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ORIGINAL ARTICLE

The role of matrix metalloproteinase-2 in the culture media in embryo implantation rate in normogonadotrophic cases undergoing ICSI

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KEYWORDS

MMP-2;
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Abstract *Objective:* The aim of the study is to correlate the changes in the biochemical marker MMP-2 in the culture media with the outcome of normogonadotrophic cases undergoing ICSI.

Methodology: A prospective study of infertile females was conducted in El-Shatby Maternity University Hospital between October 2011 and May 2012 utilizing a sample of 40 normogonadotrophic infertile women (22 females with unexplained infertility and 18 females with tubal factor infertility).

Results: Clinical pregnancy was 57.5%; 15 out of the 22 females with unexplained infertility and 8 out of the 18 females with tubal factor infertility. There was no abortion, ectopic or chemical pregnancy. Ongoing pregnancy after 14 weeks of gestational age was 100%.

Total (MMP-2) ranged between (4.1 and 21.1) and (3.5–37) ng/ml with the mean of (9.91 ± 5.48) and (13.91 ± 8.87) ng/ml for non pregnant and pregnant groups respectively. There were no statistical significant differences between the two groups regarding total MMP-2 ($P = 0.055$).

The mean of MMP-2/embryo/h ranged between (0.05 ± 0.05) and (0.06 ± 0.08) ng/ml/embryo/h for non pregnant and pregnant groups respectively. There were no statistical significant differences between the two groups regarding MMP-2/embryo/h ($P = 0.234$).

Conclusions: MMP-2 concentration in the culture media cannot be used as a biochemical marker for embryo selection or prediction of implantation in the normogonadotrophic cases undergoing ICSI.

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Recommendations: Results of the present study suggest searching for other markers in the culture media for better embryo selection and for prediction of implantation in the normogonadotrophic cases undergoing ICSI.

1. Introduction

Embryo implantation is considered the most important step in both natural and assisted human reproduction. Implantation is an active process, involving apposition, adhesion, and invasion of the embryo into the maternal endometrial stroma (1).

Failure of implantation is considered the major barrier in ICSI success. A vital embryo, a receptive endometrium and an effective molecular dialogue between both embryo and endometrium are necessary for successful implantation (2). To overcome this barrier in practice and to increase the chance of implantation, more than one embryo are transferred into the uterine cavity, but this may lead to multiple pregnancy which is considered as one of the complications of ICSI due to its detrimental effect for both the parents and society (3).

More than 500 genes which encode proteases or protease like proteins have been discovered in human genome sequence (4). Matrix metalloproteinases (MMPs) are considered as a group of them, this group contains more than 20 enzymes that cause breakdown of the extracellular matrix (ECM) and basement membrane (BM). MMPs are involved in matrix remodeling as biochemical mediators associated with perimenstrual phases in endometrium (5,6).

Although MMP expression is very low in most adult tissues, active tissue remodeling induces a significant increase in this expression (7). Few of them are expressed throughout the menstrual cycle. The maximal expression of MMPs has been observed during the perimenstrual phase and is undetectable during the proliferative and secretory phases (8).

MMPs are also known as matrixins which are found in invertebrates, vertebrates and plants. They are calcium and zinc-dependent proteases which are responsible for the breakdown and rebuilding of connective tissue components such as collagen, elastin, gelatin and casein (9). In 1962, MMPs had been discovered by Jerome Gross and Charles M. Lapiere while studying the triple-helical collagen degradation during the metamorphosis of a tadpole tail (10) MMPs family members sharing in about 40% of their primary structures. According to substrate specificity and cleavage mechanism, MMPs can be classified into six categories: collagenases, stromelysins, matrilysins, gelatinases, membrane-associated MMPs (MT-MMPs) and MMPs with no group designation. Nowadays it is possible to determine the structures of many MMPs using X-ray crystallography and nuclear magnetic resonance (NMR) studies (11). With exception of membrane associated MMPs (MT-MMPs), MMPs are commonly secreted from cells in its inactive form to prevent MMPs from degradation of the essential components in the cells. The enzyme is divided into three domains: N-terminal propeptide, catalytic domain and hemopexine like domain (12).

In mammalian species numerous MMPs are necessary for processes of implantation, placentation, and parturition. Successful pregnancy requires invasion of the embryo to maternal endometrium which requires breakdown of basement membrane and extracellular components which are degraded by the effect of some MMPs. The trophoblastic cells' invasion of the endometrium during the process of implantation and placentation requires dramatic cellular mobility of MMP, remodeling of host tissue, and development of placenta (13,14).

The expression of MMPs, which are able to breakdown basement membranes is necessary for successful implantation and trophoblast invasion. The main component of the basement membrane is collagen IV. Trophoblast cells secrete gelatinases (gelatinase A: MMP-2: 72-kDa and gelatinase B: MMP-9: 92-kDa) which degrade collagen IV, so they are considered as key enzymes in the invasion process (15). MMP-2 and MMP-9 secretion and activation by trophoblasts are necessary for invasion as approved by several studies (15–20).

However, the exact changes in the expression of protease during the first trimester are still not clear. During the first stage of trophoblast invasion, MMP-9 was found to be more pronounced in some studies (15,18,19), while MMP-2 in other studies (20–22). In a study done by Xu et al., MMP-2 found to be the main gelatinase secreted until 9 weeks of gestation by first trimester trophoblast cells, MMP-9 was found to be secreted after that (22).

The viable human embryo has a specific metabolic fingerprint, which is expressed in culture medium as a metabolic footprint (23). Embryo culture medium analysis reveals the components of the medium used or released by the embryo (24).

In recent years, the introduction of a number of new non-invasive embryo viability tests takes place. The aim of these tests is to measure specific molecules from the culture medium to determine what the embryo secreted or consumed (25–34).

2. Materials and methods

The aim of the study is to correlate the changes in the biochemical marker MMP-2 in the culture media with the outcome of normogonadotrophic cases undergoing ICSI.

To accomplish this aim, a prospective study of infertile females was conducted in El-Shatby Maternity University Hospital in Alexandria. The estimated sample size was 40 normogonadotrophic infertile women (22 with unexplained and 18 with tubal factor infertility). The field work was carried out between October 2011 and May 2012.

3. Data collection

3.1. Orientation and official approval from relevant authorities: (collaborative agreement)

In order to enable the researcher to conduct the study, the necessary permissions to conduct the study were obtained.

3.2. Ethical consideration

a. The confidentiality of collected data was stressed.
b. A written informal consent was taken from pregnant women.

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3.3. Patients and induction of ovulation:

- Ovulation induction was carried out by long down regulation protocol:
  a) Pituitary desensitization was performed by the use of gonadotrophin releasing hormone (GnRh) agonist (Suprefact®, Hoechst) which was used in all patients as a daily subcutaneous dose of 0.5 mg started on cycle day 21. Once a serum oestradiol concentration was suppressed to $\leqslant$50 pg/ml, the dose was reduced to 0.2 mg and continued until the day of (hCG) administration.
  b) Ovarian stimulation with urinary follicle stimulating hormone (u-FSH) (Fostimon®; IBSA) 75 IU as well as urinary human menopausal gonadotrophin (u-HMG))Merional®, IBSA) 75 IU. The standard initial dose was 300 IU started from the third day of the cycle till the criteria for administration of human chorionic gonadotrophin (hCG) and was monitored by serial serum oestradiol concentrations and trans-vaginal ultrasound beginning on day 5 of stimulation until the day of (hCG) administration. Based on these results, the FSH/HMG dose and subsequent monitoring was individualized. Ovarian stimulation was continued until at least two follicles reached a mean diameter of $\geqslant$18 mm.
  c) Ovulation was induced when there were two or more follicles greater than 18 mm in diameter by administration of human chorionic gonadotrophin hCG (Choriomon®, IBSA) 10,000 IU subcutaneous or intramuscular injection.
- The injected oocytes were cultured in an open system consisting of 4-well dishes. Each well contained the exact amount of 0.5 ml of ISM1™ Medicult (Origio, Måløv, Denmark) culture media covered with Liquid Paraffin. A maximum of 4 embryos were cultured per well.
- Culture media used for culturing all embryos were collected, labeled for each case and pooled together immediately after transfer of the embryos, and stored at (−20) degrees until the time of assay. The concentration of MMP-2 was measured in the thawed culture medium by the ELISA (Enzyme Linked Immuno Sorbent Assay) technique, by (RayBio® Inc., North Metro-Atlanta, Georgia) Human MMP-2 kits.
- The concentration in culture medium was determined per embryo per hour. First, concentration per patient was measured by subtracting the result from the initial concentration of the biochemical marker MMP-2 in the culture medium. This concentration was determined in quadruplicates. Then the concentration per embryo was determined by dividing the concentration per patient by the number of embryos cultured. The result was then divided by the number of hours of culture to obtain the secretion per embryo per hour.

4. Data analysis

The chi-square (x_2) test was used to analyze categorical variables which were expressed as percentage values. Continuous variables were reported as mean value and standard deviation (SD) and analyzed using the *t*-test. The continuous variables were also reported as median and analyzed using Mann–Whitney test. *P*-value <0.05 was considered statistically significant. All analyses were performed using SPSS for Windows, version 18.0.

5. Results

Out of the forty studied cases, 22 cases had unexplained infertility from which 15 cases became pregnant (65.22%) and 18 cases had tubal factor infertility from which 8 cases became pregnant (34.78%). Also out of the studied group 30 cases had primary infertility from which 16 cases became pregnant (69.57%) and 10 cases had secondary infertility from which 7 cases became pregnant (30.43%) Table 1.

The mean age of the studied group ranged between (28.68 ± 5.44) and (28.57 ± 4.06) years for non pregnant and pregnant groups respectively. There were no statistical significant differences between the two groups regarding age ($P = 0.470$) (Table 1).

The mean duration of infertility ranged between (6.84 ± 5.04) and (5.45 ± 3.61) years for non pregnant and pregnant groups respectively. There were no statistical significant differences between the two groups regarding duration of infertility ($P = 0.159$) (Table 1).

Table 1 Comparison between the two studied groups regarding demographic data.

	Non Pregnant		Pregnant		*P*
Age					
Range	21–38		24–39		
Mean	28.68		28.57		0.470
SD	5.44		4.06		
Duration of infertility					
Range	2–20		1–14		
Mean	6.84		5.45		0.159
SD	5.04		3.61		
Type of infertility	No.	%	No.	%	
Primary	14	82.35	16	69.57	0.355
Secondary	3	17.65	7	30.43	
Unexplained	7	41.18	15	65.22	0.130
Tubal	10	58.82	8	34.78	

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Table 2 Comparison between the two studied groups regarding number and quality of retrieved oocytes.

	Non pregnant	Pregnant	*P*
Oocytes			
Range	2.0–28.0	3.0–28.0	
Mean	9.24	14.61	0.008
SD	6.69	6.74	
MII			
Range	1.0–23.0	2.0–22.0	
Mean	8.12	12.96	0.010
SD	5.96	6.41	

Table 3 Comparison between the two studied groups regarding Embryo scoring.

	Non pregnant	Pregnant	*P*
Embryos			
Range	1.0–14.0	2.0–19.0	
Mean	5.29	9.04	0.008
SD	4.12	5.04	
Class A			
Range	0.0–11.0	0.0–11.0	
Mean	2.88	4.61	0.050
SD	3.28	3.19	

The mean number of oocytes retrieved ranged between (9.24 ± 6.69) and (14.61 ± 6.74) oocytes for non pregnant and pregnant groups respectively. Pregnant group had a statistically significant higher number of oocytes than the non pregnant group ($P = 0.008$) (Table 2).

The mean number of the mature metaphase II (MII) oocytes was (8.12 ± 5.96) and (12.96 ± 6.41) for non pregnant and pregnant groups respectively. Pregnant group had values statistically higher than non pregnant group ($P = 0.010$) (Table 2).

The mean number of embryos obtained after fertilization was (5.29 ± 4.12) and (9.04 ± 5.04) embryos for non pregnant and pregnant groups respectively, pregnant group had values statistically higher than non pregnant group ($P = 0.008$) (Table 3).

The mean of class-A embryos ranged between (2.88 ± 3.28) and (4.61 ± 3.19) embryos for non pregnant and pregnant groups respectively, pregnant group had also values statistically higher than non pregnant group ($P = 0.05$) (Table 3).

The mean number of embryos transferred was (3.53 ± 1.42) and (4.48 ± 0.90) embryos for non pregnant and pregnant groups respectively. Pregnant group had values statistically higher than non pregnant group ($P = 0.007$) (Table 4).

Clinical pregnancy outcome in the study was 57.5% and it was divided as; 12 singleton, 9 twin and 2 triplets which was reduced to a single pregnancy at 7th week of gestational age. There was no abortion, ectopic or chemical pregnancies. Ongoing pregnancy after 14 weeks of gestational age was 100%. (Table 5) Implantation rate ranged between 20% and 60% with the mean of 35.4 ± 13% in the pregnant group (Table 6).

The mean of total MMP-2 ranged between (9.91 ± 5.48) and (13.91 ± 8.87) ng/ml for non pregnant and pregnant groups respectively. There were no statistical significant differences between the two groups regarding total MMP-2 ($P = 0.055$) (Table 7).

The mean of MMP-2/embryo/h ranged between (0.05 ± 0.05) and (0.06 ± 0.08) ng/ml/embryo/h for non pregnant and pregnant groups respectively. There were no statistical significant differences between the two groups regarding MMP-2/embryo/h ($P = 0.234$) (Table 8).

There were no correlations between MMP-2 and MMP-2/embryo/h and between implantation and fertilization rates (Table 9).

Table 4 Comparison between the two studied groups regarding embryos transferred.

	Non pregnant	Pregnant
Embryos transferred		
Range	1–5	2–5
Mean	3.53	4.48
SD	1.42	0.90
P	0.007	

Table 5 Pregnancy outcome in the study.

Clinical Pregnancy	23/40
•Single	•12/23
•Twin	•9/23
•Triplet	•2/23
Chemical Pregnancy	0/40
Abortion	0/40
Ectopic Pregnancy	0/40
Ongoing pregnancy after 14 weeks gestational age	23/23

Table 6 Implantation rate in the pregnant group.

	Pregnant
Implantation rate	
Range	20–60%
Mean	35.4%
SD	0.13

Table 7 Comparison between the two studied groups regarding Total MMP-2.

	Non pregnant	Pregnant
Total MMP-2		
Range	4.1–21.1	3.5–37
Mean	9.91	13.91
SD	5.48	8.87
P	0.055	

6. Discussion

To the best of our knowledge, the present study is the first to detect the link between the concentration of MMP-2 in the cul-

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Table 8 Comparison between the two studied groups regarding MMP-2/embryo/h.

	Non pregnant	Pregnant
MMP2/Embryo/h		
Range	0.0–0.2	0.0–0.3
Mean	0.05	0.06
SD	0.05	0.08
P	0.234	

Table 9 Correlation between total MMP-2 and MMP-2/embryo/h and between implantation and fertilization rates.

	IR	FR
Total MMP-2		
r	.162	.184
P	.319	.255
MMP-2/embryo/72		
r	.049	−.149
P	.764	.358

ture media and its relation to the outcome of cases undergoing ICSI. Previous studies concerned only with detection and not the measurement of MMP-2 in the culture media or concerned with measurement of the concentration of MMP-2 in the follicular fluid and not in the culture media of cases undergoing ICSI.

In our study, we found that the concentration of MMP-2 in the culture media measured by enzyme linked immunosorbent assay (ELISA) did not show any correlation with the fertilization, implantation or pregnancy rates among the cases involved in the study. So the difference in these parameters is probably depending on other factors rather than the level of MMP-2 in the culture media.

The only known study to our knowledge was concerned with measuring MMP-2 in the culture media done by Lee et al., and they were concerned only with MMP-2 detection in the culture media and not to correlate its concentration with pregnancy success. Lee et al. conducted their study on 54 infertile women subjected to ART. Only 16 clinical pregnancies were observed. MMP-2 enzyme activities in the culture media from the embryo culture dishes were analyzed by gelatin zymography. MMP-2 activity was present in the culture media of sample analyzed. In the same study but in the follicular fluid of all women, gelatinase expression corresponding to MMP-2 at 72 kDa molecular weights was observed by zymography. No correlation was found between the follicular fluid concentration of MMP-2 and fertilization rates. Also no significant difference in MMP-2 expressions was found between pregnant and non-pregnant groups (35).

In another study done by Sallam et al., detection of gelatinases in the culture media and in the follicular fluid of 50 cases undergoing ICSI measured by (ELISA) and its relation to embryo selection was done, this study measured only MMP-9. They found that there is no statistical significance between MMP-9 in both the culture media and the follicular fluid with pregnancy outcome (36).

The majority of studies done on MMP-2 in the field of ART were concerned with the detection of MMP-2 in the follicular fluid in different situations. Shalev et al., in a study done on 24 normal ovulatory women as a control group and 24 women suffering from PCOS, Gelatin zymography and western blot analysis were used to detect the MMP-2 in the follicular fluid and in the cultured luteinized granulose cells. They reported for the first time higher levels of MMP-2 in the follicular fluid and in the cultured luteinized granulose cells of patients with PCOS under treatment in an IVF program compared with normally ovulating patients (37).

In another study done by Baka et al., detection of the level of MMP-2 was done using enzyme linked immunosorbent assays (ELISA), in the follicular fluid of 35 patients with polycystic ovaries, compared them with the levels found in 35 normally ovulating women enrolled in their first in vitro fertilization (IVF) cycle and then correlated them with pregnancy rates in these two groups, MMP-2 levels in women with polycystic ovaries were higher without reaching statistical significance. The two groups had no difference 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 IVF pregnancy rates (38).

7. Recommendations

Results of the present study suggest searching for other markers in the culture media for better embryo selection and for prediction of implantation in the normogonadotrophic cases undergoing ICSI.

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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