INTERNATIONAL JOURNAL OF FERTILITY AND STERILITY (Int J Fertil Steril)

Abstract & Full Text Indexing to:
1. Emerging Sources Citation Index (ESCI,ISI)
2. PubMed Central (PMC)
3. National Library of Medicine (NLM)
4. Index Medicus for the Eastern Mediterranean Region (IMEMR)
5. Index Copernicus International
6. EMBASE
7. Scopus
8. CINAHL Database
9. Google Scholar
10. Proquest
11. Directory of Open Access Journals (DOAJ)
12. Open Academic Journals Index (OAJI)
13. Directory of Research Journals Indexing (DRJI)
14. Scientific Information Database (SID)
15. Iranmedex
16. Regional Information Center for Sciences and Technology (RICeST)
17. Islamic World Science Citation Center (ISC)
18. Magiran
19. InfoBase Index
20. Science Library Index

Editorial Office Address: P.O.Box: 16635-148, Royan Institute, Tehran, Iran
(Mohammad Hossein Nasr Esfahani, Ph.D.)
Tel & Fax: +9821-22510895
Web: www.ijfs.ir
Emails: ijfs@royaninstitute.org & info@ijfs.ir

Printing Company:
Jurband Ghaemprint Co.
NO. 5, Jalil khoob alley, Niroo Havaei Street, Tehran, Iran

INTERNATIONAL JOURNAL OF FERTILITY AND STERILITY

Int J Fertil Steril, Vol 10, No 4, Jan-Mar 2017, Pages: 320-394

Contents

Original Articles

Ethics, Legal, Social, Counseling Article

Short Communication

Original Article

Clomiphene Citrate Treatment Cycle Outcomes of Polycystic Ovary Syndrome Patients Based on Basal High Sensitive C-Reactive Protein Levels: A Cross-Sectional Study

Serkan Kahyaoglu, M.D.*, Omer Hamid Yumuşak, M.D., Sebnem Ozyer, M.D., Meryem Kuru Pekcan, M.D., Merve Erel, M.D., Mahmut Nedim Cicek, M.D., Salim Erkaya, M.D., Yasemin Tasci, M.D.

Department of Obstetrics and Gynecology, Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey

Abstract

Background: Polycystic ovary syndrome (PCOS) is highly associated with an ovulatory infertility, features of the metabolic syndrome, including obesity, insulin resistance and dyslipidemia. Serum concentrations of high sensitive C-reactive protein (hs-CRP) were significantly higher in obese than in non-obese PCOS patients at baseline, suggesting a relationship between elevated hs-CRP levels and obesity. The aim of this study was to evaluate whether cycle day 3 hs-CRP levels before clomiphene citrate (CC) treatment would predict cycle outcomes in women with PCOS.

Materials and Methods: This cross-sectional study was conducted among 84 infertile women with PCOS who were treated with CC at Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey, between January 2014 and January 2015. Based on the exclusion criteria, cycle outcomes of remaining 66 infertile women with PCOS treated with CC were analyzed. The hs-CRP levels and insulin resistance indexes were evaluated on day 3 of the CC treatment cycle. The primary outcome measures were number of preovulatory follicles measuring≥17 mm and pregnancy rates.

Results: The mean ± SD age of the patients was 24.0 ± 3.8 years (range 18-36). The mean ± SD body mass index (BMI) of the patients was 25.7 ± 4.9 (range 17-43). Fifty patients developed dominant follicle (75%) and 5 patients established clinical pregnancy during the study (clinical pregnancy rate: 7%). The mean ± SD baseline hs-CRP, fasting insulin and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) values of the patients with and without dominant follicle generation during treatment cycle were 6.42 ± 7.05 and 4.41 ± 2.95 (P=0.27), 11.61 ± 6.94 and 10.95 ± 5.65 (P=0.73), 2.68 ± 1.79 and 2.41 ± 1.30 (P=0.58), respectively. The mean ± SD baseline hs-CRP, fasting insulin and HOMA-IR values of the patients with and without clinical pregnancy establishment following treatment cycle were 6.30 ± 2.56 and 5.90 ± 6.57 (P=0.89), 11.60 ± 7.54 and 11.44 ± 6.61 (P=0.95), 2.42 ± 1.51 and 2.63 ± 1.70 (P=0.79), respectively.

Conclusion: In this study, we did not observe a predictive value of cycle day 3 hs-CRP levels on preovulatory follicle development and pregnancy rates among infertile PCOS patients treated with CC. Also, no relationship between HOMA-IR values and dominant follicle generation or clinical pregnancy establishment was demonstrated in our study, confirming the previous studies emphasizing the neutral effect of metformin utilization before and/or during ovulation induction to pregnancy rates.

Keywords: Polycystic Ovary Syndrome, Ovulation Induction, Clomiphene, C- Reactive Protein

Citation: Kahyaoglu S, Yumuşak OH, Ozyer S, Pekcan MK, Erel M, Cicek MN, Erkaya S, Tasci Y. Clomiphene citrate treatment cycle outcomes of polycystic ovary syndrome patients based on basal high sensitive C-reactive protein levels: a cross-sectional study. Int J Fertil Steril. 2017; 10(4): 320-326.

Received: 10 May 2016, Accepted: 7 Jul 2016
***Corresponding Address: Department of Obstetrics and Gynecology, Zekai Tahir Burak Women's Health Education and Research Hospital, Serhat Mahallesi 1292, Sokak Nevbahar Botanik 2 Konutları C Blok No: 7/49 Yenimahalle, Ankara, Turkey**
Email: mdserkankahyaoglu@gmail.com

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 320-326

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder with a 5-10% prevalence among women of reproductive age (1-3). PCOS should be diagnosed with the presence of two of three criteria (oligo-ovulation/anovulation, hyperandrogenism, and polycystic ovarian morphology) on ultrasonography after exclusion of other endocrine disorders as determined at the 2003 Rotterdam consensus meeting (4). Hyperandrogenism has been established as a mandatory diagnostic criteria for PCOS in National Institute of Health (NIH) and Androgen Excess Society criteria unlike Rotterdam consensus. PCOS is strongly related to irregular menstrual cycles, oligo-anovulation, and infertility accompanied by metabolic disorders like obesity, insulin resistance, gestational diabetes mellitus, diabetes, and cardiovascular disease, which makes this relatively prevalent syndrome as a public health issue (5-7).

Chronic low-grade inflammation is involved in the pathogenesis of obesity-related syndromes like PCOS, which is also a proinflammatory state with an association between inflammation at the molecular level and insulin resistance (8-11). It still remains unclear that whether elevations of inflammatory markers like tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and high sensitive C-reactive protein (hs-CRP) are related to PCOS or are a function of obesity, abdominal adiposity, or both. Serum concentrations of hs-CRP were significantly higher in obese than in non-obese PCOS patients at baseline, suggesting a relationship between elevated hs-CRP levels and obesity (12). Clinical reflections of increased serum levels of hs-CRP among obese and non-obese PCOS patients are not clear and worth to investigate for determining a predictive marker for future prognosis of interventions in management of PCOS. Increased hs-CRP levels as a reflection of activated systemic inflammatory response either due to obesity or PCOS disease can be considered as increased metabolic activity of the disease. This increased inflammatory activity can theoretically affect folliculogenesis, and ovulation as the anovulation is the main defect of patients with PCOS, resulting in infertility. The aim of this study was to evaluate whether cycle day 3 hs-CRP levels before commencing clomiphene citrate (CC) treatment would predict ovulation induction cycle outcomes in women with PCOS.

Materials and Methods

This cross-sectional study was conducted at the infertility clinic of Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey, between January 2014 and January 2015. PCOS patients with tubal factor infertility, male infertility, endometriosis, and systemic disorders like overt diabetes mellitus, cardiac pathologies and thyroid diseases were excluded. The cycle outcomes of 84 infertile women with PCOS who were treated with CC+coitus or CC+intrauterine insemination (IUI) were evaluated. Eighteen patients were excluded due to use of metformin and absence of completing the treatment cycle. Remaining 66 patients' ovulation induction cycle data were assessed. Of 66 patients, 6 patients' pregnancy outcomes were not detected due to loss from follow-up. The hs-CRP levels of the patients were measured on cycle day 3 of the CC treatment cycle. The serum samples for hs-CRP levels were drawn on the day 3 of the menstrual cycle because ovulation induction stimulates an inflammatory response. hs-CRP was measured with a high sensitivity immunoturbidimetric assay (Roche Diagnostics, USA) using an automated clinical chemistry analyzer. The hs-CRP assay coefficients of variation were 2.7% at 0.12 mg/L, 3.45% at 0.41 mg/L, and 5.7% at 0.03 mg/L. The assay has a detection limit of 0.03 mg/L and a calibration range up to 300 mg/L. Normal range reference value of hs-CRP for adults was accepted as <5 mg/dL.

Insulin resistance situation of the patients was evaluated using the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) within the 2 months preceding the treatment cycle. HOMA-IR values greater than 2.5 were thought to be related to insulin resistance (13). Then, patients were given 50-100 mg CC for five days starting from day three of their menstrual cycles. A transvaginal ultrasonography was performed at day 12 and every other day until a follicle ≥ 17 mm was detected. Timely coitus or IUI procedure regarding the preference of the patients and clinicians was recommended to the patients 36 hours after triggering the ovulation with 10.000 IU human chorionic gonadotropin (hCG) intramuscular injection. Patients underwent a serum pregnancy test on the 14th day following triggering

of ovulation. Patients with positive pregnancy tests were called for evaluation of clinical pregnancy with ultrasonography at 5 weeks after the pregnancy test. Clinical pregnancy was defined as fetal heart beat detection on transvaginal ultrasonography at 7 weeks of gestation. The primary outcome measures were number of preovulatory follicles measuring≥17 mm and pregnancy rates.

Ethical considerations

An informed consent was obtained from all of participants, and the Institutional Review Board of Zekai Tahir Burak Women's Health Education and Research Hospital approved this project.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, SPSS Inc., USA) version 22.0. Normal distribution of data was evaluated using Kolmogorov-Smirnov test. The continuous variables were presented by mean ± SD and compared using the independent sample t test. The nonparametric variables without normal distribution were tested using Mann Whitney U test. Correlation analysis was performed using Pearson correlation test. The comparison of categorical values was made using Fisher's exact test or Chi-square test. Receiver operating characteristic (ROC) curve was used to compare the diagnostic performance of the diagnostic tests. $P<0.05$ were considered statistically significant.

Results

The mean ± SD age of the patients was 24.0 ± 3.8 years (range 18-36) with a mean duration of infertility of 3.0 ± 1.6 years (range 1-9). The mean ± SD body mass index (BMI) of the patients was 25.7 ± 4.9 (range 17-43). The mean ± SD cycle day 3 hs-CRP level of the patients was 5.93 ± 6.35 and the mean ± SD HOMA-IR value of the patients was 2.61 ± 1.68. Fifty patients developed dominant follicle (75%) and 5 patients established clinical pregnancy during the study (clinical pregnancy rate: 7%). All patients (5 of 5) with clinical pregnancy establishment and 33 of 55 patients (60%) without clinical pregnancy establishment underwent an IUI procedure despite the fact that male infertility was an exclusion criteria of the study (P=0.64). The baseline hs-CRP levels and HOMA-IR values of the patients were not correlated (r=0.02 using Pearson correlation, P=0.87). A significantly positive correlation was seen between baseline hs-CRP levels and BMI values of the patients (r=0.54 using Pearson correlation; P<0.001). CC treatment cycle days 3 and 5 did not influence the dominant follicle development (P=0.37) and clinical pregnancy achievement (P=0.47), respectively. Dominant follicle development following CC treatment was not related to baseline insulin resistance (P=0.64). The mean ± SD baseline hs-CRP, fasting insulin and HOMA-IR values of the patients with and without dominant follicle generation during treatment cycle were 6.42 ± 7.05 and 4.41 ± 2.95 (P=0.27), 11.61 ± 6.94 and 10.95 ± 5.65 (P=0.73), 2.68 ± 1.79 and 2.41 ± 1.30 (P=0.58), respectively.

The ovulation induction cycle outcomes of the study group according to establishment of clinical pregnancy following treatment cycle are demonstrated in Table 1. Patients' ovulation induction cycle outcomes based on BMI classification are demonstrated in Table 2.

Table 1: Cycle outcomes of patients with and without achieving clinical pregnancy with ovulation induction by administration of 5 days of oral CC treatment

Parameter	Clinical pregnancy (+) (n=5)	Clinical pregnancy (-) (n=55)	P value
Age (Y)	22.4 ± 2.5	24.6 ± 3.9	0.32*
BMI (ratio)	27.0 ± 5.0	25.1 ± 4.4	0.37*
Infertility duration (Y)	3.0 ± 1.2	3.0 ± 1.5	0.54*
Day 3			
FSH (mIU/mL)	5.8 ± 2.3	6.2 ± 1.2	0.70**
LH (mIU/mL)	7.1 ± 4.8	8.8 ± 6.4	0.43**
E_2 (pg/mL)	55 ± 35	45 ± 16	0.90**

Table 1: Continued

Parameter	Clinical pregnancy (+) (n=5)	Clinical pregnancy (-) (n=55)	P value
Cycle day of CC commencement n (%)			
Day 3	5 (100%)	47 (85.5%)	0.47***
Day 5	0	8 (14.5)	
Day 3 CRP level (mg/dL)	4.58 ± 2.25	5.94 ± 6.91	0.75
Fasting glucose level (mg/dL)	91.5 ± 8.8	89.9 ± 9.9	0.72**
2-hour postprandial glucose level (mg/dL)	106.9 ± 19.7	105.8 ± 21.8	0.91**
Fasting insulin (mIU/L)	10.2 ± 3.6	11.3 ± 6.6	0.90*
HOMA-IR (ratio)	2.25 ± 0.65	2.59 ± 1.72	0.78*
>14 mm follicle number (n)	0.8 ± 0.4	1.2 ± 1.2	0.51*
Day 12 periovulatuar E_2 level (pg/mL)	124 ± 62	550 ± 687	0.17*
hCG triggering day of cycle	17.01.4	13.52.9	0.015*
hCG day endometrial thickness (mm)	7.0 ± 1.8	7.2 ± 1.8	0.73**

Data are presented as mean ± SD, *; Mann Whitney U test, **; Independent samples t test, ***; Fisher's exact test, CC; Clomiphene citrate, BMI; Body mass index, CRP; C-reactive protein, FSH; Follicle stimulating hormone, LH; Luteinizing hormone, E_2; Estardiol, hCG; Human chorionic gonadotropin, and HOMA-IR; Homeostasis model assessment-insulin resistance.

Table 2: Patients' ovulation induction cycle outcomes based on BMI classification

BMI (kg/height²)	Cycle day 3 hs-CRP	Fasting glucose level	2-hour postprandial glucose level following 75 g oral glucose tolerance test	HOMA-IR	Number of follicle>14 mm following ovulation induction	Dominant follicle achievement n (%)	Clinical pregnancy achievement n (%)
<30 (n=52)							
Mean ± SD	5.68 ± 6.88	89.4 ± 10.0	105.4 ± 21.4	2.46 ± 1.75	1.24 ± 1.33	29 (55%)	2 (3.8%)
≥30 (n=8)							
Mean ± SD	6.79 ± 5.08	94.0 ± 7.8	108.4 ± 22.6	3.23 ± 0.62	1.13 ± 0.35	8 (100%)	3 (37.5%)
P value	0.24*	0.10*	0.62*	0.01*	0.76*	0.09**	0.01**
Total (n=60)							
Mean ± SD, n (%)	5.83 ± 6.64	90.0 ± 9.8	105.9 ± 21.4	2.56 ± 1.66	1.23 ± 1.23	37 (61%)	5 (8%)

*; Mann Whitney U test, **; Fisher's exact test, BMI; Body mass index, and HOMA-IR; Homeostatic model of assessment-insulin resistance.

Our findings showed that cycle day 3 hs-CRP levels, fasting serum glucose levels and 2-hour postprandial serum glucose levels were found to be higher among patients with BMI values ≥30 than patients with BMI values <30, indicating there were statistically insignificant differences in this regard. HOMA-IR value and clinical pregnancy rate were significantly higher in patients with BMI values ≥30 than patients with BMI values <30. Cycle day 3 hs-CRP levels showed no predictive value for dominant follicle establishment following 5 days CC treatment, [area under the curve (AUC)=0.44, 95% confidence interval (CI)=0.29-0.59, P=0.52] (Fig.1).

Later ovulation triggering cycle day by hCG utilization significantly predicted successful pregnancy outcome (AUC=0.86, 95% CI=0.74-0.99, P=0.018) (Fig.2).

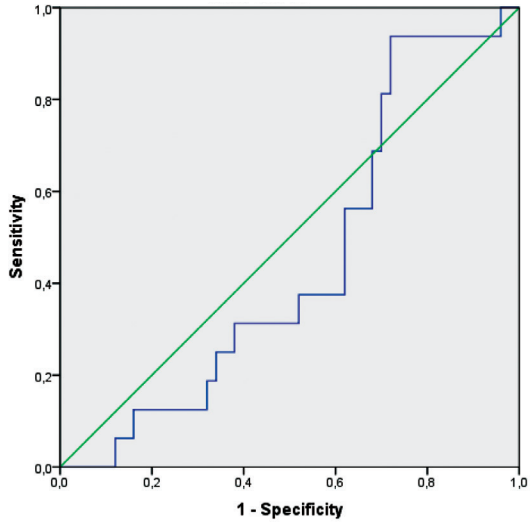

Fig.1: ROC analysis of cycle day 3 hs-CRP levels and dominant follicle establishment following 5 days CC treatment for ovulation induction (AUC=0.44, 95% CI=0.29-0.59, P=0.52).
ROC; Receiver operating curve, hs-CRP; High sensitive C-reactive protein, CC; Clomiphene citrate, AUC; Area under the curve, and CI; Confidence interval.

Later ovulation triggering cycle day by hCG utilization significantly predicted successful pregnancy outcome (AUC=0.86, 95% CI=0.74-0.99, P=0.018) (Fig.2).

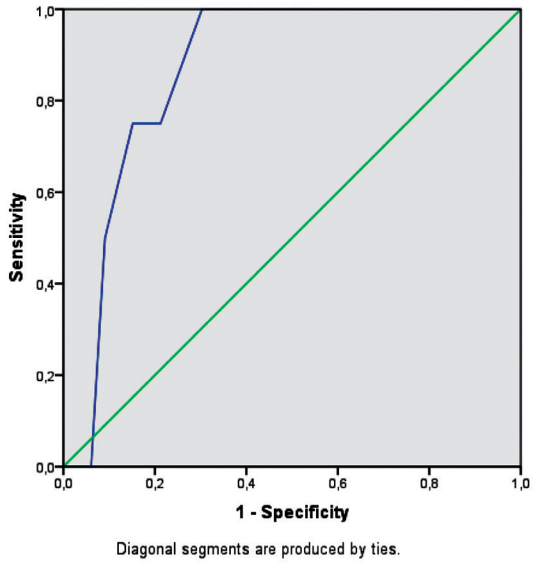

Fig.2: ROC analysis of ovulation triggering day by hCG and clinical pregnancy achievement (AUC=0.86, 95% CI=0.74-0.99, P=0.018). Cut-off value for predicting clinical pregnancy establishment by ovulation triggering day is 16.5 days with sensitivity and specificity of 75 and 85%, respectively.
ROC; Receiver operating curve, hCG; human chorionic gonadotropin, AUC; Area under the curve, and CI; Confidence interval.

Cut-off value for predicting clinical pregnancy establishment by ovulation triggering day is 16.5 days with sensitivity and specificity of 75 and 85%, respectively. When 2.5 cut-off level was considered for increased HOMA-IR ratio reflecting insulin resistance, high HOMA-IR ratio was not correlated with dominant follicle development (P=0.64) and clinical pregnancy establishment (P=0.65). No statistically significant relationship was determined between BMI and dominant follicle generation or clinical pregnancy establishment.

Discussion

PCOS is a multisystemic disorder, which includes short and long-term complications for the affected women. Exact pathophysiological mechanism and etiological factors have not been yet defined. Serum levels of inflammatory markers like hs-CRP are elevated in PCOS which demonstrates chronic low-grade inflammation state of the disorder. Serum hs-CRP level is positively correlated with adipose tissue content and BMI ratio of the body (14). In our study, we also demonstrated a significantly positive relationship between hs-CRP and high BMI levels. The short and long-term clinical effects of high hs-CRP level caused by high BMI level cannot be predicted. Success rate of ovulation induction cycles among PCOS patients are needed to be evaluated with objective laboratory or clinical markers. We hypothesized that high cycle day 3 serum level of hs-CRP would deteriorate folliculogenesis that resulted in unfavorable cycle outcomes reflected by absence of dominant follicle and implantation failure. However, we could not detect any significant relationship between serum hs-CRP levels and cycle outcomes. Later artificial ovulation triggering day with hCG was significantly associated with clinical pregnancy achievement in our study. We established 16.5 days of artificial ovulation triggering day from the beginning of the menstrual cycle as a cut-off level for successful cycle outcomes induced by CC in PCOS patients. This can be explained by relatively late maturation of oocytes in CC cycles unlike gonadotropin cycles (15).

In this study, we did not observe a predictive value of cycle day 3 hs-CRP levels on preovulatory follicle development and pregnancy rates among infertile PCOS patients treated with CC. Although weight reduction to improve folliculo-

genesis in PCOS patients is recommended as a first line management strategy, the benefits of getting rid of obesity seems to be achieved by other pathophysiological events rather than lowering serum hs-CRP levels. HOMA-IR levels and clinical pregnancy rates of the patients with BMI values ≥30 were found to be significantly higher than patients with BMI values <30, which demonstrates that HOMA-IR levels did not adversely affect pregnancy achievement among obese PCOS patients. Cycle day 3 hs-CRP levels and dominant follicle achievement rates of patients with BMI values ≥30 were found to be insignificantly higher than patients with BMI values <30, which demonstrates that cycle day 3 hs-CRP levels, as a reflection of systemic inflammatory response, did not adversely or positively affect folliculogenesis and clinical pregnancy establishment. Agacayak et al. (16) have evaluated the levels of inflammatory markers and neopterin in obese and non-obese patients with PCOS using 2 separate control groups with matching BMI. No statistically significant difference was found between obese and non-obese patients with PCOS and control subjects in neopterin, IL-6, TNF-α, and neutrophil/lymphocyte ratio levels unlike CRP levels which were significantly higher in obese patients with PCOS compared to obese control subjects, demonstrating the facilitatory effect on serum CRP levels of PCOS patients by disease itself rather than obesity. Aziz et al. (17) have also demonstrated the relationship between obesity, insulin resistance and CRP with plasma endogen thrombin generation, which reflects cardiovascular disease progression among patients with PCOS.

Orvieto et al. (18) have investigated serum and follicular fluid CRP levels in patients undergoing controlled ovarian hyperstimulation (COH) for *in vitro* fertilization (IVF)-embryo transfer cycle and their possible correlation to COH variables. In this study, a significant increase in serum CRP levels during COH, especially after hCG administration, has been detected during COH procedure which demonstrates the stimulatory effect of COH on a state of systemic inflammation. They have found that no significant correlations exist between serum and follicular fluid CRP, or between serum CRP-to-BMI ratio and serum sex steroid levels or IVF treatment variables. In another study performed by the same group, a significant increase in serum ovarian androgen levels during gonadotropin treatment has been detected in patients undergoing COH for IVF. A significant increase in the levels of both serum CRP and ovarian androgens (testosterone, androstenedione) has also been found after hCG administration, which demonstrates that ovarian androgen levels increase in correlation with the degree of inflammation, as reflected by CRP levels (19). The results of this study supported our objective regarding the association between increased systemic inflammatory response accompanied by increased ovarian androgen levels and defective folliculogenesis among patients with PCOS.

Secondly, no relationship between HOMA-IR values and dominant follicle generation or clinical pregnancy establishment were demonstrated in our study, confirming the previous studies emphasizing the absence of any beneficial effect of metformin utilization on pregnancy rates before and/or during ovulation induction (20). The number of clinical pregnancies was so low to make a conclusive statement for the relationship between baseline hs-CRP levels and pregnancy rate.

Conclusion

Both basal serum hs-CRP levels and HOMA-IR ratios showed no predictive value for development of dominant follicles and/or achievement of clinical pregnancy among infertile women diagnosed with PCOS. Further studies with larger number of patients for evaluating the predictive value of hs-CRP on cycle outcomes of infertile PCOS patients are needed.

Acknowledgements

There is no financial support and conflict of interest regarding in this article.

References

1. Boyle JA, Cunningham J, O'Dea K, Dunbar T, Norman RJ. Prevalence of polycystic ovary syndrome in a sample of Indigenous women in Darwin, Australia. Med J Aust. 2012; 196(1): 62-66.
2. Younis JS, Jadaon JE, Haddad S, Izhaki I, Ben-Ami M. Prospective evaluation of basal stromal Doppler studies in women with good ovarian reserve and infertility undergoing in vitro fertilization-embryo transfer treatment: patients with polycystic ovary syndrome versus ovulatory patients. Fertil Steril. 2011; 95(5): 1754-1758.
3. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010; 25(2): 544-551.
4. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus

Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004; 81(1): 19-25.
5. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet. 2007; 370(9588): 685-697.
6. Moti M, Amini L, Mirhoseini Ardakani SS, Kamalzadeh S, Masoomikarimi M, Jafarisani M. Oxidative stress and antioxidant defense system in Iranian women with polycystic ovary syndrome. Iran J Reprod Med. 2015; 13(6): 373-378.
7. Shen SH, Shen SY, Liou TH, Hsu MI, Chang YC, Cheng CY, et al. Obesity and inflammatory biomarkers in women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2015; 192: 66-71.
8. Escobar-Morreale HF, Luque-Ramírez M, González F. Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. Fertil Steril. 2011; 95(3): 1048-1058. e1-2.
9. González F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab. 2006; 91(1): 336-340.
10. González F, Rote NS, Minium J, Kirwan JP. Increased activation of nuclear factor kappaB triggers inflammation and insulin resistance in polycystic ovary syndrome. J Clin Endocrinol Metab. 2006; 91(4): 1508-1512.
11. González F, Rote NS, Minium J, Kirwan JP. In vitro evidence that hyperglycemia stimulates tumor necrosis factor-alpha release in obese women with polycystic ovary syndrome. J Endocrinol. 2006; 188(3): 521-529.
12. Samy N, Hashim M, Sayed M, Said M. Clinical significance of inflammatory markers in polycystic ovary syndrome: their relationship to insulin resistance and body mass index. Dis Markers. 2009; 26(4): 163-170.
13. Stovall DW, Bailey AP, Pastore LM. Assessment of insulin resistance and impaired glucose tolerance in lean women with polycystic ovary syndrome. J Womens Health (Larchmt). 2011; 20(1): 37-43.
14. Ertas IE, Kahyaoglu S, Yilmaz B, Ozel M, Sut N, Guven MA, et al. Association of maternal serum high sensitive C-reactive protein level with body mass index and severity of pre-eclampsia at third trimester. J Obstet Gynaecol Res. 2010; 36(5): 970-977.
15. George K, Kamath MS, Nair R, Tharyan P. Ovulation triggers in anovulatory women undergoing ovulation induction. Cochrane Database Syst Rev. 2014; (1): CD006900.
16. Agacayak E, Tunc SY, Sak S, Basaranoglu S, Yüksel H, Turgut A, et al. Levels of neopterin and other inflammatory markers in obese and non-obese patients with polycystic ovary syndrome. Med Sci Monit. 2015; 21: 2446-2455.
17. Aziz M, Sidelmann JJ, Wissing ML, Faber J, Skouby SO. Endogenous thrombin potential in polycystic ovary syndrome: the association to body mass index, insulin resistance, and inflammation. Gynecol Endocrinol. 2015; 31(9): 720-724.
18. Orvieto R, Chen R, Ashkenazi J, Ben-Haroush A, Bar J, Fisch B. C-reactive protein levels in patients undergoing controlled ovarian hyperstimulation for IVF cycle. Hum Reprod. 2004; 19(2): 357-359.
19. Orvieto R, Fisch N, Yulzari-Roll V, La Marca A. Ovarian androgens but not estrogens correlate with the degree of systemic inflammation observed during controlled ovarian hyperstimulation. Gynecol Endocrinol. 2005; 21(3): 170-173.
20. Huang X, Wang P, Tal R, Lv F, Li Y, Zhang X. A systematic review and meta-analysis of metformin among patients with polycystic ovary syndrome undergoing assisted reproductive technology procedures. Int J Gynaecol Obstet. 2015; 131(2): 111-116.

Original Article

Molecular Detection and Genotypic Characterization of *Toxoplasma gondii* in Paraffin-Embedded Fetoplacental Tissues of Women with Recurrent Spontaneous Abortion

Amir Abdoli, M.Sc.[1], Abdolhossein Dalimi, D.V.M., Ph.D.[1]*, Haleh Soltanghoraee, Ph.D.[2], Fatemeh Ghaffarifar, Ph.D.[1]

1. Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Abstract

Background: Congenital toxoplasmosis is an important cause of spontaneous abortion worldwide. However, there is limited information on detection and genotypic characterization of *Toxoplasma gondii (T. gondii)* in women with recurrent spontaneous abortion (RSA). The aim of this study is the molecular detection and genotypic characterization of *T. gondii* in formalin-fixed, paraffin-embedded fetoplacental tissues (FFPTs) of women with RSA that have referred to the Avicenna Research Institute in Tehran, Iran.

Materials and Methods: This experimental research was undertaken on 210 FFPTs of women with RSA. The information of the patients was collected from the archives of Avicenna Research Institute in Tehran, Iran. After DNA extraction, the presence of *T. gondii* was examined by nested polymerase chain reaction targeting the *GRA6* gene. Genotyping was performed on positive samples using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) that targeted the *GRA6* and *SAG3* genes. Sequencing was conducted on two *GRA6* positive samples.

Results: *T. gondii* DNA was detected in 3.8% (8/210) of the samples. Genotyping showed that all positive samples belonged to type III of the *T. gondii* genotype. Sequencing two genomic DNAs of the *GRA6* gene revealed 99% similarity with each other and 99-100% similarity with *T. gondii* sequences deposited in GenBank. There were six patients with histories of more than three abortions; one patient had a healthy girl and another patient had two previous abortions. Abortions occurred in the first trimester of pregnancy in seven patients and in the second trimester of pregnancy in one patient.

Conclusion: The results of this study have indicated that genotype III is the predominant type of *T. gondii* in women with RSA in Tehran, Iran. Also, our findings suggest that toxoplasmosis may play a role in the pathogenesis of RSA. However, further studies are needed to elucidate a clear relationship between *T. gondii* infection and RSA.

Keywords: *Toxoplasma gondii*, Abortion, Molecular Detection, Genotype, Iran

Citation: **Abdoli A, Dalimi A, Soltanghoraee H, Ghaffarifar F. Molecular detection and genotypic characterization of Toxoplasma gondii in paraffin-embedded fetoplacental tissues of women with recurrent spontaneous abortion. Int J Fertil Steril. 2017; 10(4): 327-336.**

Introduction

Toxoplasmosis is one of the most common parasitic diseases where approximately one-third of the world's population is affected (1, 2). Approximately 25 to 30% of the world's population is infected by *Toxoplasma gondii (T. gondii)*. Nevertheless, the most common form of infection is asymptomatic (2-4). Human infections generally occur by the consumption of undercooked meatthat contains tissue cysts or by water and food contami-

Received: 7 Dec 2015, Accepted: 11 Jul 2016
***Corresponding Address: P.O.Box: 14155-331, Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran**
Email: dalimi_a@modares.ac.ir

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 327-336

nated with oocysts present in cat feces. Congenital infection is one of the most important sequels of toxoplasmosis in pregnant women (1). Congenital transmission of *T. gondii* predominantly occurs at the first time during pregnancy (3, 5). The approximate incidence rate of congenital toxoplasmosis is 1.5 cases per 1000 live births with a global incidence rate of 190,100 cases annually (6). Frequency of transplacental transmission and severity of congenital toxoplasmosis correlates with the gestational age of infected mothers. The highest rates of transplacental transmission occur in the third trimester of pregnancy; which usually results in asymptomatic infections at birth. However, they may develop clinical signs (such as chorioretinitis, slower mental and neurological development) at a later age (1). On the other hand, the severity of congenital toxoplasmosis is highest in the first and second trimesters of pregnancy which usually results in abortion or stillbirth (1, 5, 7, 8).

Recurrent spontaneous abortion (RSA) is the loss of three or more consecutive pregnancies before 20 weeks of pregnancy (9) and affects approximately 1 to 2% of couples trying to conceive (10). Several factors-genetic background, anatomical abnormalities, endocrine disruption, autoimmune disorder, and infectious diseases have been attributed to play roles in the etiology of RSA (9-11). Infectious agents account for 0.5 to 5% of RSA (10). The most common infectious causes of RSA are *Chlamydia trachomatis, Ureaplasmaurealyticum, Mycoplasma hominis,* cytomegalovirus (CMV), and human papillomaviruses (HPV) (9, 11). Several studies have reported significantly higher seroprevalence of ToRCH infections in women with spontaneous abortion or negative obstetric history, including preterm deliveries, intrauterine deaths or growth retardation (12-16).

Although several studies have reported an association between *T. gondii* infection and spontaneous abortion (6), the role of toxoplasmosis in the etiology of RSA is less clear. Hence, we have investigated the rate of *T. gondii* infection in formalin-fixed, paraffin-embedded fetoplacental tissues (FFPTs) of women with RSA that referred to the Avicenna Research Institute in Tehran, Iran.

Materials and Methods

This experimental study was performed on archived FFPTs of women with RSA that referred to the Avicenna Research Institute in Tehran, Iran during 2013-2015. This study was approved by the Ethical Committees of Tarbiat Modares University and Avicenna Research Institute. Avicenna Research Institute was consent about the research on the archived FFPTs of women.

Patients and samples

We collected 210 FFPTs of aborted fetuses or placentas of women with recurrent abortion from the archives of the Avicenna Research Institute in Tehran, a referral center for infertile couples in Iran. Information of clinical symptoms, pathological findings, and genetic background were obtained from patients' medical records.

DNA extraction

For each FFPT, five 10 μm thick sections were cut and transferred to 1.5 ml microtubes. In order to avoid cross-contamination, we used a new, sterile disposable microtome blade for each block. Sections were deparaffinized by the addition of 1 ml xylene (Merck, Germany) for 15 minutes at 50°C. Subsequently, the tubes were centrifuged at 13000 g for 5 minutes and the supernatant was discarded. This step was repeated twice. The samples were rehydrated in a descending ethanol series (100, 90, 80, 70%) and subsequently washed with distilled water. For DNA extraction, 800 μL of lysis buffer (50 mM tris-HCl, pH=8.0, 25 mM EDTA, and 400 mM NaCl), 100 μL 10% sodium dodecyl sulfate (SDS, Merck, Germany), and 10 μL proteinase K (20 μg/μL, Thermo Fisher Scientific, Wilmington, DE, USA) were added to each tube (17). The suspension was incubated at 55ºC for 72 hours. After overnight, an additional of 10 μL proteinase K (20 μg/μL) was added to each tube (18). In order to precipitate undissolved proteins and debris, we added 300 μL of 6 M NaCl to each tube for 15 minutes at 4°C. After centrifugation (13000 g for 15 minutes), the supernatant was transferred to 1.5 ml microtubes (17). Then, 800 μl of phenol-chloroform-isoamyl alcohol (25:24:1) was added to each microtube. The microtubes were centrifuged (13000 g for 15 minutes) and we transferred the supernatants to new microtubes. Subsequently, 1 ml of chloroform was added to each microtube. The microtubes were centrifuged (13000 g for 15 minutes) and the supernatant was transferred to sterile microtubes. DNA was precipitated by the addition of one-tenth the volume of a sodium acetate solution (3 M, pH=5.2) and twice the volume of cold 100% ethanol, kept at -20°C

overnight, and subsequently centrifuged at 13000 g for 20 minutes. Finally, the pellet was washed with 70% ethanol, centrifuged (13000 g for 15 minutes), resuspended in 50 µL of distilled water, and stored at -20°C until use.In order to ensure that the DNA was extracted, we used two *T. gondii* positive tissue samples (GenBank accession numbers. KR809554 and KR809555) which had been detected in our previous study (19). These positive samples were fixed in formalin and embedded in paraffin after which the following procedure for DNA extraction was performed. We also used the Rh strain of *T. gondii* as a positive control.

Detection of *T. gondii* infection by nested polymerase chain reaction

PCR was conducted using a pair of *T. gondii*-specific primers:

GAR6-F1: 5'-ATTTGTGTTTCCGAGCAGGT-3' and
R1: 5'-GCACCTTCGCTTGTGGTT-3'.

Nested-PCR was performed with primers:

GAR6-F2: 5'-TTTCCGAGCAGGTGACCT-3' and
R2: 5'-TCGCCGAAGAGTTGACATAG-3' (20).

Amplifications were conducted ina final volume of a 20 µL reaction mixturethat contained 10 µL of 2x Taq DNA polymerase Master Mix with 2 mM $MgCl_2$ (Cat. no. A170301, Ampliqon, Denmark), 10 pmol of each primer, 5 µL of distilled water, and 3 µL of template DNA. For nested-PCR, one µL of the first round PCR product was used as the template. For each reaction, two positive controls (DNA extracted from *T. gondii* paraffin-embedded tissuesand the RH strain of *T. gondii*) and a negative control (double distilled water) were included. Amplification was performed with initial denaturation for 5 minutes at 95°C, followed by 35 cycles at 95°C for 30 seconds (denaturation), annealing at 59°C in the first round, and 57°C in nested PCR for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 10 minutes. A total of 5 µl of nested-PCR products along with a 100-bp DNA ladder were electrophoresed in 1.5% safe stain (Sinaclon, Iran) agarose gels and visualized under ultra-violet trans-illumination.

Genotyping of positive samples by restriction fragment length polymorphism

Positive samples were genotyped using *GRA6* and *SAG3* markers (21). First, we digested the nested-PCR products of *GRA6* positive samples using Tru1I (MseI) restriction enzyme (Cat. No. ER0982, Thermo Fisher Scientific, USA) as previously described (20). Digestion was conducted in a final volume of 16 µL reaction mixtures that contained 5 µL of the nested-PCR products, 1µL of Tru1I enzyme, 1 µL of 10X Buffer R, and 9 µL of nuclease-free water. Then, the reaction mixtures were incubated at 65°C for 1 hour according to the manufacturer's instructions. A total of 10 µl of restriction fragments were electrophoresed by Tris-acetate-EDTA (TAE) buffer through 3% (w/v) agarose gel stained with safe stain and visualized under UV transillumination. We conducted genotyping of the positive samples by the *SAG3* marker (21, 22). Nested-PCR was carried out for positive samples using the *SAG3* marker as previously described (22). Next, the products were digested using BcnI (NciI) restriction enzyme (Cat. No. ER0061, Thermo Fisher Scientific, USA) at 37°C for 6 hours according to the manufacturer's protocols. The restriction fragments were electrophoresed and visualized under UV transillumination. The type of *T. gondii* was determined according to the restriction patterns after digestion with restriction enzymes (21). In order to determine better illustrationpatterns of the genotypes, the *GRA6* and *SAG3* sequences of three types of *T. gondii* (RH type I, ME49 type II, and NED type III) were obtained fromGenBank and digested by their restriction enzymes using NEBcutter V2.0 (http://nc2.neb.com/NEBcutter2/).

Nucleotide sequence analysis of the *GRA6* gene

We extracted two positive nested-PCR products of the *GRA6* gene from the gel (Vivantis Gel Purification kit, Selangor DarulEhsan, Malaysia) according to the manufacturer's instructions. The products were sequenced in the forward and reverse directions by the Sequetech Corporation (Mountain View, CA, USA), edited with BioEdit software, (23) and compared with GRA6 partial sequences of *T. gondii* available in GenBank.

Results

Detection of *T. gondii* in women with recurrent spontaneous abortion

T. gondii DNA was detected in 8 out of 210 samples (3.8%) by the *GRA6* marker (Fig.1).

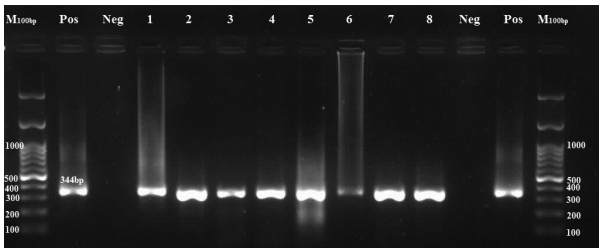

Fig.1: PCR products of the *GRA6* positive samples. *Toxoplasma gondii (T. gondii)* positive samples give a 344-bp band.
M; 100 bp DNA marker, Pos; Positive control, Neg; Negative control, and Lanes 1-8; Positive samples.

As shown in Table 1, patients had a mean age of 33.5 years (range: 28-39 years). There were seven patients with a history of previous abortion (patients 1-3, 5-8). From these, six occurred in the first trimester and one occurred in the second trimester (patient 1). One patient had a healthy girl (patient 4) with no history of previous abortion. The abortion of this patient (patient 4) was occured in the first trimester of pregnancy. Patient 1had clinical symptoms of fever and severe necrotizing chorioamnionitis before the abortion. Patient 3 reported clinical symptoms such as rapid heart beat, maternal anemia, and edema of the legs and ankles before the abortion. The edema resolved after the abortion. Patient 2 had a history of hypothyroidism. Nonspecific symptoms were reported from other patients before the abortions (Table 1).

Table 1: Information of the *Toxoplasma gondii (T. gondii)* infected women with recurrent spontaneous abortion (RSA)

Patient No.	Age (Y) City Province	Number of gestations (G), Number of abortions (AB)	Week of abortion	Chromosomal aneuploidies[§]	Pathological findings in fetoplacental tissues	Symptoms
1	36 Abhar Zanjan	G6, AB6 All pregnancies aborted at second trimester	LMP[†]: 16w (Second trimester)	Not detected	Inflammatory cell infiltration with patchy amnionic necrotizing foci in the membrane	Fever, severe abdominal and back pain, premature rupture of membranes (PPROM)
2	31 Tuyserkan Hamedan	G4, AB4 All pregnancies aborted at first trimester	LMP: 11w+2d Ultrasound: 8w (First trimester)	Not detected	No remarkable pathological findings	Hypothyroidism
3	39 Eslamshahr Tehran	G8, AB8 All pregnancies aborted at first trimester	LMP: 7w (First trimester)	Not detected	No remarkable pathological findings	No specific symptoms
4	38 Tehran Tehran	G2, AB1 She has one healthy girl	LMP: 11w (First trimester)	Not detected	No remarkable pathological findings	Rapid heartbeat, anemia and edema of the legs and ankles
5	36 Karaj Alborz	G3, AB3 All pregnancies aborted at first trimester.	Ultrasound: 6w+4d (First trimester)	MLPA findings compatible with an extra copy of chromosome 15 (trisomy 15)	The membrane showed calcification without inflammation	No specific symptoms
6	28 Kashan Isfahan	G5, AB5	LMP: 13w Ultrasound: 11w (First trimester)	Not detected	No remarkable pathological findings	No specific symptoms
7	31 Tehran Tehran	G3, AB3 All pregnancies aborted at first trimester	LMP: 11w Ultrasound: 8w+3d (First trimester)	Not detected	No remarkable pathological findings	No specific symptoms
8	29 Tehran Tehran	G2, AB2 All pregnancies aborted at first trimester	LMP: 11w Ultrasound: 8w (First trimester)	Not detected	No remarkable pathological findings	No specific symptoms

[†]; LMP: Last menstrual period and [§]; Chromosomal aneuploidies were detected using multiplex ligation-dependent probe amplification (MLPA).

Genotyping of positive samples

GRA6 completely characterized eight samples as *T. gondii* genotype III (Fig.2). Genotyping of positive samples were conducted by using the *SAG3* marker. The results showed amplification of *SAG3* in six out of eight *GRA6* positive samples. Digestion of *SAG3* PCR products by BcnI enzyme determined that all six products belonged to *T. gondii* genotype III (Fig.3).

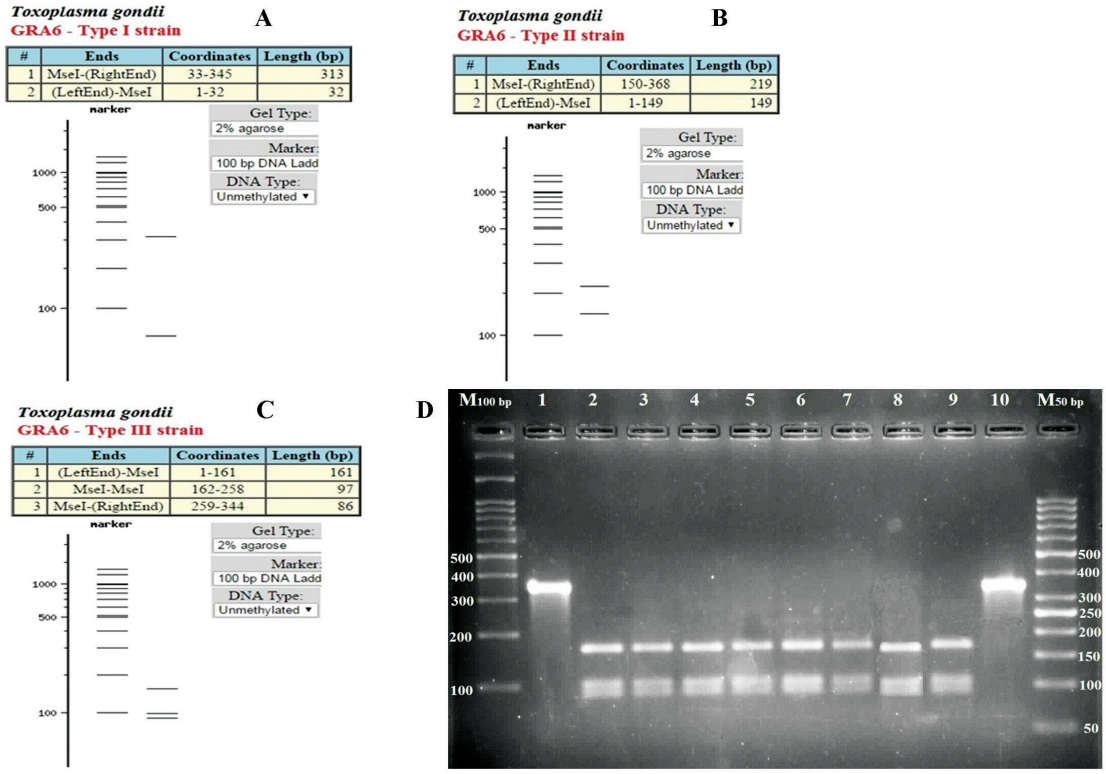

Fig.2: Genotyping of positive samples with the *GRA6* marker. The products were digested with Tru1I enzyme. **A, B, C.** Patterns of three types of *Toxoplasma gondii (T. gondii)* genotype, and **D.** Agarose gel electrophoresis of PCR products digested with Tru1I enzyme.
M; 100 and 50 bp DNA marker, Lanes 1 and 10; Undigested positive samples, and Lanes 2-9; *T. gondii* genotype type III.

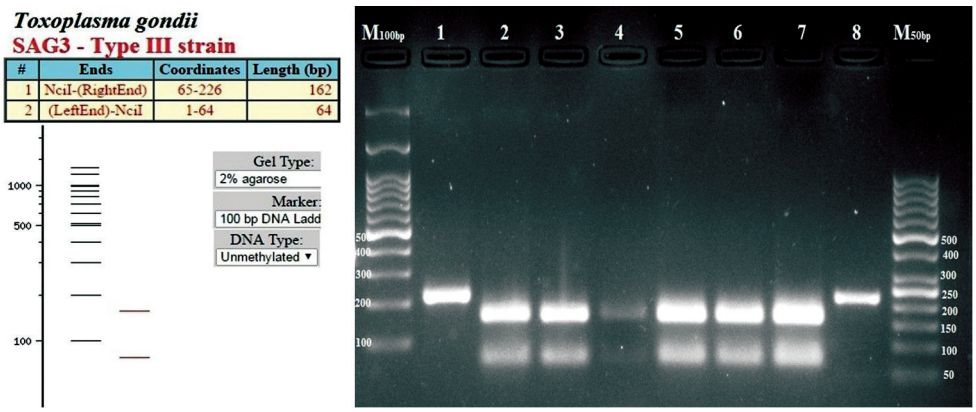

Fig.3: Genotyping of positive samples with the *SAG3* marker. The products were digested with BcnI enzyme. **A.** Patterns of *Toxoplasma gondii (T. gondii)* type III genotype and **B.** Agarose gel electrophoresis of PCR products digested with BcnI enzyme.
M; 100 and 50 bp DNA marker, Lanes 1 and 8; Uundigested positive samples, and Lanes 2-7; *T. gondii* genotype type III.

Sequencing of the *GRA6* gene

We submitted two *GRA6* nucleotide sequences with a length of 344 bp to the GenBank database (KT735111, KT735112). The alignment of our sequences revealed the highest similarity (100%) with *T. gondii* isolated from cat (KP792620, KP792621), sheep (KT735113-19), rat (KP792610, KP792613), and bird (KR809554-8, KP792606-9, KP792600-2) hosts in Iran (Table 2).

Table 2: Multiple sequence alignment of the *GRA6* gene of *Toxoplasma gondii (T. gondii)* from our samples and other hosts in Iran. Our samples are shown in red as accession numbers KT735111 and KT735112

```
KT809309  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KP792604  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTTGTGGTGC
KP792605  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTTGTGGTGC
KR809555  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTTC
KT735112  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAGAACAGCTTCGTGGTGC
KP792614  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KR809556  TTTCCGAGCAGGTGACCTGGGTCGCCTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KR809558  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGTAACAGCAGGAAAACAGCTTCGTGGTGC
KT735111  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735113  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735114  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735115  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735116  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735117  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735119  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KR809557  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KR809554  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KP792621  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KP792620  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KP792609  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KP792610  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
KT735118  TTTCCGAGCAGGTGACCTGGGTCGCTTTTTTGAAACAGCAGGAAAACAGCTTCGTGGTGC
          ************************.******.**********.********.******.*

KT809309  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792604  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792605  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KR809555  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735112  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792614  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KR809556  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KR809558  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735111  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735113  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735114  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735115  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735116  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735117  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735119  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KR809557  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KR809554  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792621  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792620  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792609  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KP792610  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
KT735118  CACGTAGCGTGCTTGTTGGCGACTACCTTTTTTTCTTGGGAGTGTCGGCGAAATGGCACA
          ************************************************************

KT809309  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTCCTGTCCTTTAACTGTCTCCACAGTTGC
KP792604  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KP792605  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KR809555  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735112  CGGTGGCACCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KP792614  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KR809556  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735112  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
```

Table 2: Continued

```
KP792614  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KR809556  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KR809558  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735111  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735113  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735114  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735115  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735116  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735117  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735119  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KR809557  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KR809554  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KP792621  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KP792620  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KP792609  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KP792610  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTTGC
KT735118  CGGTGGCATCCATCTGAGGCAGAAGCGTAACTTCTGTCCTTTAACTGTCTCCACAGTCGC
          ********.**********************.**********************.**

KT809309  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCCGTCGC
KP792604  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KP792605  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KR809555  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735112  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KP792614  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KR809556  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KR809558  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735111  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735113  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735114  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735115  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735116  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735117  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735119  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KR809557  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KR809554  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KP792621  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KP792620  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KP792609  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KP792610  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
KT735118  TGTGGTCTTTGTAGTTTTCATGGGTGTACTCGTCAATTCGTTGGGTGGAGTCGCTGTCGC
          ************************************************.*****

KT809309  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGGGGACAGCA
KP792604  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KP792605  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KR809555  AGCAGACAGCGGTGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735112  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KP792614  AGCAGACAGCGATGGTGTTAAGCAGGCCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KR809556  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KR809558  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735111  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735113  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735114  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735115  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735116  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735117  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735119  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KR809557  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KR809554  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KP792621  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KP792620  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KP792609  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KP792610  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
KT735118  AGCAGACAGCGATGGTGTTAAGCAGACCCCTTCGGAAACCGGTTCGAGCGGTGGACAGCA
          ***********.**********.**************************.*********
```

Table 2: Continued

KT809309	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KP792604	AGAAGCAGTGGGGACCCCTGAAGACTATGTCAACTCTTCGGCGA
KP792605	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KR809555	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735112	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KP792614	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KR809556	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KR809558	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735111	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735113	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735114	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735115	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735116	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735117	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735119	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KR809557	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KR809554	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KP792621	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KP792620	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KP792609	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KP792610	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
KT735118	AGAAGCAGTGGGGACCACTGAAGACTATGTCAACTCTTCGGCGA
	* *

*; Exact match between all sequences and •; Mismatch with at least one sequence.

Discussion

In the current study, we detected *T. gondii* DNA in 3.8% of women with RSA. To our knowledge, this study was the first report of molecular diagnosis of *T. gondii* in women with RSA. In previous studies in Iran, Ghasemi et al. (8, 24) detected *T. gondii* DNA in 7.3% (6/82) of women with spontaneous abortion and in 3.6% (1/28) of women with stillbirth in Tehran. Asgari et al. (25) detected *T. gondii* DNA in 14.4% (78/542) of paraffin-embedded tissue samples from women with spontaneous abortion in Shiraz, Southern Iran. Hoveyda et al. (26) detected *T. gondii* DNA in 15.48% (10/65) of paraffin-embedded tissue samples from Iranian women with abortions by PCR. Genotyping of positive samples by PCR-restriction fragment length polymorphism (RFLP) has indicated that all eight positive samples belonged to genotype III of *T. gondii*. is classified into three main genotypes (type I, II, and III) with some differences in virulence and epidemiological patterns (27, 28). Genotype III is the most prevalenttype of *T. gondii* worldwide (27, 29). However, type I has the highest virulence of among *T. gondii* genotypes (28). In Iran, genotype III is the most prevalent type of *T. gondii* (30), however genotype II (30, 31) and in some studies genotype I has been reported in different hosts (32, 33).

Association of *T. gondii* seropositivity with infertility or bad obstetric outcomes has been reported in different studies. In this regard, El-Tantawy et al. (34) observed significantly higher seroprevalcenc of *T. gondii* in infertile women. In that study, 61.85% (193/312) of infertile and 44% (44/100) of fertile women had *T. gondii* IgG seropositivity in Egypt. Malik et al. (35) demonstrated a significantly higher seroprevalence of *T. gondii* in 417 women with unfavorable obstetric history such as intrauterine deaths, intrauterine growth retardation, and preterm deliveries in India. According to the results, *T. gondii* IgM antibody was detected in 28% (120/417) of women with negative obstetric history, which 57% (68/120) had a history of previous abortion. Interestingly, *T. gondii* IgM antibody was found in 76.5% of women with two or more abortions and 23.5% of women with a single abortion. Toxoplasmosis was diagnosed in 6 out of 9 (66.7%) patients with secondary infertility and 3 (33.3%) with primary infertility (35). Aral et al. (36) did not find a significant association between *T. gondii* seropositivity and infertility in women in Turkey.

In recent years, several studies were conducted about the influences of latent (asymptomatic) toxoplasmosis on mothers and their offspring (3, 4, 37). In this regard, Kaňková and Flegr (38) reported that pregnant women with latent toxoplasmosis (IgG seropositive women) had developmentally younger fetuses (based on ultrasound scan) com-

paredto *T. gondii* negative women at week 16 of pregnancy. Kaňková et al. (39) also demonstrated that infants of mothers with latent toxoplasmosis had significantly slower postnatal motor development than mothers without latent toxoplasmosis during the first year of life. Another study by the same group revealed that *T. gondii*-infected pregnant women had used significantly more assisted reproductive technology to conceive compared to *T. gondii*-negative women. *T. gondii*-infected women had a longer time to conceive and more fertility problems than *T. gondii*-negative women (40).

This study was the first molecular detection of *T. gondii* in fetoplacental tissues of women with RSA, however it had some limitations. We did not access the previous abortion samples of the patients. In addition, we were unable to follow the patients and their future pregnancies. Hence, our study only suggested that toxoplasmosis might play a role in the pathogenesis of RSA. Additional investigations with larger groups of patients should be conducted in order to elucidate a clear relationship between *T. gondii* infection and RSA.

Conclusion

The results of this study have indicated that genotype III is the predominant type of *T. gondii* in women with RSA in Tehran, Iran. Our results also indicated that *T. gondii* infection might play a role in the pathogenesis of RSA. However, more research should be conducted in this regard to elucidate a clear relationship between *T. gondii* infection and RSA.

Acknowledgements

This study is a part of a Ph.D. thesis of the first author that financially supported by Tarbiat Modares University. The authors would like to sincerely thank Miss Azadeh Soltani (Avicenna Research Institute, Iran) for her help in preparation of tissue sections and technical assistance. The authors also appreciate all members of the Pathology and Molecular Genetics laboratories at Avicenna Research Institute for their sincere assistance throughout this study. The authors report no conflict of interest.

References

1. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004; 363(9425): 1965-1976.
2. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012; 25(2): 264-296.
3. Abdoli A, Dalimi A, Arbabi M, Ghaffarifar F. Neuropsychiatric manifestations of latent toxoplasmosis on mothers and their offspring. J Matern Fetal Neonatal Med. 2014; 27(13): 1368-1374.
4. Dalimi A, Abdoli A. Latent toxoplasmosis and human. Iran J Parasitol. 2012; 7(1): 1-17.
5. Paquet C, Yudin M. Toxoplasmosis in pregnancy: prevention, screening, and treatment. J Obstet Gynaecol Can. 2013; 35(1): 78-79.
6. Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ. 2013; 91(7): 501-508.
7. Li XL, Wei HX, Zhang H, Peng HJ, Lindsay DS. A meta analysis on risks of adverse pregnancy outcomes in Toxoplasma gondii infection. PLoS One. 2014; 9(5): e97775.
8. Ghasemi FS, Rasti S, Piroozmand A, Bandehpour M, Kazemi B, Mousavi SG, et al. Toxoplasmosis-associated abortion and stillbirth in Tehran, Iran. J Matern Fetal Neonatal Med. 2016; 29(2): 248-251.
9. Pandey MK, Rani R, Agrawal S. An update in recurrent spontaneous abortion. Arch Gynecol Obstet. 2005; 272(2): 95-108.
10. Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol. 2009; 2(2): 76-83.
11. Nigro G, Mazzocco M, Mattia E, Di Renzo GC, Carta G, Anceschi MM. Role of the infections in recurrent spontaneous abortion. J Matern Fetal Neonatal Med. 2011; 24(8): 983-989.
12. Aali bibi S, Fasihi Harandi M, Nazari E, Salari Z. Comparison of Toxoplasma gondii IgG and IgM seropositivity between women with spontaneous abortions and ongoing pregnancies. Iranian Journal of Obstetrics, Gyneocology and Infertility. 2011; 14(1): 1-6.
13. Alsamarai AM, Hassan HMM, Alsalihi FG, Alobaidi AH, Aljumaili ZKM. Toxoplasma gondii, Rubella and Cytomegalovirus co-infections as risk factors for abnormal pregnancy outcomes. Middle East Journal of Family Medicine. 2014; 12(3): 16-23.
14. Aljumaili ZKM, Alsamarai AM, Najem WS. Seroprevalence of Herpes Simplex Virus Type 2 (HSV 2) in women with bad obstetric history. American Journal of Dermatology and Venereology. 2013; 2(3): 31-38.
15. Ramana B, Reddy BK, Murty D, Vasudevanaidu K. Seroprevalance of rubella in women with bad obstetric history. J Family Med Prim Care. 2013; 2(1): 44-46.
16. Turbadkar D, Mathur M, Rele M. Seroprevalence of torch infection in bad obstetric history. Indian J Med Microbiol. 2003; 21(2): 108-110.
17. Biase FH, Franco MM, Goulart LR, Antunes RC. Protocol for extraction of genomic DNA from swine solid tissues. Genet Mol Biol. 2002; 25(3): 313-315.
18. Bonin S, Groenen PJ, Halbwedl I, Popper HH. DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissues. In: Stanta G, editor. Guidelines for molecular analysis in archive tissues. New York: Springer; 2011; 33-36.
19. Abdoli A, Dalimi A, Soltanghoraee H, Ghaffarifar F. Molecular detection of Toxoplasma gondii in house sparrow (Passer domesticus) by LAMP and PCR methods in Tehran, Iran. J Parasitic Dis. 2015 (A head of print).
20. Khan A, Su C, German M, Storch G, Clifford D, Sibley LD. Genotyping of Toxoplasma gondii strains from immunocompromised patients reveals high prevalence of type I strains. J Clin Microbiol. 2005; 43(12): 5881-5887.
21. Su C, Shwab EK, Zhou P, Zhu XQ, Dubey JP. Moving towards an integrated approach to molecular detection and

identification of Toxoplasma gondii. Parasitology. 2010; 137(01): 1-11.
22. Grigg ME, Ganatra J, Boothroyd JC, Margolis TP. Unusual abundance of atypical strains associated with human ocular toxoplasmosis. J Infect Dis. 2001; 184(5): 633-639.
23. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999; 41: 95-98.
24. Ghasemi FS, Rasti S, Bandehpour M, Kazemi B, Pirooz-mand A, Mousavi GA. Molecular diagnosis of Toxoplasma gondii in aborted women. Jundishapur J Microbiol. 2015; 8(1): e15925.
25. Asgari Q, Fekri M, Monabati A, Kalantary M, Mohammadpour I, Motazedian MH, et al. Molecular genotyping of Toxoplasma gondii in human spontaneous aborted fetuses in Shiraz, Southern Iran. Iran J Public Health. 2013; 42(6): 620-625.
26. Hoveyda L, Shanehsazzadeh M, Behbahani M. Toxoplasmosis in Iranian abortion cases with appendectomy. J Applied Biol Sci. 2012; 6(2): 31-36.
27. Sibley LD, Khan A, Ajioka JW, Rosenthal BM. Genetic diversity of Toxoplasma gondii in animals and humans. Philos Trans R Soc Lond B Biol Sci. 2009; 364(1530): 2749-2761.
28. Xiao J, Yolken RH. Strain hypothesis of Toxoplasma gondii infection on the outcome of human diseases. Acta Physiologica. 2015; 213(4): 828-845.
29. Shwab EK, Zhu XQ, Majumdar D, Pena HF, Gennari SM, Dubey JP, et al. Geographical patterns of Toxoplasma gondii genetic diversity revealed by multilocus PCR-RFLP genotyping. Parasitology. 2014; 141(4): 453-461.
30. Zia-Ali N, Fazaeli A, Khoramizadeh M, Ajzenberg D, Dardé M, Keshavarz-Valian H. Isolation and molecular characterization of Toxoplasma gondii strains from different hosts in Iran. Parasitol Res. 2007; 101(1): 111-115.
31. Saki J, Khademvatan S, Yousefi E, Tavalla M, Abdizadeh R. Detection and genotyping of Toxoplasma gondii isolated from soil in Ahvaz, southwest of Iran. J Parasit Dis. 2016 (In press).
32. Tavalla M, Oormazdi H, Akhlaghi L, Shojaee S, Razmjou E, Hadighi R, et al. Genotyping of Toxoplasma gondii isolates from soil samples in Tehran, Iran. Iran J Parasitol. 2013; 8(2): 227-233.
33. Danehchin L, Razmi G, Naghibi A. Isolation and genotyping of toxoplasma gondii strains in ovine aborted fetuses in Khorasan Razavi Province, Iran. Korean J Parasitol. 2016; 54(1): 15-20.
34. El-Tantawy N, Taman A, Shalaby H. Toxoplasmosis and female infertility: is there a co-relation? American Journal of Epidemiology and Infectious Disease. 2014; 2(1): 29-32.
35. Malik A, Rizvi M, Khan F, Khan N, Rabbani T, Khan HM. Toxoplasma gondii in women with bad obstetric history and infertility: a five-year study. Asian Pac J Trop Dis. 2014; 4 Suppl 1: S236-S239.
36. Aral AG, Elhan HA, Akarsu C. Retrospective evaluation of Toxoplasma gondii seropositivity in fertile and infertile women. Mikrobiyol Bul. 2011; 45(1): 174-180.
37. Flegr J, Prandota J, Sovičková M, Israili ZH. Toxoplasmosis-a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PloS One. 2014; 9(3): e90203.
38. Kaňková Š, Flegr J. Longer pregnancy and slower fetal development in women with latent" asymptomatic" toxoplasmosis. BMC Infect Dis. 2007; 7: 114.
39. Kaňková S, Sulc J, Křivohlavá R, Kuběna A, Flegr J. Slower postnatal motor development in infants of mothers with latent toxoplasmosis during the first 18 months of life. Early Hum Dev. 2012; 88(11): 879-884.
40. Kaňková S, Flegr J, Calda P. The influence of latent toxoplasmosis on women's reproductive function: four cross-sectional studies. Folia Parasitol (Praha). 2015; 62. pii: 2015.041.

Original Article

Association between Nutritional Status with Spontaneous Abortion

Rahimeh Ahmadi, M.Sc., Saeideh Ziaei, M.D.*, Sosan Parsay, Ph.D.

Department of Midwifery and Reproductive Health, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

Abstract

Background: Spontaneous abortion is the most common adverse pregnancy outcome. We aimed to investigate a possible link between nutrient deficiencies and the risk of spontaneous abortion.

Materials and Methods: This case-control study included the case group (n=331) experiencing a spontaneous abortion before 14 weeks of pregnancy and the control group (n=331) who were healthy pregnant women over 14 weeks of pregnancy. The participants filled out Food Frequency Questionnaire (FFQ), in which they reported their frequency of consumption for a given serving of each food item during the past three months, on a daily, weekly or monthly basis. The reported frequency for each food item was converted to a daily intake. Then, consumption of nutrients was compared between the two groups.

Results: There are significant differences between the two groups regarding consumed servings/day of vegetables, bread and cereal, meat, poultry, fish, eggs, beans, fats, oils and dairy products (P=0.012, P<0.001, P=0.004, P<0.001, P=0.019, respectively). There are significant differences between the two groups in all micronutrient including folic acid, iron, vitamin C, vitamin B6, vitamin B12 and zinc (P<0.001).

Conclusion: Poor nutrientions may be correlated with increased risk of spontaneous abortion.

Keywords: Abortion, Nutrition, Pregnancy

Citation: **Ahmadi R, Ziaei S, Parsay S. Association between nutritional status with spontaneous abortion. Int J Fertil Steril. 2017; 10(4): 337-342**

Introduction

Spontaneous abortion is the most common adverse pregnancy outcomes occuring in approximately between 12 and 15% of clinically recognized pregnancies (1). The main reasons of early pregnancy loss are still unknown. Although a number of spontaneous abortions are caused by chromosomal abnormalities, maternal factors including nutritional status, also may contribute to this occurrence. The presence of appreciable amount of folic acid and vitamins has been reported to be essential for normal embryogenesis (1-3). There is reliable evidence indicating that maternal micronutrient status contributes to pregnancy outcome (4-9). A possible link has been suggested between nutrient deficiencies and reproductive risk factors. Interest has focused mainly on the risk of malformations. An association between intake of micronutrients, such as folic acid and zinc, magnesium and iron, and pregnancy outcome has been investigated (1-9).

In addition, reduced consumption of animal fats, carotene and proteins have been associated with the risk of hydatiform mole (10). Although the effects of maternal nutrition on fetal development and birth outcomes have been obviously demonstrated in animal studies, the findings of studies in humans inconsistent.

Due to influences of health eating habits on early phases of conception and pregnancy, we aimed to explore the association between nutrient deficiencies and the risk of spontaneous abortion.

Received: 14 Dec 2015, Accepted: 23 Jun 2016
***Corresponding Address: P.O.Box: 1415-111, Department of Midwifery and Reproductive Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran**
Email: ziaei_sa@modares.ac.ir

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 337-342

Materials and Methods

This case-control study was carried out in Tehran-Iran. The case group (n=331) experienced a spontaneous abortion before 14 weeks of pregnancy, while the control group (n=331) were healthy pregnant women over 14 weeks of pregnancy. Cluster sampling was used for selection of participations. In this method, ten regions were picked out randomly from 22 regions of Tehran, one public hospital was selected randomly from each picked region, and 331 cases was recruited randomly from these ten hospitals. Each case was assigned one control from these ten hospitals matched on maternal age, duration from last delivery in multiparous women, body mass index (BMI), occupation, and educational status.

Inclusion criteria were as follow: i. Singleton pregnancy, ii. 18-35 years of age, iii. No history of chronic diseases, such as diabetes, hypertension, cardiovascular diseases and thyroid dysfunction, iv. No history of any congenital or karyotypic abnormalities in the woman, her husband or one of the immediate relatives, v. Pregnancy without use of assisted reproductive technology (ART), vi. No vaginal bleeding in the first trimester of pregnancy in the control group, vii. Lack of fetal malformations in the current pregnancy, viii. No smoking during pregnancy.

The study was approved by the Ethical Committee of Tarbiat Modares University and a written informed consent was obtained from the participants.

At enrollment, the interviewers administered a questionnaire to the women to collect baseline information on socio demographic, and obstetrics history. Then, case group filled out dietary information after abortion in the hospital while the control group completed the same questionnaire.

Dietary assessment

The assessment of dietary intake was performed using Food Frequency Questionnaire (FFQ) which was previously validated on the adult population of the city of Tehran, Iran (11). This questionnaire assesses 168 food items consumed in the preceding three months. This version of FFQ consists of a list of foods with a standard serving size commonly consumed by Iranian. For validity and reliability of FFQ, Mirmiran et al. (11) compared the dietary data collected monthly by means of twelve 24 hour dietary/recalls (24hDR) and biochemical markers with data collected from FFQ. The exact agreement rate was reasonable.

The participants were taught the food portion sizes using food photographs and measuring containers and given instructions for recording their dietary intake by a registered dietitian. The following food items were evaluated: fruits, vegetables, breads and cereals, dairy products, meat, poultry, fish, eggs, dry beans, and fats, oils, sweet. Due to presence of fats in above mentioned groups, dietary intake was calculated based on following information: 8 g fat in each ounce of sheep; 3 g fat in each ounces of veal meat; 1 g fat in each portion of bread and cereal; 5 g fat in each portion of low fat milk; 8 g fat in each portion of high fat milk .These amounts of fats were then added to the fat and oil group.

Participants were asked to report their frequency of consumption of a given serving size of each food item during the past three months, on a daily, weekly or monthly basis. The reported frequency for each food item was converted to a daily intake. Portion sizes of consumed food were then converted to gram using household measures. Also, the number of the women who consumed different numbers of food serving portion was compared between the two groups.

For assessment of micronutrients intake Mosbys Nutria Trace Nutrition Analysis Software was used.

Statistical analysis

The sample size was estimated based on a pilot study, considering a power of 80% and alpha of 5%.

Overall comparisons between two groups were performed using t test or χ^2 test. We computed the odds ratio (OR), as estimators of risks of spontaneous abortion, for the various parameters, with an approximate 95% confidence interval (CI). All statistical tests were two sided tests and were performed using a 5% significance level. The Statistical Package for the Social Sciences (SPSS, SPSS Inc, USA) version 20 was used for all statistical analysis.

Results

Table 1 shows the characteristics of the cases and controls. There are no significant differences between the two groups regarding maternal age, duration from last delivery in multiparous women, BMI, occupation, and educational status.

There are significant differences between the two groups regarding consumed servings/day of vegetables, bread and cereal, meat, poultry, fish, eggs, dry beans, nuts, fats, oils, sweet and dairy products (P=0.012, P<0.001, P=0.004, P<0.001, P0.019, respectively) (Table 2).

Table 1: Comparison of demographics and obstetric characteristics between women with and without clinical spontaneous abortion

Variables	Case group (n=331)	Control group (n=331)	P value
Maternal age (Y)*	27.79 ± 5.30	27.31 ± 4.37	N.S
Duration from last delivery (months)*	41.78 ± 50.31	49.76 ± 45.97	N.S
BMI*	24.95 ± 6.72	24.25 ± 4.63	N.S
Occupation**			N.S
Housewife	293 (88.5)	308 (93.1)	
Employed	38 (11.5)	23 (6.9)	
Educational status**			N.S
Lower than university	267 (80.7)	275 (83.1)	
University	64 (19.3)	56 (16.9)	

*; Values are given as mean ± SD using Student's t test, **; Values are given as number (%) using Chi-squared test (χ^2), N.S; Not significant, and BMI; Body mass index.

Table 2: Comparison of daily intake of food items between women with and without clinical spontaneous abortion. Values are given as number (%) $\varkappa^2$ test

Food items, portion per day	Case group (n=331)	Control group (n=331)	P value
Vegetables			0.012
(<3 parts)	282 (85.2)	253 (76.4)	
(3-5 parts)	49 (14.8)	77 (23.3)	
(>5 parts)	0 (0)	1 (0.3)	
Fruits			0.055
(<2 parts)	152 (45.9)	122 (36.9)	
(2-4 parts)	169 (51.1)	195 (58.9)	
(>4 parts)	10 (3)	14 (4.2)	
Breads and cereals			<0.001
(<6 parts)	129 (39)	88 (26.6)	
(6-11 parts)	199 (60.1)	208 (62.8)	
(>11 parts)	3 (0.9)	35 (10.6)	
Meat and beans			0.004
(<2parts)	268 (81.0)	232 (70.1)	
(2-3 parts)	63 (19.0)	98 (29.6)	
(>3 parts)	0 (0)	1 (0.3)	
Dairy products			<0.001
(<2 parts)	173 (52.3)	120 (36.3)	
(2-3 parts)	151 (45.6)	190 (57.4)	
(>3 parts)	7 (2.1)	21 (6.3)	

Table 2: Continued

Food items, portion per day	Case group (n=331)	Control group (n=331)	P value
Fats and oils			0.019
(<55 g)	77 (23.3)	50 (15.1)	
(55-66 g)	113 (34.1)	114 (34.4)	
(>66 g)	141 (42.6)	167 (50.5)	

In addition, there are significant differences between the two groups in terms of all micronutrient factors including folic acid, iron, vitamin C, vitamin B6, vitamin B12 and zinc (Table 3).

Table 3: Comparison of daily intake of micronutrients between women with and without clinical spontaneous abortion*

Variables	Case group (n=331)	Control group (n=331)
Folic acid (µg)	416.09 ± 94.36	526.49 ± 73.41
Fe (mg)	19.29 ± 4.83	22.16 ± 4.03
Vitamin C (mg)	75.63 ± 7.62	78.06 ± 5.91
Vitamin B6 (mg)	1.12 ± 0.46	1.55 ± 0.33
Vitamin B12 (µg)	2.10 ± 0.39	2.32 ± 0.25
Zn (mg)	8.06 ± 1.50	9.93 ± 0.87

Values are given as mean ± SD by the independent t student test. *; P<0.001.

Table 4 depicts the OR and the 95% CI of logistic regression models for spontaneous abortion and consumption of micronutrients. For the women consuming the micronutrients OR of spontaneous abortion was significant in acid folic (0.986, 0.984-0.988), iron (0.868, 0.837-0.900), vitamin C (0.949, 0.928-0.971), vitamin B6 (0.096, 0.064-0.144), vitamin B12 (0.129, 0.076-0.218), and zinc (0.288, 0.237-0.349).

Table 4: Logistic regression models of the association between micronutrients and spontaneous abortion*

Variables	OR (95% CI)
Folic acid	0.986 (0.984-0.988)
Fe	0.868 (0.837-0.900)
Vitamin C	0.949 (0.928-0.971)
Vitamin B6	0.096 (0.064-0.144)
Vitamin B12	0.129 (0.076-0.218)
Zn	0.288 (0.237-0.349)

OR; Odds ratio, CI; Confidence interval, and *; P<0.001.

Discussion

Our findings indicated that there are significant differences between the two groups in number of the women who consumed servings/day of vegetables, breads and cereals, meat, poultry, fish, eggs, dry beans, nuts, fats, oils, sweet and dairy products.

The incidence of pregnancy wastage is high in women from poor socio-economic groups. Maternal malnutrition is considered to be an important factor contributing to spontaneous abortions by way of altering the germ cell morphology; however, the relation between maternal nutrition and spontaneous abortion is complex and influenced by several biologic, socioeconomic, and lifestyle factors, which vary extremely in different populations (12, 13).

During pregnancy, there is an increased nutritional demand for both mother and fetus. Maternal under nutrition probably increases the risk of intrauterine death and abortion (14-17), possibly due to cellular dysfunction. Maternal nutritional deficiencies also cause serious damages on different stages of fetal development. A number of experimental animal studies and observational human studies have mentioned the consequences of malnutrition at the very earliest embryonic stages that affected fetal growth and birth outcomes (13, 18). Furthermore, evidence from animal studies have indicated that fetal growth is mostly affected by maternal under nutrition during the peri-implantation stage and the stage of rapid placental development (19, 20).

The maternal nutrition is more likely to be affected by socioeconomic and lifestyle factors in various ways. Socioeconomic status influences pregnancy dietary intake that results in multiple rather than single nutrient deficiencies. It has been also reported that cultural factors play a major role in maternal age at initiation of childbearing and interval between delivery (13, 16, 18, 21).

Karmer et al. (22) has stated that the countries

with lowest rates of adverse birth outcomes had done so not through health care interventions but rather by reducing the prevalence of socioeconomic disadvantage and poverty.

According to the results of this study, the average of all micronutrients which was consumed by the case group was significantly lower than in the control group. The observed association in our study is in agreement with the findings of several previous studies. Optimal pregnancy outcomes are dependent upon the intake of adequate nutrients, and malnutrition results from inadequate diet, is synonymous with growth failure, especially during the rapid growth phases of fetus. It was recognized that poor pregnancy outcomes results not only from a deficiency of protein and macronutrient but also from inadequate intake of micronutrients that are vital during pregnancy (15-17).

Folate deficiency during pregnancy is also associated with numerous adverse pregnancy outcomes, including spontaneous abortions. A number of studies have indicated that suboptimal vitamin B6 status and elevated plasma total homocysteine concentrations a marker of poor folate or vitamin B12 status, may increase the risk of spontaneous abortion (1, 2, 6, 23, 24), although the association is still unknown. Elevated plasma total homocysteine concentrations are caused by genetic abnormalities or suboptimal folate, vitamin B12, and/or vitamin B6 status.

We also found lower consumption of vitamin C in the women with spontaneous abortion. In a report based on the women with recurrent abortion by Vural et al. (25), they have found that antioxidant elements including vitamin C decreased in case group in comparison with healthy pregnant women.

In the present study, women with spontaneous abortion had lower consumption of iron and zinc as compared to the control women. This is consistent with other studies, in which they have found an association between poor zinc status and adverse pregnancy outcomes (22, 26-29)

The present study had several limitations. A major limitation of this study was the self-report of dietary data and the lack of precise estimation of micronutrients and macronutrients consumption. Another limitation of our study was that neither embryos nor parents were karyotyped. Aneuploidy is a common cause of spontaneous abortion and that was not excluded in our study. The third limitation was that any hospital-based case-control study on abortion includes only women with spontaneous abortion requiring hospital admission, with the consequent exclusion of women with subclinical abortions or very early pregnancy losses.

A strength of the current study was dietary data were collected approximately at same gestational ages in cases and controls groups; meaning the case group was interviewed in hospital and the control group in prenatal clinics. Further, the differences observed could not be explained by some confounding factors because these parameters were matched as far as possible between the two groups.

Conclusion

Despite potential limitations and difficulties in the interpretation, our findings illustrated that a diet poor in several nutrients may increase risk of spontaneous abortion.

Acknowledgements

This study was carried out with the kind cooperation of the participants. This study was funded by Tarbiat Modares University, Tehran, Iran. There is no conflict of interest in this article.

References

1. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Hemocystein and folate levels as risk factors for recurrent pregnancy loss. Obstet Gynecol. 2000; 95(4): 519-524
2. Ronnenberg AG, Goldman MB, Chen D, Aitken IW, Willett WC, Selhab J, et al. Preconception folate and vitamin B status and clinical spontaneous abortion in Chinese women. Obstet Gynecol. 2002; 100(1): 107-113.
3. Zetterberg H, Regland B, Palmer M, Rymo L, Zafiropoulos A, Arvanitis DA. The transcobalamin codon 259 polymorphism influences the risk of human spontaneous abortion. Hum Reprod. 2002; 17(12): 3033-3036.
4. Ziaei S, Janghorban R, Shariatdoust S, Faghihzadeh S. The effects of iron supplementation on serum copper and zinc levels in pregnant women with high-normal hemoglobin. Int J Gynecol Obstet. 2008; 100(2): 133-135.
5. Fall CH, Yajnik CS, Rao S, Davies AA, Brown N, Farrant HJ. Micronutrients and fetal growth. J Nutr. 2003; 133(5 Suppl 2): 1747S-1756S.
6. de la Calle M, Usandizaga R, Sancha M, Magdaleno F, Herranz A, Cabrillo E. Homocysteine, folic acid and B-group vitamins in obstetrics and gynecology. Eur J Obstet Gynecol Reprod Biol. 2003; 107(2): 125-134.
7. Black RE. Micronutrients in pregnancy. Br J Nutr. 2001; 85 Suppl 2: S193-197.

8. Rao S, Yajnik CS, Kanade A, Fall CH, Margetts BM, Jackson AA. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their bodies at birth: pune maternal nutrition study. J Nutr. 2001; 131(4): 1217-1224.
9. Keen CL, Clegg MS, Hanna LA, Lanoue L, Rogers JM, Daston GP, et al. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr. 2003; 133(5 Suppl 2): 1597S-1605S.
10. Di Cintio E, Parazzini F, Chatenoud L, Surace M, Benzi G, Zanconato G, et al. Dietary factors and risk of spontaneous abortion. Eur J of Obstet Gynecol Reprod Biol. 2001; 95(1): 132-136.
11. Mirmiran P, Esfahani FH, Mehrabi Y, Hedayati M, Azizi F. Reliability and relative validity of an FFQ for nutrient in the Tehran lipid and glucose study. Public Health Nutr. 2010; 13(5): 654-662.
12. Ashworth CJ, Antipatis C. Micronutrient programming of development throughout gestation. Reproduction. 2001; 122(4): 527-535.
13. Villar J, Merialdi M, Gülmezoglu AM, Abalos E, Carroli G, Kulier R, et al. Nutritional interventions during pregnancy for the prevention or treatment of maternal morbidity and preterm delivery:an overview of randomized controlled trials. J Nutr. 2003; 133(5 Suppl 2): 1606S-1625S.
14. Abu-Saad K, Fraser D. Maternal nutrition and birth outcome. Epidemiol Rev. 2010; 32: 5-25.
15. Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. J Nutr. 2004; 134(9): 2169-2172.
16. Maternal nutrition: new developments and implications. Proceedings of a symposium. Paris, France, June 11-12, 1998. Am J Clin Nutr. 2000; 71(5 Suppl): 1217S-1379S.
17. Ramachandran P. Maternal nutrition-effect on fetal growth and outcome of pregnancy. Nutr Rev. 2002; 60(5 Pt 2): S26-S34.
18. Scroll TO. Iron status during pregnancy:setting the stage for mother and infant. Am J Clin Nutr. 2005; 81 (5): 1218S-1222S.
19. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KM. Epigenetic control of fetal gene expression. BJOG. 2008; 115(2): 158-168.
20. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr. 2007; 27: 363-388.
21. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr. 2000; 71(5): 1280S-1284S.
22. Kramer MS, Seguin L, Lydon J, Goulet L. Socio-economic disparities in pregnancy outcome:why do the poor fare so poorly? Paediatr Perinat Epidemiol. 2000; 14(3): 194-192.
23. Coumans AB, Huijgens PC, Jacobs C, Schats R, de Vries JI, van Pampus MG, et al. Haemostatic and metabolic abnormalities in women with unexplained recurrent abortion. Hum Reprod. 1999; 14(1): 211-214.
24. Ronnenberg AG, Venners SA, Xu X, Chen C, Wang L, Guang W, et al. Preconception B-vitamin and homocysteine status,conception and early pregnancy loss. Am J Epidemiol. 2007; 166(3): 304-312.
25. Vural P, Akgül C, Yildirim A, Canbaz M. Antioxidant defense in recurrent abortion. Clin Chim Acta. 2000; 295(1-2): 169-177.
26. Abdul-Barry J, Al-Rubai SA, Qasim QA. Study of oxidant-antioxidant status in recurrent spontaneous abortion. Thi-Qar Medical Journal. 2011; 5(1): 35-46.
27. Picco SJ, Anchordoquy JM, de Matos DG, Anchordoquy JP, Seoane A, Mattioli GA, et al. Effect of increasing zinc sulphate concentration during in vitro maturation of bovin oocytes. Theriogenology. 2010; 74(7): 1141-1148.
28. Graham TW, Thurmond MC, Gershwin ME, Picanso JP, Garvey JS, Keen CL. Serum zinc and copper concentrations in relation to spontaneous abortion in cows:implications for human fetal loss. J Reprod Fertil. 1994; 102(1): 253-262.
29. Zare A, Saremi A, Hajhashemi M, Kardar GA, Moazzeni SM, Pourpak Z, et al. Correlation between serum zinc levels and successful immunotherapy in recurrent spontaneous abortions. J Hum Reprod Sci. 2013; 6(2): 147-151.

Original Article

Lack of Association between Cu T-380A Intrauterine Device and Secondary Infertility in Iran

Mahnaz Abdinasab, B.Sc.[1], Razieh Dehghani Firouzabadi, M.D.[1], Tahmineh Farajkhoda, Ph.D.[2*], Ali Mohammad Abdoli, M.D.[1]

1. Yazd Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstract

Background: The appropriate choice of a contraceptive method has been a major issue in reproductive health research. Cu T intrauterine device (Cu T IUD) has been introduced as one of the most effective contraceptive methods in the world, however, the relationship between prior use of Cu T IUD and secondary infertility has not been evaluated in Iran. To examine the association of Cu T-380A IUD and secondary infertility in Iran.

Materials and Methods: A retrospective cohort study was conducted from December 2010 to September 2011 in the Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. A total of 750 married women (15-49 years old) with at least one parity, whom were referred to four educational healthcare centers of Shahid Sadoughi University of Medical Sciences, were selected as participants. They were divided into two groups (case and control) based on previous history of using Cu T-380A IUD. Data were gathered using a standard reliable questionnaire along with a face-to-face interview and were analyzed with descriptive and analytical (χ^2) tests.

Results: Mean period of Cu T-380A IUD usage in the case group was 57.46 ± 47.74 months and mean time length from Cu T-380A IUD removal to pregnancy was 14.87 ± 5.18 months in this group. We observed no relationship between the use of Cu T-380A IUD and frequency of secondary infertility (3.5% in the case group versus 2.7% in the control group, P=0.52).

Conclusion: Given the relatively large sample size studied here, it is unlikely that Cu T-380A IUD results in secondary infertility and may be used by Iranian women as a safe contraceptive method.

Keywords: Copper Intrauterine Devices, Infertility, Complication, Cohort Study, Iran

Citation: Abdinasab M, Dehghani Firouzabadi R, Farajkhoda T, Abdoli AM. Lack of association between Cu T-380A intrauterine device and secondary infertility in Iran. Int J Fertil Steril. 2017; 10(4): 343-349.

Introduction

The Copper T-380A intrauterine device (Cu T-380A IUD) is one of the most effective, long-term and reversible contraceptive methods worldwide (1, 2). The American College of Obstetricians and Gynecologists (ACOG) has established Cu T-380A as a safe contraception (2). Other notable advantages of this method are low-cost, convenience and its acceptability among women (3, 4).

According to the International Planned Parenthood Federation (IPPF), more than 95% of women who use CU IUDs are satisfied with them (5-7). It is, however, important to note that the Cu T-380A does not protect against sexually transmitted diseases (STDs) and pelvic inflammatory disease (PID) (6-8). Several studies have reported an association between the use of CU IUDs and secondary infertility following STDs or PID in parous women (3, 8-12). For instance, a double risk of tubal infer-

Received: 29 Oct 2015, Accepted: 16 Jul 2016
*Corresponding Address: P.O.Box: 8916877443, Research Center for Nursing and Midwifery Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: farajkhoda_t@yahoo.com

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 343-349

tility has been associated with prior use of CU IUD in two independent studies (8, 9). On the contrary, Hubacher et al. (11) showed in a well-designed case-control study that prior use of CU IUD was not associated with subsequent infertility. Several studies have shown the rare but serious side effects of CU IUDs such as STDs or PID when used in a monogamous relationship (6, 7, 12). There are also misconceptions about secondary infertility after removing CU IUD in the mind of women who use this method (13-15). According to Mansour et al. (16), fear of subsequent infertility following the use of CU IUD is considered as one of the main concerns in use of IUD among women and thought to be one of the most important factors for cessation of IUD use in developing countries (15) where it is commonly used (17). Islamic Republic of Iran has seen significant achievements in family planning in recent years (18, 19) with the use of contraception increasing from 49.9% in 1989 to 73.8% in 2009 (20, 21). This mainly has been driven by free healthcare services for family planning methods such as Cu T-380A IUD throughout the Public Health Center Network (20). The national research of Integrated Monitoring Evaluation System in Iran (IMES) reported IUD users to comprise 11.9% of contraceptive users in Iran (22).

The prevalence of infertility is high in Iran at nearly 20% (23). While this has been reported about 10-15% worldwide (23-29). The notable percentage of Cu T-380A IUD usage by Iranian women (21), specific socio-cultural background of Iranian family regarding female infertility and cultural issues of child adoption among Iranian families (30) and lack of enough study in this regard, led us to examine the association of Cu T-380A IUD and secondary infertility in the Yazd province.

Materials and Methods

We gathered current registry information under A retrospective cohort study was carried out from December 2010 to September 2011 in the Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The Ethics Committee of Shahid Sadoughi University of Medical Sciences of Yazd approved this study (code number#823). The study was conducted in four educational health care centers (Akbari, Rahmat Abad, Maskan and Azad Shahr) of Shahid Sadoughi University of Medical Sciences of Yazd. Data regarding secondary infertility and use of Cu T-380 A IUD were gathered by a face-to-face interview along with a structured questionnaire. The sampling process has been illustrated in the flowchart below (Fig.1).

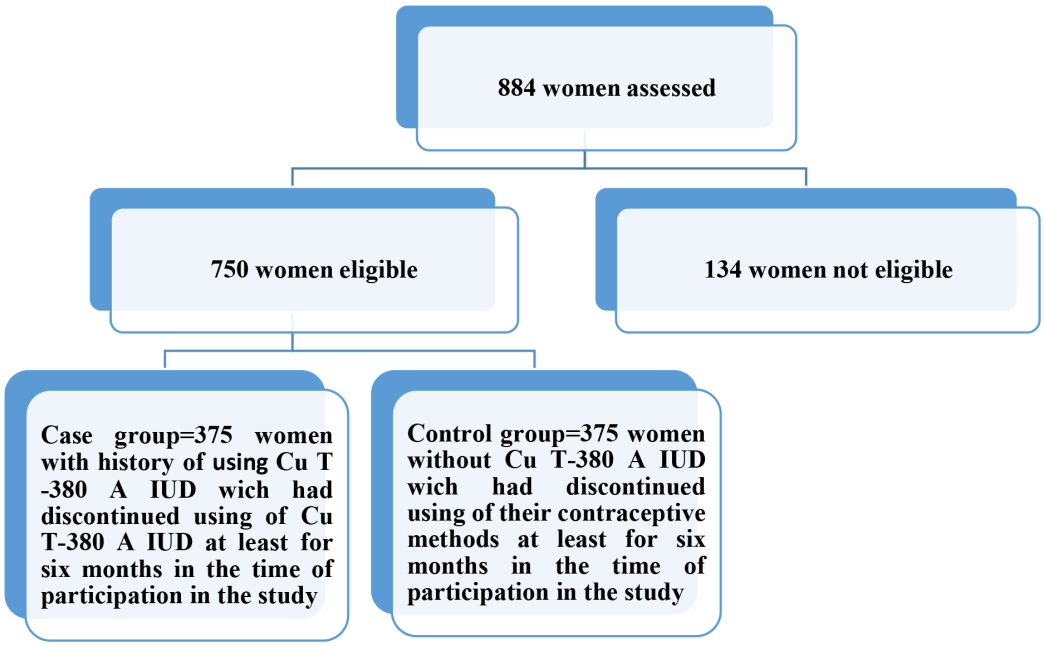

Fig.1: Sampling process for selecting the case and control groups. IUD; Intrauterine device.

After obtaining written informed consent, 750 married women in reproductive age (15-49 years old) attending one of the aforementioned healthcare centers with a history of using contraceptive methods including Cu T-380A IUD, combined oral contraceptive pill (OCP), withdrawal method and male condom, were selected as participants through conducting convenience sampling method. One hundred thirty four women were not eligible because they had not inclusion criteria or had not consent or time to participate in the study. Women with a history of using only progestin methods such as minipills, depotmedroxyprogesteroneacetate (DMPA) and Minera IUD were not eligible for participation in the study. All the inclusion criteria were a history of using Cu T-380A IUD for at least six months (only in the case group) and discontinued use of Cu T-380 A IUD for at least six months at the time of participation in the study, having regular intercourse, history of at least one pregnancy, no history of primary infertility or infertility treatments, no systematic diseases, and also no use of additional contraceptive methods at the time of sampling. Eligible women who withdrew from the study after participation were taken into account as the exclusion criterion. Participants had discontinued using their aforementioned contraceptive methods for at least six months at the time of participating in this study. The following formula shows sample size calculation.

In this study P_1=Prevalance in study population, P_2 =Variance of study population, α=The probability of making a type I error, N=Total sample size, n_1=Sample size in group 1 and n_2=Sample size in group 2. This formula is used for calculation of sample size for qualitative variable such as occurnece of pregnancy in two groups in this study.

$P_{1\ (Prevalance)}$=2%
$P_{2\ (Variance)}$=36%
α (The probability of making a type I error)=5%
N=Total sample size
$n_{1\ (Sample\ size\ in\ group\ 1)}$=375
$n_{2\ (Sample\ size\ in\ group\ 2)}$=375
$N=Z^2_{1-\frac{\alpha}{2}}\ P1(1-P1)+P2(1-P2)$

Women were divided into two groups (case group=375 women and control group=375 women) based on prior history of using Cu T-380A IUD. Participants were matched for age (+/- 2 years). The control group had a history of using other contraceptive methods such as OCP, withdrawal method and male condom. Data were gathered by a standard structured questionnaire based on scientific literature review. Experts in the related discipline approved content validity of the questionnaire (CVI=0.91). Reliability (internal consistency) of the questionnaire was calculated using Cronbach's alpha statistical test (α=0.89). The questionnaire consisted of various questions regarding personal characteristics, reproductive history and use of Cu T-380A IUD, and were completed in a face-to-face interview. Additional data were obtained from previous records in healthcare centers. Age, parity, length of using previous contraceptive methods before participation in the study, problems during use of contraceptive methods, complications needing treatment, response to treatment, hospitalization for PID, control visits for PID fallow up, date of discontinuing contraceptive methods and reasons of withdrawing were obtained from records and compared with participant answers. Also, occurrence of pregnancy was asked from the participants as and then it checked in their medical record as a variable to measure study outcome. Since recall bias is common and an inevitable factor in retrospective studies, we used both methods of data gathering and compared accuracy of obtained data from participants by the quesionnaire. History of PID was also gathered from records and questionnaires. Two questions were asked from participants such as "do you have a history of uterine infection so serious you had to behospitalized?" and "do you have a history of uterine infection with fever, infected vaginal discharge and pain in the pelvic area so serious that you had to visit a gynecologist and receive injectable or oral antibiotics?"

Statistical analysis

Data were analyzed by SPSS (version 16) using descriptive (mean and SD) and analytic (χ^2) statistical tests. Significance level was considered at $P<0.05$.

Results

All 750 women completed the questionnaires in full. Mean age difference between the case group (34.79 ± 7.41 years) and the control group (33.93 ± 7.77 years) was not statistically significantly different (P=0.123). The results showed that there were significant statistical differences in mean number of gravidity (P=0.001) and abortion (P=0.038) in the case and control groups. Mean length of Cu T-380A IUD in the case group was 57.46 ± 47.74 months and mean length from Cu T-380A IUD removal to pregnancy was 14.87 ± 5.18 months (Table 1).

Table 1: Comparison of personal characteristics in the case and control groups

Groups	Case (mean ± SD)	Control (mean ± SD)	P value	
Age (Y) t test	34.79 ± 7.41	33.93 ± 7.77	P=0.123	
Number of gravidity t test	3.12 ± 1.07	3.60 ± 1.58	P=0.001*	
Number of abortion t test	0.33 ± 0.75	0.23 ± 0.59	P= 0.038*	
Mean length of Cu T-380A IUD using (month)	57.46 ± 47.74			
Mean length from Cu T-380A IUD removal to pregnancy (month)	14.87 ± 5.18			
Educational status	**Case n (%)**	**Control n (%)**	**Total**	**P value**
Under diploma	200 (53%)	244 (65%)	444 (59%)	P=0.002*
Diploma and greater	175 (47%)	131 (35%)	306 (41%)	
Total	375 (100%)	375 (100%)	750 (100%)	
Occupational status				
Housewife	327 (87%)	338 (90%)	665 (89%)	P>0.05
Employee	48 (13%)	37 (10%)	85 (11%)	
Total	375 (100%)	375 (100%)	750 (100%)	

*; Statistically significant.

A significant statistical difference was observed in educational level between the case and control groups (P=0.002) but not in occupational status (P>0.05, Table 1).

Prior history of PID was observed in 30 women (8.1%) in the case group and in 26 women (7.1%) in the control group, with the difference not statistically significant [P=0.61, odda ratio (OR)=0.867, confidence interval (CI)=(0.50-1.49), Table 2].

Table 2: Comparison of prior history of pelvic inflammatory disease (PID) based on both self-reported data by women and their clinical records in the case and control groups

Groups	Case (n=375)	Control (n=375)	Total
With Prior history of PID	30 8%	26 6.9%	56 7.6%
Without Prior history of PID	345 92%	349 93%	694 92.4 %
Total	375 100%	375 100%	750 100%

The main aim of this study was to examine the relationship between the use of Cu T-380A IUD and secondary infertility. The results showed that there was no signifiacnt association between history of Cu T-380 A IUD use and secondary infertility [3.5% in the case group versus 2.7% in the control group, P=0.52, OR=1.31, CI=(0.57-3.03), Table 3].

Table 3: Comparison of secondary infertility based on occurrence of pregnancy at least six months after discontinuing the contraceptive method in the case and control groups

Groups	Case (n=375)	Control (n =375)
No occurrence of pregnancy	13 3.5%	10 2.7%
Occurrence of pregnancy	362 96.5%	365 97.3%
Total	375 100%	375 100%

There was also no significant difference between the case and control groups (P=0.5) in perceiving an association between use of Cu T-380A IUD and secondary infertility, with women equally divided between agreeing, disagreeing and not knowing (Table 4).

Table 4: Comparison of perception of women regarding the association between prior use of Cu T-380A intra utrine device (IUD) and secondary infertility

Groups	Case	Control	Total
I agree that prior use of Cu T-380A IUD can cause secondary infertility	133 35.5%	128 34.1%	261
I disagree agree that prior use of Cu T-380A IUD can cause secondary infertility	118 31.4%	114 30.4%	232
I don't have any idea concerning association between prior use of Cu T-380A IUD and secondary infertility	124 33.1%	133 35.5%	257
Total	375	375	750

Discussion

Conducting reproductive health research (RHR), should be done in safe practice manner and women should fulfill the benefits of scientific progress in the community including contraceptive research (31-33). The aim of this study was to assess the relationship between use of Cu T-380A IUD and secondary infertility. We observed no such association. Philippov et al. (29) showed that the prevalence of primary and secondary infertility respectively after Cu T IUD removal was 2.6 and 10.8%. Mansour et al. (16) assessed a thorough literature review for prospective studies reporting pregnancy rates in women after discontinuing contraceptive methods. One-year pregnancy rate following removal of Cu T-380A IUD was reported high, ranging from 71 to 91%, similar to other barrier methods or use of no contraceptive method. In another study, the observed fertility rate following cessation of use of Cu IUDs implied that fertility is normal after discontinuation of this method (34). These findings are consistent with those of this study.

We found the frequency of prior history of PID to be similar in the case and control groups. Several studies have assessed association between Cu IUD use and PID or salpingitis (7-9). Daling et al. (8) and Cramer et al. (9) reported a doubling in the risk of tubal infertility associated with prior use of IUD. They reported that there was not any risk of secondary infertility in women with prior use of CU IUDs even the copper device had been removed for the reason of CU IUDs complications. In this regard, Cramer et al. (9) reported that the number of partners is as an important factor, since women with only one sexual partner had no significant increase in the risk of tubal infertility, regardless of the type of contraception used. Lack of bacteriologic information in both case-control studies was an important limitation. Hubacher et al. (11), however, undertook a well-designed case-control study to determine the risk of tubal sterility associated with CU IUD use. Their results showed that use of CU IUD was not associated with subsequent infertility. They reported that history of prior infection with Chlamydia trachomatis (confirmed by antibodies against Chlamydia) was significantly associated with tubal infertility. Incontrast, use of a CU IUD showed no association with tubal infertility. These two findings showed that STDs (chlamydial infection and gonorrhea, particularly), and not plastic or copper, are the common causes of tubal infertility. Bromham (35) reported that when one controls for the confounding effect of prior STD exposure, the increase in risk associated with CU IUD disappears. Hamerlynck and Knuist (13) believe that resistance against the use of CU IUDs due to the perceived increased risk of PID and subsequent infertility is related to lack of awareness with respect to recent developments and also unpleasant experiences during the 1980s and earlier when Dalkon Shield IUDs were used. According to Huggins and Cullins (12), the risk of infertility associated with prior use of CU IUD is low. PID and its consequences are primarily a concern in the immediate post-insertion time frame or to women who have a greater risk of acquiring STDs (those with multiple partners or those with prior PID). Also, Swende et al. (17) have shown that contamination at the time of IUD insertion is responsible for PID. The excess risk of PID among IUD users, with the exception of the first few months after insertion, is related to STDs and not the IUD. Women without risk factors for STDs have little increased risk of PID or infertility associ-

ated with IUD use (12). Furthermore, Kohn et al. (2) showed that 31% of all healthcare providers recommend an IUD for clients with history of recent STD, 37% of them suggest IUD insertion for women with previous history of PID and 38% for clients not in a monogamous sexual relationship. Although 77% of healthcare providers stated that IUDs were safe for adolescents, it would be unlikely for 18% of them to recommend an IUD to a client under 20 years of age. Moreover, 86% of respondents were aware that IUDs can be used in nulliparous women, however, 25% of them would not recommend an IUD to such clients. There are no contraindications for IUD use based solely on age or parity (2, 36).

Assessing the view of women on prior use of Cu T-380A IUD and its association with secondary infertility was one of aims of this study. Although association between prior use of Cu T-380A IUD and secondary infertility was not observed between the case and control groups but 261 women from 750 women who participated in the study believed that prior use of Cu T-380A IUD can cause secondary infertility. It seems that women need to be better educated on this issue. Stoddard et al. (4) suggested that IUD should be offered as the first-line contraceptive method for most women and the misconceptions regarding IUD should be explained to women. In another study, 61% of healthcare providers believed that counseling clients about IUD would take more time than other methods but it is necessary (2). Given that recent studies have shown that Cu IUDs maybe used by eligible nulliparous women and adolescent girls (15, 37), they have been introduced as a safe contraceptive method for such individuals which have no risks factor for STDs and PID (2, 36).

The high prevalence of infertility threatens familial life, health and social status of women in our country. Primary, secondary and lifetime infertilities have been reported to be at 21.1, 7.8 and 6.4%, respectively in population-based study of Iran (38). Since prevalance of infertility is high in Iran (23, 38), the most important reason of primary and secondary infertilities is menstrual dysfunction (not use of Cu T-380A IUD). The socio-demographic factors can influence the prevalence of infertility. Providing information about significant factors on infertility may help women to increase their fertility chances (38). In developing countries like Iran, having children is greatly valued for social, cultural and economic reasons (39). From an Islamic perspective, attitude toward motherhood is extremely honored with the famous tradition stating that "heaven lies at the feet of mothers" (40). Since infertility may prevent couples to attain the desired life and cause some social and psychological problems, Iranian women with infertility may undergo several individual and social problems that may influence their quality of lives.

Given that we identified no association between prior use of Cu T-380 A IUD and occurrence of secondary infertility, introducing this method as a long-acting contraceptive for eligible women can help women and their husbands to decrease their stress level of secondary infertility and have a pleasant experience with their selected contraceptive method.

Conclusion

We observed no association between prior use of Cu T-380A IUD and occurrence of secondary infertility. Women may use currently available Cu T-380A IUD without any fear of secondary infertility. However, it should be kept in mind that this is a good contraceptive choice for eligible women only. Overall, it is recommended that the risks and advantages of using the method should be considered on an individual basis. One of the best ways for selecting eligible women for IUD use is for individuals to undergo appropriate counseling. Barriers such as pervasive myths, misconceptions, misinformation and incorrect beliefs among healthcare providers, clients and communities should be explained briefly.

Acknowledgements

The author would like to thank from Shahid Sadoughi University of Medical Sciences for financial support. There is no conflict of interest in this article.

References

1. Family planning: a global hand book for providers. Baltimore and Geneva: CCP and WHO, 2011; 131-156. Available from: http://apps.who.int/iris/bitstream/10665/44028/1/9780978856373_eng.pdf. (10 Nov 2016).
2. Kohn JE, Hacker JG, Rousselle MA, Gold M. Knowledge

and likelihood to recommend intrauterine devices for adolescents among school-based health center providers. J Adolesc Health. 2012; 51(4): 319-324.
3. Stanback J, Shelton JD. Pelvic inflammatory disease attributable to the IUD: modeling risk In West Africa. Contraception. 2008; 77(4): 227-229.
4. Stoddard A, McNicholas C, Peipert JF. Efficacy and safety of long-acting reversible contraception. Drugs. 2011; 71(8): 969-980.
5. International Planned Parenthood Federation. Intra-uterine devices. Available from: http://www.ippf.org/our-work/what-we-do/contraception/myths/Intrauterine-Devices. (18 Aug 2013).
6. Berek JS. Novak's gynecology.15th ed. Philadelphia: Lippincott Williams and Wilkins; 2012; 222-226.
7. Fritz MA, Speroff L. Clinical gynecology endocrinology and infertility. 8th ed. Philadelphia; Lippincott Williams and Wilkins; 2011; 1095-1020.
8. Daling JR, Weiss NS, Metch BJ, Chow WH, Soderstrom RM, Moore DE, et al. Primary tubal infertility in relation to the use of an intrauterine device. N Engl J Med. 1985; 312(15): 937-941.
9. Cramer DW, Schiff I, Schoenbaum SC, Gibson M, Belisle S, Albrecht B, et al. Tubal infertility and the intrauterine device. N Engl J Med. 1985; 312(15): 941-947.
10. New studies link IUDs, infertility; say copper devices safer. Contracept Technol Update. 1985; 6(6): 81-83.
11. Hubacher D, Lara-Ricalde R, Taylor DJ, Guerra-Infante F, Guzman-Rodriguez R. Use of copper intrauterine devices and the risk of tubal infertility among nulligravid women. N Engl J Med. 2001; 345(8): 561-567.
12. Huggins GR, Cullins VE. Fertility after contraception or abortion (review). Fertil Steril. 1990; 54(4): 559-573.
13. Hamerlynck JV, Knuist M. Modern intra-uterine contraception: a better option. Ned Tijdschr Geneeskd. 2001; 145(3): 1621-1624.
14. Gutin SA, Mlobeli R, Moss M, Buga G, Morroni C. Survey of knowledge, attitudes and practices surrounding the intrauterine device in South Africa. Contraception. 2011; 83(2): 145-150.
15. Aradhya K, Rahim Keita D, Mwebesa W. Repositioning of the intrauterine device continues. Africa Health. 2010: 36-37. Available from: http://www.fhi360.org/sites/default/files/media/documents/Africa%20Health,%20January%202010.pdf. (10 Nov 2016).
16. Mansour D, Gemzell-Danielsson K, Inki P, Jensen JT. Fertility after discontinuation of contraception: a comprehensive review of the literature .Contraception. 2011; 84(5): 465-477.
17. Swende TZ, Ocheke A, Mohammed H. Forgotten intrauterine contraceptive device: a rare cause of infertility. Niger Med J. 2009; 18(4): 420-421.
18. United Nations in Islamic republic of Iran. The first millennium development goals report. 2004. Available at: www.undp.org/.../MDG/.../MDG%20Country%20Reports/Iran/Iran. (18 Aug 2013).
19. Simbar M. Achievements of the family planning program in Iran. Journal of School of Public Health and Institute of Public Health Research. 2010; 8 (1): 81-92.
20. Vakilian K, Mirzaii K, NajmAbadi. Reproductive health in Iran: international conference on population and development goals. Oman Med J. 2011; 26(2): 143-147.
21. World Health Organization. WHO Statistical Information System (WHOSIS). 2010. Available at: http://www.who.int/whosis/whostat/EN_WHS2011_Part2.pdf. (20 Mar 2013).
22. Mottaghi Z, MirzaiiNajmabadi K. Women's perspective and beliefs about barriers to IUD use: a qualitative study. Journal of Knowledge and Health. 2011; 6(3): 24-30.
23. Yassini SM, Taghavi Shavazi M, TaghaviShavazi N. Factors associated with adoption acceptance rate from the view point of infertile couples. Iran J Reprod Med. 2012; 10(5): 413-418.
24. Cunningham F, Leveno K, Bloom S, Hauth J, Rouse D, Spong C. William's obstetrics. 23th ed. USA : McGraw Hill; 2009; 673-698.
25. Gibbs RS, Karlan BY, Haney AF, Nygaard IE. Danforth's obstetrics and gynecology. 10th ed. Philadelphia: Lippincott Williams & Wilkins; 2008; 705-716.
26. Tanagho EA, Mcaninch JW. Smith's general urology. 17th ed. USA: McGraw Hill; 2008; 235-237.
27. Semi-family affairs Office. Statistics of the semi-family affairs office performance. Tehran, state welfare organization, 2005. Available from: http://un.org.ir/sites/default/files/page/238/files/undaf_2012_-_2016.pdf. (19 Aug 2012).
28. Razzaghi R. Adoption. Tehran, state welfare organization; 2007. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4169677/. (10 Nov 2016).
29. Philippov OS, Radionchenko AA, Bolotova VP, Voronovskaya NI, Potemkina TV. Estimation of the prevalence and causes in of Western Siberia Infertility. Bull World Health Organ. 1998; 76(2): 183-187.
30. Bokaie M, Farajkhoda T, Enjezab B, Heidari P, KarimiZarchi M. Barriers of child adoption in infertile couples: Iranian's views. Iran J Reprod Med. 2012; 10(5): 429-434.
31. Federation International of Gynecology Obstetrics/FIGO. Ethical issues in obstetrics and gynecology by the FIGO committee for the study of ethical aspects of Human Reproduction and Women Health. 2009. Available from: http://www.figo.org/files/figo-corp/Ethical%20Issues%202009%20-%202012%20pdf.pdf. (18 Aug 2013).
32. Cook RJ, Dickens BM. Considerations for formulating Reproductive health laws. World Health Organization. 2nd ed. 2000. Available from: http://www.who.int/topics/reproductive_health/en/. (17 Aug 2011).
33. Farajkhoda T, Latifnejad Roudsari R, Abbasi M. An exploratory study to introduce a practical ethical framework for reproductive health research. Iran J Reprod Med. 2013; 11(1): 31-38.
34. Struthers BJ. Copper IUDs, PID, and fertility in nulliparous women. Adv Contracept. 1991; 7(2-3): 211-230.
35. Bromham DR. Intrauterine contraceptive devices - a reappraisal. Br Med Bull. 1993; 49(1): 100-123.
36. Smith E, Daley AM. A clinical guideline for intrauterine device use in adolescents. J Am Acad Nurse Pract. 2012; 24(8): 453-462.
37. Connor H, McGinn E, Pile JM, Babamuradova M, Ndede F, editors. The supply-demand-advocacy family planning model at work: experience in Kenya and Azerbaijan. International Conference on Family Planning; 2009 Nov 15-18; Kampala, Uganda. 2009.
38. Rostami Dovom M, Ramezani Tehrani F, Abedini M, Amirshekari G, Mehrabi Y. Prevalance of primary and secondary infertility among 18-49 years old Iranian women: a population-based study in four selected provinces. Hakim. 2014; 16 (4): 294 -301.
39. Ombelet W. False perceptions and common misunderstandings surrounding the subject of infertility in developing countries. ESHRE Monographs. 2008; (1): 8-11.
40. Bhatti LI, Fikree FF, Khan A. The quest of infertile women in squatter settlements of Karachi, Pakistan: a qualitative study. Soc Sci Med. 1999; 49(5): 637-649.

Original Article

Chances to Have A Boy after Gender Selection by Pre-Implantation Genetic Screening Are Reduced in Couples with only Girls and without A Boy Sired by The Male Partner

Soryya Panahi, M.Sc.[1, 2], Fariba Fahami, M.Sc.[2], Mohammad Reza Deemeh, M.Sc.[1], Marziyeh Tavalaee, Ph.D.[1], Hamid Gourabi, Ph.D.[3], Mohammad Hossain Nasr-Esfahani, Ph.D.[1, 4*]

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Midwifery, School of Nursing and Midwifery Care Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Isfahan Fertility and Infertility Center, Isfahan, Iran

Abstract

Background: Gender selection and family planning have their roots in human history. Despite great interest in these fields, very few scientific propositions exist which could explain why some family do not attain the desired sex. Therefore, the aim of this study was to evaluate whether sex of previous child or children could affect the outcomes of pre-implantation genetic screening (PGS).

Materials and Methods: This historical cohort study including 218 PGS cases referring to Isfahan Fertility and Infertility Center (IFIC). Couples were grouped as those who their male child passed away or her husbands' has a son(s) from their previous marriage (n=70) and couples who just have daughter (n=148). Male normal blastocysts were transferred for both groups. The outcomes of PGS including pregnancy, implantation and abortion rates, along with possible confounding factors were compared between the two groups.

Results: Significant differences in pregnancy, implantation and abortion rates were observed between couples whose their male partner had/has one boy (n=70) compared to those who have just girl(s) (n=148) despite similar number and quality of male normal blastocyst transferred in the two groups. Confounding factors were also considered.

Conclusion: The Y- bearing spermatozoa in male partners with no history of previous boy have lower ability to support a normal development to term, compared to male partners with previous history of boy requesting family balancing.

Keywords: Pre-Implantation Genetic Screening, Pregnancy, Implantation, Abortion

Citation: **Panahi S, Fahami F, Deemeh MR, Tavalaee M, Gourabi H, Nasr-Esfahani MH. Chances to have a boy after gender selection by pre-implantation genetic screening are reduced in couples with only girls and without a boy sired by the male partner. Int J Fertil Steril. 2017; 10(4): 350-356.**

Introduction

Gender selection has its roots in ancient culture and this desire has even lasted through this millennium. The alternative name for this desire is family balancing (1). Beside the cultural aspects of gender selection, communal pressures, ethnicity, social economical and educational status of couples influence parental procreative desire. In some society, urge for such a desire has led to illegal abortion of undesired sex, family pressures and even divorce (2). Considering the fact that parental procreative desire for boy is much higher than girls,

Received: 28 Apr 2016, Accepted: 25 Jun 2016
***Corresponding Address: P.O.Box: 8165131378, Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran**
Email: mh.nasr-esfahani@royaninstitute.org

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 350-356

in some rural communities, couples may continue their fertility, despite having several girls, in hope of a boy delivery. Therefore, there appear to be an obligation for these couples to bear a male heir for the family. In these communities, sometime they may name their girls as: "die di" in Hong Kong and China meaning "bring a younger brother" (3), "ghez bas" in Turkish meaning the last daughter.

Despite ancient history of gender selection, the mechanism or etiology of gender determinant in natural conception cycle remains unknown and except some equivocal hypothetical proposition, there is no solid theory, except random opportunity for X or Y bearing sperm penetrating an oocyte (4).

Iran, with high ethnic backgrounds (Fars, Lors, Kords, Turks, Arabes, Blooch and Turkamen), some families feel obliged to bear a male heir for the family. Therefore, gender selection technology, especially pre-implantation genetic screening (PGS) which has high accuracy, has provided an opportunity for these couples to fulfill this procreative paternal desire for family balancing.

Gender selection in this era has opened a hot ethical issue in different societies. Critics believe that family balancing is the right of all parents with procreative desire for certain sex (5). Therefore, based on recent epidemiological studies which show reduced general fertility rate in Iran (6) and furthermore, revealing that a small percentage of community may opt for gender selection procedure like PGS, are among the reasons why this procedure, as family balancing method, is provided by some infertility centers. Therefore, regardless of ethical issue of gender selection, which is out of scope of this study, the aim of this historical cohort study is to evaluate whether sex of previous child or children, could affect the outcomes of PGS. Therefore, the male partner of the couples were grouped to those who had/has one boy or those who have just girls.

Materials and Methods

This is a historical cohort study including 218 PGS cases based on below inclusion and exclusion criteria referring to Isfahan Fertility and Infertility Center (IFIC). In this center, couples are routinely informed and written consent are obtained to allow the usage of their data for research purposes. The study was approved by the Ethical Committee of IFIC.

Inclusion criteria

Couples who requested PGS for male gender selection for family balancing were included in this study. Therefore, PGS was carried out for the couples who had at least two children of the opposite sex, in this circumstance at least two girls and/or couples with history of having male (their son died and have two girl or have a son from their first marriage and were seeking from family balancing for their second marriage with two girls).

Exclusion criteria

i. Female with age higher than 42 years, ii. History of medical or obstetric complications within previous pregnancies, including premature delivery, low birth weight, stillbirth, gestational hypertension, gestational diabetes and abnormal uterine, iii. Maternal systemic diseases (cardio- vascular, renal, glands disease, cancer, etc) that have adverse impact on pregnancy, iv. Previous history of infertility, v. history of habitual abortion (> 2 abortions), vi. candidates of family balancing, with at least two girls, who did not undergo embryo transfer due to ovarian hyper stimulation syndrome or unsuitable endometrium (grade C or endometrium with less than 6 mm thickness), and vii. Couples who had no normal male blastocyst for transfer.

Ovulation induction, intra cytoplasmic sperm injection and pre-implantation genetic diagnostic

Briefly, following ultrasound scan on day 2 of menstrual cycle, patients underwent ovarian stimulation with gonadotropins and gonadotropin-releasing hormone (GnRH)-antagonist following serial monitoring. Human chorionic gonadotropin (HCG) was administered when at least two leading follicles measured 18 mm in diameter were observed. 36 hours later transvaginal ultrasound-guided oocyte retrieval was performed.

Intra cytoplasmic sperm injection (ICSI) was implemented for all the cases and embryo biopsy for PGS procedure performed on day 3 following oocyte retrieval by direct aspiration of a single blastomere through an opening created by Laser (Hamilton Thorne, New Zealand) in the zona pellucida. Following fixation of the biopsied blastomere, nuclear DNA was analyzed by Fluorescent In Situ Hybridization (FISH) method for 3 chromosomes (X, Y, and 18). Embryos were scored

on day 5 and stage of development were recorded (arrested, compact, early blastocyst, expanded blastocyst and hatched blastocyst) (7). One or maximum of 2 male blastocyst(s) were transferred per patients. Percentages of chemical and clinical pregnancies, implantation and abortion rates were assessed according to Deemeh et al. (8).

Variables analyzed include female and male age, semen parameters, number of retrieved oocytes, number of oocytes injected, fertilization rate, number of embryos biopsied, number of blastocysts transferred or frozen, and the fate of the remaining embryos.

Based on X, Y and 18 chromosomes, percentages of eligible male and female embryos, male and female blastocyst were assessed. In addition, percentages of eligible and abnormal male and female arrested embryos, and, not founded or not diagnosed embryos were determined.

In order to facilitate the calculation, number of PGS embryos whose biopsied blastomere had no nuclei or signals, were deducted from the number of PGS embryos. Therefore, the number of PGS embryos refers to embryos that were biopsied and had a genetic report regarding chromosomes X, Y and 18. Furthermore, in this study "normal" refers to embryos that had correct set of chromosomes follow screening for X, Y and 18.

Patient categorization

Couples seeking family balancing in the IFIC were categorized to two groups: i. Those with history of having male progeny and ii. Those that have at least two girls. Considering the limited number of couples in the first group, in order to reduce confounding effect, the couples for the second group were chosen within the period that the couples from the first group were performing PGS.

Statistical analysis

Statistical analyses were performed using Chi square and independent t test using SPSS version 16. $P<0.05$ was considered as statistically significant.

Results

Table 1 shows the clinical outcomes of normal male embryo screen by PGS between couples with history of male partner having a previous boy and couples who have girls when requesting PGS for family balancing. The chemical pregnancy (60.0 vs. 16.9%), clinical pregnancy (58.6 vs. 16.9%) and ongoing pregnancy (57.1 vs. 6.1%) rates were significantly different between the two groups. Similarly, implantations (46.9 vs.14.97%) and abortion rates (7.14 vs. 64.0%) were significantly different between the two groups.

Table 1: Comparison of clinical outcomes of normal male embryo screen by pre-implantation genetic screening (PGS) between couples with and without history of previous boy

Clinical outcomes	No history of previous boy	History of previous boy	P value
Chemical pregnancy	16.9% (25/148)	60.0% (42/70)	<0.001
Clinical pregnancy	16.9% (25/148)	58.6% (41/70)	<0.001
Ongoing pregnancy	6.1% (9/148)	57.1% (40/70)	<0.001
Non-pregnant	83.1% (123/148)	40.00% (28/70)	<0.001
Abortion rate	64.00% (16/25)	7.14% (1/41)	<0.001
Implantation rate	14.97%	46.9 %	<0.001

Following this analysis, in order to evaluate the differences observed between the two groups were not due to difference in parameters related to embryos quantity and quality (Table 2), and also other confounding parameters (Tables 3, 4), these parameters were compared between the two groups. The number of retrieved oocyte (11.1 ± 5.4 vs.10.9 ± 4.5) and injected oocyte (8.2 ± 3.5 vs. 8.4 ± 3.1) were similar between two groups and no significant differences were observed. However, a statistically significant difference ($P<0.05$) was found in the number of immature oocytes between couples without previous history of boy (7.4 ± 11.4) and couples with previous history of boy (4.2 ± 7.72). Percentage of fertilization also were similar between two groups (75.3 ± 17.6 vs. 78.6 ± 18.1). When the same analysis was performed for embryonic parameters, we observed that the number of PGD embryos (5.9 ± 3.0 vs. 6.0 ± 2.7), number of embryos not found (0.5 ± 0.8 vs. 0.7 ± 1.0) and total blastocyst (4.1 ± 2.1 vs. 4.4 ± 2.2) were similar between the groups studied and there was no statistically significant difference. However a statistically significant difference ($P<0.05$) was observed in the number ar-

rested embryos (1.3 ± 1.3 vs. 0.9 ± 1.1) between two groups. But, percentage of this parameter was not statistically significant (Table 2).

In addition, we compared normal and abnormal embryonic parameters based on X, Y and chromosome 18 analyses in the couples with and without previous history of boy. We did not observed any significant difference in male and female embryos based on day 3 report, male and female blastocyst, and also male and female embryos arrested between two groups except abnormal female blastocyst and normal female embryos arrested (Table 3).

Table 2: Description of oocyte, fertilization and embryonic parameters in the groups with and without pervious history of boy

	Parameters	No history of previous boy	History of previous boy	P value
Oocyte	Retrieved	(11.1 ± 5.4)	(10. 9 ± 4.5)	0.7
	Injected	(8.2 ± 3.5)	(8.4 ± 3.1)	0.6
	Immature	7.4 ± 11.4	4.2 ± 7.72	0.01*
	Fertilization rate	75.3 ± 17.6	78.6 ± 18.1	0.2
Embryonic parameters	PGS embryos	100% (5.9 ± 3.0)	100% (6.0 ± 2.7)	0. 8
	Arrested embryos	20.5 ± 18.1 (1.3 ± 1.3)	15.1 ± 19.0 (0.9 ± 1.1)	0.07 (0.03)*
	Not found	8.0 ± 12.6 (0.5 ± 0.8)	11.4 ± 15.7 (0.7 ± 1.0)	0.09 (0.07)
	Total blastocyst	72.7 ± 18.7 (4.1 ± 2.1)	74.2 ± 20.3 (4.4 ± 2.2)	0.6 (0.4)

Percentages are presented outsides the parenthesis and numbers in the parenthesis.
***; P Value less than 0.05 statistically significantly and PGS; Pre-implantation genetic screening.**

Table 3: Description of embryonic parameters based on X, Y and chromosome 18 analyses in the group with and without previous history of boy

	Parameters	Statues	No history of previous boy	History of previous boy	P value
Based on X, Y and 18	Male embryos based on day 3 report	Normal	40.9 (1.9 ± 1.1)	40.4 (2.02 ± 1.2)	0.9 (0.6)
		Abnormal	10.0 ± 13.1 (0.6 ± 0.9)	9.6 ± 16.9 (0.5 ± 0.9)	0.8 (0.3)
	Female embryos based on day 3 report	Normal	29.7 ± 22.4 (1.7 ± 1.6)	26.8 ± 21.0 (1.5 ± 1.8)	0.4 (0.4)
		Abnormal	19.5 ± 19.4 (1.1 ± 1.1)	23.2 ± 20.5 (1.2 ± 1.1)	0.2 (0.4)
	Male blastocyst	Normal	38.9 ± 21.1 (1.8 ± 0.9)	39.2 ±18.4 (2.0 ± 1.2)	0.9 (0.3)
		Abnormal	5.9 ± 10.3 (0.4 ± 0.6)	6.3 ± 12.1 (0.3 ± 1.0)	0.8 (0.6)
	Female blastocyst	Normal	26.0 ± 21.8 (1.5 ± 1.5)	25.4 ± 20.7 (1.4 ± 1.4)	0.8 (0.7)
		Abnormal	8.5 ± 12.9 (0.4 ± 0.7)	13.4 ± 16.3 (0.7 ± 0.8)	0.03* (0.02)*
	Male embryos arrested	Normal	2.0 ± 6.0% (0.1 ± 0.4)	1.2 ± 5.2 (0.1 ± 0.2)	0.4 (0.06)
		Abnormal	4.0 ± 8.3% (0.3 ± 0.6)	3.2 ± 10.3 (0.2 ± 0.6)	0.6 (0.3)
	Female embryos arrested	Normal	3.6 ± 8.4% (0.2 ± 0.5)	1.4 ± 5.4 (0.1 ± 0.4)	0.02* (0.04)*
		Abnormal	10.9 ± 13.8% (0.6 ± 0.7)	9.8 ± 15.4 (0.5 ± 0.7)	0.6 (0.4)

Percentages are presented outsides the parenthesis and numbers within the parenthesis.
***; P value less than 0.05 statistically significant.**

The same analysis was performed on male and female characteristics in the couples with and without previous history of boy. Female and male age, percentage of sperm motility, normal morphology, sperm concentration and number of previous spontaneous abortion were similar between two groups (Table 4).

Qualities of blastocysts were assessed according to Gardener criteria (7) and no significant difference was observed between the qualities of blastocysts transferred between the two groups (data not shown).

Table 4: Comparison of male and female characteristics in the group with and without previous history of boy

Parameters	No boys	One or more boy (s)	P value
Female age (Y)	34.7 ± 4.00	34.3 ± 4.4	0.5
Male age (Y)	40.4 ± 4.8	40.3 ± 5.0	0.9
Number of previous spontaneous abortion	15	2	0.06
Sperm motility (%)	47.0 ± 13.7	46.5 ± 12.5	0. 8
Normal sperm morphology (%)	92.3 ± 5.3	91.8 ± 6.4	0.5
Sperm concentration (10^6/ml)	55.0 ± 22.5	58.2 ± 23.7	0.4

Discussion

Importance of gender has its root in history and different reasons have been proposed for why some individuals give birth to only male or female offspring. However, scientist believe that since equal number of boys and girls are born in a society, this can be attributed to equal number of X-and Y-bearing spermatozoa produced during spermatogenesis and their random chance of fertilization. Therefore, the allocation of sex is depended on which X-or Y-bearing spermatozoa reaches the egg first (4). Withstanding this theory, some scientists believe that different factors, like nutrition, resource availability (famine), may skew the random chance of fertilization of the X- and Y-bearing spermatozoa (9, 10). To extend our understanding of the factors which may distort the random chance of fertilization and development of the X-and Y-bearing spermatozoa, we assessed the outcome of PGS sex selection in two groups of couples: those in which the male partner had/has a history of a male and those couples that had/have just female offspring.

The results revealed significant difference in terms of implantation, chemical, clinical and ongoing pregnancy rates between the two groups and these parameters were significantly higher in the group that their male partner had/has a previous boy. The rate of abortion was also substantially higher in the group that their male partner had no previous boy compared to the other group. Considering similar number or percentage of normal male blastocysts transferred in the two groups, our results suggest that it is likely that the Y- bearing spermatozoa have lower ability to support a normal development to term in couples whom their male partner had no previous boy. In order to solidify this possibility by ruling out the confounding factors, we compared both mean number and/or percentage of the various factors between the two groups. Among these factors only total number of immature oocytes, number of embryos with normal female that arrested (did not developed to blastocyst), and percentage of abnormal female embryos that did reach blastocyst stage were significantly different between the groups.

Number of immature oocyte were higher in the group with no previous boy, although this factor could have confounding effect but considering the fact that other factors like number of normal male blastocysts were similar in both groups, we believe the influence of this factor could not be substantial. If the number or percentage of female arrested embryos were higher in the group with history of previous boy, this is would be against our conclusion but since this parameter is higher in the other group, logically this does not affect our conclusion. Higher number of normal female embryo that arrested in the group with history of previous boy compared to the other group, suggest that one of the reasons for higher chance of conception for boy in this group might be related to this factor but this possibility does not rule out lower chance of implantation and pregnancy in the group with no history of boy since they have similar number of normal male blastocyst transferred between the two groups.

Therefore, based on the above evidence, we concluded that chance of a Y-bearing sperm to support normal development to birth in the couples with previous history of girl and no boy is reduced and this proposition may be considered as one but not the sole reason why in some couples attempts to

have a boy baby is reduced. Although, historically, such a hypothesis may have been theoretically proposed at scientific level, but to the best of our knowledge no scientific data has been published or provided on this subject. Study of literature on this context suggest that in some species meiotic derivers and suppressors especially those related to sex-linked meiotic drive may skew in sex ratio or induce phenotype like "hybrid male sterility". Recent report by Soh et al. (11) suggest that male-specific region of the Y (MSY) chromosome, with massively amplified gene families may have role in this process. Indeed, it has been shown that "mice Knock-down of Sly or Slx, one of the three X-Y gene pairs, also distorts sex ratio in favor of females or males, respectively". These authors state that "the mouse MSY's three acquired and massively amplified gene families and their X homologs are reminiscent of a meiotic driver and suppressor pair: in all three cases, both the X and Y genes are highly amplified, they are expressed specifically in testicular germ cells, and perturbation of gene family copy number results in sex ratio distortion". However, a long way remains ahead of researchers in this filed to evaluate whether the observation in this study may be related to massively amplified gene families which can act as repressor or drivers and may skew the sex ratio.

Study of literature based on animal studies and hypothetical propositions suggest that mothers may have some impudence over the sex of their offspring and factors like increased follicular testosterone, presence of glucose in the uterine environment and female testosterone levels rise in response to environmental stressors may skew the sex ratio (12-15). Furthermore, study of literature show that in stress condition, pregnant women disproportionately aborts male fetus (16). Although, we did not study these factors in our population but could higher stress condition in the couples who had no history of previous boy, may have influenced their implantation and pregnancy rates? This possibility remains to be determined.

One of the underlying mechanisms which may explain our proposed theory might be the difference in degree of DNA fragmentation in X- and Y-bearing sperm in individuals with no history of previous boy. Although we were not able to assess the degree of DNA fragmentation in X- and Y bearing sperm, but previous study suggests introducing sperm to stress condition, like heat stress, the chance of survival of their X-bearing sperm is higher than the Y-bearing sperm (17). Considering the fact that in our study the barriers of fertilization is bypassed by ICSI, the chance of normal male blastocyst formation is equal between the two groups, but their post blastocyst development is reduced in group with no history of previous boy. This is a hypothetical proposition and need future validation.

Another possible mechanism which may explain our observation in this study might be the bias selection or response of endometrium between the two groups of patients, despite similar number and quality of normal boy blastocysts transferred between the two groups. In line with such a possibility a recent study showed differential gene expression to Y-and X-bearing sperm population inseminated into uterine of porcine uterus (4).

Conclusion

To our knowledge this is the first report, concluding that chance of pregnancy to term is significantly higher when male normal blastocyst are transferred to couples whose male partner had/has a boy compared to couples whose male partner have just girls.

Acknowledgements

We would like to thank the Isfahan Fertility and Infertility Center for permission to use their patients' data and the Royan Institute for their kind co-operation in doing genetic tests on blastomere samples. There is no financially support and none of the authors has any conflicts of interest to disclose.

References

1. Schenker JG. Gender selection: cultural and religious perspectives. J Assist Reprod Genet. 2002; 19(9): 400-410.
2. Unnithan-Kumar M. Female selective abortion – beyond 'culture': family making and gender inequality in a globalising India. Cult Health Sex. 2010; 12(2): 153-166.
3. Chan CL, Yip PS, Ng EH, Ho PC, Chan CH, Au JS. Gender selection in China: its meanings and implications. J Assist Reprod Genet. 2002; 19(9): 426-430.
4. Almiñana C, Caballero I, Heath PR, Maleki-Dizaji S, Parrilla I, Cuello C, et al. The battle of sexes start in oviduct modulation of oviductal transcription by x and y- bearing spermatozoa. BMC Genomic. 2014; 15: 293.
5. Strange H. Non-medical sex selection: ethical issues. Br Med Bull. 2010; 94: 7-20.
6. Vahidnia F. Case study: fertility decline in Iran. Popul Environ. 2007; 28(405): 259-266.
7. Gardner DK, Schoolcraft WB. In vitro culture of human

blastocysts. Jansen R, Mortimer D, editors. Towards reproductive certainty: infertility and genetics beyond. Carnforth, UK; Parthenon Press; 1999; 378-388.
8. Deemeh MR, Tavalaee M, Nasr-Esfahani MH. Health of children born through artificial oocyte activation: a pilot study. Reprod Sci. 2015; 22(3): 322-328.
9. James WH. The variations of human sex ratio at birth during and after wars, and their potential explanations. J Theor Biol. 2009; 257(1): 116-123.
10. Hardy ICW. Possible factors influencing vertebrate sex ratios: an introductory overview. Appl Anim Behav Sci. 1997; 51(3): 217-241.
11. Soh YQ, Alföldi J, Pyntikova T, Brown LG, Graves T, Minx PJ, et al. Sequencing the mouse Y chromosome reveals convergent gene acquisition and amplification on both sex chromosomes. Cell. 2014; 159(4): 800-813.
12. Larson MA, Kimura K, Kubisch HM, Roberts RM. Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocyst and in the expression of the signaling molecule IFN-tau. Proc Natl Acad Sci USA. 2001; 98(17): 9677-9682.
13. Rosenfeld CS, Roberts RM. Maternal diet and other factors affecting offspring sex ratio: a review. Biol Reprod. 2004; 71(4): 1063-1070.
14. Grant VJ. Could maternal testosterone levels govern mammalian sex ratio deviations? J Theor Biol. 2007; 246(4): 708-719.
15. Grant VJ, Chamley LW. Can mammalian mothers influence the sex of their offspring peri-conceptually? Reproduction. 2010; 140(3): 425-433.
16. Zorn B, Sucur V, Stare J, Meden-Vrtovec H. Decline in sex ratio at birth after 10-day war in Slovenia: brief communication. Hum Reprod. 2002; 17(12): 3173-3177.
17. Pérez-Crespo M, Pintado B, Gutiérrez-Adán A. Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Mol Reprod Dev. 2008; 75(1): 40-47.

Original Article

Which Stage of Mouse Embryos Is More Appropriate for Vitrification?

Nasibeh Ghandy, M.Sc.[1*], Abbas Ali Karimpur Malekshah, Ph.D.[2]

1. Department of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Molecular and Cell Biology Research Center, Department of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Abstract

Background: Vitrification has been shown as one of the most effective methods of cryopreservation for mammalian embryos. However, there is no consensus which stage of embryonic development is the most appropriate for vitrification with subsequent maximal development after thawing. This study was carried out to explore and compare the effect(s) of vitrification on mouse 2-cell, 4-cell, 8-cell, morula and blastocyst stage embryos and subsequent blast formation and hatching after thawing.

Materials and Methods: In this experimental study, 2-cell embryos were obtained from the oviducts of super ovulated female NMRI mice. Some embryos were randomly selected and vitrified through a two-step media protocol and cryotop. Other embryos were cultured to assess their development. During the ensuing days, some of these cultured embryos were vitrified at 4-cell, 8-cell, morula and blastocyst stages. After 10 to 14 days, the embryos were thawed to assess their survival and also cultured to determine the rate of blastocyst formation and hatching. The results were analyzed using one-way ANOVA and Tukey's post-hoc tests.

Results: There was no significant difference in the survival rates of vitrified embryos at 2-cell, 4-cell, 8-cell, morula and blastocyst stages after thawing ($P>0.05$). The blastocyst formation rate of vitrified 8-cell embryos was significantly higher than that of 2-cell embryos ($P<0.05$). The hatching rate of vitrified 4-cell, 8-cell and blastocysts were significantly higher than that of 2-cell embryos ($P<0.05$).

Conclusion: Vitrification is suitable for cryopreservation of all stages of mouse embryonic development. However, the best tolerance for vitrification was observed at 4- and 8-cell stages of development. Accordingly, the development of vitrified embryos to blastocysts, following thawing, was most efficacious for 4 and 8-cell embryos. Compared to mouse 2-cell embryos, embryos vitrified as blastocysts had the highest rate of hatching.

Keywords: Vitrification, Embryo, Preimplantation

Citation: Ghandy N, Karimpur Malekshah AA. Which stage of mouse embryos is more appropriate for vitrification? Int J Fertil Steril. 2017; 10(4): 357-362.

Introduction

Cryopreservation of embryo and oocyte are two valuable methods, used to increase the success of infertility treatment. There are two common methods, used for the cryopreservation of embryos: slow freezing and vitrification (1). In slow freezing, embryos are exposed to a gradual decrease in temperature, and are then transferred to liquid nitrogen for storage.

Vitrification was initially reported in 1985 (2). In this method, the concentration of cryoprotectants is increased and embryos are directly plunged into liquid nitrogen so that they are

Received: 17 Nov 2015, Accepted: 16 Jul 2016
*Corresponding Address: P.O.Box: 4847191971, Department of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
Email: nasibeh.ghandi@gmail.com

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 357-362

cooled at a very high speed rate (over 20,000°C/minute). In this situation, intra- and extra-cellular liquids become solid without ice crystal formation. This kind of freezing, as a result, is called vitrification.

High speed cooling and warming rates are the most critical factors to preserve embryos during the process, which can prevent the formation of ice crystals in the intra- and extra-cellular space (3). This method has gained growing worldwide recognition among experts in assisted reproductive technology (ART) laboratories since it was initially used by researchers in an attempt to demonstrate the practicality of vitrification technique, which is commonly used for the cryopreservation of mammalian embryos. This can be attributed to the fact that vitrification has several noticeable merits, which can make it distinct from the conventional slow-freezing method (4). Notable advantages of vitrification over the conventional slow-freezing method are that is simpler, less expensive, more efficient and rapid. This can lead to higher survival and developmental rates when compared to the results of slow-freezing method (5, 6).

In one study has demonstrated that the efficiency of embryo cryopreservation depended not only on the cryopreservation method, but also on the developmental stage of the embryos (7).

Although several previous studies have already attempted to systematically evaluate the most suitable developmental stage for mouse embryo cryopreservation, there are conflicting results in the literature. Some studies have concluded 2-cell stage (8) is the best for vitrification while the others have noted 8-cell (4), morula (9) or blastocyst (1) as the most optimal stage for embryo cryopreservation and cryotolerance. Moreover, there have been a limited number of published studies investigating the reaction of different stages of mammalian embryo to the stress of cryopreservation and thawing (1, 4, 8-16).

With the limited number of published studies and the conflicting reports in mind, we decided to carry out our investigation. In this study, we vitrified mouse embryos at various developmental stages (2-cell, 4-cell, 8-cell, morula and blastocyst) through the cryotop method; so that we could determine the optimal embryonic developmental stage for cryopreservation. It is recognized that the results obtained through mouse embryos could not be extrapolated to human embryos. However, it is hoped that the findings of the current study could provide some empirical insights for prospective researchers with regard to the choice of an optimal developmental stage for the vitrification of human embryos.

Materials and Methods

Experimental design

In this experimental study, 2-cell stage mouse embryos were obtained from 6 to 8 weeks-old NMRI female mice superovulated with 7/5 IU of pregnant mare serum gonadotropin (PMSG, Hipra, Spain) given intraperitoneally. The process was followed by intraperitoneal injection of 7/5 IU of human chorionic gonadotropin (hCG, LG life sciences, Korea) after 48 hours. These mice were then mated with adult male mice of the same strain immediately after the injection of hCG, and were checked for mating the following day morning. The mated female mice were sacrificed by cervical dislocation 44-48 hours after the hCG injection, their oviducts were removed, and the 2-cell embryos were flushed from oviducts. This procedure was performed at room temperature (25°C) and the flushing medium was comprised of a Ham's F10 with HEPES (Ham's F10-HEPES, Sigma, Germany) and 20% Human Serum Albumin (HSA, Bio test, Germany). It is worth mentioning that only morphologically normal embryos were used for the sake of this study.

After the initial wash in flushing medium, 2-cell embryos were randomly divided into two groups. The embryos in the first group were vitrified, and those of the second group were transferred to T6 medium containing 10% HSA for continuous culture at 37°C, in the air with 6% CO_2 humidified incubator. The embryo culture process lasted for four days, following 52-54, 66-68, 72, 96 hours after hCG injection, embryos at 4-cell, 8-cell, Morula, Blastocyst stage, respectively, were isolated from culture medium, and they were subsequently vitrified.

Vitrification

Mouse embryos at 2-cell, 4-cell, 8-cell, moru-

la and blastocyst stages were vitrified by a two-steps procedure through the cryotop as a carrier, as described by Kuwayama et al. (5). Embryos were initially equilibrated in vitrification solution 1 (VS1) comprising 7.5% (v/v) ethylene glycol (EG, Sigma, Germany) and 7.5% (v/v) dimethyl sulfoxide (DMSO, Sigma, Germany) at room temperature for 8 to 10 minutes. They were subsequently placed in vitrification solution 2 (VS2) consisting of 15% (v/v) EG, 15% (v/v) DMSO and 0.5 mol/L sucrose (Sigma, Germany). In less than 2 minutes, 4-5 embryos in minimal vitrification solution were placed on the inner surface of the cryotop carrier (Kitazato, Japan). After that, the cryotop was vertically dipped into liquid nitrogen. Then it was inserted into a protective straw-cap before it was cryostored in liquid nitrogen.

Thawing

After cryo-storage for 10-14 days, the embryos were thawed. Briefly, the cryotop containing the embryos were removed from the protective straw-cap and dipped into thawing solution 1 (TS1), containing 1.0 mol/L sucrose, at 37°C. After 1 minute equilibration in TS1, the embryos were moved into thawing solution 2 (TS2), containing 0.5 M sucrose, for 3 minutes. Subsequently, embryos were washed for 5 minutes in three drops of Ham's F10-HEPES medium with 20% HSA at 37°C (4). After that, embryos were cultured in T6 medium with 10% HSA under mineral oil at 37°C, in air with 6% CO_2 humidified incubator.

The survival rate of embryos was assessed by observing the lightness and intactness of blastomers and zona pellucida. Development of the embryos were maintained and monitored until they reached the blastocyst and hatching stages. The survival rate, blastocyst formation, and the hatching rate were all recorded and compared among the groups.

Statistical analysis

The data were statistically analyzed using the Statistical Package for Social Sciences (SPSS, Version 15.0). One-way ANOVA and Tukey's post-hoc tests were used to compare and analyze the post-vitrification survival rates, blastocyst formation rates, and hatching rates among the experimental groups. Results have been reported as mean ± SD. A P value<0.05 was considered significant.

Ethical considerations

This project has been approved by the Ethic Committee of Mazandaran University of Medical Sciences (Sari, Iran) with code No. 91-108.

Results

Embryonic development, after vitrification and the subsequent thawing are summarized in Table 1. No statistically difference was observed in the post-vitrification survival rates of vitrified mouse embryos at the 2-cell (82.1%), 4-cell (83.2%), 8-cell (85.8%), morula (77%) and blastocyst (60.5%) stages (P>0.05) (Figs.1, 2). The blastocyst formation and hatching rate for the vitrified 2-cell embryos group were the lowest, 56.5% ± 23.4 and 21.2% ± 14.7 respectively (P<0.05). On the other hand, hatching rate of blastocysts were highest (59.8% ± 25.7, P<0.05). Hatching rate of vitrified 4-cell, 8-cell and blastocysts were significantly higher than that of 2-cell embryos (P<0.05).

Table 1: Mouse embryonic development after early cleavage-stage embryo vitrification-warming

Stage of vitrification	Embryos (n)	Survival rate (%) ± SD	Blastocysts formation (%) ± SD	Hatched (%) ± SD
Vitrified 2-cell	157	82.1% ± 16.8	56.5% ± 23.4	21.2% ± 14.7
Vitrified 4-cell	145	83.2% ± 11	79.8% ± 13.6	49.1% ± 25.5*
Vitrified 8-cell	166	85.8% ± 22.4	84% ± 21.2*	50.6% ± 25.7*
Vitrified morula	180	77% ± 17.8	75.8% ± 21	30% ± 16.3
Vitrified blastocyst	140	60.5% ± 22.3	-	59.8% ± 25.7*

*; Significantly different from those of 2-cell embryos within the same column (P<0.05).

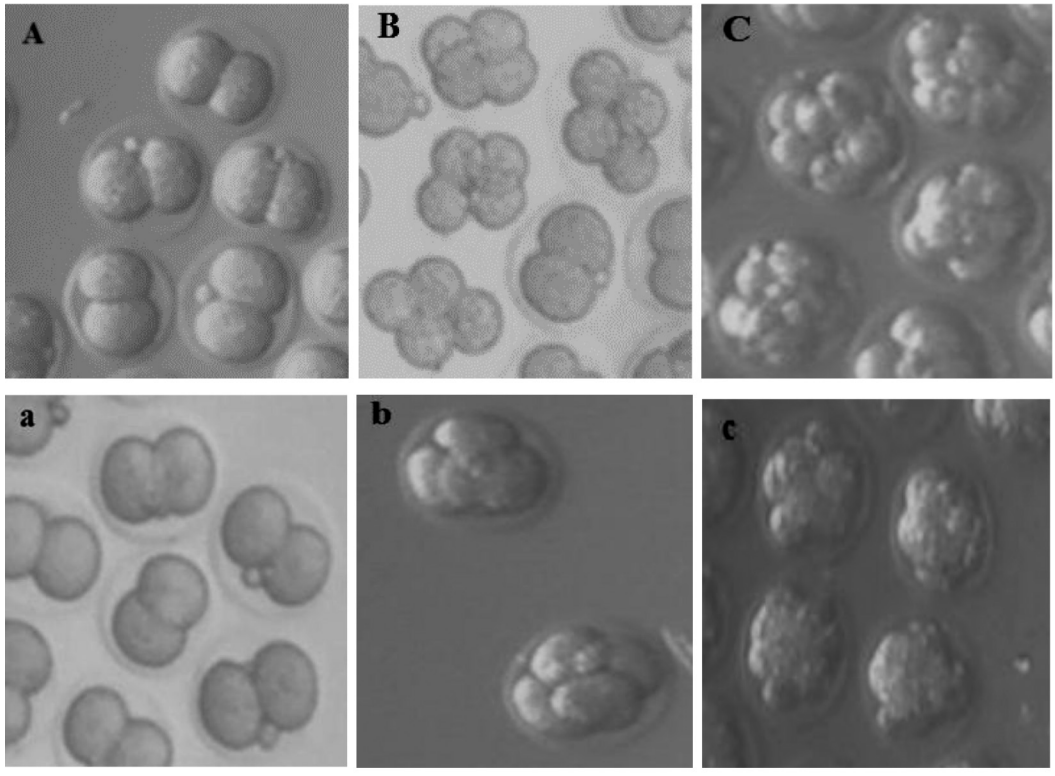

Fig.1: Different cleavage stages of fresh mouse embryos in the culture medium. **A.** 2-cell embryos, **B.** 4-cell embryos, **C.** 8-cell embryos. Different cleavage stages of vitrified mouse embryo 2 hours after thawing in the culture medium. **a.** 2-cell embryos, **b.** 4-cell embryos, and **c.** 8-cell embryos (magnification: ×200).

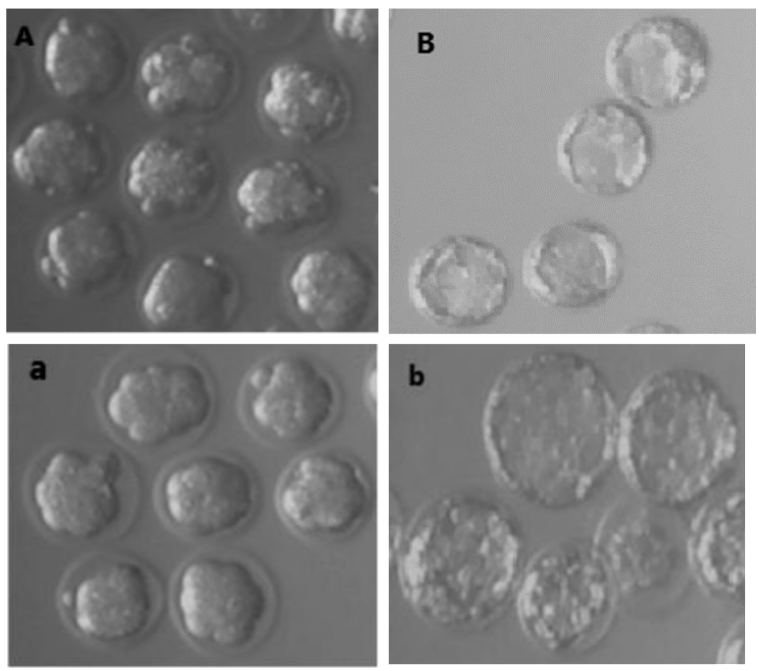

Fig.2: Different cleavage stages of fresh mouse embryos in the culture medium. **A.** Morula embryos, **B.** Blastocyst embryos. Different cleavage stages of vitrified mouse embryos 2 hours after thawing in the culture medium. **a.** Morula embryos and **b.** Blastocyst embryos (magnification: ×200).

Discussion

Despite the fact that there are many advances in the field of cryopreservation of embryo, there is no agreement on the optimal developmental stage for cryopreservation of embryo. In the current study, we vitrified different stages of mouse embryos through the Cryotop method. There was no significant difference in survival rate after thawing the vitrified embryos at 2-cell, 4-cell, 8-cell, morula and blastocyst stages. This finding is consistent with the results of studies performed by Zhou et al. (1) and Zhang et al. (4), which showed that there was no difference in survival rates among preimplantation mouse embryos. In contrast, Yan et al. (8) reported that mouse 2-cell embryos had the best tolerance for cryopreservation, but they used the open-pulled straw (OPS) method for vitrification. Differences in the survival rate may be due to the different strains of mice used in various studies. Investigators have shown that genotype could significantly influence post-thaw viability of frozen mouse embryos. In addition to genotype, such other factors as thawing temperature and the culture medium may also contribute to differences in the survival rates (17).

Zhou et al. (1) demonstrated that early blastocyst stage is the most feasible stage for mouse embryo cryopreservation. Blastocyst is more complex and different from the earlier stages of development, whereby it contains an inner cell mass, a trophoblastic layer and a blastocoelic cavity. Regarding to various cell types presented in blastocyst, there is a greater chance for different metabolic activities and permeability when compared to the earlier stages of development (18). Generally, developmental stage of blastocyst (19), types of the cryoprotectants (20), exposure time to equilibration with vitrification solutions (19) and type of the carriers (21) can all affect the survival rates of the blastocyst.

Although the survival rates for various embryonic stages were similar, in our study blastocyst formation in 2-cell stage embryos were significantly lower than that of 8-cell stage embryos. However, the rates of blastocyst formation for 4-cell, 8-cell and morula stages were similar. Zhang et al. (4), reported similar results to the demonstrated findings by Zhou et al. (1), conducted by OPS method, indicating that blastocyst rate of the vitrified 1-cell and 2-cell embryos were lower than those of the vitrified 4-cell, 8-cell and morula embryos.

Graves-Herring and Boone (22) cryopreserved mouse 2-cell and 8-cell embryos in either an open- or closed-Stripper Tip or an open- or closed-CBS nozzle. The 8-cell provided a higher blastocyst rate than the 2-cell stage embryos, vitrified using the same manner.

According to our research, hatching rate of 2-cell embryos was significantly lower than that of the 4-cell, 8-cell and blastocyst stages embryos. This finding correlates with that of Zhang et al. (4), indicating that hatching rate of the vitrified 2-cell embryos were significantly lower than that of the vitrified 8-cell embryos. In the vitrified 4-cell embryo group, hatching rate was significantly lower than that of the vitrified 8-cell embryo groups. Our study, however, showed a similar hatching rate for 4- and 8-cell embryos.

On the other hand, in a study performed by Păcală et al. (18), hatching rates of the morula stage embryos were higher than that of the blastocyst stage embryos. In this study, glycerol was used as a permeating cryoprotective agent. There is experimental proof that permeability co-efficiency of glycerol is increased from the 1-cell to the blastocyst stage in mice. After thawing, increase in glycerol absorption needs much time to efflux from the embryo (23).

It must be noted that is essential for 2-cell embryos to be kept in culture medium for several days until they reach their blastocyst and hatching stages. 2-cell embryos also need to withstand with any kind of stress, induced by cold, being out of the incubator for investigations and potential recordings, and above all, the harmful effects of culture medium during this period. It is logic that some embryos sustained damage by various prevailing peripheral stresses, leading to lower overall blastocyst formation and decreased hatching rate.

A study by Lane et al. (24) demonstrated that vitrification of hamster 2-cell embryos inhibited the activity of two transport proteins (Na+/H+ antiporter an HCO-/Cl- exchanger), which are responsible for the regulation of intracellular pH, playing a key regulatory role in metabolism, energy production, and cell division. It is proposed that similar reactions may affect mouse 2-cell embryos.

Conclusion

From the obtained results, it appears that developmental potential of mouse 2-cell embryos is more impacted by vitrification compared to 4-cell or 8-cell stage. The success of vitrification technique depends on the developmental stage of embryo and the utilized method. Cryotop method is suitable for vitrification of mouse cleavage-stage embryos from 2-cell stage to blastocyst, with the same effect on the survival rate. Embryos at 4-cell and 8-cell stages are at the most suitable options for vitrification, using cryotop method. They can show highest tolerance to the stress of freezing as well as thawing. They can also yield highest level of post-vitrification developmental competence among early cleavage stage embryos.

Acknowledgements

The authors would like to acknowledge the officials of Mazandaran University of Medical Sciences for their assistance and financial support. This paper was yielded as a research project (Consent No. 91-108) from Mazandaran University of Medical Sciences in Iran. We declare to have no conflict of interest.

References

1. Zhou GB, Hou YP, Jin F, Yang QE, Yang ZQ, Quan GB, et al. Vitrification of mouse embryos at various stages by open-pulled straw (OPS) method. Anim Biotechnol. 2005; 16(2): 153-163.
2. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at -196 degrees C by vitrification. Nature. 1985; 313(6003): 573-575.
3. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online. 2006; 12(6): 779-796.
4. Zhang J, Cui J, Ling X, Li X, Peng Y, Guo X, et al. Vitrification of mouse embryos at 2-cell, 4-cell and 8-cell stages by cryotop method. J Assist Reprod Genet. 2009; 26(11-12): 621-628.
5. Kuwayama M, Vajta G, Ieda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod Biomed Online. 2005; 11(5): 608-614.
6. Lin TK, Su JT, Lee FK, Lin YR, Lo HC. Cryotop vitrification as compared to conventional slow freezing for human embryos at the cleavage stage: survival and outcomes. Taiwan J Obstet Gynecol. 2010; 49(3): 272-278.
7. Leibo SP, Martino A, Kobayashi S, Pollard JW. Stage-dependent sensitivity of oocytes and embryos to low temperatures. Anim Reprod Sci. 1996; 42(1-4): 45-53.
8. Yan CL, Yang QE, Zhou GB, Hou YP, Zhao XM, Fan ZQ, et al. Open pulled straw (OPS) vitrification of in vitro fertilised mouse embryos at various stages. Acta Vet Hung. 2008; 56(2): 245-253.
9. Han MS, Niwa K, Kasai M. Vitrification of rat embryos at various developmental stages. Triogenology. 2003; 59(8): 1851-1863.
10. Miyake T, Kasai M, Zhu SE, Sakurai T, Machida T. Vitrification of mouse oocytes and embryos at various stages of development in an ethylene glycol-based solution by a simple method. Theriogenology. 1993; 40(1): 121-134.
11. Lian A, QU YX, Hung YX, Ma WW, Tang JM, Cheng GX. Comparison of vitrification cryopreservation among different stages of mouse embryos. Shi Yan Sheng Wu Xue Bao. 2003; 36(4): 279-282.
12. Cuello C, Gil MA, Parrilla I, Tornel J, Vázquez JM, Roca J, et al. Vitrification of porcine embryos at various developmental stages using different ultra-rapid cooling procedures. Theriogenology. 2004; 62(1-2): 353-361.
13. AbdelHafez F, Xu J, Goldberg J, Desai N. Vitrification in open and closed carriers at different cell stages: assessment of embryo survival, development, DNA integrity and stability during vapor phase storage for transport. BMC Biotechnol. 2011; 11: 29.
14. Ainul Bahiyah AB, Wan Khadijah WE, Abdullah RB. Comparison between direct plunging and vitrification techniques on development of mouse embryos at various preimplantation stages. J Anim Vet Adv. 2012; 11(2): 200-206.
15. Ling XF, Zhang JQ, Cao SR, Chen J, Peng Y, Guo X, et al. Effect of cryotop vitrification on preimplantation developmental competence of murine morula and blastocyst stage embryos. Reprod Biomed Online. 2009; 19(5): 708-713.
16. Manno FA 3rd. Cryopreservation of mouse embryos by vitrification: a mata-analysis. Theriogenology. 2010; 74(2): 165-172.
17. Valdez CA, Abas Mazni O, Takahashi Y, Hishinuma M, Kanagawa H. Effects of equilibration time, precooling and developmental stage on the survival of mouse embryos cryopreserved by vitrification. Theriogenology. 1990; 33(3): 627-636.
18. Păcală N, Ivan A, Cean A. Vitrification of mice embryos in different developmental stages using four vitrification methods. Biotechnol Biotechnol Equip. 2012; 26(5): 3324-3328.
19. Van Der Zwalmen P, Gaurois B, Ectors FJ, Touati K, Massip A, Ectors F. Some factors affecting successful vitrification of mouse blastocyst. Theriogenology. 1988; 30(6): 1177-1183.
20. Massip A, Van der Zwalmen P, Leroy F. Effect of stage of development on survival of mouse embryos frozen--thawed rapidly. Cryobiology. 1984; 21(5): 574-577.
21. Kader A, Choi A, Sharma RK, Falcone T, Agarwal A. Effect of varying equilibration time in a two-step vitrification method on the post-warming DNA integrity of mouse blastocysts. Fertil Steril. 2010; 93(8): 2640-2645.
22. Graves-Herring JE, Boone WR. Comparison of open and closed systems for vitrification. The Journal of Clinical Embryology. 2009; 12(3): 12-15.
23. Rall WF. Factors affecting the survival of mouse embryos cryopreserved by vitrification. Cryobiology. 1987; 24(5): 387-402.
24. Lane M, Lyons EA, Bavister BD. Cryopreservation reduces the ability of hamster 2-cell embryos to regulate intracellular pH. Hum Reprod. 2000; 15(2): 389-394.

Original Article

The Efficacy of Well-Being Therapy for Depression in Infertile Women

Majid Moeenizadeh, Ph.D.*, Haniyeh Zarif, M.A.

Department of Psychology, Faculty of Education and Psychology, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

Background: Infertility is a major public health problem with physical, psychological and social dimensions. High prevalence of psychological problems has been reported in infertile women. The objective of this study was to examine the effectiveness of well-being therapy (WBT) for depression in infertile women who were referred to an infertility center in Mashhad, Iran.

Materials and Methods: This preliminary trial was conducted at the Montasariya Infertility Center, Mashhad, Iran, between July and October 2011. A group of 22 infertile women were randomly assigned into experimental (n=11) and control groups (n=11). Patients were assessed with two self-rating inventories including the Psychological Well-being (PWB) and the Depression, Anxiety and Stress Scale-21 (DASS-21) before and after the interventions and the waiting-list period. WBT was performed in 8 to 10 sessions according to the published protocol.

Results: Analysis of covariance (ANCOVA) showed a significant difference regarding the depression scores of experimental group between pre- and post-treatment as compared to control subjects.

Conclusion: The results suggested the feasibility and clinical advantages of adding WBT to repertoire of the treatment techniques for depression in infertile women.

Keywords: Infertility, Depression, Psychological Well-Being, Well-Being Therapy

Citation: Moeenizadeh M, Zarif H. The efficacy of well-being therapy for depression in infertile women. Int J Fertil Steril. 2016; 10(4): 363-370.

Introduction

Infertility is a stressor that affects not only women but also their husbands. Infertility is more stressful and unacceptable for women, although the signs of depression in men/husbands have been indicated (1). In a study by Litt et al. (2), they have measured optimism, specific expectancies for fertilization success, coping strategies, and distress levels in people whose attempts were unsuccessful, approximately 8 weeks before the attempt. The results of this experimental study have showed that the distress returned just two weeks after notification of a negative pregnancy test.

In another recent study by Bleil et al. (3), they have examined the influence of optimism on infertility treatment among 198 women. The treatment results were categorized as successful and failed *in vitro* fertilization (IVF) treatment cycle. By the end of the 18-month study, the participants were divided into two groups of having delivered a baby and being pregnant due to cycle. At baseline, optimism and pessimism were also measured. They have indicated pessimism as a risk factor in failure of IVF treatment.

Lancastle and Boivin (4) have studied psychological variables on reproductive health on 97 women. Their primary outcomes were optimism, anxiety, and coping with problems three months before fertility treatment. The secondary outcomes were the biological response to treatment (e.g. es-

Received: 28 Aug 2014, Accepted: 19 Apr 2015
*Corresponding Address: P.O.Box:1518, Department of Psychology, Faculty of Education and Psychology, Ferdowsi University of Mashhad, Mashhad, Iran
Email: moeinizadeh@um.ac.ir

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 363-370

tradiol level). Their findings have shown that optimism is the result of its shared variance with neuroticism. They have concluded that psychological variable were significant indicators of a single concealed structure. In another study conducted in Greece, 137 infertile women were evaluated to measure stress (infertility-related stress, anxiety, symptoms of depression, and mood states), character peculiarities (neuroticism, extraversion, optimism) and coping strategies using the Appraisal of Life Events (ALE). The finding of study confirmed that ALE had a satisfactory reliability and convergent validity (5).

Although one of the main problems of the couples is primary infertility worldwide, the rate of secondary infertility increases in the third world countries, in which the cost of treatment as well as social and cultural factors are considered as the important factors in restricting access to fertility treatment (6).

The global infertility prevalence rates are difficult to determine, but according to the report by World Health Organization (WHO), published at the end of 2012, one in every four couples in developing countries had been found to be affected by infertility. Marriage as a social phenomenon is negatively affected by infertility that leads to poor physical and mental health of individuals (7).

Physical health and welfare

Positive emotions are the key points in improving well-being. According to Layous et al. (8), when moderating variables, such as positive behavior, positive thoughts and positive emotions, are accompanied by with positive activities, they diminish depressive symptoms and improve well-being. Such results led to represent the following model (Fig.1) which points out the effectiveness of cognitive interventions in reducing depressive symptoms and increasing positive well-being in the clients.

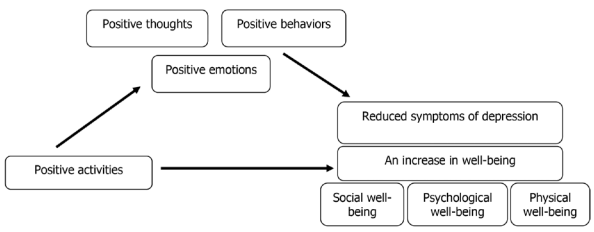

Fig.1: The effectiveness of positive psychology interventions in reducing depressive symptoms and increasing well-being.

It seems that there is an undeniable and clear relationship between positive emotions with physical health and well-being because illnesses are accompanied by pain and unhappiness. Disease is often recognized as an increasingly negative effect, while it may cause movement disorder which also decreases the positive effect and satisfaction (9).

Rayan and Frederick (10) have reported positive emotion of vivacity and vitality. According to their findings, mental vigor is the sign of organismic well-being that should be accompanied with both psychological and physical factors affecting the supply of energy to the individual.

Well-being therapy

Well-being therapy (WBT) enhancing positive psychology intervention was developed by Fava et al. (11-17) in Italy and validated in a number of controlled trials. WBT is based on an educational model which is structured, directive, and problem-oriented to present problems and situations. The duration of each session is 45 to 60 minutes. The therapy is over a period of eight weeks. WBT includes technique of self-observation along with the use of a structured diary and interaction between patients and therapists. The therapy sessions are divided into three phases-initial, intermediate and final. The first two sessions (the initial phase) are for identifying incidences of well-being and applying the rules into situational context regardless of its short period (13). Patients are requested to maintain report in the form of a structured diary to explain the circumstances surrounding the episodes of well-being, rated on a scale of 0 to 100, with 0 indicating absence of well-being and 100 indicating the most intense well-being. In the 3rd to 5th sessions (the intermediate phase), patients are encouraged to recognize thoughts and beliefs that lead to early cutting off of well-being. In the 6^{th} to 8^{th} sessions (final phase), patients are assessed according to following Ryff 's six dimensions of psychological well-being (PWB) scales: i. Environmental mastery, ii. Personal growth, iii. Purpose in life, iv. Autonomy, v. Self-acceptance, and vi. Positive relations with others (18). Therefore, the objective of this study was to examine the effectiveness of WBT for depression in infertile women who were referred to an infertility center in Mashhad, Iran.

Materials and Methods

In this preliminary trial, patients (n=22) were randomly assigned to an experimental group (n=11) and a control group (n=11) to find out the efficacy of WBT for depression in infertile women, with assessment before and after therapy.

Samples

The sample consisted of infertile women (20 to 40 years old) who visited the Montasariya Infertility Center, Mashhad, Iran, between July and October 2011. Twenty-two infertile women suffering from depressive disorders as per Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) criteria were chosen for the study using Krejcie and Morgan (19) sample size table.

Ethical consideration

We obtained the permission of the Montasariya Infertility Center to conduct this study. We also explained our aim to participants separately before they signed an informed consent, although we did not ask them to include their names in the questionnaire.

Assessments

PWB and the Depression, Anxiety and Stress Scale-21 (DASS-21) were used by the researcher prior to the treatment. The first and second sessions were spent on administrating Persian version of DASS-21 including a quantitative measure of distress along with the three axes of depression, anxiety and stress (20) and PWB consisting of subjective, social and psychological dimensions as well as health-related behaviors (21). The subjects were reassessed with the DASS-21 and PWB after treatment (8 sessions) by the same researcher who had performed the previous evaluations and who was blind to the treatment assignment. According to Samani and Jokar (20), the reliability and validity of DASS-21 ranged from 0.74 to 0.81. They also showed that the test-retest reliability values for depression, anxiety and stress were 0.80, 0.76, and 0.77, respectively, while Cronbach's alpha values for depression, anxiety and stress were 0.81, 0.74, and 0.78, respectively. Furthermore, for the validity of this scale, the confirmatory factor analysis and principal components method were utilized. Kaiser-Meyer-Olkin (KMO) measure was equal to 0.9012 and the Bartlett's test of sphericity (x2) was equal to 3092.93.

The second scale with 84 items was utilized to evaluate six dimensions of PWB as follows: i. Autonomy, ii. Environmental mastery, iii. Personal growth, iv. Positive relationships with others, v. Purpose in life, and vi. Self-acceptance. Each dimension was measured with 14 items. The original 20-item per scale version of the PWB was validated in a community-based sample of 321 men and women from multiple age groups (22). Analyses represented that each of the six scales had high levels of internal consistency with alpha coefficients ranging from 0.86 to 0.93. Test-retest reliability (over 6 weeks) was acceptable, ranging from 0.81 to 0.88 for the six scales. The scales also showed good construct validity with significant correlation with Bradburn's Affect Balance Scale (correlation coefficients ranged from 0.25 to 0.62), Neugarten's Life Satisfaction Index (LSI) (correlation coefficients ranged from 0.26 to 0.73, all $P<0.001$) and Rosenberg Self-Esteem Scale (RSES) (correlation coefficients ranged from 0.36 to 0.62, $P<0.001$). This scale was translated into Persian by this investigator. The reliability was calculated (n=30 students) and Chronbach coefficient alpha ranged from 0.88 to 0.96.

Procedure

In this 8-week program, the period of each session was 45-60 minutes. Self-observation accompanied with programmed diary and interaction between patients and therapists were employed in WBT group therapy. The treatment sessions were classified into three phases, initial, intermediate, and final. The first two sessions (the initial phase) were involved in identifying incidences of well-being as well as applying the instructions into situational context regardless of its short period (13). The patients were also asked to give a daily report in which the circumstances of well-being incidences, ranged from 0-100; 0 indicates lack of well-being and 100 illustrates full well-being. In the 3rd to 5th session (the intermediate phase), the patients were encouraged to recognize the thoughts and belief which led to early cutting off of well-being. In the 6th to 8th session (final phase), the patients were assessed by Ryff's dimensions of PWB scales as follows: i. Environ-

mental mastery, ii. Personal growth, iii. Purpose in life, iv. Autonomy, v. Self-acceptance, and vi. Positive relations with others (18). Therefore, the therapist's objective was to direct patients from a dysfunctional level to an optimal one based on the above-mentioned six dimensions.

During the therapy, the experimental group (n=11) was treated with standard WBT techniques, while the control group (n=11) was on the waiting list for therapy. Therefore, at the end of the sessions, post-assessment was conducted for each client using DASS-21 and PWB.

Statistical analysis

The obtained data from experiential and control groups were analyzed for normally distributed variables using Levene's test, and analysis of covariance (ANCOVA) was then utilized to assess the depression scores. Statistical significance was defined by $P<0.05$.

Results

Twenty-two infertility women were assigned into the WBT intervention group. Eleven (50%) women considered as experimental group had the mean age of 27.45 (SD=3.62), while the other 11 women (50%) presented as control group had the mean age of 28.18 (SD=4.29). Descriptive statistics and demographic characteristics are presented in Tables 1 and 2.

According to Levene's test, there are no significant differences regarding the scores of PWB between experimental and control groups, showing the equality of variances (Table 3). Therefore, presumption of ANCOVA for the depression score was established, indicating there are significant differences regarding depression scores between experimental and control groups (P=0.000, Table 4).

Table 1: Descriptive statistics for age and duration of infertility

	Demographic characteristics	n	Range	Minimum	Maximum	Mean	SD	SE
Experimental group	Age	11	11	23	34	27.45	3.62	1.09
	Duration of infertility	11	4	3	6	4.18	1.27	0.38
Control group	Age	11	15	22	37	28.18	4.29	1.29
	Duration of infertility	11	4	2	6	3.73	1.17	0.35

Table 2: Frequencies and percent statistics for age rate and education level

Age			Education level	Education	
Age rate	Experimental	Control		Experimental	Control
22-26	6 (55%)	5 (45%)	Diploma	7 (64%)	7 (64%)
27-31	3 (26%)	4 (36%)	Advanced diploma	2 (18%)	1 (9%)
32-37	2 (19%)	2 (19%)	Bachelor	2 (18%)	3 (27%)
Sum	11 (100%)	11 (100%)	Sum	11 (100%)	11 (100%)

Table 3: Data analysis using Levene's test for equality of variances and ANCOVA for PWB scales

Levene's test				ANCOVA					
F	df1	df2	Sig	Source changes	Sum squares	df	Mean squares	F value	P value
0.896	1	20	0.355	Pretest	12758.188	1	12758.188	41.110	0.000
				Group* pretest	25.113	1	25.113	0.081	0.779

ANCOVA; Analysis of covariance, F; Function, df; Degree of freedom, Sig; Significant level, *; Correlation is significant at the 0.05 level, and PWB scales; Psychological Well-being scales.

Table 4: Data analysis using Levene's test for equality of variances and ANCOVA for depression scores

Levene's test				ANCOVA					
F	**df1**	**df2**	**Sig**	**Source changes**	**Sum squares**	**df**	**Mean squares**	**F value**	**P value**
0.268	1	20	0.610	Pretest	159.381	1	159.381	25.827	0.000
				Group*pretest	0.638	1	0.638	0.103	0.751

ANCOVA; Analysis of covariance, F; Function, df; Degree of freedom, Sig; Significant level, and *; Correlation is significant at the 0.05 level.

The Cohen's effect size (23) and Cohen's d values were calculated for pre- and post- treatment for experimental and control groups (0.646 and 1.693, respectively), suggesting that the experimental group had greater effect than control group. The dimensions of PWB were also subjected to ANCOVA, indicating that the two groups showed significant differences in all dimensions (Table 5, Fig.2).

According to Table 5, it is clearly showed an increase in well-being state causes a decrease in depression, depicted in Table 6, as well.

It indicates that well-being state increased after treatment by WBT (Table 7).

Our findings are in agreement with the previous sources, reviewed in detail elsewhere (24). The present study showed that all infertile women who took part in the eight-session program improved more and showed close relationships, optimistic, happiness, innocence, success, and socialization than the infertile women who did not participate in the program. Therefore, the findings suggested that WBT intervention could be more effective. Furthermore, the correlation between post DASS-21 and post PWB scores was calculated. Also, Pearson correlation revealed (Table 8) that there is a significant negative relationship between (r=-0.601, P=0.003) well-being and depression scores, meaning that when well-being increases after treatment, depression decreases.

Table 5: Comparison of the Ryff's dimensions of PWB scales for pre- and post-treatment between experimental and control groups

Group	Pre exper		Pre control		Post exper		Post control		Significant	
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F value**	**P value**
Total PWB	297.72	32.64	300.45	27.63	383.45	35.02	289.90	25.79	41.11	0.000
Autonomy	44.36	5.85	45.54	5.69	59.72	7.26	42.54	4.56	16.951	0.001
Environment	44.36	5.85	45.54	5.69	59.72	7.26	42.54	4.56	15.288	0.001
Positive relation	54.72	7.73	54.45	3.41	67.63	7.47	53.45	4.74	6.132	0.023
Purpose in life	51.36	7.71	50.90	6.10	64.81	5.26	49.00	6.58	50.224	0.000
Personal growth	52.18	6.08	50.18	7.99	65.45	5.42	49.63	9.16	44.404	0.000
Self-acceptance	45.63	8.65	48.54	7.98	59.63	8.74	47.63	7.94	100.19	0.000

F; Function, df; Degree of freedom, Sig; Significant level, PWB scales; Psychological Well-being scales.

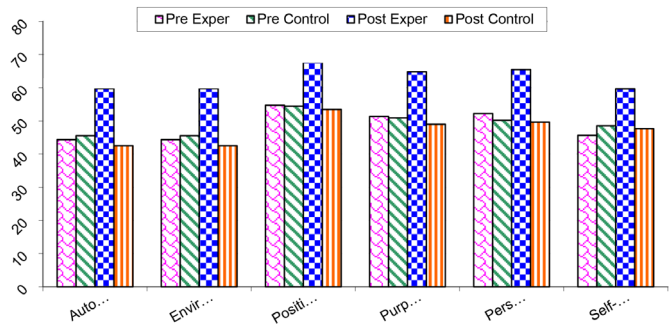

Fig.2: The differences regarding PWB scale pre- and post-treatment between experimental and control groups. PWB scales; Psychological well-being scales.

Table 6: Comparison of PWB and DASS-21scales for pre- and post-treatment between experimental and control groups

Groups	n=11	PWB scales		DASS-21 scale	
Experimental		Pre	Post	Pre	Post
	Mean	297.72	383.45	20.90	7.27
	SD	32.64	35.02	1.86	3.60
Control	Mean	300.45	289.90	21.09	23.09
	SD	27.63	25.79	2.87	4.03

All data are presented as mean ± SD. PWB scales; Psychological Well-being scales, and DASS-21 scale; Depression, Anxiety and Stress Scale-21 scale.

Table 7: Comparison of the effect size values of PWB and DASS-21scales between two groups

	Experimental group	Control group
Cohen's d	2.8559257217923335	8.10277696158679
Cohen's r (effect size)	0.8191171025474095	0.9708625601696644

PWB scales; Psychological Well-being scales, and DASS-21 scale; Depression, Anxiety and Stress Scale-21 scale.

Table 8: Comparison of Pearson correlation coefficients between scores of pre-post Dass21 and pre-post PWB scales

	Pre PWB	Post PWB	Pre DASS	Post DASS
Pre well-being Pearson correlation	1	0.497*	0.170	-0.217
Sig. (2-tailed)		0.019	0.448	0.332
N	22	22	22	22
Post well-being Pearson correlation	0.497*	1	0.066	-0.601**
Sig. (2-tailed)	0.019		0.771	0.003
N	22	22	22	22
Pre DASS Pearson correlation	0.170	0.066	1	0.448*
Sig. (2-tailed)	0.448	0.771		0.037
N	22	22	22	22
Post DASS Pearson correlation	-0.217	-0.601**	0.448*	1
Sig. (2-tailed)	0.332	0.003	0.037	
N	22	22	22	22

DASS21; Depression, Anxiety and Stress Scale, PWB scales; Psychological Well-being scales, *; Correlation is significant at the 0.05 level (2-tailed), and **; Correlation is significant at the 0.01 level (2-tailed).

Discussion

In a study by Fava et al. (25), they have evaluated the efficacy of WBT in patients with recurrent major depression disorder (MDD). Their results indicated that the level and severity of depressive symptoms in MDD patients after treatment with WBT were substantially reduced and a 6-year follow-up was also confirmed this content. In the latest research by Moeenizadeh and Kumar (26), it was pointed out that the application of WBT reduced the symptoms of depression and increased the psychological well-being. A question then arises that why the growth on positive psychology leads to a reduction in depressive symptoms in patients. The answer to this question should be searched in a study that examines the benefits of positive emotions and other feeling. In this regard, it should be noted that positive emotions not only make people feel good about themselves, but also influence the various aspects of a person's life such as marital satisfaction, interpersonal relationships, career success and good physical conditions (27). Lyubomirsky et al. (28) have shown a positive association between cognitive interventions and reduced symptoms of depression, leading to presence of positive emotions. Therefore, when moderating variables such as positive behavior, positive thoughts and positive emotions are accompanied by positive activities, depressive symptoms are replaced with happiness and feeling of well-being. Such results lead to represent the fol-

lowing model by Layous et al. (8), in which they have indicates the effectiveness of cognitive interventions in reducing depressive symptoms and increasing positive well-being in the clients.

In a meta-analysis of 51 studies, Sin and Lyubomirsky (29) have found that positive psychotherapy interventions were effective prospectively in increasing the well-being and reducing depressive symptoms. In another study by Wood and Joseph (30), they have shown that people earning low scores in PWB scales (regardless of the general structure or one of its constituent dimensions) are at the risk of depression. Their model has revealed that people with lower well-being are depressed seven times more than those with higher well-being in the next 10 years.

In Iran, the relationship between well-being and depression was assessed on 410 women in a study conducted by Ghodsi and Hojjatoleslami (31). The results of this study have revealed that there was no depression among women who had a high sense of well-being. According to their results, an increase in well-being is likely to elicit a reduced risk of depression. The researcher utilized a new method of therapeutic intervention of WBT on women suffering from infertility problems. Their findings are in line with other studies applying WBT to enhance psychological well-being in order to reduce emotional disorders and depression (11, 12).

As in current study, the use of WBT intervention caused to reduce the negative emotions such as depression and increase well-being. According to this treatment, the clients were encouraged to identify their positive emotions in different situations and try to improve them. Our findings revealed that the infertile women in experimental group showed a significant decrease in depression. About the effect sizes, experimental group also showed greater symptom reduction than control group, meaning that there is significant differences in PWB scores. Our results, therefore, indicated that treatment group showed significant improvements in all six dimensions of PWB, confirming the efficacy of this novel technique.

Perhaps the greatest limitation of this study is related to the lack of a follow-up program. Follow-up program could not be conducted because some of infertility women were in sensitive condition of infertility which prevented researchers from contacting them, or there were some limitations coming from their husbands/spouses. Furthermore, we only included the infertile women referred to Montasariya Infertility Center, so our findings cannot be generalized to other population.

Conclusion

The objective of this study was achieved through helping infertile women to reach optimal level of well-being and realize their true potential. Being pessimistic may be a risk factor for IVF treatment failure. Our results suggested the feasibility and clinical advantages of adding WBT to repertoire of the treatment techniques for depression in infertile women. The future studies are required to explain how pessimism can have a negative effect on results of therapeutic methods through biological and behavioral mechanisms.

Acknowledgements

The authors acknowledge the Montasariya Infertility Center for their support and cooperation to apply the modern technique of WBT for infertile women. Furthermore, we would like to extend our special thanks to all participants. There is no financial support and conflict of interest in this article.

References

1. Jordan C, Revenson TA. Gender differences in coping with infertility: a meta-analysis. J Behav Med. 1999; 22(4): 341-358.
2. Litt MD, Tennen H, Affleck G, Klock S. Coping and cognitive factors in adaptation to in vitro fertilization failure. J Behav Med. 1992; 15(2): 171-178.
3. Bleil ME, Pasch LA, Gregorich SE, Millstein SG, Katz PP, Adler NE, et al. Fertility treatment response: is it better to be more optimistic or less pessimistic? Psychosom Med. 2012; 74(2): 193-199.
4. Lancastle D, Boivin J. Dispositional optimism, trait anxiety, and coping: unique or shared effects on biological response to fertility treatment? Health Psychol. 2005; 24(2): 171-178.
5. Gourounti K, Anagnostopoulos F, Vaslamatzis G. Primary appraisal of infertility: evaluation of the psychometric properties of a Greek version of the Appraisal of Life Events scale (ALE) in a Sample of infertile women undergoing fertility treatment. Women Health. 2010; 50(7): 688-704.
6. Lunenfeld B, Van Steirteghem A; Bertarelli Foundation. Infertility in the third millennium: implications for the individual, family and society: condensed meeting report from the Bertarelli Foundation's second global conference. Hum Reprod Update. 2004; 10(4): 317-326.
7. Sadeghi S, Babapoor J, Salehi M. The comparison of marital satisfaction in fertile and infertile women. Nursing & Midwifery. 2007; 2(6): 25-30.

8. Layous K, Chancellor J, Lyubomirsky S, Wang L, Doraiswamy PM. Delivering happiness: translating positive psychology intervention research for treating major and minor depressive disorders. The Journal of Alternative and Complementary Medicine. 2011; 17(8): 675-683.
9. Ryan RM, Deci EL. On happiness and human potentials: a review of research on hedonic and eudaimonic well-being. Annu Rev Psychol. 2001; 52: 141-166.
10. Ryan RM, Frederick C. On energy, personality, and health: subjective vitality as a dynamic reflection of well-being. J Pers. 1997; 65(3): 529-565.
11. Fava GA, Rafanelli C, Grandi S, Conti S, Belluardo P. Prevention of recurrent depression with cognitive behavioral therapy: preliminary findings. Arch Gen Psychiatry. 1998; 55(9): 816-820.
12. Fava GA, Rafanelli C, Cazzaro M, Conti S, Grandi S. Well-being therapy. A novel psychotherapeutic approach for residual symptoms of affective disorders. Psychol Med. 1998; 28(2): 475-480.
13. Fava GA. Well-being therapy: conceptual and technical issues. Psychother Psychosom. 1999; 68(4): 171-179.
14. Fava GA, Mangelli L. Assessment of subclinical symptoms and psychological well-being in depression. Eur Archiv Psychiatry Clin Neurosci. 2001; 251 Suppl 2: II47-52.
15. Fava GA, Ruini C. Development and characteristics of a well-being enhancing psychotherapeutic strategy: well-being therapy. Journal of Behavior Therapy and Experimental Psychiatry. 2003; 34(1): 45-63.
16. Fava GA, Ruini C, Rafanelli C, Finos L, Salmaso L, Mangelli L, et al. Well-being therapy of generalized anxiety disorder. Psychotherapy and Psychosomatics. 2005; 74(1): 26-30.
17. Fava GA, Tomba E. Increasing psychological well-being and resilience by psychotherapeutic methods. J Pers. 2009; 77(6): 1903-1934.
18. Ryff CD, Singer B. Psychological well-being: meaning, measurement, and implications for psychotherapy research. Psychother psychosom. 1996; 65(1): 14-23.
19. Krejcie RV, Morgan DW. Determining Sample Size for Research Activities. Educational and Psychological Measurement. 1970; 30(3): 607-610.
20. Samani S, Jokar B. A study on the reliability and validity of the short form of the depression anxiety stress scale (DASS-21). Semi-annually Journal of Social Sciences and Humanities of Shiraz University. 2007; 26(3): 65-77.
21. Ryff CD. In the eye of the beholder: views of psychological well-being among middle-aged and older adults. Psychol Aging. 1989; 4(2): 195-201.
22. Ryff CD. Happiness is everything, or is it? Exploration on the meaning of psychological well-being. J Pers Soc Psychol. 1989; 57(6): 1069-1081.
23. Cohen JC, Schall R. Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. Am J Clin Nutr. 1988; 48(4): 1031-1034.
24. Ruini C, Fava GA. Well-being therapy for generalized anxiety disorder. J Clin Psychol. 2009; 65(5): 510-519.
25. Fava GA, Ruini C, Rafanelli C, Finos L, Conti S, Grandi S. Six-year outcome of cognitive behavior therapy for prevention of recurrent depression. Am J Psychiatry. 2004; 161(10): 1872-1876.
26. Moeenizadeh M, Kumar K. Well-being therapy (WBT) for depression. Int J Psychol Stud. 2010; 2(1): 107-115.
27. Lyubomirsky S, King L, Diener E. The benefits of frequent positive affect: does happiness lead to success? Psychol Bull. 2005; 131(6): 803-855.
28. Lyubomirsky S, Dickerhoof R, Boehm JK, Sheldon KM. Becoming happier takes both a will and a proper way: an experimental longitudinal intervention to boost well-being. Emotion. 2011; 11(2): 391-402.
29. Sin NL, Lyubomirsky S. Enhancing well-being and alleviating depressive symptoms with positive psychology interventions: a practice-friendly meta-analysis. J Clin Psychol. 2009; 65(5): 467-487.
30. Wood AM, Joseph S. The absence of positive psychological (eudemonic) well-being as a risk factor for depression: a ten year cohort study. J Affect Disord. 2010; 122(3): 213-217.
31. Ghodsi Z, Hojjatoleslami S. Depression in women and its relation with well-being, individual and social factors in Iran. Procedia - Social and Behavioral Sciences. 2011; 28: 553-557.

Original Article

Psychometric Properties of The Fertility Quality of Life Instrument in Infertile Iranian Women

Saman Maroufizadeh, M.Sc., Azadeh Ghaheri, M.Sc., Payam Amini, M.Sc., Reza Omani Samani, M.D.*

Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Background: Infertility and its treatment can have a considerable effect on a person's quality of life (QoL). The Fertility QoL (FertiQoL) questionnaire is currently the most frequently used instrument to measure QoL in people with fertility problems. This study aims to examine the reliability and validity of the FertiQoL in infertile Iranian women.

Materials and Methods: This cross-sectional study included 155 women with fertility problems in a referral fertility clinic in Tehran, Iran from January to March 2014. A battery of instruments was used: FertiQoL, Satisfaction with Life Scale (SWLS), Hospital Anxiety and Depression Scale (HADS), and a demographic questionnaire. Construct validity of the scale was evaluated using confirmatory factor analysis (CFA). We assessed internal consistency with Cronbach's alpha and convergent validity was examined by correlating the FertiQoL with SWLS and HADS.

Results: The results of the CFA generally supported the four-factor model of Core FertiQoL and two-factor model of Treatment FertiQoL. Both FertiQoL modules and their subscales revealed acceptable internal consistency that ranged from 0.643 to 0.911. However, the FertiQoL might be improved if Q15 and T2 items were removed from the scale. These items had low loadings on the Relational and Environment factors which decreased their internal consistency. The FertiQoL and their subscales significantly correlated with both SWLS and HADS, which confirmed convergent validity.

Conclusion: The Persian version of the FertiQoL is a valid, reliable instrument to measure QoL in infertile women and seems to perform as well as the original English Version.

Keywords: Infertility, Quality of Life, Validity, Reliability

Citation: Maroufizadeh S, Ghaheri A, Amini P, Omani Samani R. Psychometric properties of the fertility quality of life instrument in infertile Iranian women. Int J Fertil Steril. 2017; 10(4): 371-379.

Introduction

Infertility is a global public health issue that affects approximately 10-15% of reproductive-aged couples worldwide (1). It reduces quality of life (QoL), especially through negative psychosocial and cultural consequences. Often-cited repercussions of infertility are depression, anxiety, social isolation and deprivation, marital instability, loss of self-esteem and self-confidence, loss of gender identity, loss of control, and feeling of self-blame and guilt (2-4). Growing bodies of research have shown that infertility and its treatments have a significant negative impact on a person's QoL (5-10). Due to this impact, assessing QoL in infertile patients, especially for women is important (7).

The World Health Organization (WHO) has defined QoL as 'an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns' (11). QoL can be assessed by both generic and disease-specific tools (12). Previously, various generic self-reported instruments have been used to assess QoL in infertile patients (13, 14). Recently, an international group of experts in several countries and from various profes-

Received: 6 Feb 2016, Accepted: 11 Jul 2016
*Corresponding Address: P.O.Box: 16635-148, Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: samani@royaninstitute.org

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 371-379

sions developed the Fertility QoL (FertiQoL) tool, which is disease-specific and assesses QoL in men and women that suffer from infertility (15).

The FertiQoL tool consists of two modules: Core FertiQoL and Treatment FertiQoL. The Core FertiQoL module assesses the impact of infertility in diverse life areas such as general health, self-esteem, emotions, partnership, family and social relationships, work life, and future life plans. The optional Treatment FertiQoL module assesses the burden or tolerability of fertility treatment. The FertiQoL has been used in various cultures and populations, and has been translated into 26 languages. However, few studies examined the psychometric properties of the FertiQoL. Initial psychometric properties of the FertiQoL were evaluated by Boivin et al. (15) in the USA, Australia/New Zealand, Canada and the UK. Their study demonstrated acceptable validity and reliability. In another study, reliability and convergent validity of the Dutch version of the FertiQoL were evaluated in infertile women. The Dutch version of the FertiQoL showed satisfactory internal consistency and had a negative correlation with anxiety and depression, which indicated acceptable convergent validity (16). In the Portuguese population, the results of confirmatory factor analysis (CFA) showed a good fit to the original measurement model and all FertiQoL domains were reliable (Cronbach's alpha: 0.72 to 0.90) (17).

To the best of our knowledge, no studies evaluated the psychometric properties of the Persian version of the FertiQoL. Therefore, the present study aimed to examine the reliability and validity of the FertiQoL in infertile Iranian women.

Materials and Methods

Patients

We conducted this cross-sectional study at Royan Institute, Tehran, Iran from January 2014 to March 2014. The Infertility Clinic of Royan Institute is a referral infertility center which provides comprehensive treatment, including assisted reproduction techniques (ART). The inclusion criteria for this study were as follows: i. Women aged 18-45 years; ii. Diagnosed with couple infertility; and iii. Ability to read and write in Persian. Participants were selected through convenient sampling from infertile women in the embryo transfer stage of ART cycles at Royan Institute. The sample size was calculated at 120 patients, considering that 5 patients were necessary for each item (subject-to-item ratio: 5:1). As a rule of thumb, a minimum sample size of 100 would be enough for a psychometric study (18). In total, 155 women agreed to participate and completely filled out the questionnaires.

Ethical approval

The Ethics Committee of Royan Institute, Tehran, Iran approved the study protocol. All participants were fully informed about the study's scope and purpose, and the confidentiality of the data. Eligible women were also assured that the data would be used only for the purpose of the study and acceptance or refusal to participate in the research had no influence on their current or future treatments. A verbal informed consent was obtained from all participants before data collection.

Instruments

Fertility Quality of Life Tool

FertiQoL is a self-report instrument that assesses QoL in individuals with fertility problems (15). FertiQoL is composed of two modules: the Core FertiQoL and Treatment FertiQoL. The Core FertiQoL module consists of 26 items. Two items are general and 24 items specific to infertility that cover four subscales of the QoL (i.e., 6 items per subscale). The four subscales are as follows: Emotional, Mind-Body, Relational, and Social. The optional Treatment FertiQoL module is composed of ten items that assess the following two subscales: Environment (6 items), and Tolerability (4 items). The FertiQoL yields 6 subscales and 2 total scores with a range of 0-100, with higher scores indicative of better QoL. The FertiQoL is a free to use instrument and the Persian version of FertiQoL is available at: www.fertiqol.org. The translation from English to Persian was performed by professional translators from Cardiff University. This paper's first author assisted in the translation process by checking the Cardiff researchers' word usage against local word use.

Satisfaction with Life Scale

The Satisfaction with Life Scale (SWLS) is a short 5-item instrument designed to measure global cogni-

tive judgments of satisfaction with one's life. Each item was scored on a 7-point Likert scale that ranged from 1 (strongly disagree) to 7 (strongly agree). Scale scores range from 5-35, with higher scores indicative of greater life satisfaction (19). The Persian version of the SWLS had adequate psychometric properties in the Iranian populations (20). The Cronbach's alpha coefficient for the SWLS was 0.872 in the present study.

Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a 14-item self-report inventory composed of two subscales: Anxiety (HADS-A) and Depression (HADS-D). Both subscales of HADS consist of 7 items with each item scored on a 4-point Likert scale that ranges from 0 to 3. Subscale scores range from 0-21, with higher scores indicating higher level of anxiety and depression, respectively. We have used the Persian version of HADS in the present study. This version has previously been shown to have satisfactory reliability and validity (21). The Cronbach's alpha coefficient for HADS-A was 0.840 whereas for HADS-D, it was 0.733 in the present study.

Statistical analysis

The factor structure of the Core FertiQoL and Treatment FertiQoL were examined by CFA. These models were tested using covariance matrices and the maximum likelihood estimation method. Goodness of fit of models were assessed using the chi-square (χ^2), relative chi-square (χ^2/df), the comparative fit index (CFI), the root mean square error of approximation (RMSEA), and the standardized root mean square residual (SRMR). The χ^2 statistic is the classical measure for evaluating model fitness, but it is highly sensitive to sample size (22). Therefore we have used χ^2/df as an alternative index to examine the model fit. A χ^2/df ratio of less than 3 is considered indicative of a good fit (23). For other goodness of fit indices, acceptable thresholds are CFI>0.90, RMSEA<0.07 and SRMR<0.08 (24). We have used Cronbach's alpha to measure the internal consistency of the FertiQoL. Values above 0.80 were considered excellent, 0.70-0.80 satisfactory, and 0.60-0.70 acceptable (25). Convergent validity of the FertiQoL was assessed by calculating its Pearson correlation coefficients with SWLS and HADS.

All data analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA), except for the CFAs, which were performed using Lisrel 8.80 (Scientific Software International, Inc., Lincolnwood, IL, USA). All statistical tests were two-tailed and a P value of less than 0.05 was considered statistically significant.

Results

Participant characteristics

The demographic and fertility characteristics of the women are presented in Table 1. The mean age of women was 31.03 ± 5.89 years. Among all participants, 45.8% had male factor infertility, 43.2% had a university education, 40.6% had no previous treatments, and 82.6% had no history of abortion. The mean duration of infertility was 6.25 ± 4.36 and 78.7% of women had primary infertility.

Table 1: Demographic and fertility characteristics of the participants (n=155)

	Mean ± SD or n (%)
Age (Y)	31.03 ± 5.89
Duration of infertility (Y)	6.25 ± 4.36
Cause of infertility	
Male factor	71 (45.8)
Female factor	29 (18.7)
Both	26 (16.8)
Unexplained	29 (18.7)
Type of infertility	
Primary	122 (78.7)
Secondary	33 (21.3)
Education level	
Primary	29 (18.7)
Secondary	59 (38.1)
University	67 (43.2)
Failure of previous treatment	
0	63 (40.6)
1	41 (26.5)
2	20 (12.9)
3	20 (12.9)
≥4	11 (7.1)
History of abortion	
No	128 (82.6)
Yes	27 (17.4)

Confirmatory factor analysis

We used the CFAs to determine the goodness of fit for the four-factor model of Core FertiQoL and two-factor model of Treatment FertiQoL. Although the χ^2 value of the Core FertiQoL model was not satisfactory (χ^2=410.80, df=252, P<0.001), the relative chi-square (χ^2/df=1.63) was satisfactory. Examination of other goodness of fit indices indicated that the model provided an acceptable fit to the data, with CFI=0.96, RMSEA=0.064, and SRMR=0.067. All factor loadings were significant, except for Q15 (0.16), which ranged from 0.36 to 0.97 (Table 2).

Table 2: Confirmatory factor analysis (CFA) of the Core Fertility Quality of Life (FertiQoL) in infertile women

	Subscale-item	Factor loading (SE)
	Emotional	
Q4	Do you feel able to cope with your fertility problems?	0.36 (0.10)
Q7	Do your fertility problems cause feelings of jealousy and resentment?	0.68 (0.09)
Q8	Do you experience grief and/or feelings of loss about not being able to have a child (or more children)?	0.91 (0.09)
Q9	Do you fluctuate between hope and despair because of fertility problems?	0.82 (0.08)
Q16	Do you feel sad and depressed about your fertility problems?	0.93 (0.08)
Q23	Do your fertility problems make you angry?	0.92 (0.09)
	Mind/body	
Q1	Are your attention and concentration impaired by thoughts of infertility?	0.75 (0.09)
Q2	Do you think you cannot move ahead with other life goals and plans because of fertility problems?	0.76 (0.09)
Q3	Do you feel drained or worn out because of fertility problems?	0.97 (0.09)
Q12	Do your fertility problems interfere with your day-to-day work or obligations?	0.61 (0.09)
Q18	Are you bothered by fatigue because of fertility problems?	0.89 (0.09)
Q24	Do you feel pain and physical discomfort because of your fertility problems?	0.65 (0.08)
	Relational	
Q6	Are you satisfied with your sexual relationship even though you have fertility problems?	0.50 (0.08)
Q11	Are you and your partner affectionate with each other even though you have fertility problems?	0.54 (0.10)
Q15	Have fertility problems strengthened your commitment to your partner?	0.16 (0.11)
Q19	Have fertility problems had a negative impact on your relationship with your partner?	0.82 (0.08)
Q20	Do you find it difficult to talk to your partner about your feelings related to infertility?	0.52 (0.09)
Q21	Are you content with your relationship even though you have fertility problems?	0.57 (0.09)
	Social	
Q5	Are you satisfied with the support you receive from friends with regard to your fertility problems?	0.51 (0.08)
Q10	Are you socially isolated because of fertility problems?	0.94 (0.10)
Q13	Do you feel uncomfortable attending social situations like holidays and celebrations because of your fertility problems?	0.95 (0.09)
Q14	Do you feel your family can understand what you are going through?	0.35 (0.11)
Q17	Do your fertility problems make you inferior to people with children?	0.84 (0.10)
Q22	Do you feel social pressure on you to have (or have more) children?	0.82 (0.09)

SE; Standard error.

The CFA for two-factor model of Treatment FertiQoL showed a significant $\chi 2$ value ($\chi 2$=64.35, df=34, P=0.001). The relative chi-square was 1.89, which indicated that the model was an acceptable fit to data. The other fit indices were CFI=0.91, RMSEA=0.076, and SRMR=0.071. All factor loadings were significant, except for T2 (0.02), which ranged from 0.35 to 0.74 (Table 3).

Reliability analysis

Table 4 shows Cronbach's alpha coefficients of the Core FertiQoL, Treatment FertiQoL, and their subscales. Both module of FertiQoL and their subscales revealed acceptable internal consistency that ranged from 0.643 to 0.911.

Convergent validity

In order to examine the convergent validity of the FertiQoL, we calculated Pearson correlation coefficients between FertiQoL, SWLS, and HADS (Table 5). As expected, the Core FertiQoL and their subscales showed significant positive correlation with the SWLS (range: 0.375 to 0.488) and negative correlation with the HADS-A (range: -0.488 to -0.632) and the HADS-A (range: -0.501 to -0.662), which indicated acceptable convergent validity.

Table 3: Confirmatory factor analysis (CFA) of the Treatment Fertility Quality of Life (FertiQoL) in infertile women

	Subscale-item	Factor loading (SE)
	Environment	
T2	Are the fertility medical services you would like available to you?	0.02 (0.10)
T5	Do you feel the fertility staff understand what you are going through?	0.46 (0.08)
T7	Are you satisfied with the quality of services available to you to address your emotional needs?	0.65 (0.07)
T8	How would you rate the surgery and/or medical treatment(s) you have received?	0.57 (0.06)
T9	How would you rate the quality of information you received about medication, surgery and/or medical treatment?	0.60 (0.07)
T10	Are you satisfied with your interactions with fertility medical staff?	0.54 (0.08)
	Tolerability	
T1	Does infertility treatment negatively affect your mood?	0.66 (0.11)
T3	How complicated is dealing with the procedure and/ or administration of medication for your infertility treatment(s)?	0.35 (0.09)
T4	Are you bothered by the effect of treatment on your daily or work related activities?	0.73 (0.10)
T6	Are you bothered by the physical side effects of fertility medications and treatment?	0.74 (0.11)

SE; Standard error.

Table 4: Descriptive statistics and Cronbach's alpha coefficients of the Core Fertility Quality of Life (FertiQoL) and Treatment FertiQoL in infertile women

		Reliability analysis		Descriptive statistics	
	Subscale	Number of items	Cronbach's alpha	Mean	SD
Core FertiQoL	Emotional	6	0.817	53.4	21.4
	Mind/Body	6	0.821	62.1	21.4
	Relational	6	0.643	70.9	16.5
	Social	6	0.750	63.9	21.1
	Total scale	24	0.910	62.6	16.9
Treatment FertiQoL	Environment	6	0.672	61.3	14.2
	Tolerability	4	0.643	54.0	19.2
	Total scale	10	0.693	58.4	12.9

Table 5: Pearson correlation coefficients between FertiQoL and the SWLS, HADS-A, and HADS-D in infertile women (n=155)

			HADS	
	Subscale	**SWLS**	**HADS-A**	**HADS-D**
Core FertiQoL	Emotional	0.375***	-0.503***	-0.529***
	Mind/body	0.421***	-0.576***	-0.622***
	Relational	0.440***	-0.488***	-0.501***
	Social	0.410***	-0.550***	-0.562***
	Total scale	0.488***	-0.632***	-0.662***
Treatment FertiQoL	Environment	0.251**	-0.146	-0.157
	Tolerability	0.246**	-0.262***	-0.382***
	Total scale	0.313***	-0.253**	-0.332***

FertiQoL; Fertility Quality of Life, HADS; Hospital Anxiety Depression Scale, SWLS; Satisfaction with Life Scale, HADS-A; HADS-Anxiety, HADS-D; HADS-Depression, **; P<0.01, and ***; P<0.001.

Relationship of the FertiQoL with demographic characteristics

As presented in Table 6, there were no significant relationships between Core FertiQoL and age (P=0.620), durations of infertility (P=0.165), and history of abortion (P=0.927). A significant difference existed among the groups in terms of their treatment failures on the Core FertiQoL; Duncan's post hoc test revealed that women with two failures in treatment had lower QoL than women without failure and women with ≥4 failures (P<0.05). There was a direct relationship between Core FertiQoL and educational level (P=0.009). Regarding the cause of infertility, the mean Core FertiQoL was lower among women who had both factors and unknown cause of infertility than other participants (P<0.05). The relationships between Treatment FertiQoL and demographic characteristics are shown in Table 6.

Table 6: Relationship of Fertility Quality of Life (FertiQoL) with demographic and clinical characteristics in infertile women

	Core FertiQoL					**Treatment FertiQoL**		
	Emotional	**Mind/Body**	**Relational**	**Social**	**Total**	**Environment**	**Tolerability**	**Total**
Age (Y)								
<30	49.6 ± 18.7	60.8 ± 19.2	72.3 ± 14.7	62.6 ± 18.8	61.3 ± 14.2	61.9 ± 13.1	54.1 ± 17.3	58.8 ± 11.8
30-35	55.6 ± 22.5	60.7 ± 24.5	72.4 ± 15.9	64.0 ± 25.3	63.2 ± 19.7	61.2 ± 13.1	55.2 ± 20.7	58.8 ± 12.7
≥35	58.9 ± 24.2	66.6 ± 22.1	66.2 ± 19.9	66.4 ± 20.9	64.5 ± 19.0	60.2 ± 17.7	52.4 ± 21.5	57.1 ± 15.3
P value	0.070	0.356	0.146	0.664	0.620	0.854	0.809	0.790
Duration of infertility (Y)								
<3	56.5 ± 17.9	66.4 ± 19.0	72.2 ± 17.4	69.2 ± 20.4	66.1 ± 15.8	60.1 ± 11.6	52.8 ± 17.9	57.2 ± 10.8
3-6	53.3 ± 22.8	61.7 ± 21.4	71.9 ± 17.9	64.4 ± 20.5	62.8 ± 17.3	57.9 ± 15.1	51.8 ± 19.8	55.5 ± 13.8
≥6	51.0 ± 22.3	59.2 ± 23.0	68.6 ± 14.0	59.1 ± 21.6	59.5 ± 17.1	66.0 ± 14.1	57.4 ± 19.4	62.6 ± 12.4
P value	0.459	0.266	0.480	0.064	0.165	0.008	0.266	0.010
Cause of infertility								
Male factor	55.8 ± 21.4	65.7 ± 20.4	72.5 ± 16.4	65.2 ± 20.4	64.8 ± 16.7	62.6 ± 14.1	58.0 ± 18.5	60.7 ± 13.0
Female factor	59.6 ± 22.2	67.0 ± 24.2	75.3 ± 15.8	70.7 ± 21.9	68.1 ± 17.8	64.5 ± 14.8	53.0 ± 21.0	59.9 ± 13.6
Both	47.9 ± 20.6	53.2 ± 19.7	66.5 ± 18.3	62.5 ± 20.7	57.5 ± 16.4	61.4 ± 12.5	49.3 ± 19.6	56.5 ± 12.1
Unexplained	46.3 ± 19.1	56.5 ± 19.7	66.2 ± 14.4	55.2 ± 20.2	56.0 ± 14.3	54.9 ± 14.1	49.4 ± 17.2	52.7 ± 11.1
P value	0.038	0.017	0.074	0.037	0.010	0.046	0.090	0.028

Table 6: Continued.

	Core FertiQoL					Treatment FertiQoL		
	Emotional	**Mind/Body**	**Relational**	**Social**	**Total**	**Environment**	**Tolerability**	**Total**
Type of infertility								
Primary	52.8 ± 21.2	62.0 ± 21.5	71.2 ± 17.0	63.5 ± 22.1	62.4 ± 17.3	62.7 ± 13.4	54.9 ± 19.4	59.6 ± 11.9
Secondary	55.7 ± 22.1	62.8 ± 21.6	69.4 ± 14.5	65.4 ± 16.9	63.3 ± 15.4	56.1 ± 16.2	50.8 ± 18.3	53.9 ± 15.3
P value	0.494	0.850	0.580	0.645	0.776	0.017	0.277	0.026
Educational level								
Primary	48.7 ± 21.0	58.8 ± 22.8	66.2 ± 13.6	58.0 ± 22.6	57.9 ± 16.6	66.7 ± 12.1	56.0 ± 20.9	62.4 ± 12.5
Secondary	50.1 ± 21.7	58.1 ± 21.9	68.6 ± 16.5	61.2 ± 20.7	59.5 ± 16.5	60.0 ± 14.5	53.5 ± 21.1	57.4 ± 14.5
University	58.4 ± 20.5	67.2 ± 19.6	74.8 ± 17.0	68.8 ± 20.0	67.3 ± 16.4	60.1 ± 14.5	53.5 ± 16.8	57.5 ± 11.4
P value	0.038	0.037	0.027	0.033	0.009	0.078	0.819	0.173
Failure of treatment								
0	59.7 ± 17.9	68.8 ± 19.7	70.4 ± 15.3	67.9 ± 19.4	66.7 ± 14.6	63.7 ± 13.3	60.2 ± 17.0	62.3 ± 11.9
1	52.0 ± 21.3	60.2 ± 22.0	70.5 ± 18.9	61.7 ± 23.2	61.1 ± 18.5	58.5 ± 14.3	45.6 ± 18.9	53.4 ± 12.0
2	39.8 ± 23.6	51.5 ± 21.0	67.1 ± 18.6	56.3 ± 23.9	53.6 ± 18.0	60.0 ± 17.5	47.5 ± 18.4	55.0 ± 12.3
3	45.2 ± 19.9	55.6 ± 18.3	72.8 ± 13.2	61.7 ± 16.2	58.8 ± 14.4	60.8 ± 13.9	53.4 ± 15.2	57.9 ± 11.7
≥4	62.1 ± 23.8	62.1 ± 25.2	78.0 ± 15.5	67.0 ± 22.8	67.3 ± 19.1	61.0 ± 13.7	62.5 ± 25.9	61.6 ± 17.9
P value	0.001	0.008	0.493	0.211	0.019	0.480	0.001	0.006
History of abortion								
No	53.1 ± 22.0	62.3 ± 22.2	70.9 ± 17.0	63.9 ± 22.0	62.5 ± 17.5	62.8 ± 13.5	54.9 ± 19.5	59.6 ± 12.2
Yes	55.1 ± 18.5	61.4 ± 17.9	70.8 ± 14.0	64.0 ± 16.6	62.8 ± 13.9	54.2 ± 15.5	49.8 ± 17.5	52.4 ± 14.7
P value	0.656	0.852	0.993	0.969	0.927	0.004	0.209	0.008

Values are mean ± SD.

Discussion

The present study has aimed to evaluate the psychometrics properties of the FertiQoL in a sample of infertile women. FertiQoL is an infertility-specific questionnaire. In contrast to similar generic measures, it limits the factors that affect QoL to only infertility and no other stressful events. To our knowledge, this is the first study that has evaluated the factor structure of FertiQoL after a study by Melo et al. (17). The four-factor model of Core FertiQoL and two-factor model of Treatment FertiQoL were tested. In general, the Core and Treatment FertiQoL provided an acceptable fit to data. All factor loadings were significant, except for Q15 and T2. The model fit indices were acceptable similar to a study conducted by Melo et al. (17). The Core FertiQoL and their subscales showed satisfactory internal consistency, except for the Relational subscale (0.643) which had better reliability after removal of Q15 (0.689). The Treatment FertiQoL and their subscales showed acceptable internal consistency (0.6-0.7); at the same time reliability of Environment subscale improved after we removed item T2 (0.771). These findings indicated that some modifications for item Q15 and T2 might be needed in the scale to yield better internal consistency. A cross-cultural difference might contribute to these results.

Our finding confirmed the expected direct relationship between Core FertiQoL and SWLS, which indicated an acceptable convergent validity. As anticipated, the Core FertiQoL and its subscales negatively correlated with anxiety and depression. Infertile women with a high Core FertiQoL score reported lower levels of anxiety or depression and vice versa. These results supported previous studies and confirmed the convergent validity of Core FertiQoL (16, 26).

We also investigated the relationship between demographic characteristics and QoL. Although the difference was not statistically significant, on average, older women reported higher Core FertiQoL, Mind-Body, Emotional and Social subscales. Conversely, older women have reported lower Relational scores than younger women, but this difference was not significant. In general therefore, as women with infertility over 35 are considered old to be pregnant, their sexual relationship seems more pointless. This finding was roughly consistent with Aarts et al. (16). The results of this study did not show a significant relationship between Core FertiQoL and duration of infertility. The same results were reported by Rashidi et al. (13) and Keramat et al. (27). In contrast, women with lower infertility duration had lower Treatment FertiQoL. This result might be explained by the fact that infertile women become more aware of the treatment process over time.

A direct relationship was found with the Core FertiQoL and its subscales in terms of education level; in other words, the higher the education level, the greater the QoL. This result agreed with previous findings from Chachamovich et al. (7) and Rashidi et al. (13). Conversely, we observed lower Treatment FertiQoL among women with higher education. This result was inconsistent with the findings of Karabulut et al. (28). Women with two failures scored lower than other women on both Core and Treatment modules and their subscales, except for the Relational and Environment subscale. This results indicated that women with two failures might suffer from lower QoL and need to be supported by family, friends, and society (29). Psychological intervention, especially those that emphasize stress management and coping-skills training, might improve QoL in these women through affecting bio-psychological dimensions. We have found worse QoL in women whose source of infertility was both and unknown. Possibly when the problem is attributable to both there is no hope for gamete donation anymore. When the cause of infertility is unknown the roles are vague so the supportive role cannot be played by either of the couples to improve their QoL. Our study has found no association between Core FertiQoL and history of abortion. In contrast, women with abortion reported lower Treatment FertiQoL score than women with no abortion. This result may be explained by the fact that centers explain neither details of procedures nor the success rate of each procedure to the patients properly; this fact is what women with abortions know better. On the other hand these women are less assured about successful deliveries and expect the centers follow them until delivery rather than just releasing them when they are diagnosed pregnant.

Limitations of this study should be considered. First, the FertiQoL can separately assess the QoL in both women and men. Due to practical reasons, only the infertile women included in the study and their partners did not participate. We have only included women who were undergoing *in vitro* fertilization (IVF) treatment in the study. Those in the pre-treatment, diagnostic phase, or other ART were not investigated. Hence, generalization of the results might be affected by the sample. Second, this was a cross-sectional study and the causal relationship between QoL, SWLS, anxiety, depression, and infertility could not be established. Third, we did not examine test-retest reliability in this study.

Conclusion

The Persian version of FertiQoL is a valid, reliable instrument for measuring QoL in infertile women that provide an exhaustive and comprehensive assessment of QoL related to fertility problems across diverse life areas. However, further psychometric studies are needed in diverse populations, especially in infertile men, including test-retest reliability.

Acknowledgements

This study was funded by Royan Institute, Iran. The authors wish to thank the women for their participation in this study and the staff at the Infertility Center of the Royan Institute for their contribution to this study. The authors declare that they have no conflicts of interest.

References

1. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007; 22(6): 1506-1512.
2. Cousineau TM, Domar AD. Psychological impact of infertility. Best Pract Res Clin Obstet Gynaecol. 2007; 21(2): 293-308.

3. van Balen F, Bos HM. The social and cultural consequences of being childless in poor-resource areas. Facts Views Vis Obgyn. 2009; 1(2): 106-121.
4. Maroufizadeh S, Karimi E, Vesali S, Omani Samani R. Anxiety and depression after failure of assisted reproductive treatment among patients experiencing infertility. Int J Gynaecol Obstet. 2015; 130(3): 253-256.
5. Verhaak CM, Smeenk JM, Evers AW, Kremer JA, Kraaimaat FW, Braat DD. Women's emotional adjustment to IVF: a systematic review of 25 years of research. Hum Reprod Update. 2007; 13(1): 27-36.
6. Dancet EA, Nelen WL, Sermeus W, De Leeuw L, Kremer JA, D'Hooghe TM. The patients' perspective on fertility care: a systematic review. Hum Reprod Update. 2010; 16(5): 467-487.
7. Chachamovich JR, Chachamovich E, Ezer H, Fleck MP, Knauth D, Passos EP. Investigating quality of life and health-related quality of life in infertility: a systematic review. J Psychosom Obstet Gynaecol. 2010; 31(2): 101-110.
8. Schmidt L. Psychosocial burden of infertility and assisted reproduction. Lancet. 2006; 367(9508): 379-380.
9. Ghaheri A, Shojaei Shahrokhabadi M, Zayeri F, Maroufizadeh S, Karimi M. Relationship among life satisfaction, anxiety and fertility quality of life in women. Koomesh. 2016; 17(4): 957-965.
10. Maroufizadeh S, Ghaheri A, Omani Samani R. Factors associated with poor quality of life among Iranian infertile women undergoing IVF. Psychol Health Med. 2016: 1-7 (ahead of print).
11. The World Health Organization quality of life assessment (WHOQOL): position paper from the World Health Organization. Soc Sci Med. 1995; 41(10): 1403-1409.
12. Luckett T, King M, Butow P, Friedlander M, Paris T. Assessing health-related quality of life in gynecologic oncology: a systematic review of questionnaires and their ability to detect clinically important differences and change. Int J Gynecol Cancer. 2010; 20(4): 664-684.
13. Rashidi B, Montazeri A, Ramezanzadeh F, Shariat M, Abedinia N, Ashrafi M. Health-related quality of life in infertile couples receiving IVF or ICSI treatment. BMC Health Serv Res. 2008; 8: 186.
14. Chachamovich JL, Chachamovich E, Ezer H, Cordova FP, Fleck MM, Knauth DR, et al. Psychological distress as predictor of quality of life in men experiencing infertility: a cross-sectional survey. Reprod Health. 2010; 7: 3.
15. Boivin J, Takefman J, Braverman A. The fertility quality of life (FertiQoL) tool: development and general psychometric properties. Fertil Steril. 2011; 96(2): 409-415. e3.
16. Aarts JW, van Empel IW, Boivin J, Nelen WL, Kremer JA, Verhaak CM. Relationship between quality of life and distress in infertility: a validation study of the Dutch FertiQoL. Hum Reprod. 2011; 26(5): 1112-1118.
17. Melo C, Gameiro S, Canavarro M, Boivin J. Does the FertiQoL assess quality of life? Results from the validation of the Portuguese version of the FertiQoL. Hum Reprod. 2012; 27 Suppl 2: P-396.
18. Tabachnick BG, Fidell LS. Using multivariate statistics. 5th ed. Boston: Pearson/Allyn & Bacon; 2007.
19. Diener E, Emmons RA, Larsen RJ, Griffin S. The satisfaction with life scale. J Pers Assess. 1985; 49(1): 71-75.
20. Maroufizadeh S, Ghaheri A, Omani Samani R, Ezabadi Z. Psychometric properties of the satisfaction with life scale (SWLS) in Iranian infertile women. Int J Reprod Biomed (Yazd). 2016; 14(1): 57-62.
21. Montazeri A, Vahdaninia M, Ebrahimi M, Jarvandi S. The Hospital Anxiety and Depression Scale (HADS): translation and validation study of the Iranian version. Health Qual Life Outcomes. 2003; 1: 14.
22. Hu Lt, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Struct Equ Modeling. 1999; 6(1): 1-55.
23. Kline RB. Principles and practice of structural equation modeling. 3rd ed. New York: Guilford press; 2011.
24. Steiger JH. Understanding the limitations of global fit assessment in structural equation modeling. Pers Individ Dif. 2007; 42(5): 893-898.
25. Nunnally JC, Bernstein IH. Psychometric Theory. 3rd ed. New York: McGraw-Hill; 1994.
26. Kahyaoglu Sut H, Balkanli Kaplan P. Quality of life in women with infertility via the FertiQoL and the Hospital Anxiety and Depression Scales. Nurs Health Sci. 2014 (A head of print).
27. Keramat A, Masoomi SZ, Mousavi SA, Poorolajal J, Shobeiri F, Hazavhei SM. Quality of life and its related factors in infertile couples. J Res Health Sci. 2013; 14(1): 57-64.
28. Karabulut A, Özkan S, Oğuz N. Predictors of fertility quality of life (FertiQoL) in infertile women: analysis of confounding factors. Eur J Obstet Gynecol Reprod Biol. 2013; 170(1): 193-197.
29. Martins MV, Peterson BD, Almeida VM, Costa ME. Direct and indirect effects of perceived social support on women's infertility-related stress. Hum Reprod. 2011; 26(8): 2113-2121.

Ethics, Legal, Social, Counseling Article

Permissibility of Multifetal Pregnancy Reduction from The Shiite Point of View

Atefeh Zabihi Bidgoli, M.D.[1*], Faezeh Azimzadeh Ardbili, Ph.D.[2]

1. Department of Family Law, Faculty of Law, Imam Sadiq University, Tehran, Iran
2. Department of Theology and Law, Imam Sadiq University, Tehran, Iran

Abstract

Background: Advancements in medical technology have significantly increased the possibility of successful infertility treatment. Medical interventions in the initial process of pregnancy that intend to increase the chances of pregnancy create the risk of multifetal pregnancies for both mothers and fetuses. Physicians attempt to reduce the numbers of fetuses in order to decrease this risk and guarantee the continuation of pregnancy. The aim of this paper is to understand the Shiite instruction in terms of the risks multifetal pregnancies have for fetuses and if it is permissible to reduce the numbers of fetuses. An affirmative answer will lead to the development of Islamic criteria for reduction of the number of embryos.

Materials and Methods: This analytical-descriptive research gathered relevant data as a literature search. We reviewed a number of Islamic resources that pertained to the fetus; after a description of the fundamentals and definitions, we subsequently analyzed juridical texts. The order of reduction was inevitably determined by taking into consideration the rules that governed the abortion provisions or general juridical rules. We also investigated the UK law as a comparison to the Shiite perspective.

Results: The primary ordinance states that termination of an embryo is not permitted and is considered taboo. However, fetal reductions that occur in emergency situations where there is no option or ordinary indication are permitted before the time of ensoulment. The goal of reduction can be chosen from different ways.

Conclusion: According to Shiite sources, fetal reduction is permitted. Defective fetuses are the criteria for selective reduction. If none are defective, the criteria are possibility and facility. But if the possibility of selection is equally for more than one fetus, the criterion is importance (for example one fetus is healthier).

Keywords: Fetal Reduction, Multifetal Pregnancy, Embryo

Citation: Zabihi Bidgoli A, Azimzadeh Ardbili F. Permissibility of multifetal pregnancy reduction from the shiite point of view. Int J Fertil Steril. 2017; 10(4): 380-389.

Introduction

Due to advancements in technology and the emergence of innovative technology in the field of infertility, currently few spouses are unable to fulfill their aspirations to produce children. Although these methods are efficient, they raise many juridical questions. Answering these questions impacts the quality and methods of treatment.

One of these problems is that physicians encounter multiple pregnancies. This possibility increases as a result of ovarian stimulation and infertility treatments. Recent decades show these statistical trends where, from 1980 to 2009, only in America have the numbers of twins or triplets increased to 76%, an increase from 18.9 to 33.3 for each 1000 births (1).

Received: 4 Nov 2015, Accepted: 17 Jun 2016
*Corresponding Address: P.O.Box: 14655-159, Department of Family Law, Faculty of Law, Imam Sadiq University, Tehran, Iran
Email: a.zabihi1001@gmail.com

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 380-389

A risk of multiple pregnancies exists both for the mother and the fetuses. Hence, selective reduction of the fetuses is a strategy suggested by physicians. The aim of this paper is to investigate the permissibility or impermissibility of fetal reduction from the Shiite view. However, it is necessary to define selective reduction or multifetal pregnancy reduction (2). The meaning of selective reduction is the removal of one or several fetuses in a pregnancy that has greater than one fetus with the intent to achieve a pregnancy with twin fetuses or one fetus. In this step, the physician aborts the surplus fetuses that have been implanted to the patient's womb to increase her chances of fertility or these fetuses formed because of stimulating drugs that caused the release of several ovules.

One method has been used for more than two decades to prevent morbidity (3). Usually, this method requires injection of potassium chloride (KCl) into the heart of one or more fetuses (4), among other methods. Before investigating the ordinance of different features of reduction, it is necessary to clarify the general ordinance that pertains to the Shia regarding reduction of fetuses.

Materials and Methods

This analytical-descriptive research was undertaken at Imam Sadiq University in October, 2015. We reviewed a number of Islamic resources about the fetus, its life, and conducted a bibliography study on the basis of medical and legal resources. The resources included books, articles, and internet sources. The study was based on comparative and annalistic studies.

This analytical-descriptive research gathered relevant data in the form of a literature search. After a description of the fundamentals and definitions, juridical texts were subsequently analyzed and the order of reduction inevitably determined by taking into consideration the rules that governed the abortion provisions or general juridical rules and principles which exist in Islamic sources. Finally, we compared the Shiite perspective with the UK law.

In some cases reduction is permitted according to Islamic instructions. In the current research we sought to enumerate the cases permitted by Islam. We have determined the criteria for selection of the fetuses for the reduction process by investigating the primary ordinance of Islamic jurisdiction. The primary ordinance is introduced beside the secondary ordinance. It is an ordinance that law makers will generate according to the discretion and the loss that has originated in the issue, such as prohibition of wine or murdering. The secondary ordinance is an ordinance issued when there is an emergency, loss, or necessity such as removal of the ordinance of prohibition of wine at the time of an emergency (5). The second ordinance is studied according to the relationship of the conditions that the reduction has suggested.

Results

A study of the permissibility of multifetal pregnancy reduction is dependent upon investigation into the primary and secondary ordinances of fetal reduction followed by the jurisprudence position. In each case of embryo reduction the conditions should be defined. Criteria for selection in fetal reduction and conducting a comparative study of the Shiite perspective versus UK law were the final steps to remove possible legal gaps in this area.

Investigation about the primary and secondary ordinances of fetal reduction

Fetal reduction and abortion have one consequence-cessation of a life. However in fetal reduction, unlike abortion, the pregnancy is not terminated. Hence it seems that specifying the first and second ordinances of abortion would be sufficient. Hence, we can transmit this ordinance to fetal reduction which is a new phenomenon.

Primary ordinance

In view of Imamiyah jurisdiction, the fetus is a respectful existence. The Quran as well as the Prophet and Imams (pbut) behavior that are defined as "tradition, logical reason, and consensus" state that abortion is illegal.

According to Imamiyah jurisdiction, even "blood money" is defined for the seed of a human being (sperm) prior to entry into a woman's womb (6, 7). Then, this sum of money differs when the fetus grows in the mother's womb where five stages (seed, clot, lump, bones, and bone clothed by flesh) are defined (7). Therefore, abortion is not

permitted in Islam.

According to verses 12 to 14 of Sura Al-Mumenoon in the Quran, fetal life includes two basic stages: i. From the beginning of combining the ovule with the sperm until ensoulment (the time when the fetus possesses a sprit) and ii. From ensoulment until birth. There is no unique belief about the time when a fetus possesses a spirit. In the view of most jurisdictions, when the embryo is in the fourth month, he or she possesses a sprit (8).

In the first stage before ensoulment the fetus is similar to a plant rather than a human being. However, its killing is taboo according to narratives. In the second stage, killing of the fetus is also taboo. Although there is no difference among the Shia about the illegality of an abortion after ensoulment (9), its reasons are various. Some jurists, according to Surah Al-Anaam verse 151 of the Quran (8, 10), consider the fetus to be human. In this case, an abortion is regarded as a murder and meets the criteria for retaliation (11-13). In this verse Allah says: "You do not kill the soul which God has sanctified- except in the course of justice".

Others deny this justification and retaliation against abortion. They believe that abortion is illegal because according to certain narratives (14), the blood money of a fetus after ensoulment is equal to a human. This group claims that the mother's and fetus's lives are the same and there is no preference between them.

Other justifications have been mentioned about the illegality of abortion. Each contains impediments. The authors (as some jurists have said) believe that the mentioned reasons are not acceptable for proving equality between the mother's and fetus's lives. This statement is also confirmed by the fact that jurists prefer the mother's life when the her life conflicts with an abortion. In this case the abortion is permitted. If the value of the fetus's and the mother's lives are equal, there is no rationale for this judgment. On the one hand, what is said in the Quran cannot be true about a fetus since it is talking about murdering a human. On the other hand, the equality of blood money for the mother and fetus cannot prove the similarity between a mother and a fetus. Therefore the only justifications about the illegality of abortion are what our Imams have said in the narratives. After all, abortion is illegal and taboo in Shia where there is a consensus. Abortion is taboo is enough to prove that despite specified cases, abortion is not permissible in other cases.

Logical thinking also prohibits oppression and it is likely to say that abortion is a type of oppression. Abortion is a form of aggression toward a person who cannot defend him- or herself (15). According to Islam, the fetus is respected from the beginning because of its genesis and it enjoys the right to life. Agreement of the spouse is not enough for fetus reduction. Creation of the fetus is the result of parental sexual flow and not enough to allow them to destroy the fetus after reproduction.

Although fetus reduction in Islam is prohibited, some cases known as secondary ordinances are permitted as discussed below.

Secondary ordinance

Although abortion is taboo in Islam, it is permitted in cases of emergency, hardship, and loss. Islamic jurisdiction has defined two specific cases in which abortion is permitted. First, when maintaining the fetus endangers the mother's life. This case is known as "tazaahom" means the possibility of gathering two ordinances simultaneously while they are in existence but there is an inability to obey both ordinances (16). The ordinance "necessity to protect the mother's life" contrasts the ordinance of "necessity to protect the fetus's life", as the mother's life is more important than the fetus's life. Therefore the mother's life is preferred (17) because the mother is a real human being whereas the fetus is potentially a human. This preference is enough to save the mother's life. Likewise, crimes against the mother deserve "retaliation" whereas those against the fetus deserve "blood money". This is the reason that the mother's life is preferred (15). Other reasons, for example, consider abortion to be a means of self-defense for the mother. According to the defense theory, saving the mother's life is preferred, because if the mother dies, the fetus will also die (18, 19).

Of note, in cases where the survival of the fetus results in birth defects or intolerable pain for the mother and protecting the fetus outside of the womb is not possible, the following rules (a and b) permit abortions as tolerating this loss is difficult for the mother (20). Rule a states: "juridical regulation of negating difficulties and troubles". This

rule says that whatever causes a problem or risk is negated by Islamic law makers. The best evidence comes from verse 87 of Surah Al- Mumanoon in the Quran: "There is no difficulty in your religion" (21).

Rule b is the "rule of negation of loss". The contingency of this act is that no man is permitted to cause loss to others in order to prevent his own loss. It is not permissible to cause loss to him to prevent loss to others (22).

The second case occurs when the fetus is deformed and defective. In such cases, since the birth of a deformed baby will create a severe hardship for the parent, abortion is permitted before ensoulment. However another perspective exists. According to this view, although tolerating an illness is difficult, it is a "test" for the parents and the fetus. According to Islamic view, Allah tests his slaves (verse 145 of Surah Al-Baqara) (23). Then, as killing patients and defective people is not permitted, killing a defective fetus is also not permissible.

Permission to kill the fetus in such cases is limited to the stage where the fetus does not have a spirit. After ensoulment, as this act considers the rights of all assignees, one cannot derive a benefit for someone (mother) and cause loss for others (fetus) (15, 20, 24). Therefore, it should be considered that in all cases where selective reduction is permitted, this permission is limited to the stage where the fetus does not possess a spirit. After this stage, fetus reduction is illegal with the exception of tazaahom.

By taking into consideration the abovementioned, if cases of multiple fetuses where continuation of pregnancy causes any hazard for the mother, the fetus reduction is considered secondary and it is not necessary to obtain the husband's permission.

To the extent possible, the number of transferred fetuses should be minimal so that selective decrease of fetuses will be unnecessary. In order to prevent multifetal pregnancy, the number of transferred fetuses should be minimal or small doses of medication (stimulating drugs that caused the release of several ovules) should be prescribed. However this will decrease the chances of fertilization. For example, if after ovary stimulation, more than three follicles have matured, the cycle should be cancelled at both ovaries to prevent multiple pregnancies. In assisted reproductive technology (ART) implantation, fewer fetuses are recommended. In elective single embryo transfer (eSET) it is recommended to choose only one high quality fetus. Unfortunately, the high cost of infertility treatment and small success rate result in the implantation of multiple fetuses in the mothers' womb to increase the chances of childbirth (25). In this regard, to counteract, some countries have approved acts in which under any circumstances, it is not permissible to transfer more than one fetus by the physicians (26). However, the duration of treatment following the implantation of one fetus and high treatment cost encourage the implantation of more fetuses.

Even in cases where the transfer of more than one embryo to the mother's womb is justifiable, it is clear that most mothers prefer not to be faced with reduction of the fetuses. In such cases the mothers may encounter extreme psychological problems because of the length of time spent waiting to have children (27). In such cases, embryo implantation should be completely controlled in a way that the possibility of multiple pregnancies decreases. When fertility centers compete to attract more patients and increase the rate of fertility and their own success, it is necessary to prevent them from misusing patients by persuading them to implant more embryos, after which reduction of the fetuses becomes necessary.

Defining the position of jurisprudence for each case of embryo reduction by taking into consideration the conditions

Considering the risk of continuation of multiple pregnancies for fetuses, jurisprudence have posed the following assumptions (28).

1. The conditions that reduction of embryos will not endanger the mother's life for continuation of pregnancy and the fetuses do not have any defect and no damage entreats them.

In these cases, fetal reduction is illegal. Physicians and well-informed parents are persecuted for their civil and penal responsibilities. Hence, reduction of fetuses in cases where there is no risk for the mother or the fetus is not permitted. The general ordinance of fetus reduction is considered.

As previously stated in the primary ordinance, according to Islam, the fetus is respected from the beginning of its genesis and it has the right to live. Therefore abortion and reduction are illegal.

2. During the pregnancy, the medical test shows the existence of abnormalities. In this case, reduction is approved in some fetuses. In such cases, the fetus deficiency is the reason for reduction. After approval of damage and hardship for the parents, an abortion is permitted according to the criterion of the secondary ordinance because the juridical evidences of prohibition of abortion do not include these cases according to some jurisprudence (15).

3. Continuation of multiple pregnancies is dangerous for the fetuses. According to resources, cases where a deficiency in the fetus is the reason for reduction are defined as "selective termination of pregnancy" whereas cases that the reason of reduction is the risk for mothers and fetuses are called "fetal reduction of multiple pregnancies" (29).

Cases where reduction of the fetus is chosen as a guarantee for healthiness of the pregnancy have different ordinances according to whether multiple pregnancies occurred naturally or by implantation and the physician. Natural multiple pregnancies occur when the number of fertilized ova are more than one or when there is a mutation in embryo's cells during division. Artificial multiple pregnancy occurs when the physician decides to implant several fetuses as a cure for infertility. The physicians, after receiving permission from the patient, stimulate the ovary with medications or implant several fetuses into the womb. Then, permission of reduction of the fetuses under two conditions is assumed.

For example, multiple pregnancies are natural and preservation of surplus embryos endangers the lives of all embryos. Here, keeping each embryo conflicts (in the exact meaning of tazaahom) with the lives of the others. Because the situation is equal for all, the priority of one embryo over the others is considered "preference without any logical reason". Preference without any logical reason means that one thing is preferred over the other in instances where both have equal features and without any special goal. This is an impossible issue. Most philosophers assume this is a reasonable rule and obvious, in a way that obviousness is evident such as the "premise of unity is half of two" (30). In such cases no embryo is preferred over another. In such cases one cannot delay and endanger the life of all fetuses. So, referring to the jurisprudence rule of "Al-maysoure la yasqoto bel-masoure" we cannot eliminate all embryos. Inevitably we should act according to an acceptable criterion. According to this rule, when someone is obliged to do something, he or she should do it according to his or her capabilities. It is mentioned as "what one cannot do completely, he should not abandon it completely" (31). In another interpretation, the lawmaker orders to something that is composed of several parts, and some parts have obstruction and some parts do not. In cases where the parts do not have any obstruction, they should not avoided (32).

In the second situation where multiple gestation is artificial and maintaining all the embryos would endanger the lives of all embryos. In this case, continuing with the pregnancy is a conflict between the mother's life and multiple synthetic pregnancies, which has emerged as a result of the decision of the parents. This can induce doubt that this agreement is subject to the legal rule of "emergency optionally does not negate arbitration". The purpose of this rule is that of someone who is obliged engages in a taboo activity, in a way that he or she cannot act according to the rule. Regarding that his inability to perform the task that has been caused by incorrect disposal (conscious possession), his or her responsibility will not decrease (21). In this way, by an incorrect decision, the mother's situation is so that she cannot preserve her fetus. Thus, fetal reduction is not possible for her. If she does so, she will be prosecuted. We can say that conscious multiple pregnancies will put her in a situation that cannot defend herself against the emergency of abortion.

In response to this question it is accepted that "regarding legal reasons and principles, if the situation of a person is a result of an adverse selection, this situation puts him out of the emergency, whereas in such circumstances it is impossible to have a bad choice" (28). It should be added that, according to the aforementioned rule, the distinction between "those emergency situations that cause irresponsibility" and "avoidance willfully" is that the person with the bad choice cannot fulfill her duties, whereas in multiple pregnancies the physician and parents try to achieve the best re-

sults for the treatment of infertility.

In this case, as with the first one, reduction of the fetuses is permitted unless the mother is in a position in which she is forced to reduce the fetus because of her previous adverse possession.

Even when the parents demand implantation of more embryos to enhance the chances for fertility, it is the responsibility of the physician to implant fewer embryos to avoid fetal reduction. Implantation of surplus embryos by the physician is the exact case for adverse selection that results in prohibition of the fetus reduction.

Of note, according to the fact that the result of both fetal reduction and abortion is the same (fetal deaths), fetal reduction (which is fully justified medically) is legally permitted in countries where abortion is not illegal, even without any medical reason.

Criterion for selection in fetal reduction

The reasons for permission of reduction of the fetuses include: deficiency, importance, ease, random choice, parents' right, and governmental ordinance.

According to the deficiency criterion in cases where reduction of the fetus is necessary, if a fetus is defective, this fetus would be the goal for reduction. This case has been discussed previously.

Importance. In terms of the ordinance that each fetus is preserved by reduction of another fetus, these ordinances conflict (in the exact meaning of tazaahom) with each other because a pregnant women should choose one of two ways: i. Keep all fetuses which ultimately results in the deaths of all the fetuses or ii. Reduction of some fetuses that ends in the deaths of those fetuses and continuation of pregnancy for the other fetuses.

When there is a conflict (in the exact meaning of tazaahom) between two or more discretions, it is important to consider the most important discretion (33). In order to enforce the rule of the most important discretion it is supposed that if it is possible to predict the importance of survival of one fetus over the others, the protection of that fetus is preferred (28). In cases that there is a considerable defect or deformity in the fetus compared to the others, the criterion is to reduce this fetus and keep the others. However the meaning of defection is not those defections that lawmakers have issued regarding the rule of reduction, but the purpose is that some fetuses are less healthy. Realization of such criterion is the decision of the physician.

Ease. This criterion is defined as "regarding the risk of reduction of the fetuses, when the above criteria are not met, the fetus that its reduction is easier will be chosen for the purpose of reduction". The assumption that the criterion of ease as an independent criterion comparing importance is not true. The reason is that the ease of reduction of one fetus over the other fetus in fact is the criterion of choosing the most important one. Therefore, we cannot think of the ease criterion as an independent criterion. This criterion depends on the specialist perspective.

Random choice or parental rights. If the conditions are equal, choice of one fetus over the others is a preference without a logical reason. If the above conditions are not met, the physician or the parents can select the fetus for reduction just by random selection (lottery) or "absence of constraints rule". The meaning of "absence of constraints rule" is that in cases where no difference exists between two affairs and one is not preferred over the other and there is no possibility of collection of those two affairs, then the person who is obliged to choose is free to choose according to wisdom (16).

The jurisprudence perspectives differ regarding random choice and picking up. According to recent perspective, the parents are free to choose the gender of the fetus, but It seems that random choice is more common among jurisprudences (34-36). Of note, the number of reductions is a specialized issue and the parents can only comment about the choice. Therefore, the parents' demands for reduction of more fetuses to reach one fetus pregnancy is not acceptable. Of course, choosing according to gender can cause serious ethical and even legal challenges in the field of gender discrimination. In other countries, this issue is also considered and although the parents have the right to choose the gender of the fetus when they are deciding to reduce the number of fetuses, considering other discretions such as the necessity of gender balance in society is accounted as an obstruction for the right of choosing according to gender (37).

Governmental criterion. Those previously mentioned criteria refer to the situations where the governor (religious ruling) did not order any writ. Protection of public rights makes governments ignore individual discretion. If the governor orders any writ, none of these criteria will be enforced. One example of such cases is that the number of genders is not equal. This issue causes many problems. Therefore, selecting gender to stabilize the society is chosen by the government (38).

In cases that the doctor legally allowed elimination of one or more embryos in multiple pregnancies, deficiency in the fetuses (if one is defective) and importance (if there is any reason to prefer one fetus) can be criterions for selection of the goal of reduction.

If the conditions of all fetuses are equal, the parents or the physician choose the fetuses by lottery or by picking them up (absence of constraints rule). Each perspective has its own devotees. Of note, if parents are permitted to choose, the recognition of the number of reductions is due to the physician. It seems that to prevent parents and physicians from misusing this situation in favor of single fetus pregnancy, passing an act by lawmakers is necessary.

Comparative study (Shiite perspective versus the UK law)

The first references to abortion in UK law appeared in the 13th Century. The law followed the Church's teachings that abortion was acceptable until 'quickening' (which, it was believed was when the soul entered the fetus). In the Ellenborough Act (1803), the punishment of abortion after 'quickening' (i.e., when movement is felt at 16-20 weeks) was the death penalty although previously the punishment had been less severe. The legal situation remained the same for centuries.

In 1861 (The Offences Against the Person Act), this penalty was reduced to life imprisonment. In 1929, Infant Life Preservation Act created a new crime of killing a viable fetus (at that time fixed at 28 weeks) in all cases except when the woman's life was at risk.

These laws caused thousands of women to resort to back-street abortions to prevent unwanted pregnancies or the need for abortions which led to permanent damage or death. For example, in 1923-33, 15% of maternal deaths were due to illegal abortions. In 1936, the Abortion Law Reform Association (ALRA) was established with the aim to campaign for the legalization of abortion. In 1967, the Abortion Act sponsored by David Steel, MP became a law and came into effect on April 27, 1968. This Act legalized abortion under certain conditions. In 1990, the 1967 Act was amended by the Human Fertilization and Embryology Act, which reduced the original time limit of 28 weeks to 24 weeks for most abortions (39).

Currently, abortion is legal on a wide number of grounds in England, Wales, and Scotland since the Abortion Act of 1967, which is one of the most liberal abortion laws in Europe. Grounds for abortion under this Act include: i. Situations where the continuance of the pregnancy would involve risk to the life of the pregnant woman greater than if the pregnancy were terminated, ii. Termination is necessary to prevent grave permanent injury to the physical or mental health of the pregnant woman, iii. The pregnancy has not exceeded its 24th week and continuance of the pregnancy would involve risk, greater than if the pregnancy were terminated, of injury to the physical or mental health of the pregnant woman, iv. Pregnancy has not exceeded its 24th week and continuance of the pregnancy would involve risk, greater than if the pregnancy were terminated, of injury to the physical or mental health of any existing children of the family of the pregnant woman, v. There is a substantial risk that if the child were born it would suffer from physical or mental abnormalities as to be seriously handicapped, vi. To save the life of the pregnant woman, and vii. To prevent grave permanent injury to the physical or mental health of the pregnant woman (40).

In 1999 approximately 8000 women who lived abroad travelled to England to have an abortion (41). The Law that regulates selective Reduction is contained in four separate statutes: Offences Against The Person Act 1861 (OAPA), Infant Life Preservation Act 1929 (ILPA), Abortion Act of 1967, and Human Fertilization and Embryology Act of 1990, section 37 (5) which amended the provisions of the Abortion Act sensibly to legalize the practice of selective reduction. With the exception of the 1990 Act, when these laws were framed, it was impossible to imagine that one or

more fetuses might be destroyed, allowing others to survive to term. Section 58 of the OAPA prohibits the performance of an Act "with intent to procure the miscarriage of a woman with child" (42).

In a comparison between the UK law and Shiite perspective, two points can be made. First, it is clear that in the UK the cases in which abortion is allowed are much more than allowed by Shia law. According to the Shia law, as previously mentioned, only the mother's survival and fetus's disability are the examples of harm that justify abortion. While in the first chapter of the Abortion Act of 1968 in the UK, mental or physical health of the mother or other children in the family are permissions for abortion. Generally it can be claimed that by the year 1929 the English parliament had a penal view toward abortion and the maximum protection was devoted to the fetus. However, the future laws, especially in 1990, legislations have preferred the mother's life to the fetus. In Shia, the mother's life preference over the fetus's life is only legitimate when mother's life would be at risk with continuation of pregnancy. Therefore, the Shia's point of view is closer to the former English laws. The reason can be the effects of religion on these former regulations and the further amendments have followed the aim of decreasing illegal abortions and its fatal results.

Second, despite the different perspective about abortion mentioned above, it is important to note that reduction of fetuses is specified in English law while Shia resources have not discussed it as a treatment. The authors of this article have attempted to determine the regulations of reduction in Shia books and their general principles and rules (such as abortion rules). Therefore, this issue should be studied by Shia in detail and the result should be passed as a law. However, no important contrast can be seen between Shia and English points of view about reduction. Two reasons prove this claim. First, treatment and saving other fetuses are the basic goals in reduction. Second, the main difference between Shia and English view in the field of abortion concerns the mother's and other children's mental and physical health. However mental and physical health has no place in reduction.

Discussion

Briefly, arbitrary choice is a strategy that the physicians suggest to eliminate the danger of multifetal pregnancy for mothers and the fetuses, that cause failure of the pregnancy. The aim of arbitrary choice is to reduce the number of fetuses to only one or two. Any order is not provided specifically about the criteria for embryo reduction in juridical sources. Therefore, it will inevitably be determined by the rules that govern abortion provisions or general juridical rules and principles which exist in Islamic sources (in primary and secondary ordinances).

The primary ordinance of intentional death of a fetus in Shiite view is sanctity and its prohibition. However if there is an emergency, loss or hardship for the mother, the ordinance is secondary. As some jurists have stated, reduction of the fetuses before ensoulment of the fetus is permitted (10, 14), but the authors believe that when the fetus endangers the mother's life, there is no different between before and after ensoulment.

The jurisprudence position in each example of fetus reduction defines reduction according to three premises: i. The conditions that multiple pregnancies will not entreat the mother or continuation of pregnancy and the fetuses are free of deficiency and there is no risk for them. In such cases reduction of embryos is forbidden, ii. Some of the fetuses are defective where, according to the secondary ordinance, reduction is permitted, and iii. Continuation of multiple pregnancies has risks for the fetuses. Such case raise the following assumptions: in a natural multiple pregnancy, if protection of the surplus fetuses endangers the life of all fetuses, we cannot act according to the lack of preference and cause the death of all fetuses. Therefore we should act according to an acceptable criterion. In artificial multiple pregnancies, the physicians are obliged to reduce the number of fetuses to avoid the situation of reduction, otherwise reduction of the fetuses is not permitted. If they do so, they will be prosecuted.

A comparison between English and Shia rules about abortion and reduction indicates that in England abortion is more widely accepted and apart from mother survival or fetus disability, the mother's or other children's mental and physical health can justify abortion. However, the Shia consider abortion cases other than mother survival or fetus disability to be forbidden and taboo. Disregarding this difference, reduction by the aim of sav-

ing other fetuses is permitted in both systems and similarities can be seen in this field.

Conclusion

In an emergency situation, reduction of the fetuses is permitted. To choose the goal of reduction, the criterion is defection in fetuses or importance. The importance means if there is a reason to protect one fetus, (for example one of them is healthier); the other fetuses will be the goal of reduction. If the conditions are equal, the parents or the physicians can choose the fetus. It is noteworthy that if the choice of parents is permitted, choosing the number of them is due to the physician's discretion. Finally the ordinance of the government and the existence of a rule are superior to other rules.

Lack of any resource about various aspects of reduction (such as legal, moral and jurisprudence) is one of the most important limitations of this study. Therefore, an investigation into the relevant provisions of reduction in details such as civil liability of the physician and medical staff in embryo transfer or fetal reduction can be a suitable topic for future research.

Acknowledgements

We acknowledge the conductors of "The Second Congress of Therapeutic Abortion and Fetal Reduction from Medical, Theological, Legal and Ethical Approaches" held at Royan Institute (2012). The authors have no financial support and conflict of interest in this article.

References

1. Martin JA, Hamilton BE, Osterman MJ. Three decades of twin births in the United States, 1980-2009. NCHS Data Brief. 2012; (80): 1-8.
2. Rorty MV, Pinkerton JV. Elective fetal reduction: the ultimate elective surgery. J Contemp Health Law Policy. 1996; 13(1): 53-77.
3. Evans M, Britt DW. Fetal reduction. Semin Perinatol. 2005; 29(5): 321-329.
4. Mittal S, Kumar S, Vimala N, Dadhwal V. Multifetal pregnancy reduction - a method to improve perinatal outcome in higher order pregnancies. J Obstet Gynecol Ind. 2004; 54(4): 351-354.
5. Valaei I. Theology principleanatomical terminology. 2nd ed. Tehran: Ney publication; 2001; 177.
6. Musavi khoi A. Mosuato Al-emam Al-khoi. 1st ed. Qom: Khoi publication; 1997; 115.
7. Yazdi Tabatabai SMK. Al-orva al-vothqa. 2nd ed. Beirut: Al-aalami publication; 1987; 809.
8. Najafi MH. Javaher al-kalam fi sharhesharaee al-Islam. 7th ed. Beirut: Ehya al-torath al- arabic publication; 1971; 43: 381, 2: 354.
9. Toosi M. Al-mabsoot fi fegh al-emamia. 3rd ed. Tehran: Al-maktabato al-martazavia leehya al-athr al-jafaria; 2008; 200.
10. Helli (allame) H. Qavaed al-ahkam fi marefate al-halal va al-haram. 1st ed. Qom: Islamic publication; 1992; 699.
11. Sallar Deilami H. Al-marasem al-alavia va al-ahkaam al-nabavia. 1st ed. Qom: Al-haramein publication; 1983; 242.
12. Tabrizi J. Tanqih mabaani va al-ahkaam. 1st ed. Qom: Dar Al-seddiqa Al-shahida publication; 2007; 329.
13. Musavi khoi A. Mabaani Takmelato al-menhaj. 1st ed. Qom: Khoi publication; 2001; 418.
14. Toosi M. Al-estebsar fima ekhtalaf men al-akhbar. 1st ed. Tehran: Islamic book publication; 2011; 298.
15. Makarim Shirazi N. Important juridical discussions. Qom: School of Imam Ali IbnAbi Talib; 2001: 286-294.
16. Meshkini MA. Terminology of and the Supreme expressions. 6th ed. Qom: Alhadi publication; 1995; 50-108.
17. Hosseini Shirazi M. Theology the book of obligatories. 2nd ed. Beirut: Daralalom; 1988; 82.
18. Tabrizi J. Serat Al-nejat . 1st ed. Qom: Al-montakhab publication; 1995; 326.
19. Musavi khoi A. Fegh Al-aazar Al-shariia va Al-masael Al-tebbia. Qom: Al-sediq al-shahida publication; 2006; 146.
20. Mohseni MA. Fiqh and medical issue. 1st ed. Qom: Bustan book; 2003; 66.
21. Mostafavi MK. Hundreds of Islamic rule. 4th ed. Qom: Islamic Publications Office affiliated to the society of the teachers in hawzah of Qom; 2000; 69, 191, 296.
22. Makarem Shirazi N. The rules of jurisprudence. 3rd ed. Qom: school of Imam Ali IbnAbiTalib; 1990; 92.
23. Beheshti A. Training in the view of Isla]. 1st ed. Qom: publishing office Payam; 1983; 161.
24. Hosseini Sistani A. Matters elected. Qom: Mehr; 1993; 407.
25. Qiong Z, Yanping L, Deep JP, Lin Z. Treatment of cornual heterotopic pregnancy via selective reduction without feticide drug. J Minim Invasive Gynecol. 2011; 18(6): 766-768.
26. Gleicher N. The irrational attraction of elective single-embryo transfer (eSET). Hum Reprod. 2013; 28(2): 294-297.
27. Ogilvie CM. Multiple pregnancy, fetal reduction and selective termination. Reprod Biomed Online. 2013; 26(6): 522-524.
28. Gurabi H, editor. The Second Congress of Therapeutic Abortion and Fetal Reduction from Medical, Theological, Legal and Ethical Approaches; 2012 Dec 19-21; Tehran, Iran. Education - Research Department of Royan Institute; 2012: 25.
29. Legendre CM, Moutel G, Drouin R, Favre R, Bouffard C. Differences between selective termination of pregnancy and fetal reduction in multiple pregnancy: a narrative review. Reprod Biomed Online. 2013; 26(6): 542-554.
30. Fakhrrazi M. Collector ideas of applicants and latecomers scientists and the Wise and speakers. Beirut: Taha Abdul RaufSaad; 1984: 110.
31. Kashani H. Perceptions of rules. 2nd ed. Qom: scientific publication: 1983: 283.
32. Bjnordy H. The rules of jurisprudence. 1st ed. Qom: Al-hadi; 1998; 125.
33. SobhaniTabrizi G. To guide the minds Investigation assets. 1st ed. Qom: Imam sadiqInstitue; 2003; 265.

34. Karimi Qomi H. Al-qorah rule. 1st ed. Qom: jurisprudential Etharimams Center peace be upon them; 1402; 25.
35. Firosabadi M, HosseiniYazdi. Jurisprudence and ijtihad and imitation (Enaya al-osul). 4th ed. Qom: Firosabadi; 2003; 270.
36. Shobeiri Zanjani M. The book of marriage. 1st ed. Qom: RayPardaz Research Institute; 1998; 17:5674.
37. Committee on Ethics, American College of Obstetricians and Gynecologists. ACOG Committee Opinion No. 360: Sex selection. Obstet Gynecol. 2007; 109(2 Pt 1): 475-478.
38. Gurabi H, editor. The Second Congress of Therapeutic Abortion and Fetal Reduction from Medical, Theological, Legal and Ethical Approaches; 2012 Dec 19-21; Tehran, Iran. Education - Research Department of Royan Institute; 136.
39. Fabbrini F. Fundamental Rights in Europe. 1st ed. Oxford University Press; 2014: 201.
40. Furedi A. About abortion in Britan. J Med Ethics. 2001; 27 Suppl 2: ii33-34.
41. Johnston WR. Historical abortion statistics. United Kingdom; 2016: 2.
42. Hall A. Selective reduction of pregnancy: a legal analysis J Med Ethics. 1996; 22(5): 304-308.

Short Communication

Screening of Two Neighboring *CFTR* Mutations in Iranian Infertile Men with Non-Obstructive Azoospermia

Somayeh Heidari, M.Sc., Zohreh Hojati, Ph.D.*, Majid Motovali-Bashi, Ph.D.

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Abstract

The genetic association between cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations and male infertility due to congenital bilateral absence of vas deferens (CBAVD) is well established. Mutant *CFTR*, however may also be involved in the etiology of male infertility in non-CBAVD cases. The present study was conducted to estimate the frequency of ΔI507 and ΔF508 *CFTR* gene mutations in Iranian infertile males. We undertook the first study of association between these *CFTR* mutations and non-obstructive azoospermia in Iran.

In this case-control study, 100 fertile healthy fathers and 100 non-obstructive azoospermia's men were recruited from Isfahan Infertility Center (IIC) and Sari Saint Mary's Infertility Center, between 2008 and 2009. Screening of F508del and I507del mutations was carried out by the multiplex-ARMS-PCR. Significance of differences in mutation frequencies between the patient and control groups was assessed by Fisher's exact test. The ΔF508 was detected in three patients. However there are no significant association was found between the presence of this mutated allele and infertility [OR=9.2 (allele-based) and 7.2 (individual-based), P=0.179]. None of the samples carried the ΔI507 mutation.

Altogether, we show that neither ΔI507 nor ΔF508 is involved in this population of Iranian infertile males with non-obstructive azoospermia.

Keywords: *CFTR*, Mutation, Azoospermia, Male Infertility

Citation: Heidari S, Hojati Z, Motovali-Bashi M. Screening of two neighboring CFTR mutations in Iranian infertile men with non-obstructive azoospermia. Int J Fertil Steril. 2017; 10(4): 390-394.

Introduction

Reproductive failure is associated with various genetic disorders, mainly numerical and structural chromosome abnormalities and gene mutations. At the genic level, male infertility has been linked with protamine gene mutations (1, 2), 5-alpha reductase deficiency (3), androgen receptor gene mutations (4, 5) and cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutations (6-14). More than 1950 *CFTR* variants have been identified in different ethnic populations, as curated in the cystic fibrosis genetic analysis consortium database. Many of these mutations are associated with a wide spectrum of phenotypes, including respiratory distress, chronic pancreatitis and male infertility (11). Male infertility caused by congenital bilateral absence of vas deferens (CBAVD) has been reported in more than 95% of men with cystic fibrosis (CF) (7, 10). The genetic association between CFTR mutations and male infertility due to CBAVD is well established (10-12, 15). Several studies have revealed involvement of *CFTR* mutations in other forms of male infertility due to defective spermatogenesis (10, 16-20). Although a two-five fold increased in *CFTR* mutation rate in males with non-obstructive azoospermia has been reported (REF), a number of reports did not find any association between them (10, 17, 20-23). van der Ven et al. (20) investigate the possible involvement of *CFTR* in the etiology of non-CBAVD male infertility. Semen specimens from 127 unrelated healthy males with various diagnoses of reduced sperm quality were screened for a panel of 13 *CFTR* mutations. Fourteen of 80 (17.5%) infertile men due to reduced sperm quality and 3 of

Received: 20 Dec 2015, Accepted: 24 July 2016
***Corresponding Address: P.O.Box: 81746-73441, Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran**
Email: z.hojati@sci.ui.ac.ir

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 4, Jan-Mar 2017, Pages: 390-394

21 (14.3%) men with azoospermia had at least one *CFTR* mutation (one azoospermic male was a compound heterozygote). No mutations were found in the control group of 26 individuals with normal semen parameters. This overrepresentation of *CFTR* mutations in men with reduced sperm quality and in men with azoospermia without CBAVD suggests that CFTR protein may be involved in the process of spermatogenesis or sperm maturation apart from playing a critical role in the development of epididymal glands and the vas deferens (20). Schulz et al. (10) investigated the frequency of *CFTR* mutations in 597 males with reduced sperm quality and 34 (5.70%) carried a mutation, indicating a two-fold higher frequency than in the general population. Boucher et al. (22) screened 39 patients with azoospermia without CAVD and 37 patients with severe oligozoospermia for a panel of 10 *CFTR* mutations. None of the *CFTR* mutations were observed in the patient group and suggested that *CFTR* gene is not involved in spermatogenesis.

Some studies have reported expression of the *CFTR* gene in human Sertoli cells, germ cells and testes, suggesting its possible involvement in spermatogenesis (24-26). Based on these investigations, to date, it remains uncertain whether screening of *CFTR* mutations should be recommended for infertile males with non-obstructive azoospermia during assisted reproduction technology. There are just a few studies reporting the association between *CFTR* mutations and non-obstrucive azoospermia, especially in the Iran. The aim of this study was to estimate the frequency of ΔI507 and ΔF508 *CFTR* mutations in Iranian infertile males with non-obstructive azoospermia. We undertook the first study of association between *CFTR* gene mutations and non-obstructive azoospermia in Iran.

This study was a case-control study. Blood samples were collected from 100 males with non-obstructive azoospermia in the Isfahan Infertility Center (IIC) and Sari Saint Mary's Infertility Center, Iran, between 2008 and 2009, and from 100 normal men (men with normal fertility with at least one child and normal sperm parameters). All the patients and control individuals gave informed written consent to be included in the study. The diagnosis of non-obstructive azoospermia was based on the following examinations: normal semen volume, normal testicular size, presence of the vas deferens by clinical examination, normal levels of serum follicle-stimulating hormone (FSH), azoospermia, absence or low levels of fructose and absence of spermatozoa in sample extracted by percutaneous testicular sperm aspiration (TESA). No symptoms of CF including chronic lung inflammation/infection, pancreatic insufficiency and intestinal obstruction were reported in the clinical files of the patients. The mean age of the patients and controls were 31.5 and 30 years, respectively. In this study, all patients were azoospermic with an absence of spermatozoa in the semen and sample extracted by TESA.

The ΔF508 and ΔI507 mutations in exon 10 of *CFTR* were selected for screening. The specificity of the primers were analyzed using Oligo®7 software (Version 7.0, Rychlik, 2007) (27). Further comparison of designed primers with the exon 10 sequence of *CFTR* was performed by CLC software (www.clcbio.com/genomics).

Two ml blood was collected from each patient and normal control in tubes containing EDTA. Leukocyte genomic DNA was extracted from blood samples using the standard method of salting out with slight modification (28). Genomics DNA samples were stored in -20˚C after determining their relevant concentrations and quality on gels.

Multiplex-ARMS-PCR was carried out in two separate reactions. This method is schematically drawn in the Figure 1.

	Normal sequence	**F508 sequence**	**I507 sequence**
DF-N primer	← _____	**X** ← _____	← _____
DF-M primer	**X** ← _____	← _____	**X** ← _____
IF-N primer	→ _____	→ **X** _____	→ **X** _____
IF-M primer	→ **X** _____	→ _____	→ _____

Fig.1: Visial representatin of wild-allele, ΔF508 and ΔI507 mutant ARMS primers for the amplification of the target sequence. The diagrams in the boxes align the normal and mutant ARMS primers (5' to 3') with the normal, ΔF508, and ΔI507 target DNA sequences.

The genotype of an individual can be determined by analysing of the amplification products. Sequences of all primers were shown in Table 1.

Table 1: List of primer sequences

Primer name	Sequences (5´ to 3´)
FC	GGT TTT ATT TCC AGA CTT CAC TTC ATA T
RC	TGC ATA ATC AAA AAG TTT TCA CAT AGT T
DFN	GTA TCT ATA TTC ATC ATA GGA AAC ACC ACA
DFM	GTA TCT ATA TTC ATC ATA GGA AAC ACC AAT
IFN	CTG GCA CCA TTA AAG AAA ATA TCA TCT T
IFM	CTG GCA CCA TTA AAG AAA ATA TCA TTG G

Wild-allele specific primers (DFN and FC primers) and ΔF508 mutant-allele specific primers (DFM and FC primers) produce 173 bp and 170 bp fragments, respectively. Amplification of sequence by wild-allele specific primers (IFN and RC primers) and ΔI507 mutant-allele specific primers (IFM and RC) give 123 bp and 120 bp fragments, respectively (Fig.2).

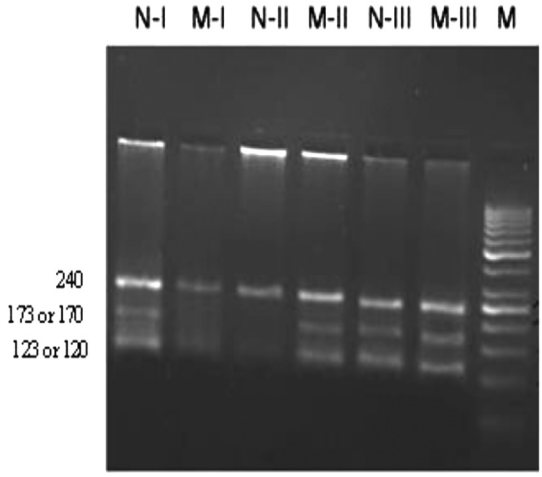

Fig.2: Detection of ΔF508 *CFTR* mutation in infertile men by using multiplex-ARMS-PCR. The amplification products of normal primers sets are shown here (N). M indicates the amplification products by IFM and DFM primers. The resulted amplified fragments for a normal and ΔF508 mutant are shown here. Sample I is a wild homozygote, sample II is a mutant homozygote (ΔF508) and sample III is a heterozygote (ΔF508). Fragment sizes are in the base pairs (bp). Marker; 50 bp DNA ladder. N-I; Normal primer sets for sample I, M-I; Mutant primer sets for sample I, N-II; Normal primer sets for sample II, M-II; Mutant primer sets for sample II, N-III; Normal primer sets for sample III, and M-III; Mutant primer sets for sample.

DNA amplification was carried out in duplicate for all samples. Each 25 µl reaction mixture contained 3 µl template DNA (50-100 ng), 1.6 µl $MgCl_2$ (2.0 mM), 2.5 µl 10X PCR buffer, 1.5 µl FC (20 pmol ml^{-1}), 1.5 µl RC (20 pmol ml^{-1}), 0.75 µl DFN (20 pmol ml^{-1}), 0.75 µl IFN (20 pmol ml^{-1}), 1 µl dNTP mix (10 mM), 0.4 µl Taq polymerase (5 U) and 12 µl ddH_2O. The reaction mixtures were prepared and kept on ice until the heating block of the thermal cycler reached the denaturation temperature (94°C). The PCR amplification was carried out at 94°C for 10 minutes and then followed with 32 amplification cycles of 40 seconds at 94°C, 1 minute at 58.8°C, 1 minute at 72°C, and a final extension at 72°C for 10 minutes. Amplification products were separated by electrophoresis using a 2.5% Metaphor agarose gel, stained with ethidium bromide and visualized by ultraviolet illumination.

Significance of differences in mutation frequencies between the patient and control groups was assessed by Fisher's exact test (SPSS software version 16.0) and P values<0.05 were considered statistically significant.

The percentage difference of ΔF508 mutation between the patient and control groups was although not statistically significant (P=0.179), possibly due to the relatively small sample size, it displayed a large effect size [OR=9.2 (allele-based) and 7.2 (individual-based)]. No individuals carried the ΔI507 mutation.

The genetic link between *CFTR* mutations and a genital form of male infertility (CBAVD) is well established. Thus, screening of *CFTR* mutations is proposed for all infertile men with CBAVD, however, the association of *CFTR* mutations and non-CBAVD male infertility, is uncertain. Recently Xu et al. (29) demonstrated *CFTR*-dependent regulation of CREB in human Sertoli cells which suggests that its defective regulation may cause spermatogenesis failure as seen in non-obstructive azoospermia. This seems to be the possible mechanism by which *CFTR* may be involved in spermatogenesis. There are a few studies which have specifically looked at the frequency and role of *CFTR* mutations in azoospermic men without CBAVD (20, 23, 30-32). Many reports to date have shown conflicting results. Some studies

showed significant association between *CFTR* mutations and non-obstructive azoospermia while the other investigations ruled out this association (14). In the present study, ΔF508 mutation was observed twice in heterozygous form (2%) and once in homozygous form among the 100 non-obstructive patients. This result is consistent with previous studies. For instance, Safinejad et al. (6) evaluated five common *CFTR* mutations (ΔF508, G542X, R117H, W1282X and N1303K) in Iranian infertile men with non-CAVD obstructive azoospermia. The common *CFTR* mutations were found in 9.43% (5.53%) patients for ΔF508 mutation. Another study by Sharma et al. (11) analyzing the frequency of *CFTR* mutations in infertile Indian males with non-obstructive azoospermia (n=60) and spermatogenic failure (n=150), showed that ΔF508 mutation was observed in 3.6% of patients with non-obstructive azoospermia.

However, some reports revealed the absence of ΔF508 mutation among all infertile patients (7, 22, 33). Ravnik-Glavac et al. (23) screened 80 men with idiopathic azoospermia, 50 men with severe oligozoospermia, 70 men with oligoasthenoteratozoospermia, and 7 men with CBAVD as well as 95 controls from Slovenia for mutations in 10 *CFTR* exons where the majority of the common CF disease causing mutations have been detected. The frequencies of *CFTR* mutations did not differ significantly between the control group and men with idiopathic nonobstructive azoospermia and subfertility, suggesting that *CFTR* mutations are not associated with spermatogenic failure and non-obstructive pathology of urogenital tract in men.

Moreover, we did not observed the ΔI507 mutation among all infertile patients and control individuals suggesting its rarity as a cause. In conclusion, our results did not show any significant association between these two mutations and non-obstructive azoospermia.

The studied population is informative but its size is not large enough to make definitive inferences about the involvement of these two mutations in non-obstructive azoospermia.

Further studies with greater number of *CFTR* mutations and patients are therefore needed to examine the role of *CFTR* in non-obstructive azoospermia.

Acknowledgements

This study was carried out at University of Isfahan and was financially supported by the Graduate Studies Office. The authors are grateful to the Graduate Office of the University of Isfahan for their support. We would also like to thank Isfahan Infertility Center and Sari Saint Mary's Infertility Center. The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Horsthemke B, Ludwig M. Assisted reproduction: the epigenetic perspective. Hum Reprod Update. 2005; 11(5): 473-482.
2. Oliva R. Protamines and male infertility. Hum Reprod Update. 2006; 12(4): 417-435.
3. Baldinotti F, Majore S, Fogli A, Marrocco G, Ghirri P, Vuerich M, et al. Molecular characterization of 6 unrelated Italian patients with 5alpha-reductase type 2 deficiency. J Androl. 2008; 29(1): 20-28.
4. Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. Eur J Hum Genet. 2002; 10(5): 303-312.
5. Galani A, Kitsiou-Tzeli S, Sofokleous C, Kanavakis E, Kalpini-Mavrou A. Androgen insensitivity syndrome: clinical features and molecular defects. Hormones (Athens). 2008; 7(3): 217-229.
6. Safinejad K, Darbouy M, Kalantar SM, Zeinali S, Mirfakhraie R, Yadegar L, et al. The prevalence of common CFTR mutations in Iranian infertile men with non-CAVD obstructive azoospermia by using ARMS PCR techniques. J Assist Reprod Genet. 2011; 28(11): 1087-1090.
7. Ghorbel M, Baklouti-Gargouri S, Keskes R, Sellami-Ben Hamida A, Feki-Chakroun N, Bahloul A, et al. Screening of ΔF508 mutation and IVS8-poly T polymorphism in CFTR gene in Tunisian infertile men without CBAVD. Andrologia. 2012; 44 Suppl 1: 376-382.
8. Lu S, Yang X, Cui Y, Li X, Zhang H, Liu J, et al. Different cystic fibrosis transmembrane conductance regulator mutations in Chinese men with congenital bilateral absence of vas deferens and other acquired obstructive azoospermia. Urology. 2013; 82(4): 824-828.
9. Ferlin A, Arredi B, Foresta C. Genetic causes of male infertility. Reprod Toxicol. 2006; 22(2): 133-141.
10. Schulz S, Jakubiczka S, Kropf S, Nickel I, Muschke P, Kleinstein J. Increased frequency of cystic fibrosis transmembrane conductance regulator gene mutations in infertile males. Fertil Steril. 2006; 85(1): 135-138.
11. Sharma H, Mavuduru RS, Singh SK, Prasad R. Increased frequency of CFTR gene mutations identified in Indian infertile men with non-CBAVD obstructive azoospermia and spermatogenic failure. Gene. 2014; 548(1): 43-47.
12. Cuppens H, Cassiman JJ. CFTR mutations and polymorphisms in male infertility. Int J Androl. 2004; 27(5): 251-

256.
13. Schwarzer JU, Schwarz M. Significance of CFTR gene mutations in patients with congenital aplasia of vas deferens with special regard to renal aplasia. Andrologia. 2012; 44(5): 305-307.
14. Chen H, Ruan YC, Xu WM, Chen J, Chan HC. Regulation of male fertility by CFTR and implications in male infertility. Hum Reprod Update. 2012; 18(6): 703-713.
15. Yu J, Chen Z, Ni Y, Li Z. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Hum Reprod. 2012; 27(1): 25-35.
16. Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod. 2002; 17(1): 13-16.
17. Jakubiczka S, Bettecken T, Stumm M, Nickel I, Müsebeck J, Krebs P, et al. Frequency of CFTR gene mutations in males participating in an ICSI programme. Hum Reprod. 1999; 14(7): 1833-1834.
18. Stuppia L, Antonucci I, Binni F, Brandi A, Grifone N, Colosimo A, et al. Screening of mutations in the CFTR gene in 1195 couples entering assisted reproduction technique programs. Eur J Hum Genet. 2005; 13(8): 959-964.
19. Tamburino L, Guglielmino A, Venti E, Chamayou S. Molecular analysis of mutations and polymorphisms in the CFTR gene in male infertility. Reprod Biomed Online. 2008; 17(1): 27-35.
20. van der Ven K, Messer L, van der Ven H, Jeyendran RS, Ober C. Cystic fibrosis mutation screening in healthy men with reduced sperm quality. Hum Reprod. 1996; 11(3): 513-517.
21. Cruger DG, Agerholm I, Byriel L, Fedder J, Bruun-Petersen G. Genetic analysis of males from intracytoplasmic sperm injection couples. Clin Genet. 2003; 64(3): 198-203.
22. Boucher D, Creveaux I, Grizard G, Jimenez C, Hermabessière J, Dastugue B. Screening for cystic fibrosis transmembrane conductance regulator gene mutations in men included in an intracytoplasmic sperm injection programme. Mol Hum Reprod. 1999; 5(6): 587-593.
23. Ravnik-Glavac M, Svetina N, Zorn B, Peterlin B, Glavac D. Involvement of CFTR gene alterations in obstructive and nonobstructive infertility in men. Genet Test. 2001; 5(3): 243-247.
24. Gong XD, Li JC, Cheung KH, Leung GP, Chew SB, Wong PY. Expression of the cystic fibrosis transmembrane conductance regulator in rat spermatids: implication for the site of action of antispermatogenic agents. Mol Hum Reprod. 2001; 7(8): 705-713.
25. Hihnala S, Kujala M, Toppari J, Kere J, Holmberg C, Hoglund P. Expression of SLC26A3, CFTR and NHE3 in the human male reproductive tract: role in male subfertility caused by congenital chloride diarrhoea. Mol Hum Reprod. 2006; 12(2): 107-111.
26. Boockfor FR, Morris RA, DeSimone DC, Hunt DM, Walsh KB. Sertoli cell expression of the cystic fibrosis transmembrane conductance regulator. Am J Physiol. 1998; 274(4 Pt 1): C922-930.
27. Rychlik W. OLIGO 7 primer analysis software. Methods Mol Biol. 2007; 402: 35-60.
28. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16(3): 1215.
29. Xu WM, Chen J, Chen H, Diao RY, Fok KL, Dong JD, et al. Defective CFTR-dependent CREB activation results in impaired spermatogenesis and azoospermia. PLoS One. 2011; 6(5): e19120.
30. Meng MV, Black LD, Cha I, Ljung BM, Pera RA, Turek PJ. Impaired spermatogenesis in men with congenital absence of the vas deferens. Hum Reprod. 2001; 16(3): 529-533.
31. Tuerlings JH, Mol B, Kremer JA, Looman M, Meuleman EJ, te Meerman GJ, et al. Mutation frequency of cystic fibrosis transmembrane regulator is not increased in oligozoospermic male candidates for intracytoplasmic sperm injection. Fertil Steril. 1998; 69(5): 899-903.
32. Larriba S, Bonache S, Sarquella J, Ramos MD, Giménez J, Bassas L, et al. Molecular evaluation of CFTR sequence variants in male infertility of testicular origin. Int J Androl. 2005; 28(5): 284-290.
33. Jarvi K, Zielenski J, Wilschanski M, Durie P, Buckspan M, Tullis E, et al. Cystic fibrosis transmembrane conductance regulator and obstructive azoospermia. Lancet. 1995; 345(8964): 1578.

Advisory Board of International Journal of Fertility and Sterility (Int J Fertil Steril)
Vol 10, No 1-4, 2016-2017

Advisory Board of International Journal of Fertility and Sterility (Int J Fertil Steril)
Vol 10, No 1-4, 2016-2017

Index by Authors in International Journal of Fertility and Sterility (Int J Fertil Steril) Vol 10, No 1-4, 2016-2017

Index by Authors in International Journal of Fertility and Sterility (Int J Fertil Steril) Vol 10, No 1-4, 2016-2017

International Journal of Fertility and Sterility (Int J Fertil Steril)
Guide for Authors

Aims and Scope: The ***"International Journal of Fertility & Sterility"*** is a quarterly English publication of Royan Institute of Iran. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on Fertility and Sterility and other related topics. ***Int J Fertil Steril*** has been certified by Ministry of Culture and Islamic Guidance since 2007. It has also been accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission since 2008. **This open access journal holds the membership of the Committee on Publication Ethics (COPE).**

1. Types of articles
The articles in the field of Fertility and Sterility can be considered for publications in ***Int J Fertil Steril***. These articles are as below:
A. Original articles are scientific reports of the original research studies. The article consists of English Abstract (structured), Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, and References.
B. Review articles are the articles written by well experienced authors and those who have excellence in the related fields. The corresponding author of the review article must be one of the authors of at least three articles appearing in the references. The review article consists of English Abstract (unstructured), Introduction, Discussion, Conclusion, and References. Please refer to journal sample for other types of review (Systematic review, Pictorial review and also Debates).
C. Short communications are the articles containing new findings. Submissions should be brief reports of ongoing researches. The short communication consists of English Abstract (unstructured), the body of manuscript (should not hold heading or subheading), Acknowledgements, and References.
D. Case reports are published if only the report is of exceptional interest. It consists of English Abstracts (Unstructured), Introduction, Case Report, Discussion, Acknowledgements, and References.
E. Editorial should be written by either the editor in chief or the editorial board.
F. Imaging in reproductive medicine should focus around a single case with an interesting illustration such as a photograph, histological specimen or investigation. Color images are welcomed. The text should be brief and informative.
G. Letter to the editors are comments made by our readers on recently published articles.

2. Submission Process
A. Cover letter
Each article should be accompanied by a cover letter, signed and dated by corresponding author specifying the following statement: The manuscript has been seen and approved by all authors and is not under active consideration for publication. It has neither been accepted for publication, nor published in another journal fully or partially (except in abstract form). I hereby assign the copyright of the enclosed manuscript to ***Int J Fertil Steril.*** Corresponding author can also suggest three peer reviewers in the field of their article.
B. Copyright
All listed authors must sign the "Copyright Transfer Agreement". To the same token, the responsibility for the article's content should be borne by each one of the authors. No article will be published without a copyright transfer agreement signed by all the listed authors. Any use, distribution, reproduction or abstract of this publication in any medium, with the exception of commercial purposes, is permitted if the original work is properly cited.
C. Manuscript preparation
Authors whose first language is not English, encouraged to consult a native English speaker in order to confirm his manuscripts to US or British (not a mixture) English usage and grammar. Manuscript should be prepared in accordance with the "International Committee of Medical Journal Editors (ICMJE)". Before publishing author's article, it would be the author's responsibility to pay for the expenses, if the editor feels the level of English used in the manuscript requires editing. The manuscript must be typed in a font size of at least 12 points, double spaced with margins of at least 2.5 cm (1 inch). Please send your article in two formats (word and Pdf). The abstract and text pages should have consecutive line numbers in the left margin beginning with title page and continuing through the last page of the written text. Each abbreviation must be defined in the abstract and text when they are mentioned for the first time. Avoid using abbreviation in title. Please use the international and standard abbreviations and symbols.
It should be added that an essential step toward the integration and linking of scientific information reported in published literature is using standardized nomenclature in all fields of science and medicine. Species names must be italicized (e.g., *Homo sapiens*) and also the full genus and species written out in full, both in the title of the manuscript and at the first mention of an organism in a paper.
It is necessary to mention that genes, mutations, genotypes, and alleles must be indicated in italics. Please use the recommended name by consulting the appropriate genetic nomenclature database, e.g., HUGO for human genes. In another words; if it is a human gene, you must write all the letters in capital and italic (e.g., *OCT4, c-MYC*). If not, only write the first letter in capital and italic (e.g., *Oct4, c-Myc*). **In addition, protein designations are the same as the gene symbol, but are not italicized**.
Each of the following manuscript components should begin in the following sequence:
Title is providing the full title of the research (do not use abbreviations in title), full name(s), highest awarded academic degree(s), email(s), and institutional affiliation(s) of all the authors in English. Also you must send moblie number and full postal address of corresponding author.
Running title is providing a maximum of 7 words (no more than 50 characters).
Abstract must include: Background, Materials and Methods, Results, and Conclusion.
Keywords, three to five, must be supplied by the authors at the foot of the abstract chosen from the Medical Subject Heading (MeSH). Therefore; they must be specific and relevant to the paper.
The following components should be identified after the abstract:
Introduction: This part includes the purpose and the rationale of the study. It should neither review the subject extensively, nor have data or conclusions of the study.
Materials and Methods: It should include the exact methods or observations of experiments. If an apparatus is used, its manufacturer's name and address should be stipulated in parenthesis. If the method is established, give reference but if the method is new, give enough information so that another author can perform it. If a drug is used, its generic name, dose and route of administration must be given. Standard units of measurements and chemical symbols of elements do not need to be defined.
Statistical analysis: Type of study and statistical methods should be mentioned and specified by any general computer program used.
Ethical considerations*:* Please state that informed consent was obtained from all human adult participants and from the parents or legal guardians of minors and include the name of the appropriate institutional review board that approved the project. It is necessary to indicate in the text that the maintenance and care of experimental animals complies with National Institutes of Health guidelines for the humane use of laboratory animals, or those of your Institute or agency.
Results: They must be presented in the form of text, tables and figures. The contents of the tables should not be all repeated in the text. Tables and figures must be numbered consecutively as appeared in the text and should be organized in separate pages at the end of article while their location should be mentioned in the main text. Long articles may need sub-headings within some sections (especially the Results and Discussion parts) to clarify their contents.
Legends of Tables: A short descriptive heading should be given above each table and any footnotes and explanations underneath.
Figures: They must be sent in color and also in GIF or JPEG format with 300 dpi resolutions.
Discussion: It should emphasize the present findings and the variations or similarities with other researches done by other researchers. The detailed results should not be repeated in the discussion again. Emphasize the new and important aspects of the study.
Conclusion: It emphasizes the new and important aspects of the study. All conclusions are justified by the results of the study. It must be mentioned whether the hypothesis mentioned in the article is true, false or no conclusions can be derived.
Acknowledgements: This optional part should include a statement thanking those who contributed substantially with work relevant to the study. It should include persons who provided technical help, writing assistance and name of departments that provided only general support. Grant support should be included in this section.

Conflict of Interest: Any conflict of interest (financial or otherwise) and sources of financial support must be listed in the Acknowledgements. It includes providers of supplies and services from a commercial organization. Any commercial affiliation must be disclosed, regardless of providing the funding or not.
References The references must be written based on the Vancouver style. Thus the references are cited numerically in the text and listed in the bibliography by the order of their appearance. The titles of journals should be abbreviated according to the style used in the list of Journals Indexed in PubMed. Write surname and initials of all authors when there are six or less. In the case of seven or more authors, the names of first six authors followed by "et al." should be listed. The reference of information must be based on the following order:
Article:
Surname(s) and first letter of name & middle name(s) of author(s). Manuscript title. Journal title (abbr).publication date (year); Volume (Issue): Page number.
Example: Manicardi GC, Bianchi PG, Pantano S, Azzoni P, Bizzaro D, Bianchi U, et al. Presence of endogenous nicks in DNA of ejaculated human spermatozoa and its relationship to chromomycin A3 accessibility. Biol Reprod. 1995; 52(4): 864-867.
Book:
Surname(s) and first letter of name & middle name(s) of author(s). Book title. Edition. Publication place: publisher name; publication date (year); Page number.
Example: Edelman CL, Mandle CL. Health promotion throughout the life span. 2nd ed.ST Louis: Mosby; 1998; 145-163.
Chapter of book:
Surname(s) and first letter of name & middle name(s) of author(s). Chapter title. In: Surname(s) and first letter of name &middle name(s) of editor(s), editors. Book title. Edition. Publication place: publisher name; publication date (year); Page number.
Example: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995; 465-478.
Abstract book:
Example: Nabavi SM. Stem cell therapy for multiple sclerosis. Cell J. 2013; 5 Suppl 1: Os-13.
Thesis:
Name of author. Thesis title. Degree. City name. University. Publication date (year).
Example: Eftekhari Yazdi P. Comparison of fragment removal and co-culture with Vero cell monolayer's on development of human fragmented embryos. Presented for the Ph.D., Tehran. Tarbiyat Modarres University. 2004.
Conferences:
Name(s) of editor(s). Conference title; Holding date; Holding place. Publication place; Publisher name; Publication date (year).
Example: Harnden P, Joffe JK, Jones WG, editors. Germ cell tumors V.Proceedings of the 5th Germ Cell Tumors Conference; 2001 Sep 13-15; Leeds,UK. New York: Springer; 2002.
Internet References
Article:
Surname(s) and first letter of name & middle name(s) of author(s). Manuscript title. Journal title (abbr). publication date (year); Volume (Issue): Page number. Available from: URL link. (Observation date).
Example: Jahanshahi A, Mirnajafi-Zadeh J, Javan M, Mohammad-Zadeh M, Rohani M. Effect of low-frequency stimulation on adenosineA1 and A2A receptors gene expression in dentate gyrus of perforant path kindled rats. Cell J. 2008; 10 (2): 87-92. Available from: http://www.celljournal.org. (20 Oct 2008).
Book:
Example: Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology.[CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.
Law:
Example: Embryo donation law. Iran Judicature, Official Gazette of the Islamic Republic of Iran. Available from: http://www.dastour.ir/Brows/?lid=245069 .(20 Jul 2013).
D. Proofs: Proofs are sent by email as PDF files and should be checked and returned within 72 hours of receipt. It is the authors' responsibility to check that all the text and data as contained in the page proofs are correct and suitable for publication. The authors are requested to pay particular attention to author's names and affiliations as it is essential that these details be accurate when the article is published.
E. Pay for publication: Publishing any article is free of charge.
F. Ethics of scientific publication: Plagiarism of text from a previously published manuscript by the same or another author is a serious publication offence. Some parts of text may be used, only where the source of the quoted material is clearly acknowledged.
G. Clinical trial registration: All of the Clinical Trials performed in Iran must be registered in Iranian Registry of Clinical Trials (www.irct.ir), in order to be considered for publication even if they register in other registration site. The clinical trials performed abroad, could be considered for publication if the authors register in a website approved by WHO. This includes all of the clinical trials conducted.

3. General information
A. You can send your article via online submission system which is available at our website: **http://www.ijfs.ir.** If the article is not prepared according to the format of ***Int J Fertil Steril***, it will be returned to authors.
B. The order of article appearance in the Journal is not demonstrating the scientific characters of the authors.
C. Int J Fertil Steril has authority to accept or reject the articles.
D. The received articles will be evaluated by one epidemiologist. Then associate editor will determine its reviewers. If three reviewers pass their judgments on the article, it will be presented to the editorial board of ***Int J Fertil Steril.*** If editorial board has a positive judgment about the article, reviewers' comments will be presented to corresponding author (the identification of the reviewers will not be revealed). The executive member of journal will contact the corresponding author directly within 7-8 weeks by email. If authors do not receive any reply from journal office after the specified time, they can contact journal office. Executive manager will respond promptly to authors' message.
The Final Checklist
The authors must ensure that before submitting the manuscript for publication, they have to consider the following parts:
1. Title page should contain title, name of the author/coauthors, their academic qualifications, designation & institutions they are affiliated with, mailing address for future correspondence, email address, phone, and fax number.
2. Text of article and References prepared as stated in the "guide for authors" section.
3. Tables should be in a separate page. Figures must be sent in color and also in GIF or JPEG format with 300 dpi resolutions.
4. Covering Letter

The Editor-in-Chief: Mohammad Hossein Nasr Esfahani, Ph.D.
International Journal of Fertility and Sterility (Int J Fertil Steril)
P.O. Box: 16635-148, Iran
Tel/Fax: + 98-21-22510895
Emails: ijfs@royaninstitute.org
info@ijfs.ir