International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Khoury F et al. Int J Reprod Contracept Obstet Gynecol. 2022 Sep;11(9):2352-2356
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20222294

Original Research Article

The protein C deficiency and its effects on *in-vitro* fertilization outcome and recurrent pregnancy loss: a Syrian cohort study

Fadi Khoury[1], Haya Deeb[1], Rana Jarouj[1], Hanna Khoury[1], Nawras Alhalabi[1], Marwan Alhalabi[2,3]*

[1]Faculty of Medicine, Damascus University, Damascus, Syria
[2]Division of Reproductive Medicine, Embryology and Genetics, Faculty of Medicine, Damascus, Syria
[3]Assisted Reproductive Unit, Oriental Hospital, Damascus, Syria

Received: 05 September 2021
Accepted: 30 May 2022

***Correspondence:**
Dr. Marwan Alhalabi,
E-mail: profalhalabi@icloud.com

ABSTRACT

Background: Protein C deficiency is a rare disease; it could be primary or secondary. Female patients with protein C deficiency may develop pregnancy-associated thrombosis. The clear association between the protein C deficiency and fertility or *in-vitro* fertilization (IVF) results has not been known yet. In our study, we aimed to determine the prevalence of protein C deficiency in Syrian infertile women and to assess the impact of protein C deficiency on abortion, recurrent IVF Failure, and IVF results.
Methods: the electronic patients' results were retrospectively screened. A total of 238 women, who had IVF cycles between January 2012 and December 2017 in a tertiary care hospital, met our including criteria.
Results: the prevalence of protein C deficiency was 13.45%. There was a significant association between the protein C level and recurrent pregnancy loss (RPL). Furthermore, the protein C deficiency has an impact on the IVF results. Additionally, we did not find any association between protein C deficiency and age or number of previous IVF cycles.
Conclusions: the protein C could be the hidden factor that affects IVF results. More research should be done to better understanding the role of protein C in infertility and IVF.

Keywords: Protein C deficiency, IVF, RPL, Infertility, Syria

INTRODUCTION

Protein C is vitamin K-dependent serine protease proenzyme synthesized in the liver and converted to its active form activated protein C (APC) by thrombinin complex with thrombomodulin.[1,2]

Protein C deficiency is a rare disease with an incidence rate ranging from 1 in 200 to 1 in 500, it could be primary (congenital) or secondary (acquired).[1-4] Due to their inability to control blood coagulation, the patients with protein C deficiency classically present with deep venous thrombosis or pulmonary embolism.[1]

Additionally, female patients with protein C deficiency may develop pregnancy-associated thrombosis.[5] The relationship between protein C and fertility has not been determined yet. Some studies have found that there is a signaling system including APC involved in progesterone biosynthesis regulation; this system allows optimal luteinization during pre-ovulatory phase.[6] Furthermore, other researchers found that protein C inhibitors decrease fertilization by inhibiting the binding and the penetration of oocytes by sperms, or it can be associated with placenta mediated vascular complications during pregnancy.[7-9]

Very few studies have examined the association between protein C levels and it impact on infertility, RPL, or IVF.[10-

[13] Therefore, the aim of this study was to determine the prevalence of protein C deficiency in Syrian infertile women receiving IVF treatment and to assess the impact of protein C deficiency on RPL, recurrent IVF Failure, and IVF results.

METHODS

Data collection

All patients who underwent IVF treatment at Orient hospital between January 2012 and December 2017 were retrospectively studied.

Data collected included the patients' demographics, protein C laboratory values, history of previous abortions, history of IVF, number of oocytes obtained and number of collected embryo transfers, were obtained.

All patients who had an IVF cycle and a screening test for protein C levels were included in our study. If the included patients had previous IVF cycles, only their last IVF outcome was included in this study due to missing data in their medical files. However, patients who had a cancelled IVF cycles were excluded because the effect of protein C deficiency on the IVF outcomes could not be studied as the IVF was terminated medically.

The ethical approval was obtained from ethics committee at faculty of medicine, Damascus university, in addition to the approval of orient hospital board of directors.

Protein C assay

The blood samples for this study were collected and tested at the same laboratory. Protein C was tested using enzyme-linked immunosorbent assay (ELISA) by using AESKU Diagnostics kits©. The normal value of protein C activity in our laboratory is 70-140%. All values that were recorded below the normal range were considered as protein C deficiency.

IVF protocol

All the patients who performed the IV were treated by using long Gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist for pituitary suppression. All the details of this protocol had been discussed and explained.[14-16] The women were given human menopausal gonadotropin (HMG), or recombinant follicular stimulating hormone (rFSH), or both, to induce the growth of ovarian follicles. After proving the growth of follicles using ultrasound in conjunction with estradiol levels in the blood, human chorionic gonadotropin (HCG) was given. When 3 leading follicles reached a suitable size 17-19 mm, 10000 IU of HCG were administrated. After 36 hours, the transvaginal ultrasound guided oocytes extraction (retrieval) was done. Then for fertilization, the sperm was injected into the cytoplasm.

In day 3 (cleavage stage), with the guidance of transvaginal ultrasound, three embryos were transferred to the uterus cavity. The result of IVF was considered positive through urine HCG test and the presence of the gestational sac by the transvaginal-ultrasound procedure. On the other hand, the outcomes were considered negative (failure) in case no gestational sac or no evidence of pregnancy was detected.

Statistical analysis

The statistical analysis was made by using the statistical program for social sciences (Version 25; SPSS Inc., Chicago, IL, USA). Only the women with the previous criteria were included in the analysis. The results were expressed as mean ± standard deviation (SD). One-way ANOVA Test and Chi-square were used as appropriate. $P<0.05$ was considered as a significant statistical result.

RESULTS

A total of 238 women were included in the analysis. The patients were categorized into three groups depending on the protein C levels (Table 1). The mean age of the patients was 36.10±5.12 (mean ± SD, range: 22-47).

Table 1: Describing the age and the protein C level between the three groups.

Variables	Group 1: Protein C deficiency	Group 2: Normal protein C levels	Group 3: High protein C
Frequency (%)	32 (13.45)	195 (81.93)	11 (4.62)
Protein C level (mean ± SD)	35.91±4.61	34.64±6.12	36.21±5.16

While comparing the means of the age of the three groups, there was no significant difference between the groups (Table 2). The mean number of the oocytes obtained after the IVF stimulation was 9.71±7.62 (mean ± SD, range: 0-55). However, the mean number of the embryos that had been transferred to the patients was 3.44±2.11 (mean ± SD, range: 0-8). When comparing the means of oocytes and embryos transfers between the three groups of the patients, there was no significant association between the oocytes or the embryo transfer with the protein C levels ($p>0.05$) (Table 2).

For studying the effects of the protein C levels on RPL, the patients were categorized based on their abortion history; group 1 had no abortion history, group 2 had one or two abortions, group 3 three or more abortions (RPL). By comparing the means of protein C levels between those three groups, there was a significant difference between the groups ($p=0.041$) (Table 3). This shows a significance of protein C levels' effect on the abortion history of the

patients, especially those with RPL. On the other hand, there are 50 patients with a history with one or more abortions, 10 out of 50 (10%) have Protein C deficiency.

Table 2: Comparing the means of age, Oocytes obtained and the embryo transfers between the three groups using one-way ANOVA.

Variables	Group 1: Protein C deficiency	Group 2: Normal protein C levels	Group 3: High protein C	P value
Age (mean ± SD)	35.91± 4.61	34.64± 6.12	36.21± 5.16	NS*
Oocytes (mean ± SD)	9.03± 5.38	9.90± 8.081	8.36± 4.13	NS*
Embryo transfer (mean ± SD)	3.25± 2.14	3.50± 2.11	2.82± 2.09	NS*

*NS= Not Significant

Table 3: Comparing the means of protein C levels by using one-way ANOVA.

Variables	Group 1: no H/O of abortions	Group 2: <3 abortions	Group 3: 3 or more abortions	P value
Protein C levels (mean ± SD)	103.12± 40.73	114.24± 55.03	64.75± 36.29	0.041

By studying the effect of protein C deficiency on the number of previous IVF cycles, we excluded 11 patients with high level of protein C. Statistically; we did not find any association (p>0.05) (Table 4).

Table 4: Studying the relationship between protein C deficiency and the number of IVF cycles by using the Chi-square test.

Variables		Number of IVF cycles		P value
		No history	**Pre IVF cycles**	
Protein C levels	Deficiency (Frequency)	12	20	NS*
	Normal (Frequency)	84	111	
Total		96	131	227

*NS: Not significant

In addition to excluding the patients with high level of protein C deficiency, we also excluded the patients who had canceled IVF cycles. A total of 202 patients were studied. There was a significant association between the IVF result and the protein C levels.

Table 5: Studying the relationship between protein C levels and IVF results by using Chi-square test.

Variables		IVF results		P value
		Positive	**Negative**	
Protein C levels	Deficiency (frequency)	6	21	0.010
	Normal (frequency)	85	90	
Total		91	111	202

DISCUSSION

To the best for our knowledge, this is the first study in Syria that investigates the prevalence of protein C and its effects on RPL, previous IVF cycles and on the IVF results in infertile women.

By summarizing the results, we found a statistical correlation between having a previous history of abortion or RPL and protein C deficiency in infertile women. This finding is concurrent with other studies' findings, which reported that protein C deficiency is an independent factor that decreases the rate of pregnancy in IVF. Some studies indicate that the levels of protein C were low in infertile women who underwent IVF cycles.[11-13,17] When comparing the prevalence of protein C deficiency among women with history of abortions in other studies, we found that the prevalence in our study is higher than some previous studies, and it is also lower than others.[11,12,17,18] This disparity in the prevalence of protein C deficiency may be due to differences in the sample size, the inclusions' criteria of the patients or the types of protein C assay.

Although the test of protein C has been evaluated in different samples of women with RPL, and women with recurrent IVF failure, very few studies has evaluated protein C levels in relation to the success or failure of IVF.[11-13,19] Another significant finding in this study showed that the level of protein C affects the results of IVF cycles' outcomes. This is compatible with the findings of El Masry el al.[13] There are many contradictions and differences in the medical literature about the association between IVF failure and thrombophilia, and especially regarding protein C. Some studies have documented this association, while others did not find any association.[20-25] Furthermore, other studies did not find any protein C deficiency in women with recurrent IVF failure.[19] In contrast, we found 12 women with protein C deficiency who underwent IVF cycles.

The pathophysiology of the protein C effects on pregnancy or IVF results is still not clear. It is possible that the protein C abnormalities could lead to a hypercoagulation state which could causes placental microthrombi or impairs the

initial vascularization needed for implantation and the success of the IVF.[13]

Based on the findings of this study, we highly recommend doing the protein C laboratory assay as an essential test to all women with a previous history of abortions or in women who will undergo an IVF cycle. However, more studies should be done to conclude whether protein C assay could be a coagulation factor that affects the IVF results, and to study whether antithrombotic therapy may be considered as a therapeutic option in women with IVF failure to improve the success of the IVF.

Our major limitation in this study is that this sample did not demonstrate the exact number of infertile Syrian women in need of IVF cycles due to the high costs of IVF compared to the national average income.

CONCLUSION

The protein C deficiency affects the IVF results and has impact on RPL. Therefore, it could be the hidden factor that affects the rate of pregnancy and infertility. And it could also make a hypercoagulation state which cause placental microthrombi and impairs the vascularization needed for implantation. So, we highly recommend doing the Protein C assay to every woman with history of abortions or she undergoes an IVF cycle.

ACKNOWLEDGEMENTS

Authors would like to thank to Dr. Adnan Alkhatib for assay the protein C levels in Alkhatib laboratory.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Gupta A, Patibandla S. Protein C Deficiency. Treasure Island (FL): StatPearls Publishing: StatPearls Publishing. 2021.
2. Mannucci PM, Franchini M. Classic thrombophilic gene variants. Thrombosis haemostasis. 2015;114:885-9.
3. Tait RC, Walker ID, Reitsma PH. Prevalence of protein C deficiency in the healthy population. Thrombosis haemostasis 1995;73:87-93.
4. Alhenc-Gelas M, Gandrille S, Aubry ML, Aiach M. Thirty-three novel mutations in the protein C gene. French INSERM network on molecular abnormalities responsible for protein C and protein S. Thrombosis haemostasis. 2000;83:86-92.
5. Croles FN, Nasserinejad K, Duvekot JJ, Kruip MJ, Meijer K, Leebeek FW. Pregnancy, thrombophilia, and the risk of a first venous thrombosis: systematic review and bayesian meta-analysis. BMJ (Clinical research ed). 2017;359:j4452.
6. Cheng Y, Kawamura K, Deguchi M, Takae S, Mulders SM, Hsueh AJ. Intraovarian thrombin and activated protein C signaling system regulates steroidogenesis during the periovulatory period. Molecular endocrinol. (Baltimore, Md). 2012;26:331-40.
7. España F, Navarro S, Medina P, Zorio E, Estellés A. The role of protein C inhibitor in human reproduction. Seminars thrombosis hemostasis. 2007;33:41-5.
8. Elisen MG, van Kooij RJ, Nolte MA. Protein C inhibitor may modulate human sperm-oocyte interactions. Biol Reproduct. 1998;58:670-7.
9. Brenner B, Shapira A, Bahari C, Haimovich L, Seligsohn U. Hereditary protein C deficiency during pregnancy. Am J Obstetr Gynecol. 1987;157:1160-1.
10. Younis JS, Ben-Ami M, Izhaki I, Brenner B, Sarig G. Reduced protein C Global assay level in infertile women prior to IVF-ET treatment. J Assisted Reproduct Genetics. 2014;31:101-7.
11. Miyaji M, Deguchi M, Tanimura K. Clinical factors associated with pregnancy outcome in women with recurrent pregnancy loss. Gynecological Endocrinol. 2019;35:913-8.
12. Besharat M, Tabandeh A, Keshtkar A, Mobasheri E, Besharat S, Joshaghani H. Evaluation of Some Plasma Coagulation Factors in Women with Spontaneous Miscarriage. Int J Fertil Steril. 2015;9:309-12.
13. El Masry S, Azzam H, Youssef H, Othman M, Awad M. Reduced Protein C Global Assay Levels in Infertile Women with in vitro Fertilization Failure: A Pilot Study. Acta Haematologica. 2018;139:1-6.
14. Yu Ng EH, Yeung WS, Yee Lan Lau E, So WW, Ho PC. High serum oestradiol concentrations in fresh IVF cycles do not impair implantation and pregnancy rates in subsequent frozen-thawed embryo transfer cycles. Human Reproduct (Oxford, England). 2000;15:250-5.
15. Blockeel C, Engels S, De Vos M. Oestradiol valerate pretreatment in GnRH-antagonist cycles: a randomized controlled trial. Reproduct Biomed Online. 2012;24:272-80.
16. Albuquerque LE, Tso LO, Saconato H, Albuquerque MC, Macedo CR. Depot versus daily administration of gonadotrophin-releasing hormone agonist protocols for pituitary down regulation in assisted reproduction cycles. Cochrane Database Systematic Rev. 2013;2013:Cd002808.
17. Ayadurai T, Muniandy S, Omar SZ. Thrombophilia investigation in Malaysian women with recurrent pregnancy loss. J Obstetr Gynaecol Res. 2009;35:1061-8.
18. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertil steril. 2002;77:342-7.
19. Azem F, Many A, Ben Ami I. Increased rates of thrombophilia in women with repeated IVF failures. Human reproduction (Oxford, England). 2004;19:368-70.
20. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations are risk

factors for implantation failure. Reproduct Biomed Online. 2006;12:322-7.
21. Qublan HS, Eid SS, Ababneh HA. Acquired and inherited thrombophilia: implication in recurrent IVF and embryo transfer failure. Human Reproduct (Oxford, England). 2006;21:2694-8.
22. Bellver J, Soares SR, Alvarez C. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. Human Reproduct (Oxford, England) 2008;23:278-84.
23. Vaquero E, Lazzarin N, Caserta D. Diagnostic evaluation of women experiencing repeated in vitro fertilization failure. Eur J obstetr Gynecol Reproduct Biol. 2006;125:79-84.
24. Rudick B, Su HI, Sammel MD, Kovalevsky G, Shaunik A, Barnhart K. Is factor V Leiden mutation a cause of in vitro fertilization failure? Fertil steril. 2009;92:1256-9.
25. Simur A, Ozdemir S, Acar H. Repeated in vitro fertilization failure and its relation with thrombophilia. Gynecol obstetric investigation. 2009;67:109-12.

Cite this article as: Khoury F, Deeb H, Jarouj R, Khoury H, Alhalabi N, Alhalabi M. The protein C deficiency and its effects on in-vitro fertilization outcome and recurrent pregnancy loss: a Syrian cohort study. Int J Reprod Contracept Obstet Gynecol 2022;11:2352-6.