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# Obesity is associated with a higher level of pro-inflammatory cytokines in follicular fluid of women undergoing medically assisted procreation (PMA) programs

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**Abstract. – *Introduction:*** **Cytokines are glycoproteins that modulate reproductive function through a series of various mechanisms (by both conditioning gonadal steroidogenesis and contributing to the preservation of an inflammatory microenvironment).**

***Aim of the Study:*** **To evaluate the impact of certain clinical variables (i.e., age, obesity, insulin resistance index, serum antithyroid antibodies serum levels) on the serum concentrations of cytokines TNF-alpha, IL-6, and IL-10 in the follicular fluid of women undergoing a medically assisted procreation (PMA) cycle.**

***Materials and Methods:*** **A total of 40 female patients undergoing an intracytoplasmic sperm injection (ICSI) in oocytes, following ovarian stimulation by purified FSH and hCG carried out after suppression of ovarian function. The follicular fluid, obtained by surgical ultrasonography-guided withdrawal, was stored at –30 degrees C. Subsequently the cytokines were assayed by ELISA technique.**

***Results:*** **Women suffering from class II obesity showed follicular levels of TNF-alpha significantly higher ($p<0.05$) than women with a normal body mass index (BMI). Significantly higher concentrations of TNF-alpha and IL-6 were found in women with HOMA index >2.5. Women clinically presenting with concomitant obesity and high serum levels of antithyroid antibodies were found to have higher follicular levels of TNF-alpha and IL-6 ($p<0.05$) in comparison with women suffering from obesity only or low antithyroid antibodies levels only, or from both these conditions.**

***Conclusion:*** **Obesity is a common clinical condition associated with a higher concentration of inflammatory substances in the follicular fluid of infertile women. It is not understood, as yet, the possible pejorative role exerted by the presence of other clinical conditions, such as insulin resistance and high levels of antithyroid antibodies, that are conditions frequently encountered in the clinical practice.**

*Key Words:*

Cytokines, Follicular fluid, Obesity, Assisted procreation programs.

## Introduction

Ovarian function can be influenced by the biological action of cytokines, which are able to interfere with the signal coming from the pituitary gland as well as from the locally generated ovulatory phase, the luteal phase and the steroidogenesis. A regulatory effect by interleukin 1 (IL-1) and tumor necrosis factor-alpha (TNF-α) on the pituitary axis has been documented, both *in vitro* and *in vivo*. In fact, in the experimental animal, IL-1 administration can suppress GnRH release. In addition, IL-1 shows a direct action (i.e., inhibition) on testicular and ovarian steroidogenesis. IL-1 inhibitory effects are potentiated by TNF. Similar effects to those produced by IL-1 are ascribed to IFN-gamma[1-7].

TNF-α influences sexual hormone biosynthesis, by modulating receptor function of gonadotropins and by modifying ovarian folliculogenesis[8-10]. TNF-α, in association with IFN-gamma, decreases estradiol and progesterone secretions from luteal cells[11-14]. *In vivo* studies show the presence of higher levels of TNF-α in the follicular fluid of women with infertility of immune origin, as compared with other women presenting with an isolated tubal factor[15].

IL-6 is characterized by a pleiotropic action and by its involvement in the regulation of immune response[16]. *In vitro* studies indicate that its produc-

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tion is balanced by IL-1 alpha/beta production, whereas it is not influenced by TNF-alpha and is inhibited by IFN-gamma[17]. This cytokine could be involved in the mechanism of oocyte selection[18] and its concentration in follicular fluid appears to be related with the degree of oocyte maturation[19]. The increase in IL-6 level in the follicular fluid of women with an immunologic factor of infertility reflects a function of modulation and functional antagonism towards TNF-α[20,21]. Cytokine production in the follicular fluid is a clear example of self-regulation aimed at decreasing the impact of a pronounced inflammatory response due to high levels of TNF-α; such findings are typically found in women with immune infertility[15].

IL-10 reflects an immunosuppressant function, with a functional antagonism towards the main pro-inflammatory cytokines (i.e., IFN-gamma and TNF-α)[22]. It is not entirely clear the biological role of IL-10 in the follicular fluid, with special reference to its possible involvement in folliculogenesis[23]. A recent study by Cerkiene et al[24] carried out on 121 women shows that IL-10 follicular levels fail to interfere with the outcome of medically assisted procreation (PMA) programs. Although a decreased local production of IL-10, as a result of high TNF-α high levels, appears to be one of the mechanisms involved in generating infertility of immune origin.

Based on these data, we evaluated the concentrations of three different cytokines, i.e. TNF-α, IL-6 and IL-10, in the follicular fluid of obese women undergoing a medically assisted procreation (PMA) cycle, in relation to certain clinical variables, such as age, body mass index (BMI), insulin resistance index and antithyroid antibodies serum levels.

## Material and Methods

A total of 40 women from 28 to 43 years old (mean age: 34 years) were selected and then enrolled in the study after obtaining their signed informed consent. All the women afferent for couple infertility, in particular secondary to isolated tubal pathology in 10 cases (25%), chronic anovulation in 6 cases (15%), cervical inflammation in 8 cases (20%), documented male factor in 8 cases (20%), and apparent idiopathy in the remaining 8 cases (20%). After their physical examination, the women were grouped into clinical categories according to the following parameters: age, BMI (kg/m$^2$), insulin resistance index, presence or absence of serum antithyroid antibodies. After the initial endocrinologic evaluation, all the women underwent the following fasting blood tests: glycemia [enzymatic method; Roche Diagnostics, Monza (MI), Italy], insulin [ECLIA; Roche Diagnostics, Monza (MI), Italy] for determining HOMA index (fasting blood glucose (nmol/l) × basal insulin (nmol/l)/22.5)[25] and serum antithyroid antibodies [ECLIA; Roche Diagnostics, Monza (MI), Italy]. Following ovarian axis suppression by GnRH analogue (*Decapeptyl*® Ipsen S.p.A., Milan, Italy), all the women were treated with purified FSH (*Puregon*® Organon Italia, Rome, Italy) and hCG (*Gonasi HP*® Amsa, Rome, Italy) according to a superovulation protocol. Following ovarian pick-up, aliquots of follicular fluid were stored at –30 degrees C and IL-6, IL-10 and TNF-alpha levels were assayed by ELISA technique (sandwich ELISA R&D System Europe Ltd, Abingdon, UK). The protocol was approved by Institutional Ethics Committee and all patients signed informed consent.

### Clinical Criteria for Exclusion

Autoimmune factors, endometriosis.

### Clinical Criteria for Inclusion

*Concerning to the couple* – desire to procreate of at least two years duration, recurrent failure following induction of ovulation and planned sexual intercourse, failure of fecundation following first level technique (3-6 months);

*Congerning to the woman* – severe peritoneal factor, poor oocyte response following therapy with high doses of gonadotropins (<3 oocytes or >40 years old with <5 oocytes); and

*Concernig to the man* – severe oligospermia including cases of cryptozoospermia, severe or total asthenozoospermia, severe or total teratozoospermia, retrograde ejaculation.

### Follicular Fluid Withdrawal

Follicular fluid withdrawal was carried out by transvaginal route under ultrasonographic guide (Esaote GPX, Genova, Italy, Megas, 7.5 MHz, biplane biconvex probe). Each follicle was separately suctioned, collected into a 15-ml test tube and dipped into the culture medium. Following hCG administration, the follicles showed a mean 16 mm diameter, the mean estradiol concentration was 7.1 ± 0.6 nmol/l. Oocyte recovery took place 36 hours following hCG administration.

**Table I.** Concentration of cytokines in ovarian follicolar fluid in relation to age of patients.

| Group | Age | Number of patients | IL 6 (pg/ml) | TNF-α (pg/ml) | IL 10 (pg/ml) |
|---|---|---|---|---|---|
| 1 | < 30 | 6 | 17.1 ± 1.6 | 19.1 ± 2.1 | 6.3 ± 0.6 |
| 2 | 30-35 | 15 | 21.7 ± 1.3 | 27.6 ± 2.5* | 2.9 ± 0.3 |
| 3 | 35-40 | 12 | 14.9 ± 1.7 | 16.3 ± 1.9 | 3.7 ± 1.1 |
| 4 | > 40 | 7 | 18.4 ± 2.5 | 19.6 ± 3.2 | 3.1 ± 1.1 |

Values expressed as mean ± SEM.

### Statistical Analysis

Statistical analysis was obtained by means of ANOVA followed by the use of Newman Keuls test (SNK). *P* values <0.05 were considered as a statistically significant difference.

## Results

Patients were divided into 4 groups according to age (<30 years, 30-35 years, 35-40 years, >40 years). Patients belonging to the 30-35 years age group showed intrafollicular TNF-α levels significantly higher ($p$ <0.05) compared to patients belonging to 35-40 years age group.

Patients were divided also into 4 groups according to BMI (i.e., 18.5-24.9; 25-29.9; 30-34.9; 35-39.9 kg/m$^2$); the patietns with a BMI between 35 and 39.9 kg/m$^2$ showed intrafollicular TNF-α levels significantly higher ($p$ <0.05) compared to remaining groups (Table II).

Patients with a HOMA index >2.5 showed intrafollicular IL-6 and TNF-α levels significantly higher ($p$ <0.05) compared to patients with a HOMA index <2.5 (Table III).

No significant differences were found between patients with positive or negative serum antithyroid antibodies (Table IV).

Patients with BMI >25 kg/m$^2$ and concomitant positive serum antithyroid antibodies showed intrafollicular TNF-α and IL-6 levels significantly higher ($p$ <0.05) compared to patients with isolated obesity and/or negative antithyroid antibodies (Table V).

## Discussion

### Obesity and Female Reproduction

Female obesity is associated with higher risk of pathological endocrine conditions, such as amenorrhoea and infertility[26-30;33,34]. In addition, obesity influences negatively the outcome of medical treatments for infertility[32,35]. The risk of a miscarriage following fertilization is higher in obese women[31]. Hormonal profile, especially when there is a concomitant amenorrhoea, is characterized by a high rate of hyperandrogenism, increased aromatization, altered production of proteins that are linked to sexual hormones[26-35]. The present study confirms that obesity is a clinical condition associated with worse reproductive profile[36,37]; as demonstrated by higher rate of intrafollicular oxidative stress which was observed before fertilization in a group of patients undergoing to medically assist-

**Table II.** Concentration of cytokines in ovarian follicolar fluid in relation to body mass index of patients.

| Group | Body mass index | Number of patients | IL 6 (pg/ml) | TNF-α (pg/ml) | IL 10 (pg/ml) |
|---|---|---|---|---|---|
| 1 | 18.5-24.9 | 8 | 14.2 ± 0.6 | 15.2 ± 0.6 | 4.8 ± 0.9 |
| 2 | 25-29.9 | 16 | 20.5 ± 0.7* | 23.1 ± 0.6 | 4.4 ± 1.1 |
| 3 | 30-34.9 | 10 | 21.1 ± 1.9* | 24.7 ± 1.3 | 2.8 ± 0.7 |
| 4 | 35-39.9 | 6 | 23.1 ± 0.8* | 29.8 ± 2.5* | 2.7 ± 0.5 |

Values expressed as mean ± SEM.

**Table III.** Concentration of cytokines in ovarian follicolar fluid in relation to HOMA index of patients.

| Group | HOMA index | Number of patients | IL 6 (pg/ml) | TNF-α (pg/ml) | IL 10 (pg/ml) |
|---|---|---|---|---|---|
| 1 | > 2.5 | 14 | 22.8 ± 0.4* | 22.2 ± 1.7* | 3.9 ± 0.2 |
| 2 | < 2.5 | 26 | 15.7 ± 0.8 | 16.3 ± 1.1 | 5.4 ± 0.6 |

Values expressed as mean ± SEM.

**Table IV.** Concentration of cytokines in ovarian follicolar fluid in relation to positivity or negativity serum thyroid antibodies.

| Group | AAT ATPO | Number of patients | IL 6 (pg/ml) | TNF-α (pg/ml) | IL 10 (pg/ml) |
|---|---|---|---|---|---|
| 1 | ++ | 10 | 19.7 ± 1.9 | 23.1 ± 2.9 | 2.8 ± 0.5 |
| 2 | +/- | 10 | 16.6 ± 3.3 | 18.2 ± 3.8 | 3.9 ± 2.1 |
| 3 | -/- | 20 | 16.1 ± 1.1 | 17.9 ± 1.5 | 5.4 ± 0.8 |

Values expressed as mean ± SEM.

ed procreation[38]. In particular, insulin resistance is associated with proinflammatory balance of cytochemical ovarian microenvironment[39]. Insulin resistance is a consequence of obesity, and in women it is often linked with ovarian function leading to clinical reproductive manifestations such as early menarche onset, subfertility and polycystic ovary syndrome[40]. Likewise, the dramatic fall in oestrogen production after menopause may contribute to weight gain and changes in adipose tissue distribution[41]. Overall, women who are obese, especially those with reproductive complications including polycystic ovary syndrome, have been identified as specific high risk subgroups for further progression through to impaired glucose tolerance, type 2 diabetes mellitus and potentially cardiovascular disease[42]. Obese women exhibit an altered ovarian follicular environment, particularly increased metabolite, C-reactive protein, and androgen activity levels, which may be associated with poorer reproductive outcomes typically observed in these patients[43].

### Thyroid and Female Reproduction

The presence of high serum levels of antithyroid antibodies may be associated with a premature interruption of pregnancy as well as a failure in medically assisted procreation[44-47]. Furthermore, antithyroid antibodies positivity is often found in healthy women, who have never suffered from thyroid disease in the past. Studies carried out by Geva et al. showed that the rate of patients presenting with positive antithyroid antibodies was very high among infertile women. Moreover, a low number of them was successfully treated with medically assisted procreation. In 1998, Kim et al. reported that patients with positive serum antithyroid antibodies, together with either a tubal factor or an unclear infertility fac-

**Table V.** Concentration of cytokines in ovarian follicolar fluid in obese patients and associated serum thyroid antibodies positivity.

| Group | Clinical profile | Number of patients | IL 6 (pg/ml) | TNF-α (pg/ml) | IL 10 (pg/ml) |
|---|---|---|---|---|---|
| 1 | S + TA | 18 | 21.1 ± 1.1* | 23.1 ± 2.9* | 4.1 ± 1.9 |
| 2 | O + TA | 10 | 23.1 ± 0.4* | 28.5 ± 1.9* | 2.5 ± 0.3 |
| 3 | N/NTA | 12 | 15.3 ± 0.8 | 16.9 ± 1.1 | 4.7 ± 0.7 |

Values expressed as mean ± SEM. OW = overweight; O = obesity; TA = thyroid antibodies; N = normal weight; NTA = negatives for thyroid antibodies.

tor, showed a lower rate of success following the application of medically assisted procreation. Although a series of controversial data are found in the literature on such a subject, a relative influence is generally accepted[46, 48-54], the antioxidant enzymatic or not systems become altered during thyroid dysfynction[55]. During autoimmune thyroid dysfunction, in particular Basedow's disease, it has been observed that monocyte and macrophage activation cause an increased IL-1, IL-10 and TNF-alpha production[56]. The data obtained in the present study support an antithyroid antibodies role as a possible co-factor capable of contributing to increase the rate of oxidative stress in ovarian microenvironment, in combination with a traditional risk factor as obesity. Instead, the isolated presence of such antibodies is not associated with an increase in pro-inflammatory ovarian cytokine concentration.

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