Diagnostic therapeutic thyroidal-reproductive integrated location in patients candidate to programs of assisted medical procreation

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SUMMARY: Diagnostic therapeutic thyroidal-reproductive integrated location in patients candidate to programs of assisted medical procreation.

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Objective. The aim of this study is to evaluate the thyroid state influence on oocytes and embryo formation until the establishment of biochemical pregnancy.

Materials and methods. Medical records of women that underwent assisted reproductive techniques were examined (ART). The only inclusion criteria was the presence of a documented evaluation of thyroid functionality. The following assisted reproductive techniques were used: ICSI (intracytoplasmic sperm injection); FIV-ET (in vitro fertilization and embryo transfer); AIH (Artificial Insemination Homologous).

Results and conclusions. Based on our clinical observations it is assumable that thyroid homeostasis affects implant phase fertility and following embryo growth, rather than the first pre-implant phase considered in this study (oocytes and zygotes development).

KEY WORDS: PMA - Hypothalamus hypophyse ovary axis - Hypothalamus hypophyse thyroid axis.

Introduction

Procreation is an essential evolutionary process for life; it involves endocrine, cellular and molecular well-regulated events and space-temporally interconnec-tions. The immune and endocrine systems play a leading role in all stages of pregnancy, from oocyte maturation, to implantation and following embryo development. The close connection between thyroidal functionality, fertility (1) and pregnancy (2-4), thinking of

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the reciprocal influence between thyroidal hormones and steroidal hormones (estrogens and progesterone), seems so clear. Thyroidal dysfunctions are often linked to menstrual irregularities and to higher incidence of morbidity during and after pregnancy (congenital malformation, low birth weight, preterm birth, spontaneous abortion, postpartum hemorrhages, pregnancy hypertension with or without preeclampsia); on the other hand pregnancy is itself a cause of physiological modification of thyroidal functionality.

Anomalies of both the endocrine system and the immune system, which normally underpin reproductive difficulties, converge in autoimmune thyroiditis (the most frequent cause of hypothyroidism in reproductive age women) (5). A correlation among thyroiditis and/or sub-clinical hypothyroidism and abortions was already underlined in ‘90s, however studies in this context are incomplete and partially conflicting until today (6-8). Clinical evidence shows an increase from 3 to 5 times of abortion risk in women with autoimmune thyroiditis (9-11).

The association between thyroiditis and/or hypothyroidism and unsuccessful pregnancy was object of study even in the field of assisted reproductive technology (ART), as it was demonstrated that controlled ovarian hyperstimulation significantly affects thyroidal function (12), especially in women suffering from autoimmune thyroiditis. Controlled ovarian hyperstimulation combines the hypophyseal-ovarian axis down regulation with GnRH agonists or antagonists and the subsequent ovarian stimulation with recombinant FSH: such procedure leads to a substantial increment of estrogen levels and, therefore, of TBG (thyroxine binding globulin), which is able to significantly decrease the thyroidal hormones free fractions. FT₄ levels, in particular, play an important role in the placental (13) and fetal (14, 15) development, especially in the first trimester of pregnancy, when product of conception does not have functional thyroid tissue yet.

However, studies that value connection analytically between success of IVF (In Vitro Fertilization) and thyroid function are occasional in the international literature.

Considering the importance of thyroid function for reproduction prognosis (both natural and medically assisted), it seems necessary a screening of thyroid function and of antithyroid antibodies presence as preconceptional and pregnancy study.

**Hypothalamus hypophysis ovarian axis**

Gonadotropin releasing stimulating hormone (GnRH) represents the main regulator of reproductive function.

Neurohormone assigned to gonadotropin control is called Gonadotropin Releasing Hormone (GnRH). The releasing pulsatile nature of hypothalamic GnRH determines occasional secretion of hypophysal gonadotropins. The characteristic of GnRH self-induction effect on his receptors present on the gonadotrophic cells expresses itself only in presence of a physiological periodicity (60-90 minutes) by up-regulation of GnRH receptors; lower frequencies cause anovulation and amenorrhoea; while, higher frequencies or a constant exposure to GnRH induce refractoriness of response to gonadotropins which derives from down-regulation state.

**Hypothalamic hypophysal thyroid axis**

The synthesis and the secretion of thyroidal hormones are regulated by hypothalamic-hypophysal-thyroid axis that exerts main control through negative feedback, by intra-thyroidal processes mainly depending on iodic contribution, by influences of sympathetic system, in particular adrenergic and by factors that influence the extra thyroidal production of triiodothyronine.

The hypothalamic hypophysal thyroid axis is a paradigmatic example of negative feedback control. The reduction of thyroid hormones in circulation determines an increase of Thyrotropin Releasing Hormone (TRH) and of TSH, while the increase determines a reduction. The feedback loop occurs as at the level of parcellular neurons of TRH releasing paraventricular nucleus, as at the level of thyrotropic cells by locally generated T₃ from T₄ by type II 5’-deiodinase. This phenomenon leads to an increase of TSH during the initial stages of hypothyroidism, when circulating T₃ is still inside the limit of standard, while T₄ is already reduced.

**Thyroid hormone action**

The majority of thyroid hormone effect is referable to the activation genic transcription. Indeed, thyroid hormones modulate a wide number of metabolic processes which control synthesis and activity of enzymes, production and metabolism of other hormones and use of substrates, vitamins and minerals. Moreover thyroid hormones play a key role in neuronal and skeletal development of fetus. The lack of T₃ during fetal life leads to cretinism (intellectual disability and dwarfism). Thyroid hormone do not pass placental barrier so fetal thyroid has to synthetize thyroid hormones. This process starts at the 11th week of pregnancy.

The aim of this study is to evaluate the influence of
thyroidal status on the development of oocytes and embryo until the establishment of biochemical pregnancy

Materials and methods

Medical records of women who underwent assisted reproductive technology (ART) at infertility and FIVET UOC of “Policlinico Umberto I” of Rome, “Sapienza” University of Rome, were retrospectively examined from 2006 to 2010. The only inclusion criteria was the presence of documented evaluation of thyroidal functionality. 195 medical records, making a total of 263 procedures (as many women underwent subsequent cycles of PMA), were taken into consideration.

The data which were taken into consideration were: TSH levels, considered a reliable standard of thyroidal functionality; AbTPo and/or AbTG levels; a possible LT4 therapy; age of women; medical therapy for controlled ovarian hyperstimulation; peak of estradiol; number of taken oocytes; development stage of taken oocytes; numbers of gained embryo; possible biochemical pregnancy (positivity to β-hcG until a value of 50 mIU/ml).

Anti-thyroid peroxidase antibodies (AbTPo) were available in 117 procedures and were positive in 24 (20.5%) and negative in 93 (79.5%) cycles. Therefore, procedures were divided in two groups: group A, AbTPo+, group B, AbTPo-. Anti-thyroglobulin antibodies (anti-Tg Ab) were not because they were present in a small number of procedures.

The mean age of patients was 37.92 years (range 24-48 years), with no statistical difference between groups: group A 37.83 years (range 28-42 years); group B 37.46 years (range 27-45 years).

L-thyroxine was given in 43 procedures.

Thyroid status was evaluated by: TSH, FT3, FT4 hormone dosages determined by radioimmunological and immunoenzymatic methods; anti-thyroid peroxidase (Ab anti-TPO), anti-thyroglobulin (Ab anti-TG) antibodies dosages were determined by chemiluminescence technique.

Reference values:
- TSH [0.26÷4.94] mcUI/ml
- FT3 [0.23÷0.57] ng/dl or [3.8÷6.0] pmol/L
- FT4 [0.70÷1.48] ng/dl or [7.8÷14.3] pmol/L
- AbTPo [0÷100] IU/ml
- AbTG [0÷100] IU/ml

The following techniques of assisted reproduction were used:
- ICSI (intracytoplasmic sperm injection) in 67.3% of cycles;
- FIV-ET (in vitro fertilization and embryo transfer) in 9.5% of cycles;
- AIH (homologous intrauterine insemination) in 23.2% of cycles, as second instance after the failure of controlled ovarian hyperstimulation.

“Long” or “short” protocol was used to induce the ovarian hyperstimulation in the analyzed procedures. Long protocol contemplates the use of analogues GnRH in an only administration (Triptorelina: 3.75 mg fl i.m.) on the 21st day of previous menstrual cycle, or a daily administration from 21st day of menstrual cycle (Triptorelina: 0.1 fl s.c.), followed by the administration of gonadotropins until the day before the administration of hCG (which corresponds to the estradiol peak). The short protocol contemplates, instead, the use of analogues GnRH during the early follicular stage in association with gonadotropins; united administration lasts till the day before the administration of hCG.

The gonadotropins which were used are: recombinant FSH: ampoule that contains 100 UI of FSH in 0.5 ml physiologic solution; highly purified human urinary FSH, urofollitropin: the ampoule contains 75 UI of FSH; highly purified, human menopausal gonadotropins, HM, menotropin: the ampoule contains 75 UI of FSH and 75UI of LH; clomiphene citrate: used only in one cycle?

The hCG used was Gonasi: HP 5000 fl i.m.

The adopted therapeutic schema (kind of medicine, dosage and time of administration) was obviously personalized, basing on clinical history and particularly on patient ovarian response.

Pre-ovulatory stage monitoring was done by seriate transvaginal echography and by seriate dosage of plasmatic estradiol. The presence of a prevalent follicle of 18-20 mm and of at least two follicles of 15-18 mm (united to an appropriate value of 17-β-estradiol, at least more than 1000 pg/ml) was considered an index of appropriate follicular maturation. At this point hCG (Gonasi) was given to induce follicular dehiscence.

After about 36 hours the pick-up (oocytes taking) was done in loco-regional analgesia.

The oocytes, once detected in follicular fluid, were valued for maturity degree and quality. Those which were fit were used for in vitro fertilization.

Formed embryos were classified for their maturity degree. The number of suitable transferred embryos was valued in base of different parameters, mainly the age of patient.

In the end blood quantitative value of β-hcG was estimated, after 14 days from transfer, in woman in order to detect “biochemical” pregnancy.

To analyze data, a linear regression model was used:
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- TSH values and dose of used gonadotropins (UI of FSH);
- TSH values and dose of used urofollitropin (UI of FSH);
- TSH values and dose of used menotropin (UI of FSH);
- TSH values and dose of used recombinant FSH (UI of FSH);
- TSH values and number of taken oocytes;
- TSH values and quality of the best taken oocytes;
- TSH values and number of gained embryos;
- TSH values and quality of the best gained embryo.

To compare the mean value of TSH among patients with effective ovarian stimulation and those that needed AIH T Student Test was used.

To compare group A (AbTPO+) and group B (AbTPO-) χ² test was used.

Results

Any statistical association was discovered, from the analysis of effected elaborations, between:
- TSH values and dose of gonadotropins required to induce ovarian hyperstimulation (UI of FSH) (Figure 1);

The dispersion graph derived from the association among serum TSH values (independent variable) shows an inclination of circles (procedures) to arrange themselves according to a straight line (regression line) that is addressed to descending direction towards right (p=0.665). A positive association would have determined, instead, an ascended line towards right.

- TSH values and used drug doses to induce ovarian hyperstimulation, individually considered in order to avoid confusing factors (Figure 2);

The dispersion graph shows a regression line with descending direction towards right and respective coefficient of determination (r²) of 0.017 (p=0.280) (Figure 3).

Concerning menotropin, graph shows a r² (coefficient of determination) = 0.019, with a no significant association (p=0.493) (Figure 4).

About recombinant FSH there is no statistically significant association with TSH values (p=0.978).

- TSH values and number of taken oocytes (p=0.578)
- TSH values and quality of the best taken oocytes (p=0.325)
- TSH mean value in group with effective ovarian stimulation respect group ended in AIH (considered as failure of hyperstimulation). In this comparison an higher number of AIH was discovered for lower values of TSH, an opposite outcome respect the awaited one, but not statistically significant (p=0.298) (Figure 5)
- TSH values and number of given embryos (p=0.421)
- TSH values and quality of the best given embryo (p=0.183).

Further variables was considered in order to achieve a complete analysis, free from corrupting elements. Therefore, the various parameters (taken oocytes, trans-
ferred embryos, detected “biochemical” pregnancy, AIH done) were valued and compared between groups that included procedures with higher or lower TSH than the cut-off suggested by some Authors of 2.5 mIU/L; procedures with or without levothyroxine treatment; procedures with Ab anti-TPo positivity or negativity. There were no significant statistical differences in the various comparisons.

To underline, despite not significant association, the absence of biochemical pregnancy in group A (Ab anti-TPO+) respect the 9% detected in group B (Ab anti-TPO).

Discussion and conclusion

Despite the high frequency of thyropathy in infertile patients, the cause-effect linkage has not been yet
The results of the present study did not confirm the hypothesis, supported by clinical evidences, that the thyroidal status directly influences fertility, in terms of oocytes and embryos development, or at least not at a level that produces measurable effects on a clinical context of medically assisted reproduction.

This study has several limits that may have conditioned, in various measures, the results among which retrospective design, patients' age, the absence of a routinely valuation of thyroidal set-up and the etiological variability of infertility.
As for the age factor, we have to underline opportunely that it represents a predictive factor of pregnancy failure (both spontaneous and medically assisted). Actually, fertility has an acme at 25 years and it declines gradually after 32 years both in men and women. Age acts on women fertility mainly through aging of oocytes, as the results obtained in fertilization programs using oocytes of donors showed. In these programs, in fact, the abort rate in receivers changes from 14 to 45% according to whether age of oocytes donor is between 20-24 years or above 35 years. Aging, in fact, causes an increased prevalence of aneuploidy in oocytes which is the result of alterations of controlling mechanisms that rules the meiotic process. The consequent hormonal modifications, that characteristically begin to show themselves round 35-40 years, consist in a slight but real increase of TSH and in a diminution of inhibine, as a demonstration of rapid diminution of number and of worse quality of primordial follicles still present in ovary in this period. Age may, moreover, act even indirectly favoring the possibility to contract sexual transmitted diseases, increasing of benign pelvic pathology as endometriosis and through reduction of intercourse frequency. The mean age of included patients in this study is 37.92 years: a rather high value even if greatly predictable, both considering a general tendency to postpone the age of first pregnancy, and, especially, considering the particular setting of the study, concerning medically assisted procreation, to which for definition infertile couples, who attempt a pregnancy for a long time, turn to.

Another limit to take in count in a retrospective study as this is, that focuses the attention on the linkage between thyropathy and infertility, is the absence of an evaluation of thyroid set-up during the routine screening of pregnancy women (both spontaneous and medically assisted). Even if in the last few years a major attention of gynecologists concerning thyroidal functionality is recorded, it results still insufficient. In particular, thyroidal autoimmunity is often neglected, despite autoantibody positivity may be present even in a euthroid state. The difficulty noticed to include procedures in the study (due the lack of a thyroidal valuation), averted, from having at disposal a larger case histories.

At last, even if not for importance order, it must be considered as limit (hardly eliminable) the extreme etiological variability of sterility. Sterility recognizes, actually, a multifactorial etiology that includes anatomical, hormonal, thrombotic, autoimmune, genetic, infective or unknown causes. Sterility can be female, male, couple or idiopathic. In this study these variables, that however play a determinant role in this ailment, were not considered.

In conclusion, despite limits above mentioned surely invalidated the results, it is conjecturable that thyroidal homeostasis influences fertility during implant phase and following development of embryo, rather than the first stage of pre-implant that was considered in this study (oocytes and zygotes development).

Pregnancy, in fact, must be considered an endocrine-immune condition of special exceptionality, that requires the greatest efficiency of both systems, in particular for receptivity of endometrium and for the following embryonic development. Further investigations, therefore, should be addressed to early stages of pregnancy (first trimester), focusing the attention on the possible role of thyroid hormone and anti-thyroid antibody on physiological mechanism oriented to the maintenance and development of embryo-trophoblastic unit and fetoplacental, in case potentiated by vascularizing treatment and luteal stimulus.

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