Original Article / Özgün Makale

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.735162
J Health Sci Med 2020; 3(3): 301-306

Do midkine levels in serum and follicular fluid affect IVF-ICSI outcome?

Serum ve foliküler sıvıdaki midkin düzeyleri IVF-ICSI sonuçlarını etkiler mi?

Ayse Z. Özdemir[1], Pervin Karlı[2], Bahattin Avcı[3]

[1]Ondokuz Mayıs University IVF Center, Ondokuz Mayıs University Hospital, Samsun, Turkey
[2]Department of Obstetrics and Gynecology, Amasya University Research Hospital, Amasya, Turkey
[3]Department of Biochemistry, Ondokuz Mayis University, Faculty of Medicine, Samsun, Turkey

Cite this article as / Bu makaleye atıf için: Özdemir AZ, Karlı P, Avcı B. Do midkine levels in serum and follicular fluid affect IVF-ICSI outcome? J Health Sci Med 2020; 3(3): 301-306.

ABSTRACT

Objective: Current studies have demonstrated that midkine (MK) inhibits the apoptosis in granulosa cells and is responsible for follicular angiogenesis. The aim of this study was to investigate the relation of MK levels and ART outcome.

Material and Method: The study included a total of 99 patients who underwent IVF-ICSI at Ondokuz Mayis University between February 2019 and April 2019. Of 99 patients, no embryo development was formed in 1 patient, no oocytes were obtained by oocyte pick up (OPU) in 14 patients and the oocyte cryopreservation was established in 17 patients. In total, 67 patients underwent fresh embryo transfer after IVF-ICSI. On the day of OPU, the level of midkine in serum and follicular fluids were examined. The primary outcome was determined as pregnancy and secondary outcome was determined as blastocyst-stage embryos.

Results: The level of midkine in serum and follicular fluid was found to be higher in pregnant women compared with non-pregnant women (p=0.042 and p=0.01 respectively). Midkine levels in follicular fluid and serum lead to an increase in blastocyst development.

Conclusion: Midkine levels in follicular fluid and serum may lead to an increase in blastocyst development. The level of midkine is higher in pregnant subjects than in non-pregnant subjects.

Keywords: In vitro fertilization, pregnancy, blastocyst, midkine

ÖZ

Amaç: Güncel çalışmalar, midkinin (MK) granüloza hücrelerinde apoptozu inhibe ettiğini ve foliküler anjiyogenezden sorumlu olduğunu göstermiştir. Bu çalışmanın amacı, midkin seviyeleri ile ART sonuçları arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntem: Çalışmaya Şubat 2019 ve Nisan 2019 tarihleri arasında Ondokuz Mayıs Üniversitesi'nde IVF-ICSI uygulanan toplam 99 hasta dahil edildi. Çalışmadaki 99 hastanın birinde embriyo gelişimi sağlanamadı ve 14 hastada oosit toplanmasıyla (OPU) oosit elde edilmedi. ve oosit kriyoprezervasyon 17 hastada oluşturuldu. Toplam 67 hastaya IVF-ICSI sonrası taze embriyo transferi yapıldı. OPU gününde serum ve foliküler sıvılardaki midkin seviyesi incelendi. Primer sonuç gebelik, sekonder sonuç blastosist-aşamalı embriyolar olarak belirlendi.

Bulgular: Serum ve foliküler sıvıdaki midkin düzeyi gebelerde gebe olmayanlara göre daha yüksek bulundu (p=0,042, p=0,01). Foliküler sıvı ve serumdaki midkin seviyeleri blastokist gelişiminde artışa neden olduğu görülmüştür.

Sonuç: Foliküler sıvı ve serumdaki midkin seviyeleri blastokist gelişiminde artışa neden olabilir. Midkin seviyesi gebelerde gebe olmayanlara göre daha yüksek bulunmuştur.

Anahtar Kelimeler: İn vitro fertilizasyon, gebelik, blastokist, midkin

Corresponding Author / Sorumlu Yazar: Pervin Karlı, Amasya Üniversitesi Sabuncuoğlu Şerafeddin Eğitim ve Araştırma Hastanesi, Kadın Hastalıkları ve Doğum Kliniği, Kirazlıdere Mah.,Terminal Cad., No:37, 05200, Merkez, Amasya, Türkiye
E-mail / E-posta: parpi2300@hotmail.com
Received / Geliş: 10.05.2020 **Accepted / Kabul:** 02.06.2020

INTRODUCTION

Midkine (MK) is a protein involved in oncogenesis, inflammation and tissue repair in adult rats and human tissues. It is also responsible for cell migration, proliferation and angiogenesis (1). It is also called as a neurotrophic factor and promoting cytoplasmic maturation of oocytes (2). It is produced by granulosa cells as well as other follicular cells and can be found in follicular fluid (3). Midikine is a heparin-binding polypeptide that binds to cell-surface heparan sulfate proteoglycans. It inhibits the apoptosis of bovine cumulus cells in in vitro maturation (IVM) medium and these cells promote the developmental competence of oocytes (4).

Oocyte maturation refers to completion of Meiosis 1 by the oocytes arrested at Prophase 1 to reach metaphase 2 (M2) and completion of necessary nuclear and cytoplasmic changes for fertilization. It is revealed that 70 % of subjects do not exhibit blastocyst development after IVM of oocytes (5). This is because the physiological substances in the follicular fluid provide the cytoplasmic maturation of oocytes. In addition, the oocyte maturation in the antral follicle occurs with the communication of the oocyte with the cumulus graulosa cells (6). Midkine inhibits the apoptosis of granulosa cells so it has a role in oocyte maturation.(7).

The study demonstrated that blastocyst development rate was increased by the midkine administration in IVM media, which was thought to be due to its effects in cytoplasmic maturation (8).Current studies (7) have demonstrated that MK inhibits the apoptosis of mice granulosa cells and is responsible for follicular angiogenesis.

In this study, we aimed to investigate the relation of midkine levels and ART outcome. As far as we know, there is no study that investigates the correlation between midkine and invitro fertilization (IVF) outcomes in the literature.

MATERIAL AND METHOD

Our study was designed as a prospective cohort study. This study was approved by the university /local human research ethics committee and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was carried out with the permission of Ondokuz Mayıs University Ethics Committee (Permission granted /Decision no: 2019/12). Written informed consent was obtained from all participants who participated in this study. The study included a total of 99 patients who underwent IVF-ICSI at 19 Mayıs University between February 2019 and April 2019.

Inclusion criteria: The patients aged between 18 and 42 years of age with regular menstruation and no endocrinologic diseases were included in this study.

Exclusion criteria: Severe male factor such as oligoasthenoteratozoo-spermia, patients with hydrosalpinx, endometriosis and polycystic ovary syndrome were excluded from the study.

Of 99 patients, no embryo development was formed in 1 patient, no oocytes were obtained by OPU in 14 patients and the oocyte cryopreservation was established in 17 patients. In total, 67 patients underwent fresh embryo transfer after IVF-ICSI.

Ovulation Induction: All patients underwent ovulation induction with the standard antagonist protocol. Oocyte pick-up (OPU) was performed 36 hours following the HCG treatment and intracytoplasmic sperm injection (ICSI) was performed 4-6 hours after that. Cleavage embryos are determined 32-36 hours and blastocyst are determined 5 days after ICSI. The patients are classified according to blastocyst formation.

Starting from the day of OPU, progesterone was administered intramuscularly (progestan 50 mg; Koçak, Turkey) and estrogen (estrofem 2 mg; NovoNordisk, Denmark) was administered orally as luteal support. Patients with a positive β-HCG level on day 14 of embryo transfer were evaluated as pregnant.

Sample Collection: The follicle fluid was obtained by centrifugation of the remaining liquid after the isolation of the oocytes from the first aspirated dominant follicle fluid and storage at -80 °C. Samples contaminated with blood were not included.

Serum samples (5 mL) were obtained from the patients simultaneously on the day of OPU. The samples were centrifuged and stored at -80 °C.

Analysis of Samples: Serum and follicular fluid concentrations of MK were analyzed in the Research Laboratory of Department of Medical Biochemistry, Faculty of Medicine Ondokuz Mayıs University using the commercially available Human MK ELISA kit (Sun-RedBioCompany, Cat no. 201-12-1025, Shanghai, China) with the method of double-antibody sandwich enzyme-linked immunosorbent assay. In the study, all solutions were prepared fresh and kept in room temperature (25^0C) before use.

Five standard solutions (S1-50 ng/L, S2-100 ng/L, S3-200 ng/L, S4-400 ng/L and S5-800 ng/L) were prepared through the method of serial dilution according to the standards of Human MK. The wells were marked on ELISA plate for blanks, standards and samples. Only Chromogen A, Chromogen B and Stop solution were added to blank well. Same procedures were applied for

the standards and samples. A 50µL of Standard (S1-S5) was pipetteed into each well and 40 µL+10 µL MK antibody was pipetteed into each sample. Then, a 50 µL of treptavidin-Horseradish Peroxidase was added to the standards and samples and they were incubated at 37 °C for 60 min. After the incubation, the plate was washed 5 times with a 350 µL of washing solution using an automatic washer. 50 µL of Chromogen A and 50 µL of Chromogen B were added to each well, then the wells were left to incubate for 10 min at 37 °C. The reaction was stopped by adding a 50 µL of Stop solution. In the end, the absorbances in wavelength 450nm were read using the TECAN Micro platereader.

Sample Human MK concentrations were calculated in accordance with the standard curve obtained by the standard values and were expressed as ng/L. Kit sensitivity was defined as 4,006 ng/L with an assay range of 5 ng/L - 1500 ng/L. The samples with high concentration were verified with double examination.

Statistical Analysis

The sample size was determined according to the inputs of 80% power and a type-I error of 5%. Descriptive statistics for continuous(quantitative) variables were expressed as median, mean, standard deviation, minimum and maximum. The Kolmogorov-Smirnov (N>50) test was used to check whether the continuous variables were distributed normally. Then, nonparametric tests were used to analyze the data as the variables did not exhibit a normal distribution. The Mann-Whitney U test was used in the comparison of the parameters according to IVF outcome. The Binary Logistic Regression analysis was used for the variables that were thought to be associated with "pregnancy ". The Spearman correlation coefficient was calculated to determine the correlation between the variables. The Chi-square test was used to determine the correlation between the categorical variables. Statistical significance level was accepted as (a) %5 and the data were analyzed using the SPSS (IBM SPSS for Windows, Ver. 24) Statistics software.

RESULTS

The number of embryos and pronucleus (PN) were significantly higher in pregnant women than in non-pregnant women (p <0.05). The level of midkine in serum and follicular fluid was found to be higher in "pregnant" women compared with "non-pregnant" women (P=0.042 and P=0.01 respectively). All other parameters did not exhibit a statistically significant difference according to pregnancy status (p> 0.05) (**Table 1-2**).

Table 1. Comparison of pregnant and nonpregnant groups

		Not Pregnant		Pregnant		*p
		N	%	N	%	
Cause of Infertility	Unexplained infertility	21	46.8	13	61.9	.447
	Poor ovarian reserve	9	19.1	2	9.5	
	Male factor	16	34.0	6	28.6	
Number of embryos transferred	1	26	59.6	16		.358
	2	20	43.4	5	76.2	
Grade	1	26	56.5	14	23.8	.580
	2	12	26.0	3	66.6	
	3	5	11	3	14.3	
	4	3	6.5	1	14.3	

Table 2. Demografic comparisions

	Not Pregnant (n=46)		Pregnant (n=21)		*p
	Mean	Std.Dev.	Mean	Std.Dev.	
Age	30.00	5.76	29.71	5.45	.115
Duration of infertility (year)	6.83	5.28	5.95	3.88	.821
Number of attempts for IVF	1.55	.85	1.67	.91	.592
FSH (IU/ml)	7.72	3.28	8.54	4.37	.628
Day 2 estradiol (pg/ml)	44.51	33.58	46.57	27.27	.705
Estradiol on OPU day (pg/ml)	1329.26	884.45	1169.90	631.92	.637
Progesterone (ng/l)	.64	1.20	.42	.30	.542
LH (IU/l)	2.74	2.55	1.92	1.77	.178
Gonadotrophin (IU)	380.85	108.62	361.90	88.96	.664
Induction time (day)	9.26	2.44	9.62	2.25	.722
Endometrial thickness (mm)	8.38	1.51	8.81	1.86	.390
Number of retrieved oocytes	7.55	5.44	8.43	3.67	.129
M2 oocytes	5.66	3.96	7.14	3.42	.055
Number of embryos	3.91	3.66	5.38	2.60	.015
Pronucleus (PN)	4.30	3.71	5.48	2.52	.025
Level of follicular fluid midkine (ng/L)	789.53	314.90	1049.11	476.19	.042
Level of blood midkine (ng/L)	275.44	241.75	932.79	622.24	.001

***Statistical significance level in the Mann-Whitney U Test**
*Significance level in Chi-Square test

There was no statistically significant difference in pregnancy rates associated with an increase in the parameters of "Age", "Pn" and "Serum midkine" (Odds=1.001, 0.183,1.004) (**Table 3**).

Table 3. Binary logistic regression analysis of the factors associated with pregnancy

	p	Odds Ratio (%95 confidence interval)
Age	.985	1.001
Number of embryos	.256	5.277
PN	.249	.183
Follicular fluid midkine	.426	.999
Serum midkine	.001	1.004
Constant	.432	.090

p: Significance level in logistic regression; Method: Enter;
-2 Log likelihood: 42,58; Cox & Snell R Square: 0.45

Serum and follicular midkine levels are not correlated with the number of oocytes retrieved, number of embryos and PN. (r=0.170, 0.123,0.134) (**Table 4**).

There was a strong positive correlation between the parameters of blastocyst formation and follicular fluid midkine levels and the parameters of blastocyst formation and serum midkine levels (r= 0.654 , r= 0.541) (**Table 5**).

Table 5. Correlation between blastocyst formation and level of midkine

	Level of follicular fluid midkine	Level of blood midkine	Blastocyst formation
Follicular fluid midkine	r=1	r=0.705 *	r=0.654 *
Blood midkine level	r=0.705 *	r=1	r=0.541 *
Blastocyst formation	r=0.654 *	r=0.541 *	r=1

DISCUSSION

Main result of our study is that higher levels of MK in serum and follicular fluid were detected in pregnant patients. And we find that the blastocyst formation rate is higher in patients with higher levels of serum and follicle MK.

Midkine is a polypeptide involved in various cellular processes such as proliferation, cell migration, angiogenesis and fibrinolysis. It is responsible for oncogenesis, inflammation and tissue regeneration (9).

In the reproductive system, MK was found in high amounts in bovine follicular fluid (10). Midkine mRNA was also detected in mice granulosa cells (11). MK was also detected in human ovary at high concentrations (9).

Oocyte cytoplasmic maturation is a critical step for embryo formation. Oocyte cytoplasmic maturation is defined as the whole events which is necessary for fertilization, embryogenezis, implantation and fetal development (12,13). This events are consist of accumulation of mRNAs, proteins, substrats and nutrients (14). MK exerts pro-survival effects on the cumulus-granulosa cells, thus promoting cytoplasmic maturation of oocytes (4,8).

In mammals, oocytes are arrested in meiotic prophase I. Meiosis resumes in response to a surge of LH. Chromosomes begin to condense, nuclear membrane breaks down and spindle forms during meiosis 1. Spindle fibres attach to chromosomes. Chromosomes align in cell centre, then they separate which leads to chromosome migration. Chromosome segregation occurs during asymmetric cell division and the extrusion of the first polar body. Oocyte maturation consists of a series of nuclear and cytoplasmic changes occurring until the resumption of meiosis to the M2 stage (15), which is necessary for embryo development.

Table 4. The correlation between midkine levels and other parametres

		FSH	Day2-3 Estradiol	OPU day estradiol	OPU day progesteron	OPU day LH	Number of collected oocyte	M2	Embryo	PN	Follicular fluid midkine
Day 2-3 estradiol	r	-.030									
OPU day estradiol	r	-.387**	.426**								
OPU day progesteron	r	-.272**	.204*	.619**							
OPU day LH	r	.293**	-.220*	-.262**	-.186						
Number of collected oocytes	r	-.479**	.364**	.717**	.401**	-.399**					
M2 oocyte	r	-.450**	.228*	.575**	.359**	-.361**	.941**				
Embryo number	r	-.216	.137	.444**	.267*	-.146	.759**	.824**			
PN	r	-,255*	.135	.421**	.236	-.166	,810**	.854**	.956**		
Follicular fluid midkine	r	-.205	.012	.146	.039	-.125	.170	.212	.123	.134	
Serum midkine	r	.003	-.002	.067	.089	-.102	.108	.188	.187	.174	.594**

*p<0.05 **p<0.01 r=Spearman's rhonon parametric correlation coefficient

MK is thought to be involved in the process of cytoplasmic maturation of oocytes (4).

Although oocyte maturation is a highly complex phenomenon, nuclear maturation can occur spontaneously in vitro. However, some substances in the follicle fluid have been shown to be necessary for blastocyst formation, postfertilization development and oocyte developmental competence (8)

In one of the studies, in bovine IVM, heparin binding follicle fluid is used in one group and follicular fluid that did not bind heparin in the other group. Blastocyst formation was found to be higher in heparin-bound follicle fluid group (16). Heparin binding proteins in follicle fluid are asic fibroblast growth factor (bFGF), midkine and pleiotrophin (PTN) (17,18).

Studies have shown that defect-induced mice for PTN and MK have reduced mature oocytes and most are infertile (7).

In other study, oocytes covered with bovine granulosa cells were taken and divided into two groups. In one group, IVM medium contained the midkine, but not in the other group. Nuclear maturation was not affected at the end of the study but blastocyst formation was more common in IVM medium containing MK (8)

In another study, denuded oocytes that were removed with granulosa cells have used. Blastocysts did not occur in groups with and without MK in IVM fluid in denuded oocytes. This study is very important in terms of showing that MK contributes to blastocyst formation in the presence of granulosa cells (4).

Hirota et al have found a positive correlation between MK level and estrogen level in follicle fluid in IVF patients. They found an inverse correlation between oxygenation and MK level. In addition, it was observed that the MK increased the mitogenic activity in granulosa cells. MK mRNA was detected in both granulosa and theca cells (9).

In our study, there was no correlation between MK levels and M2 counts, number of retrieved oocytes, number of embryos in pregnant patients however higher levels of midkine in serum and follicular fluid were detected in pregnant patients. This may be due to the fact that blastocyst formation rate is higher in patients with higher levels of midkine in follicular fluid and serum. Cytoplasmic oocyte maturation is necessary for blastocyst formation. And high levels of MK provide cytoplasmic maturation.

In other studies, midkine was also detected in the endometrium. The level of midkine was found to be lower in ectopic endometrium compared to eutopic endometrium (19). Our study did not include the evaluation of endometrium midkine levels. One of the limitations of our study is that we did not evaluate the correlation between perinatal outcomes and MK levels in patients.

Our study is the first study in the literature to investigate the relationship between IVF success and MK levels in serum and follicle fluid. Although the patient population is small, it shows that MK level is effective on blastocyst formation and pregnancy outcome. Larger prospective studies are needed for a clearer understanding on the subject.

CONCLUSION

This study has a significant value in terms of demonstrating the necessity of MK for the success of IVF and blastocyst formation

Abbreviations: MK, Midkine; M2, metaphase 2; IVM, in vitro maturation; IVF, invitro fertilization; OPU, Oocyte pick-up; ICSI, intracytoplasmic sperm injection; PN, pronucleus; LH, Luteinizing Hormon; FSH, follicle stimulating hormone; PTN, pleiotrophin; bFGF, asic fibroblast growth factor

ETHICAL DECLARATIONS

Ethics Comittee Approval: The study was carried out with the permission of Ondokuz Mayıs University Ethics Committee (Permission granted: 2019, Decision no: 2019/12).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Acknowledgments: The authors would like to thank the staff of the IVF center for the benefits.

REFERENCES

1. Muramatsu T. Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. J Biochem (Tokyo) 2002; 132: 359–71.

2. De Sousa PA, Da Silva SJ, Anderson RA. Neurotrophin signaling in oocyte survival and developmental competence: a paradigm for cellular toti-potency. Cloning Stem Cells 2004; 6: 375–85.
3. Hirota Y, Osuga Y, Nose E, et al. The presence of midkine and its possible implication in human ovarian follicles. Am J Reprod Immunol 2007; 58: 367–73.
4. Ikeda S, Saeki K, Imai H, Yamada M. Abilities of cumulus and granulosa cells to enhance the developmental competence of bovine oocytes during in vitro maturation period are promoted by midkine; a possible implication of its apoptosis suppressing effects. Reproduction 2006; 132: 549–57.
5. Leidenfrost S, Boelhauve M, Reichenbach M, et al. Cell arrest and cell death in mammalian preimplantation development: lessons from the bovine model. Plos ONE 2011; 6: e22121.
6. Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat Rev Mol Cell Biol 2013; 14: 141–52.
7. Muramatsu H, Zou P, Kurosawa N, et al. Female infertility in mice deficient in midkine and pleiotrophin, which form a distinct family of growth factors. Genes Cells 2006; 11: 1405–17.
8. Ikeda S, Ichihara-Tanaka K, Azuma T, Muramatsu T, Yamada M. Effects of midkine during in vitro maturation of bovine oocytes on subsequent developmental competence. Biol Reprod 2000; 63: 1067–74.
9. Hirota Y, Osuga Y, Koga K, et al. Possible implication of midkine in the development of endometriosis. Hum Reprod 2005; 20: 1084–9.
10. Ohyama Y, Miyamoto K, Minamino N, Matsuo H. Isolation and identification of midkine and pleiotrophin in bovine follicular fluid. Mol Cell Endocrinol 1994; 105: 203–8.
11. Karino S, Minegishi T, Ohyama Y, et al. Regulation and localization of midkine in rat ovary. FEBS Lett 1995; 362: 147–50.
12. Brevini-Gandolfi, T. A. L., and F. Gandolfi. The maternal legacyto the embryo:cytoplasmic components and their effects on earlydevelopment. Theriogenology 2001; 55: 1255–76.
13. Sirard MA, Richard F, Blondin P, Robert C. Contribu-tion of the oocyte to embryo quality. Theriogenology 2006; 65: 126–36.
14. Krisher RL. The effect of oocyte quality on development. J Anim Sci 2004; 82 (Suppl. E): E14–E23.
15. Kupker W, Diedrich K, Edwards RG. Principles of mammalian fertilization. Hum Reprod 1998; 13 (Suppl 1): 20–32.
16. Ikeda S, Azuma T, Hashimoto S, Yamada M. In vitro maturation of bovine oocytes with fractions of bovine follicular fluid separated by heparin affinity chromatography. J Reprod Dev 1999; 45: 397–404.
17. Seli E, Zeyneloglu HB, Senturk LM, Bahtiyar OM, Olive DL, Arici A. Basic fibroblast growth factor: peritoneal and follicular fluid levels and its effect on early embryonic development. Fertil Steril 1998; 69: 1145–8.
18. Ohyama Y, Miyamoto K, Minamino N, Matsuo H. Isolation and identification of midkine and pleiotrophin in bovine follicular fluid. Mol Cell Endocrinol 1994; 105: 203–8.
19. Chung HW, Wen Y, Choi EA, et al. Pleiotrophin (PTN) and midkine (MK) mRNA expression in eutopic and ectopic endometrium in advanced stage endometriosis. Mol Hum Reprod 2002; 8: 350–5.