Intrafollicular Levels of Matrix Metalloproteinases-2 and -9 in Patients with Polycystic Ovaries Are Not Associated with Pregnancy Rate During IVF Cycle

STAVROULA BAKA1,2, KONSTANTINA ZOURLA1, ARIADNE MALAMITSI-PUCHNER2, EVANGELOS MAKRAKIS2, GEORGE KAPAROS1, STELLA DEMERIDOU1, THEODORE MOUSTAKARIAS2, DESPOINA TZANAKAKI2, DIMITRIS HASSIAKOS2 and EVANGELIA KOUSKOUNI1

1Department of Biopathology, and 2IVF Unit, Second Department of Obstetrics and Gynecology, Aretaion University Hospital, Athens, Greece

Abstract. This study aimed to detect the levels of matrix metalloproteinases (MMP)-2 and -9, using enzyme-linked immunosorbent assays, in the follicular fluid of 35 patients with polycystic ovaries, compare them with the levels found in 35 normally ovulating women enrolled in their first in vitro fertilization (IVF) cycle and then correlate them with pregnancy rates in these two groups. Levels of MMP-9 were found significantly increased in women with polycystic ovaries when compared with the controls, while MMP-2 levels were higher in women with polycystic ovaries without reaching statistical significance. The two groups did not differ in age, in the number of embryos transferred or in pregnancy rates. In conclusion, the results indicated an increased gelatinolytic activity in patients with polycystic ovaries after ovarian stimulation for IVF treatment without detecting any association between levels of MMP-2 and 9 and IVF pregnancy rates.

The initiation of pregnancy in an IVF cycle is dependent on a plethora of factors such as follicular development, number of retrieved oocytes, fertilization, embryo development and finally, implantation. All these processes require continuous changes, first in the ovaries and then in the uterus. Human ovaries are subjected throughout the entire reproductive life to extensive tissue remodeling secondary to functional changes during follicular growth, ovulation and atresia. The profound remodeling of extracellular matrix, comprising degradation and recomposition, is determined greatly by the activity of metalloproteinases (MMPs), a group of proteolytic enzymes (gelatinases). Thus, the presence of MMPs in the follicular microenvironment is important for the subsequent follicular development. Decreased levels of MMPs in preovulatory follicular fluid (FF) have been associated with deficient follicular growth and/or ovulation (1).

The role of MMPs in ovarian tissue remodeling has already been demonstrated. MMP-2 and MMP-9 were isolated in FF, in culture media and granulosa cell homogenates from follicular aspirates collected at oocyte recovery for in vitro fertilization (IVF) (2) and in human preimplantation embryos (3).

Gelatinases seem to contribute to the stabilization of the extracellular matrix, an important process at the initiation of pregnancy, after rescuing the corpus luteum (2). Interestingly, in a recent study, the expression of MMP-9 in the FF and culture media was a prerequisite for successful IVF pregnancy (4).

The polycystic ovary syndrome (PCOS) is associated with persistent anovulation as a result of inappropriate follicular growth followed by failure to select the dominant follicle and follicular arrest. By definition, PCOS is associated with the presence of multiple cystic follicles bilaterally and many other morphological changes in the ovaries which result in a continuous and increased remodeling of the ovarian stroma. Recently, elevated serum concentrations of MMP-2 and MMP-9 have been reported in women with PCOS (5, 6). Similarly, higher levels of MMP-9 and MMP-2 were found in granulosa cells from PCOS patients compared to normal ovulatory women, so that MMP-9 and MMP-2 may be associated with inappropriate atresia in PCOS (7).

This study was based on the hypothesis that since MMP-9 is present in the FF and was associated with successful IVF outcome, some differences in the activity of this enzyme are expected in women with polycystic ovaries where tissue remodeling is increased. Therefore, the aim was to investigate MMP-2 and MMP-9 levels in the FF of PCOS patients, compare them with those in normally ovulating women all undergoing controlled ovarian hyperstimulation.
for their first IVF attempt and to detect possible relationships between these levels and IVF pregnancy rates.

**Materials and Methods**

The Ethical Committee of Aretaieion Hospital approved the study and informed consent forms were obtained from all participants.

Women presenting for their first IVF cycle with no previous pregnancy after at least 3 IUI attempts were invited to participate in the study. The study population included 35 women with diagnosed PCOS, based on the evidence of any two of three features – hyperandrogenism, menstrual irregularity and polycystic ovary morphology, according to the Rotterdam consensus criteria (8) and 35 normally ovulating women. Controlled ovarian hyperstimulation was achieved in all individuals with a long protocol: daily administration of gonadotrophin releasing hormone (GnRH) (beta-follitropin, Puregon, Organon, The Netherlands) in a daily dose of 75-300 IU (depending on the patient’s characteristics) when down-regulation was confirmed (serum estradiol <50 pg/ml). Follicular development was monitored with serial ultrasonographic examinations and serum estradiol measurements. When 3 or more follicles 18 mm in diameter were detected on transvaginal ultrasonography, 10,000 IU of human chorionic gonadotropin (hCG) were administered. Follicular fluids were collected during oocyte pick-up which was scheduled 35-36 h post-hCG and was performed transvaginally under ultrasonography. After removing the oocytes, blood-free FF samples were centrifuged at 600 xg for 10 minutes and supernatants were stored at –80˚C until processing. IVF was performed with the respective husband’s spermatozoa and all embryo transfers were performed on day 3 with a Wallace catheter under ultrasound guidance.

The concentrations of MMP-2 and MMP-9 were determined in duplicate using the respective RayBio® Human MMP-2 and MMP-9 ELISA kits (RayBiotech, Inc, USA) designed to measure their pro and active forms. The minimal detectable dose was less than 80 pg/ml for MMP-2 and less than 10 pg/ml for MMP-9. The intra-assay and inter-assay CV for both enzymes were <10% and <12%, respectively.

Statistical analysis of the data was performed using t-test, Fisher exact test and Mann-Whitney test. Data are expressed as means±SD. P-values of <0.05 were considered to be significant.

**Results**

In this study, 35 women with PCOS were analyzed and compared with 35 normal ovulatory women who served as controls. All FF were collected from follicles reaching about 18 to 20 mm in diameter. In each patient FF samples were pooled and a volume of about 5 ml (after centrifugation) was cryostored until analysis.

Patient characteristics can be seen in Table I. There were no significant differences in patients’ age or in the number of embryos transferred. Moreover, the two groups did not differ in body mass index, duration of infertility, or the total gonadotrophins administered (data not shown). Activity of both MMP-2 and MMP-9 varied greatly between patients. A trend towards higher MMP-2 concentrations was noted in the PCOS group, but the difference did not reach significance (p=0.06). A significant increase in MMP-9 levels (p<0.001) was found in women with polycystic ovaries when compared to the controls.

Even if the number of achieved pregnancies in the PCOS group was higher in comparison with the controls (14 versus 7), the difference was not statistically significant (p=0.06). Finally, there were no significant differences when comparing the MMP-2 and MMP-9 FF concentrations between the pregnant and non-pregnant groups in both the study population as well as in the controls (Table II).

**Discussion**

The ovarian tissue is submitted to major remodeling processes of the extracellular matrix throughout reproductive life, together with structural and functional modifications (9). In the present study, it is demonstrated that MMP-2 and MMP-9 concentrations were higher in the FF of IVF patients with PCOS compared to the levels found in normally ovulating women undergoing IVF treatment. To date, there are a limited number of published studies regarding the intrafollicular levels of MMP-2 and -9 in PCOS patients undergoing IVF and this study is probably the first to measure them in the patients’ first IVF attempt.

MMPs are present throughout follicular development (10). Specifically, MMP-2 was detected in similar amounts at different phases of follicular growth (11) and was proposed as a marker of follicular health (12). Likewise, MMP-9 was also present in the follicular fluid and its levels increased significantly when follicular volume increased (11, 13); in particular when follicular diameter was over 25 mm, MMP-9 was associated with the detection of a follicular cyst (12). At ovulation, the increase in ovarian collagenolysis was associated with an increase in MMP expression and activity (9). In order to overcome possible variations between different-sized follicles, pooled FF were used for all patients so a more representative sample for each patient was obtained.

The presence of MMP-2 and -9 was confirmed in freshly prepared granulosa cells, culture media and samples of corpora lutea (2). Interestingly, D’Ascenzo et al. (14) demonstrated for the first time that MMP-2 and -9 levels were much lower in FF of IVF patients compared with normally ovulating women. Furthermore, they presumed that MMP-2 is involved in follicular development while MMP-9 expression is recruited in follicular breakdown. Similarly, no statistically significant differences were observed in MMP-2 and MMP-9 FF levels between natural cycle women and different stimulation protocols (15).

Gelatinolytic activity was observed throughout the formation of the corpus luteum. Significant increase in the
activity of the MMPs was associated with the regression of the corpus luteum (10).

The passage from normal follicles to atresia by performing hypophysectomy determines an increase in intrafollicular levels of MMP-2 and -9. Therefore, these MMPs were associated with inappropriate follicular development in PCOS (10). Lahav-Baratz et al. (16) found similar activity of MMP-2 and MMP-9 in the FF of leading follicles in normally ovulating and women with PCOS. The explanation for these results was based on the fact that MMP-2 is less influenced by a specific endocrine milieu (9). On the contrary in their study group, Nikolettos et al. (17) found a significant positive correlation between MMP-2 and peak estradiol levels.

On the other hand, MMP-9 seems to be influenced by hormones and it has even been hypothesized that MMP-9 may have an important modulatory effect on progesterone secretion (18). In women with polycystic ovaries, the increased levels of luteinizing hormone (LH) can influence changes in the estradiol to progesterone ratio and then changes in the MMP levels compared with that of normally ovulating women (19).

Shalev et al. (7) reported higher levels of MMP-2 and MMP-9 in the FF and in the cultured luteinized granulosa cells of patients with PCOS under treatment in an IVF-embryo transfer (IVF-ET) program compared with normally ovulating patients. In PCOS women undergoing IVF treatment, the administration of exogenous gonadotropins during controlled ovarian hyperstimulation may affect the MMP levels since changes in the cellular response to hormones as well as hormonal production are different in these patients compared to non-PCOS women (20).

In a recent study, the possibility of detecting gelatinases in human embryos was investigated. MMP-2 was detected in embryos at all stages, while MMP-9 was detected in only a minority of human embryos (3).

Pregnancy represents a period of major changes so variations in MMP levels could be expected. Nevertheless, studies revealed that MMP-2 levels in serum (21) or in FF (4) were not different between pregnant and non-pregnant women, suggesting that evaluation of this MMP is not useful for the management of infertile women after IVF-ET. In contrast, higher MMP-9 levels in FF and culture media were found in the pregnant group compared to the non-pregnant group in IVF cycles (4). Since no differences in FF MMP-2 and -9 levels were observed in these patients in relation to pregnancy, there is agreement with the hypothesis presented by Stamouli et al. (2) stating that the rescue of the corpus luteum in early pregnancy involves the maintenance of cellular function through the stabilization of the extracellular matrix. It seems that at least at the initiation of pregnancy, the modifications that are required in the extracellular matrix do not involve great variations in MMP levels. The presented results do not encourage the consideration of either of the MMPs studied as predictors for the establishment of pregnancy.

In summary, levels of MMP-2 and MMP-9 in the first IVF cycle were not associated with a higher pregnancy rate and MMP-9 levels were increased in the PCOS group. Since considerable variations were observed in their levels, both between the two groups of the study, as well as between women of the same group, it is hypothesized that levels are modulated by the hormonal environment within each patient. Further studies focusing on the tissue MMP levels in PCOS patients are needed for a better understanding of the mechanisms involved in ovarian reconstruction, follicular development during controlled ovarian hyperstimulation and possible implication in the initiation of pregnancy through a direct effect on the uterus.

References


Received July 14, 2008
Revised December 2, 2008
Accepted December 8, 2008