Original Research Article **Urology**

International Journal of Life science and Pharma Research

ISSN 2250-0480

THE EFFECT OF SEMEN PARAMETERS ON THE OUTCOME OF INDUCED IN VITRO FERTILIZATION (IVF) IN INFERTILE COUPLES

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ABSTRACT

Infertility is one of the most important crises in the lifestyle, leading to many problems, for couples. Infertility may be associated with many factors. The aim of this study is to determine the effect of semen parameters on the outcome of Induced in-Vitro Fertilization in infertile women. This study was performed as a retrospective cohort study using infertile couple's data that was provided by IVF in Hamadan University of Medical Sciences Endometrial and Endometriosis Research Center in 2016. Couples who had a normal semen parameter were compared with couples who had an abnormal semen parameter in terms of the outcome of the intervention, which included the positive increase of the β-HCG titer in two time measurements. The total number of subjects studied was 289, with mean age 30.49 years. The percentage of positive β-HCG (31.21%), incidence of fertilization (30.54%) and pregnancy (29.86%). The frequency of abnormal semen parameters based on WHO criteria in term of mobility was 53.98%, sperm number 32.22%, volume 4.45% agglutination 4.53% and leukocytospermia 12% respectively. There were a significant relationship between morphology>20% (p=0.022) and normal sperm adhesion (p=0.041) with pregnancy incidence. The viscosity and morphology of sperm, are two semen parameters that effects on the success rate of fertility Induced in-Vitro Fertilization method.

KEYWORDS: *Induced in-Vitro Fertilization (IVF), seminal parameters, infertility, sperm, pregnancy.*

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Received on: 28-08-2018
Revised and Accepted on: 10-12-2018
DOI: http://dx.doi.org/10.22376/ijpbs/lpr.2019.9.1.L34-44

INTRODUCTION

Infertility refers to the inability of a woman to become pregnant after one year of unprotected sex, which affects about 15 percent of couples in reproductive age.[1] It is estimated that 60 to 80 million couples each year suffer from infertility in the world, an issue that is an important issue in reproductive health.[2] For many years, the cause of infertility was attributed to women, but according to research, it has been found that 30 to 50 percent of infertility caused by factors relating to men and it is reported that male factor affects half the infertility rate and affects one in 20 people.[3] Understanding the root cause of infertility and choosing the appropriate treatment that would suit the patient's affordability and availability is important for designing treatment plans and directing diagnostic and therapeutic priorities.[4] Induced in Vitro Fertility (IVF) plays an important role in the treatment of various types of infertility, including tube causes, endometriosis, immunological, male causes, and so on. Today, intracytoplasmic sperm injection (ICSI) is used to treat severe infertility.[5] However, since these methods are expensive and require advanced laboratory equipment, knowing the predictive factors plays an important role in the outcome of the treatment.[6] In the study of infertile couples, semen test is the most important and fundamental method for assessing the cause of infertility, and the choice of treatment method.[7] Therefore, couples who treat infertility should be regularly monitored for semen parameters.[8] Various studies have been done on the role of different parameters of semen on fertilization and post IVF pregnancies. Sperm morphology is one of the most important parameters. Sperm deformity is a reliable predictor of fertility success in patients undergoing IVF.[9] In this regard, the criterion of Kruger is significant and has a great biomarker in determining the ability to fertilize without affecting the mobility and number of sperm.[10] According to this criterion, when the natural sperm morphology is less than 14%, the pregnancy rate is reduced. So that in cases with less than 4% the result of the treatment may be very poor.[11] Various studies have been performed on the sperm morphology and many in vitro fertilization results have shown contradictory results. A number of these studies have shown that abnormal morphology of sperm can negatively affect the fertility rate of IVF, while other studies have shown contradictory results.[12-22] Another parameter whose effect on the reproductive assisted reproductive techniques has been studied is the age of males, which is significantly associated with decreasing semen volume, sperm count, motility and morphplogy.[23] Some studies over the effect of age have shown significant reductions in fertility, live birth, embryo quality and implantation,[24-,30] but others have suggested that age does not affect fertility, abortion and live birth.[31-35] According to Atkinson *et al*., concentration and sperm motility do not affect the fertility and delivery efficacy in IVF and IUI[36], but another study suggests that very low sperm motility reduces fertility rates in IVF and ICSI.[37] A number of studies have also shown that leukocytospermia has a negative effect on the efficiency of IVF and ICSI,[38-44] but some studies have also shown that leukocytospermia has no effect on the incidence of pregnancy and fertility after IVF and ICSI.[45-52] Considering the different results obtained from the studies and in view of the prevalence of infertility and the methods of assisted reproduction available and also the failure to carry out a similar study in this regard, this study was conducted to determine the relationship between semen parameters and success rates IVF.

MATERIAL AND METHODS

A retrospective cohort study was conducted on a total of 298 couples undergoing *invitro* Fertilization (IVF) at Endometrial and Endometriosis Research Center of Hamadan University of Medical Sciences during 2015-2016. The experimental group consist of women whose sperm analysis of their spouses was abnormal, and the control group whose sperm analysis of their spouses was normal. These two groups were compared with regard to fertility success. The goals of the study and its effects on future studies were explained to the selected patients and they were also ensured that an anonymous checklist is designed and in the final report, the patient's name will not be given. The ethics committee of Hamadan University of Medical Sciences approved this protocol. Inclusion criteria includes indication of IVF, the age of wife 18 to 42 years and spouse of 18 years old or older and exclusion criteria includes are those not willing or able to sign a consent form, medical conditions interfere with IVF, abnormal screening tests including complete blood count, Varicella titer, Syphilis, hepatitis B, hepatitis C, HIV 1 and 2, Gonorrhea and Chlamydia, PAP smear. The success of the IVF method is to increase the β-HCG titer between two measurements. The data of these individuals included the characteristics of male semen analysis, the initial and processed tests, the success of in-vitro fertilization (as mentioned), and

changes in the β-HCG titer. Semen volume, number of sperms (count), sperm motility, morphology, agglutination time and leukocytospermia were compared in both groups based on WHO criteria.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS16 software and tests for comparing natural and abnormal parameters of semen (nominal qualitative variable) with positive β-HCG and pregnancy (nominal qualitative variable) of chi-square. The significance level in this study was considered to be 0.05.

RESULTS

Two hundred and ninety-eight cases of IVF were performed, 109 cases in 2015 and 189 in 2016. The mean (SD) age of women and their spouses was 30.94 ± 6.89 and 35.19 ± 7.11 years, respectively (Table 1). The percentage of positive β-HCG (31.20%), incidence of fertilization (30.54%) and pregnancy (29.8%).

Table 1
Previous pregnancy records in women studied

Variable	Variable levels	Frequency	Percentage
Number of Children	0	158	53
	1	51	17.11
	2	4	1.33
	3	1	0.33
	7	1	0.33
	Unknown	83	27.9
	Total	298	100
Previous Pregnancy	Normal	53	92.99
	IVF	3	5.26
	ICSI	1	1.75
	Total	57	100
Previous IVF number	0	17	5.7
	1	206	69.1
	2	58	19.5
	3	17	5.7
	Total	298	100

IVF: in-Vitro Fertilization, ICSI: intracytoplasmic sperm injection

Table 2
Frequency distribution of increase β-HCG titers, fertilization success and Pregnancy rate in infertile women under IVF.

	Positive β-HCG	Fertilization rate	Pregnancy rate	
Successful IVF n.(%)	93(31.21%)	91(30.54%)	89(29.86%)	93(31.21)
Unsuccessful IVF n.(%)	-	-	-	205(68.79)
				298(100%)

IVF: in-Vitro Fertilization, β-HCG: beta-human chorionic gonadotropin

The success rate of IVF in β-HCG incremental titers in two measurements was 31.21%. The incidence of fertilization in women with an increase β-HCG titer was 97.85%. The incidence of pregnancy was 97.8% in women with a success fertilization. (Table 2).

Table 3
Frequency of pregnancy success rate in IVF method in terms of morphology of sperm at different cutting points.

Cutting point	Variable levels	pregnancy		P-Value
		Yes	No	
4%	< 4%	2	7	0.465
	≥ 4%	87	202	
10%	< 10%	21	47	0.473
	≥ 10%	68	162	
15%	< 15%	38	83	0.362
	≥ 15%	51	126	
20%	< 20%	72	144	0.022
	≥ 20%	17	65	
25%	< 25%	88	161	0.015
	≥ 25%	8	41	
30%	< 30%	83	172	
	≥ 30%	6	37	0.008

According to the results of Table 3, normal morphology of sperm 20% and above significantly increases the success rate of IVF (P <0.05). The findings indicate that 76.4% (68 cases) of pregnancy cases in morphology were higher than 10% and 57.3% in morphology more than 15% (Table 3).

Table 4
IVF pregnancy success rate based on sperm motility

Cutting point	Variable levels	pregnancy		P-Value
		Yes	No	
15%	< 15%	29	73	0.401
	≥ 15%	60	136	
20%	< 20%	32	89	0.174
	≥ 20%	57	120	
25%	< 25%	38	99	0.270
	≥ 25%	51	110	
30%	< 30%	44	115	0.224
	≥ 30%	45	94	
35%	< 35%	49	122	0.343
	≥ 35%	40	87	
40%	< 40%	59	145	0.374
	≥ 40%	30	64	

There was no significant difference between the success rate of IVF according to the rate of semen motility in the cutting points of 15, 20, 25, 30, 35 and 40% (P = 0.> 0.05). The findings indicate that about 67.4% (60 cases) of cases leading to pregnancy occurred in motility more than 15% and 57.3% in 25% motility or higher (Table 4).

Table 5
The success rate of pregnancy in the IVF method in terms of the sperm counts. (Million in milliliters)

Cutting point	Variable levels	pregnancy		P-Value
		Yes	No	
15 million	< 15	2	14	0.095
	≥ 15	87	195	

20 million	< 20	3	15	0.159
	≥ 20	86	194	
25 million	< 25	3	17	0.101
	≥ 25	86	192	
30 million	< 30	5	19	0.223
	≥ 30	84	190	

There was no statistically significant difference between the success rates of pregnancy by IVF with the sperm count in cutting points from 15 to 30 million per ml (P> 0.05). Findings indicate that 96.6% (86 cases) of pregnancy-related cases occurred at concentrations higher than 20 million per ml and 94.4% at a concentration of more than 30 million per mL. (Table 5).

Table 6

The success rate of pregnancy in the IVF method in terms of the volume of semen (mL)

Cutting point	Variable levels	pregnancy		P-Value
		Yes	No	
1.5	< 1.5 ml	3	8	0.574
	≥ 1.5 ml	86	201	
.0	< 2 ml	27	71	0.319
	≥ 2 ml	62	138	
	< 2.5 ml	29	73	0.401
	≥ 2.5 ml	60	136	

Mean (±SD) of semen volume in successful pregnancy was 3.14 (±1.31) and in unsuccessful pregnancy was 3.32 (±1.15) mL (P = 0.334). There was no significant difference between IVF success rate in terms of semen volume at 1.5, 2 and 2.5 mL shear points (P> 0.05). The findings indicate that 96.6% (86 cases) of cases leading to pregnancy have fallen in excess of 1.5 mL (Table 6).

Table 7

The IVF success rate in terms of semen agglutination time.

Agglutination	pregnancy		Total	P-Value
	No	Yes		
Abnormaln.(%)	6(46.2)	7(53.8)	13	
Normal n.(%)	203(71.5)	82(28.5)	284	0.068
Total	209(70.1)	89(29.9)	298	

Frequency of pregnancy was 29.9% in women with normal semen agglutination and 53.8% in women with abnormal spermatic semen agglutination (P = 0.068) (Table 7).

Table 8

Frequency of IVF success rate in terms of severity of leukocytospermia

Cutting point	Variable levels	pregnancy		P-Value
		Yes	No	
3	< 3	73	170	0.515
	≥ 3	16	39	
	< 5	83	185	
5	≥ 5	6	24	0.150
7	< 7	86	198	0.355
	≥ 7	3	11	

There was no statistically significant difference between the success rate of IVF with severity of leukocytospermia at cut points 3, 5 and 7 (P => 0.05) (Table 8).

Table 9
The IVF success rate in terms of couple's age

Variable	Incidence of pregnancy	Number	Age Yrs (±SD)
Female	No	206	30.86±6.73
	Yes	89	29.77±7.28
Husband	No	206	35.71±7.50
	Yes	89	34.17±6.05

p Value for female=0.216, p value for male=0.088

There was no statistically significant difference between the success rate of pregnancy on the age of women (t = 1.23, p = 0.216) and spouse's age (t = 1.71, p = 0.088) (Table 9).

Table10
Logistic regression results, the effect of semen parameters on pregnancy in IVF-induced women.

Predictive variables	B	S.E.	Wald	df	Sig.	Exp (B)
Percentage of sperm motility more than(30%)	0.553	0.294	3.553	1	0.59	1.739
Number of sperm in one milliliter (Less than 20 million)	- 0.25	0.306	0.669	1	0.413	0.779
Morphology of sperm (abnormal)	-0.018	0.010	3.177	1	0.075	0.982
Semen volume	-0.122	0.115	1.120	1	0.290	0.885
Agglutination (abnormal)	-1.241	0.607	4.179	1	0.041	0.289
Leukocytospermia	-0.442	0.469	0.886	1	0.347	0.643
Age of the woman (years)	-0.011	0.025	0.212	1	0.645	0.989
Age of the man (years)	-0.028	0.026	1.199	1	0.273	0.972
Constant	2.536	1.087	5.440	1	0.020	12.626

According to the results of the statistical test of logistic regression using "Enter" method, the predictive parameters of pregnancy in infertile women under IVF showed only abnormal spermatic agglutination time had a significant predictive potential for pregnancy (P=0.041). In general, the above parameters could predict 69.9% of pregnancy success in IVF (Table 10).

DISCUSSION

In this study, independent variables such as female age, male age and semen parameters based on WHO criteria only showed significant statistical relation between percent agglutination time of semen with pregnancy. In this research, with increasing leukocytospermic severity, the success rate of IVF was decreased, but the difference was not statistically significant, which is consistent with the results of studies.[45-52] In another study by Hasani et al., the effect of leukocytospermia in infertile men on sperm parameters was investigated. The results showed that the sperm motility in the leukocytospermic group had a significant difference between the non-leukocytospermic group and the healthy subjects. According to the direct effect of sperm parameters on fertility and fertilization, it was concluded that leukocytospermia probably leads to impaired fertility in men.[53] In the present study, the success rate of IVF was decreased with an increase in leukocytospermia, but the difference was not statistically significant. The reason for the difference between the results of this study and the findings of Hasani et al. is probably due to differences in sample size. In the present study, the total number of samples with abnormal leukocyte was 12 cases. In the study of Hasani et al., 45 cases of abnormal leukocytospermia were studied. In the present study, there was no significant relationship between sperm morphology and IVF success rate. Our results show that 76.4% (68 cases) of

pregnancy cases in morphology were higher than 10% and 57.3% in morphology more than 15%. Shi et al., showed that normal sperm morphology affects IVF-ET and that normal morphology 5% is 4% higher than normal morphology in predicting c-IVF efficacy.[18] He and colleges observed that if normal morphology of sperm is less than 4%, it can affect total fertility, but if normal morphology of sperm is less than 3%, the normal fertility rate in IVF decreases.[54] Li et al., showed that in ICSI compared to IVF, the fertility rate of subjects with normal morphology was less than 4% higher and the abortion rate was significantly lower. Another study also found that the incidence of pregnancy with IVF in normal sperm is higher than that of abnormal sperm.[22] Nouri et al., concluded that the post-IVF pregnancy process was complex, but the embryo's return seems to be better than ICSI.[55] In the study of Shu et al., on determining the effect of sperm morphology on the success rate of in vitro fertilization, the results showed that the fertilization rate in the group with moderate morphology was significantly less than the mild group, but there was no significant difference between the normal groups. In the end, the researchers concluded that the abnormal morphology of sperm had a moderate difference in the rate of fertilization but significantly reduced the quality of the fetus.[19] There was no meaningful relationship between the success rates of IVF with morphology based on WHO criteria, which is in line with the findings of Shu et al. But at a cutting point of 20% and above, the success rate of IVF was significantly increased. Feng et al., in their study on the effect of sperm morphology, based on the World Health Organization's Criteria. Congenital defects were not observed in any of the newborns and there was no significant difference during pregnancy, preterm birth and low body weight and sperm morphology, given the WHO criteria, only has limited value for predicting the outcome and status of the newborn in IVF.[19] In the present study, the implications of postpartum pregnancy were not studied, but there was no significant relationship with the findings of Feng et al., between the semantic morphology and the cutoff point determined by the World Health Organization and the incidence of pregnancy. In the study of Obara et al., according to WHO criteria, there was a significant difference between the frequency and rate of fertilization in normal and abnormal morphology groups. But other sperm parameters were not associated with the frequency and fertility rate. Overall, the researchers concluded that STRICT's criteria for predicting success in IVF were credible criteria.[56] In the study of Oehninger et al., fertility results in the IVF method in patients with severe morphological disorder in sperm were studied. In this study, increasing the concentration of seminal fluid increased fertilization but had no effect on the prognosis of gestation. In the end, the researchers concluded that patients with severe morphologic disorders of sperm had a small ability to have successful pregnancy.[57] In the present study, consistent with the results of Obara et al. study, with increasing natural morphology, the success rate of IVF was significantly increased. In our study, there was no significant difference between age of couples and fertility. The results of this study are in line with the results of studies.[23,32-35] A study by Wu et al., showed that there was no difference between the amount of pregnancy and implantation among different male age groups[35] while De la Rochbrochard *et al.,* reported that the age of parents (fathers over 40 years old and mothers over the age of 35) significantly affects fertility in IVF.[29] In this study, there was no significant relationship between sperm motility and IVF. The study by Atkinson et al., showed that mobility significantly affects fertility, but fertility and delivery rates in IVF and IUI are not affected by this parameter,[36] which is consistent with the results of our study. Based on the results of our study, we recommend that those who are selected for IVF method have at least the following criteria in their semen parameters: sperm count>15 m/mL, normal morphology>20%, progressive motility >15% and normal agglutination time.

CONCLUSION

Based on semen parameters determined by the World Health Organization, semen parameters other than sperm agglutination time had no significant effect on the success rate of fertility assisted inoculation, which may be normal and abnormal due to the inappropriate selection of cutting points for the above parameters. The second reason for not observing the significant difference in this study may be due to the low abnormal samples in each of the subgroups of semen parameters which reduces the ability to study.

AUTHOR CONTRIBUTION STATEMENT

This article is based on the thesis of R. Omrani's general medical course, to master the guidance of M. Alemi. Both J. Tarhani and B. Khodadadiauthors contributed to the final version

of the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

FUNDING ACKNOWLEDGEMENT

We acknowledge the resources and financial support for the study was provided by Endometrial and Endometriosis Research Center of Hamadan University of Medical Sciences (Grant Number-9511056663).

ACKNOWLEDGMENT

We would like to thank all our colleagues who helped us with this research.

CONFLICT OF INTEREST

Conflict of interest declared none.

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