or with TAI.

Conclusion

The mechanisms that intervene with thyroid function during and after OH are complex, but our results indicate that the effect of OH during ART on thyroid function is dependent on the increment of E_2 levels. Larger prospective studies are needed to further elucidate the relation between OH and thyroid function in subfertile, euthyroid women with or without TAI, untreated subclinical hypothyroidsm, and treated hypothyroidism. These studies should investigate thyroid parameters during OH and during the subsequent pregnancy, and they should also explore the development of the children.

Author Contributions

Conceived and designed the experiments: AFM, FPH, JSEL. Analyzed the data: KF, MJCE. Wrote the first draft of the manuscript: KF. Contributed to the writing of the manuscript: KF. Agree with manuscript results and conclusions: KF, MJCE, AFM, FPH, JSEL, FHdJ, BCF. Jointly developed the structure and arguments for the paper: KF, JSEL. Made critical revisions and approved final version: FHdJ, MJCE, JSEL, FHdJ, BCF. All authors reviewed and approved of the final manuscript.

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