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Thyroid and ovarian function in infertile women

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The aim of this study was to examine more closely the interaction between thyroid function and pituitary–ovarian axis in infertile women. In 185 infertile women without clinical signs of thyroid dysfunction, TRH-tests (TSH basal and 30 min after 200 μg TRH i.v.) were performed in the early follicular phase in addition to routine hormonal checks (gonadotrophins, oestradiol, DHEAS, testosterone, prolactin). The women were classified as euthyroid (n = 74; TSH stim 5–20 mU/l), latent hyperthyroid (n = 31; TSH stim <5 mU/l), and preclinical hypothyroid (n = 80; TSH stim >20 mU/l). From frozen serum, the following determinations were performed: TSH IRMA, laevothyroxine (T4), thyroxine binding globulin (TBG), microsomal (Mab) and thyroglobulin (Tab) antibodies. Various correlations between the thyroid parameters and the pituitary–ovarian axis were demonstrated. With increasing TBG concentrations, the interval between menses decreased. Overall and spontaneous pregnancy rates were highest in women with normal (<75th perc.) basal and stimulated TSH, high (>75th perc.) T4 and low (<25th perc.) Mab. Women with normal Tab or high TBG experienced the highest delivery rate (77 versus 30%), while in women with low Tab or high Mab abortion and tubal pregnancies were most frequent. As only 25 women exhibited elevated Mab (>500 U/ml) or Tab (>200 U/ml) which correlated with elevated TSH and normal T4, the routine determination of thyroid antibodies was not necessary. The TRH-test, however, should be included in infertility work-up.

Key words: thyroid function/ovarian function/infertile women

Introduction

In ~20% of infertile women, thyroid disturbances can be diagnosed by thyrotropin (TSH) stimulation tests (Gerhard *et al.*, 1990b). Pacchiarotti *et al.* (1986) observed lowered thyroxine (T4) concentrations in 60% of the women with preclinical hypothyroidism. In addition, Betterle *et al.* (1987) demonstrated increasing concentrations of thyroid antibodies in asymptomatic patients with pathological thyrotropin releasing hormone (TRH) tests. The way in which thyroid disorders might influence ovarian dysfunction and infertility rate is still obscure, though in some cases, TRH-induced hyperprolactinaemia has been proven. Therefore, the aim of this study was to examine more closely the connection between thyroid and ovarian function in infertile women.

Materials and methods

Since 1982, TRH tests have been performed on a routine basis in infertility work-up at the University Women's Clinic in Heidelberg. For the study presented here, the sera were used from 185 infertile women without clinical signs of thyroid dysfunction, and frozen in 1986 and 1987. Blood samples were chosen from 80 women with preclinical hypothyroidism and 31 women with latent hyperthyroidism (definition given below). Seventy-four randomly chosen blood samples from euthyroid women served as controls.

The following diagnostic procedures were performed in all the women: during the early follicular phase (2–5 days following spontaneous or induced menstruation) 10 ml of blood were taken three times at 15-min intervals between 8.00 and 10.00 a.m., and pooled for determination of basal concentrations. In the pooled blood follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol-17β (E_2), prolactin (Prol), testosterone (T), dehydroepiandrosterone sulphate (DHEA-S), sexual hormone binding globulin (SHBG) and TSH were determined. Following the third sampling, patients received an intravenous bolus injection of 200 μg TRH (Antepan 200[R]). Ten millilitres of blood were again taken after 15 and 30 min for determination of Prol and TSH respectively. In the second phase of the cycle, between the 5th and 10th day of raised basal body temperature (BBT), patients had blood taken three times for determination of progesterone (P) and E_2. On one of these days, between 8.00 and 10.00 a.m., the metoclopramide test was performed which comprised: basal blood sampling for Prol, intravenous injection of 10 mg metoclopramide (Paspertin[R]), renewed sampling after 30 min for determination of Prol concentrations. The test was carried out between the 21st and 23rd day of the cycle in anovulating patients. Endocrinological diagnosis was completed for ovulatory patients by means of an endometrial biopsy taken 1–3 days before expected menstruation; the endometrial strip was removed from the anterior uterine wall with a Nowak curette and subsequently examined histologically according to the criteria of Noyes *et al.* (1950).

In accordance with the endocrine check-up of the women, partners underwent andrological examination including sperm analysis. In the cycles following endocrinological diagnosis, the uterine and tubal factors were studied by hysterosalpingography and/or laparoscopy and the sperm–mucus interaction with the Sims–Huhner postcoital test (PCT). In cases where the PCT was either negative (≤1 propulsive motile spermatozoon per high-

Table I. Sensitivity and inter-assay variations in the hormone assays used. Intra-assay variation was below 10% for all methods The normal range was given by the respective companies

Hormone	Units	Method	Normal range	Sensitivity	Interassay variation %
FSH	ng/ml	RIA, IRE, Fleur Belgium MRC 78/549, 1 ng = 3.26 mU	1–5	0.23	6.8
LH	ng/ml	RIA, IRE, Fleur Belgium MRC 68/40, 1 ng = 4.20 mU	1–6	0.26	8.2
Prolactin	ng/ml	RIA, IRE, Fleur Belgium MRC 75/504, 1 ng = 34 μU	basal <20 30′ after Metocl. <250	1 5	8 3
Oestradiol-17β	pg/ml	RIA, Travenol	25–150	3	7.6
Testosterone	pg/ml	RIA, Serono	>500	50	12 4
DHEA-S	ng/ml	RIA, Diagn. Prod. Corp.	>4000	50	10 3
Progesterone	ng/ml	RIA, Diagn. Prod. Corp	>10 luteal phase	0.25	7.2
SHBG	nmol/l	RIA, Farmos Diagn. Turku	10–400	0.5	11.2
TSH	mU/l	RIA, Behring Comp	basal <6 30′ after TRH 2–20	0.14	8.8
TSH	mU/l	Irma, Henning Comp.	0.3–4	0.03	5
T4	ng/ml	RIA, Henning Comp.	45–120	12	18
TBG	mg/l	RIA, Henning Comp.	11.3–28 9	5	14
Mab	U/ml	RIA, Henning Comp.	<300 neg.; >500 pos.	146	18
Tab	U/ml	RIA, Henning Comp.	<100 neg.; >200 pos.	72	18

power field) or poor (2–6 propulsive motile spermatozoa), it was repeated in the following cycle after pretreatment with ethinyloestradiol (80 μg per day from the 3rd to 9th day of the cycle); the same regimen was followed in anovulating patients.

After termination of the diagnostic procedures, the women were treated individually as follows: in hyperandrogenaemic states with dexamethasone and clomiphene, in hyperprolactinaemia with bromocriptin, in luteal insufficiency or unexplained infertility with clomiphene or gonadotrophins (for further details, see Gerhard *et al.*, 1990a). Women with latent and preclinical hypothyroidism were substituted with levothyroxine 100 μg/day for 3 months. After this time, stimulation with clomiphene, or in cases without adequate response, with gonadotrophins was carried out to improve follicular maturation. Women with hyperthyroidism were transferred to specialists for further diagnostics, operations, or thyreostatic treatment. The pregnancy rate over the next 2 years was recorded.

The methods used for hormone determinations are summarized in Table I. Thyroid function disturbances were defined as follows: latent hyperthyroidism [TSH 30′ minus basal TSH (= delta TSH) under 5 mU/l], latent hypothyroidism (basal TSH over 6 mU/l), preclinical hypothyroidism (basal TSH within norm, stimulated TSH over 20 mU/l).

While in previous years TSH RIA (Behringwerke) assay was used, TSH determinations were repeated from frozen serum with a TSH IRMA (Firma Henning) assay. As demonstrated in Figure 1 (top) the correlation between basal TSH RIA and IRMA values was low due to the increased sensitivity of IRMA. The correlation was good in stimulated TSH concentrations (Figure 1 bottom). However, the comparison of the TSH RIA values >20 mU/l showed that TSH IRMA measured ~5 mU/l less than RIA, so that the definition of preclinical hypothyroidism was different between IRMA and RIA TSH. In pooled serum, levothyroxine (T4), thyroxine binding globulin (TBG), microsomal (Mab) and thyroglobulin (Tab) antibodies (ab) were measured (Table I). For all hormones and thyroid parameters percentiles were constructed and used for statistics. In addition,

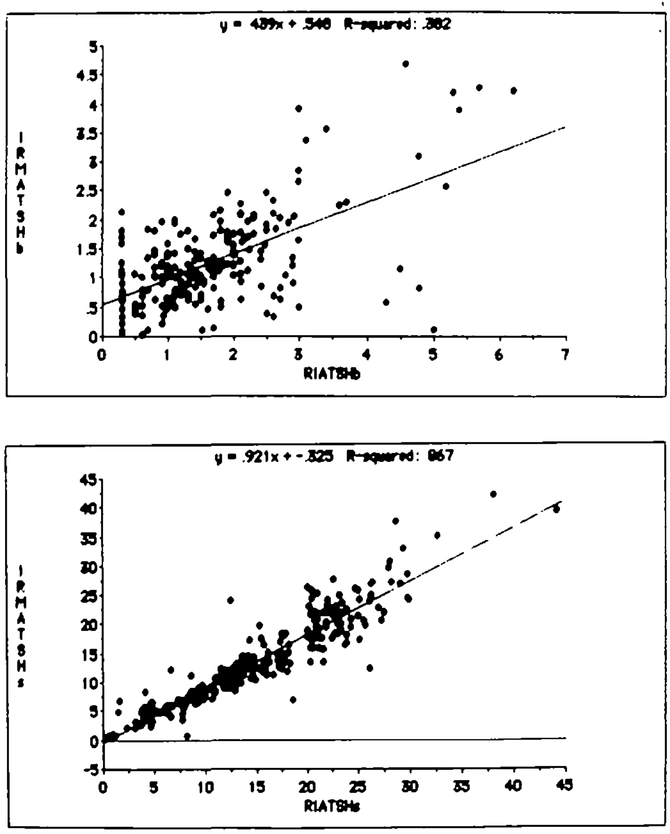

Fig. 1. Correlation between TSH IRMA and TSH RIA (mU/l) concerning the basal TSH concentrations (upper panel) and the stimulated TSH levels (lower panel).

Mab and Tab were divided into two groups with borderlines at 500 and 200 U/ml, respectively. As the borderline for Mab given by the company might have been set too high, statistical calculations were also performed for a borderline of 400 U/ml.

Patients' data were encoded and processed at the University Computer Centre, Heidelberg. SAS was used for the statistical

Table II. Parameters of thyroid function (percentiles) and correlation with hormone concentrations in the follicular and luteal phase

Thyroid parameter	FSH[1] LH[2]	Oestradiol fol[3] lut[4]	Testosterone[5] DHEAS[6] SHBG[7]	TSH basal	TSH +30	Prolactin basal	Prolactin +15	Prolactin +30
TSH <25	*54[2]	*78[4]	*54[7]					
25–75	68	57	37					
>75	81	58	48					
			C = 0.1723[5]					
TSH <25		*71[3]				**67	+58	++53
+30 25–75		60				89	87	87
>75		55				67	82	86
T4 <25	*57[2]		*67[5]	<*M93	++106	*68	<*M94	**97
25–75	91		93	>M77	86	95	>M79	76
>75	71		90		64	88		
			C = 0.1732[5]	C = −0.3253				
TBG <25	**120[1]	**107[3]	+63[5]				*105	**113
25–75	82	87	96				87	86
>75	91	66	92				64	66
			C = 0.2718[5]				C = −0 1829	C = −0.1764
T4/ <25		**65[3]	*71[6] *47[7]		++94			
TBG 25–75		92	88 66		95			
>75		99	97 67		59			
		C = 0.2124[3]	C = 0.1710[6]		C = −0.3155			
		C = 0.1567[4]	C = 0.2108[7]					
Mab <25					++60	**77		
25–75					97	82		
>75					87	106		
					C = 0.1974	C = 0.2362		
Tab <M		**79[3]					**78	
>M		96					95	
		C = 0.1657[3]					C = 0.1672	C = 0.1591

Mean score as calculated with the Kruskal–Wallis test is given.
$*P < 0.05$, $**P < 0.01$, $^{+}P < 0.005$, $^{++}P < 0.001$.
M = Median; C = correlation coefficient (Spearman) between the two hormones.

evaluation. The Kruskal–Wallis test, the Wilcoxon test for dependent samples, the Wilcoxon test for independent samples, the Chi-square test and the Spearman correlation coefficient were applied. Though large numbers of comparisons were made, the tables only provide those results which were significant at a level of $P < 0.05$. As research data on the link between thyroid and ovarian function in infertile women are scarce, we present all the significant results of our exploratory statistical analysis in order to construct some hypotheses which, of course, have to be proven in future prospective studies.

Results

Correlation between thyroid parameters, pituitary and ovarian hormones

Between the various thyroid parameters the following significant correlations ($P < 0.01$) were found (Spearman correlation coefficient): basal TSH with stimulated TSH 0.8050, with TBG 0.2762, with Mab 0.3029, stimulated TSH with T4 0.3065 and Mab 0.2354, T4 with TBG 0.4627, Mab with Tab 0.5045. Mab >400 U/ml was found in 29 patients, 21 of whom displayed values >500 U/ml. Tab concentrations >200 U/ml were determined in 10 women. Twenty-five women exhibited elevated Mab (>500 U/ml) or Tab (>200 U/ml). In the latter group stimulated TSH was significantly more frequent above the 50th percentile (68%) than in patients without ab (47%, $P = 0.05$). In addition, T4 was usually within the norm (25th–75th percentile, 71%) compared to patients without ab (47%, $P = 0.05$).

Table II shows the significant correlations between the thyroid parameters and the hormones. Thyroid parameters were classified according to their percentiles in low (<25th percentile), normal (25th–75th percentile) and elevated or high (>75th percentile) categories. In addition, some correlation coefficients (C) are shown. Basal TSH was positively correlated to LH and T in the early follicular phase. Women with low basal TSH concentrations exhibited the highest E_2 concentrations in the luteal phase and the highest SHBG concentrations. As for stimulated TSH, a significant negative correlation to E_2 in the follicular phase was also seen. Women with low TSH response to TRH showed the lowest Prol response to TRH. Women with low T4 had the lowest LH and T concentrations in the early follicular phase. Their LH/FSH ratio was lowest too (0.61, versus 0.85 in normal T4 values). In the luteal phase their basal Prol concentrations were low but after stimulation with metoclopramide they exhibited higher Prol concentrations than women with high T4. TBG showed an inverse relationship to FSH, E_2 in the follicular phase and Prol after stimulation with TRH. In women with low TBG the T concentrations were lower than in women with normal or elevated TBG. These women exhibited a higher P/E_2 ratio in the luteal phase (80) than women with normal (50) or elevated (70) T4 values. The T4/TBG ratio showed a positive interaction with E_2 in the follicular phase, DHEAS, SHBG and a negative correlation with stimulated TSH. Patients with low Mab had low stimulated TSH and low basal Prol values. Patients with Mab >400 U/ml exhibited significantly elevated E_2 concentrations in the early follicular phase (P = 0.0264) and Prol values in the luteal phase (P = 0.0458), while P concentrations in the early luteal phase were significantly lower (P = 0.0378) than in women with Mab >400 U/ml. Women with low Tab (< median) had lower E_2 and stimulated Prol concentrations than women with high Tab (> median). The P concentrations of women with elevated Tab were lower (7.5 ng/ml) than in women with normal (9.9 ng/ml) or low (8.3 ng/ml) Tab (P = 0.01). In women with elevated Tab and Mab (>500, >200) concentrations were above the 50th percentile in 26%, and in women without ab in 52% (P = 0.027). At the end of the luteal phase the values were 27% as opposed to 55% (P = 0.018).

Correlation of the thyroid parameters with clinical data

In women with high TBG concentrations the body mass index (BMI) was higher (39.3 kg/m^2) than in the other patients (35.2 and 35.6 kg/m^2 in low and normal TBG groups respectively). The T4/TBG ratio was negatively related to the BMI, demonstrating highest values in patients with a low ratio (39.5 kg/m^2, versus 35.5 and 36.5 kg/m^2 in normal and high T4/TBG ratio groups). In women with elevated Mab (>500 U/ml) and Tab (>200 U/ml) body weight and body surface were significantly higher than in women without ab (P < 0.05).

Out of all thyroid parameters only TBG exhibited several correlations with the menstrual history and the BBT. With increasing TBG concentrations, menstrual intervals became shorter (menstrual interval ≤25 days in 24% of the patients with low TBG, in 40% with normal and in 60% with elevated TBG respectively, P = 0.02). Seventy per cent of the women with low TBG concentrations had eumenorrhoea, but only 37% of the women with elevated TBG concentrations had eumenorrhoea (P = 0.008). In women with low and high TBG levels a reduced duration of the luteal plateau phase (≤9 days) was less frequent (24%) than in women with normal TBG concentrations (44%, P = 0.029). A slow and stepwise increase of the BBT after ovulation was more frequent in women with low (74%) than in women with elevated TBG concentrations (36%, P = 0.008).

Stimulated TSH showed some correlations with the cervical factor at midcycle and with the histological dating of the endometrial biopsy (Table III). Women with high stimulated TSH had the highest frequency of delayed endometria. The cervical index was better in women with elevated stimulated TSH levels than in women with normal and low TSH. However, PCT was significantly worse in women with exaggerated TSH response. Normal PCT was less frequent in women with low Tab (<25th percentile) than in women with normal and high Tab (45% versus 71% and 69%). Women with high Mab (>75% percentile) had the highest rate of normal PCTs (53% versus 30%) and the lowest rate of pathological andrological factors. In women with elevated Mab and Tab (>500, >200 U/ml respectively) no correlation with immunological infertility could be demonstrated (Table IV). Their cervical index at ovulation was better (P = 0.0249). Endometriosis was proven by laparoscopy in 44% of the women with ab and only in 9% of women without ab (P = 0.004).

Table III. Stimulated TSH and correlation with endometrial dating, cervical factor at midcycle and postcoital test (PCT) correlation

		No of patients	TSH stim. IRMA (%) <25 Percentile	25–75	>75	P
Endometrial	Out of phase	27	9	19	32	
biopsy	Normal	37	34	24	26	0.05
n = 136	Rest	72	57	57	42	
Cervical	Poor	44	48	48	23	
factor	Medium	24	24	27	13	0.026
n = 103	Good	35	28	25	64	
PCT	Positive	83	57	75	39	
n = 149	Poor	33	35	14	33	0.004
	Negative	19	8	11	27	

Values given are the correlation %

Table IV. Thyroidal antibodies and postcoital test (PCT)

PCT result	No. of patients	Correlation % Mab >500 Tab >200	Mab <500 Tab <200	P
Immunological factor	19	0	17	
Cervical factor	34	17	27	
Andrological factor	29	17	23	0.0037
Normal	50	67	33	

Table V. Significant correlations of thyroid parameters with pregnancy rates

Variable	Percentile	Pregnancy % (n = 91)	P	Spontaneous pregnancy % (n = 42)	P
TSH basal	<50	64	0.006		
(IRMA)	>50	41			
TSH stim	<50	65	0.000	35	0.001
(IRMA)	>50	35		12	
	<25	57		31	
	25–75	56	0.017	27	0.05
	>75	31		10	
T4	<25	46		13	
	25–75	44	n.s.	21	0.05
	>75	63		35	
Mab	<50	57		29	
	>50	41	0.028	17	0.05
	<25	65		37	
	25–75	44	0.039	17	0.04
	>75	43		20	

Out of 186 patients, 91 conceived. Nearly half of these pregnancies (n = 42) occurred independently of the treatment. Basal TSH was lower in fertile than in infertile patients (Table V). Women with high stimulated TSH had lower pregnancy rates than women with normal or low stimulated TSH. The inverse correlation was true for T4. Women with low Mab had a higher pregnancy rate than women with normal and high Mab. The same significant correlations were found for these thyroid parameters and the spontaneous pregnancy rate. In women with Tab >200 U/ml, the conception rate was 70%, while in the rest of the patients it was 47% (NS).

Table VI. Correlations of Tab and TBG with pregnancy (n = 91) and abortion (n = 24) rates

Thyroid parameter percentile		Total abortion rate (n = 24)	Tubal pregnancy (n = 12)	Early abortion (n = 12)	Delivery >32 week (n 67)	Delivery >36 week (n = 57)
Tab	<10	67	44	22	33	33
	10–90	20	10	9	80	68
	>90	33	8	25	67	58
Level of significance		0.01	0.05	n.s.	0 01	0.05
TBG	<10	M34	18	18	64	45
	10–90	M16	14	13	73	63
	>90		0	11	89	89
Level of significance		0.05		0.05		0.05

Frequencies % are given. M = Median.

Table VII. Concentrations of thyroid parameters in hyper-, eu- and hypothyroid patients, classified according to the TRH-test

Thyroid parameters	Level of signif.	Hyperthyroid (n = 31)	Euthyroid (n = 80)	Hypothyroid (n = 74)	Total (n = 185)
TSH basal R mU/l	0.0001	0.5 (0.3–2.6)	1.2 (0.3–5.2)	2.1 (0.3–9.9)	1 4 (0.3–9.9)
TSH + 30′ R mU/l	0.0001	4.0 (0 3–4.9)	12.0 (5.2–19.5)	23.0 (20–47 2)	14.4 (0.3–47.2)
△ TSH R mU/l	0 0001	3.0 (0.0–4.6)	10.5 (3.3–17.2)	21 (14.8–46.6)	14.7 (0 0–46 6)
TSH basal I mU/l	0.0001	0.5 (0.05–1.12)	1 08 (0.22–4.03)	2.0 (0 9–9.9)	1.2 (0.05–9 9)
TSH + 30′I mU/l	0 0001	4 0 (0.5–8 3)	10 9 (0.8–23.9)	21 7 (1.1–73.4)	12.9 (0.5–73.4)
△ TSH I mU/l	0.0001	3.6 (0.4–7.1)	9.5 (0.1–22.9)	19.2 (13.2–63 5)	10.2 (0.0–63 5)
T4 ng/ml	0.0001	90.5 (66.9–129)	77.9 (55.7–125)	73.5 (35.4–134)	77.6 (35.4–134)
TBG mg/l	n.s.	24.5 (14.4–33.6)	24.7 (15.0–40.3)	25.3 (12 0–46 9)	24 8 (12.0–46.9)
T4/TBG × 10^{-3}	0.001	3.8 (2 4–6.3)	3.2 (2.3–6.0)	3.0 (1.7–5.5)	3.2 (1.9–6.3)
Mab U/ml	0.0001	20 (0–5479)	168 (0–30548)	113 (6.9–17604)	109 (0.0–30548)
Tab U/ml	n.s.	14.7 (0.0–727)	19.1 (0–1031)	18 (0.0–899)	18.3 (0.0–1031)

Median values and range are given. I = IRMA, R = RIA.

Women with normal Tab had the highest delivery rate (Table VI). On the other hand, women with low Tab had the highest abortion rate due mostly to a high tubal pregnancy rate. In women with Mab >500 U/ml, a tendency towards an increased abortion rate (25%) and decreased delivery rate (58%) was demonstrated compared with women with low Mab (12% and 75% respectively). Women with high TBG concentrations had the highest delivery rate, while early and late abortions and premature deliveries were most frequent in women with low TBG concentrations.

Level of thyroid parameters in eu-, hyper- and hypothyroid patients

Only one patient exhibited an elevated basal TSH RIA value of 6 mU/l, thus presenting with latent hypothyroidism. Three patients of the so-called hyperthyroid group had basal TSH RIA concentrations ≤0.3 mU/l. In all the other 105 patients TRH-testing revealed mild thyroid dysfunction. As patients were classified according to their basal and stimulated TSH concentrations, hypothyroid patients exhibited the lowest basal and stimulated TSH concentrations and the hyperthyroid women the highest (Table VII). TSH IRMA measured significantly lower basal and stimulated TSH concentrations than TSH RIA. Delta TSH IRMA was higher in hyperthyroid patients and lower in eu- and hypothyroid patients compared with delta TSH RIA. Hyperthyroid women had the highest T4 and T4/TBG concentrations and the lowest Mab. Hypothyroid patients had the lowest T4 concentrations. The highest Mab values were measured in euthyroid patients. As for TBG and Tab, no significant differences between the three thyroid function groups were observed.

Hormones and pregnancy rates in the three thyroid function groups

In euthyroid patients, luteal insufficiency with a shortened luteal phase, stepwise BBT increase and low P concentrations in the early luteal phase were more frequent than in the two other groups (Table VIII). In hyperthyroid patients, menarche was earlier than in the other groups. Their basal and stimulated Prol was lower, DHEAS higher, and P in the late luteal phase and mean P concentrations lower. In the early and mid-luteal phase, hyperthyroid patients exhibited lower E_2 concentrations than the other two groups. A close look at concomitant sterility factors revealed that the husbands of the hypothyroid women had the lowest sperm count and reduced sperm motility (P = 0.009). Therapeutic pregnancies in this group were mostly induced by levothyroxine (alone 14%, in combination with other drugs 6%) and clomide. In euthyroid patients, the early abortion rate was higher in women with Mab >500 U/ml (83%) than in women with lower Mab concentrations (25%, P = 0.005). Euthyroid patients had the highest overall and spontaneous pregnancy rate with husbands producing the best sperm parameters. Therapeutic pregnancies were often induced by bromocriptine (alone 4%, in

Table VIII. Significant correlations between the three thyroid function groups and menstrual cycle or various hormones, respectively

Variables	Percentile	Correlation % Hyperthyroid (n = 31)	Euthyroid (n = 80)	Hypothyroid (n = 74)	P
Early menarche (< 12 years)		24	3	5	0.004
Stepwise BBT increase		25	47	24	0.024
Luteal phase < 12 days		50	73	46	0.018
Spontaneous pregnancy		19	29	8	0.003
No pregnancy		42	40	65	
Prol	>75	23	33	29	0.017
Prol 15'TRH	<10	30	7	9	0 019
DHEAS	>50	67	58	37	0.004
Prol luteal phase	>50	43	69	51	0.027
P 5th luteal day	>75	35	2	24	0.006
	<25	13	33	25	
10th luteal day	<10	13	0	8	0.029
	>90	10	3	12	
mean value	<10	13	5	11	
luteal phase	10–90	70	94	80	0.029
	>90	17	2	9	
E_2 5th luteal day	>50	79	43	32	0.022
7th luteal day	<25	15	–	30	0.031
	>75	46	40	4	

Only significant rows of the chi-square tests are given, thus the numbers do not add up to 100%.

combination with other drugs 7%). In the hyperthyroid group of patients, dexamethasone was often effective in inducing a pregnancy (alone 3%, in combination with other drugs 7%).

Discussion

The clinical relevance of subclinical thyroid disturbances is still controversial. Owing to the increased discriminating potency of the newly developed TSH assays, TRH testing in women without clinical signs of thyroid dysfunction is usually omitted. However, there are several reports in which treatment of subclinical dysfunctions is demanded. Vardali *et al.* (1989) showed significant differences in TSH patterns in latent hyperthyroidism compared to euthyroid individuals, proposing that latent hyperthyroidism might represent a first stage of hyperthyroidism which should be treated. In subclinical hyperthyroidism, LDL/HDL changes were observed suggesting risk factors for coronary heart disease (Gräni *et al.*, 1981; Teuber *et al.*, 1981; Althaus *et al.*, 1988). In a double-blind placebo-controlled study, Nyström *et al.* (1988) observed significant alterations in psychometric and neurophysiological tests in laevothyroxine-treated preclinical hypothyroid patients. In subjects from psychiatric institutions suffering from depression and altered moods, preclinical hypothyroidism was frequently diagnosed and substitution with thyroxine improved the psychiatric symptoms. In women suffering from premenstrual syndrome (Brayshaw and Brayshaw, 1986) and in women with anovulation (Louvet *et al.*, 1979) who exhibited an exaggerated response of TSH to TRH, substitution with thyroxine proved to be most effective.

Women with elevated basal TSH exhibited lower E_2 concentrations in the follicular phase and a higher incidence of retarded premenstrual endometrial biopsies (Gerhard *et al.*, 1990a). In women with low basal TSH, LH and T were low too, and E_2 elevated, thus resulting in a higher pregnancy rate. The same was true for stimulated TSH. The correlation between low incremental increase of TSH and a high spontaneous pregnancy rate was more obvious. There was a significant inverse relationship between stimulated TSH and T4 and T4/TBG. This result is not surprising, since it is in good agreement with the concept that T4 plays the dominant role in the regulation of TSH secretion, because T4 is rapidly converted to T3 within the pituitary cells. Thus total pregnancy rate and spontaneous pregnancy rate were highest in women with high T4 concentrations. An explanation might be found in the in-vitro studies of Maruo *et al.*, (1987) where steroid production of granulosa cells was stimulated more when FSH was added in combination with T4 than with FSH alone. Women with low T4 concentrations had low LH and T concentrations and higher stimulated Prol values. Pacchiarotti *et al.* (1986) observed low T4 concentrations in many patients with so-called subclinical hypothyroidism (>60%) thus showing that in fact, they were already mildly hypothyroid.

Women with low TBG concentrations had higher FSH, E_2 and stimulated Prol values and low T concentrations. Normal intervals between menstruations were more frequent in these women with high TBG, who often experienced polymenorrhoea and a prompt shift in BBT. The highest delivery rate was found in women with high TBG. No significant differences of the TBG concentrations could be demonstrated between the three clinical thyroid dysfunction groups. This is in contradiction with the results of Pacchiarotti *et al.* (1986), who reported slightly increased TBG concentrations in subclinical hypothyroid subjects. In pregnancy and during treatment with contraceptive pills, Sparre *et al.* (1987) and Röcker *et al.* (1988) found that increasing E_2 concentrations were followed by increasing TBG levels and decreasing T4/TBG ratios. It can be assumed, however, that unphysiological doses of oestrogen exert different effects on TBG metabolism than do normal E_2 concentrations as in our study. In addition, the influence of the BMI must be taken into account, as shown for TBG in this paper and for SHBG previously (Gerhard *et al.*, 1989).

Mab and Tab are frequently found in sera of women with goitre and hypothyroidism and women with Graves' disease. Betterle *et al.* (1987) proved that asymptomatic patients with thyroid antibodies showed increasing pathological results for TRH testing. The titre of Mab correlated with the degree of lymphoid infiltration of the thyroid gland. However, the presence of Mab in the serum does not always imply active tissue destruction. For example, 20% of healthy middle-aged women have Mabs (Hamada *et al.*, 1987). In the present study 10% of the women had Mab, 5% Tab and 3% of the women had Mab as well as Tab. Pacchini *et al.* (1988) gave an incidence of 5% thyroid ab in young patients increasing to 23% in subjects over 60 years old. Mab concentrations correlated positively with stimulated TSH, with E_2 in the follicular phase, Prol in the luteal phase and negatively with P in the early luteal phase. As the Mab concentrations were positively correlated with the body surface area, decreased P concentrations might be more dependent on body mass as previously described (Gerhard *et al.*, 1989). Though a connection between thyroid ab and an immunological cervical factor could be excluded in our study, the high frequency of endometriosis in Mab-positive patients awaits further clarification. The higher rate of spontaneous early abortion in women with positive Mab might be explained by the low progesterone concentrations in the luteal phase and the presence of endometriosis.

Women with low Mab experienced the highest spontaneous pregnancy rate. Mab concentrations were highest in euthyroid patients and lowest in hyperthyroid women. The choice of a threshold of 500 U/ml yielded no significantly different frequencies in the three thyroid dysfunction groups. Though it is thought that Mab damages thyroid cells by binding to the cell surface, it could be demonstrated that Mabs are heterogeneous and can be separated into specific subgroups with differing characteristics depending on the epitope to which they bind (Hamada *et al.*, 1987). Saller *et al.* (1988) examined the sera of 13 patients with long-term remission of Graves' disease which were all Mab-positive. These antibodies, however, did not interact with the active site of the thyroid peroxidase complex.

Tab was positively correlated with E_2 and with stimulated Prol. Women with low Tab had the highest abortion rate, above all a high rate of tubal pregnancies. This correlation cannot be interpreted as a result of the Tab, but can be explained by low E_2 concentrations and ovulatory dysfunction.

In the present study, women were assigned to three thyroid function groups according to their TRH test. Within these groups, significant differences could be proven for menstrual disorders, various hormones and pregnancy rate. Most of these differences could not have been demonstrated if only basal TSH had been measured. This agrees well with the results of Schneider (1987) who compared different TSH methods, concluding that the incremental increase of TSH on TRH improves differential diagnosis of thyroid function even when using highly sensitive IRMAs. In addition, Davis *et al.* (1988) found less complications in pregnancies of women with subclinical hypothyroidism when substituted with levothyroxine compared to those without substitution treatment. In the present study, hypothyroid patients had the lowest pregnancy rate despite levothyroxine substitution. However, in this group of patients, the sperm parameters were significantly worse than in the other groups, E_2 in the luteal phase was lower than in hyper- and euthyroid patients. As we demonstrated in a previous study (Gerhard *et al.*, 1990b), levothyroxine substitution induces an increase of E_2. Optimal corpus luteum function cannot be achieved in all patients, thus making clomiphene treatment necessary. Stimulated prolactin was higher in hypothyroid patients and correlated to incremental TSH increase and low T4 concentrations. After levothyroxine replacement therapy in preclinical hypothyroid patients, plasma prolactin and TSH secretion in response to metoclopramide and TRH is significantly inhibited (Lombardi *et al.*, 1986; Gerhard *et al.*, 1988).

The hyperthyroid patients exhibited the best luteal function. In this group, mostly elevated DHEAS concentrations were observed so that, in many cases, pregnancies were able to be induced by dexamethasone treatment. It is not clear whether there is a link between thyroid and adrenal function. Euthyroid patients exhibited the highest basal prolactin concentrations in the follicular and the luteal phase. Although the spontaneous pregnancy rate was highest in this group, several patients conceived after bromocriptine treatment.

Owing to the frequency of preclinical hypothyroidism in Central Europe, the lack of side effects of levothyroxine substitution and the proven prophylactic importance for the prevention of neonatal goitre, the TRH test is suggested as a screening examination for thyroid function disturbances in infertile women. The routine determination of T4, TBG, Mab and Tab appears to be unnecessary. Further clinical and experimental studies must be conducted to elucidate the role of Mab in women with endometriosis and early abortion.

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