Debate

► Male Infertility during Antihypertensive Therapy: Are We Addressing Correctly The Problem?
Antonio Simone Laganà, Salvatore Giovanni Vitale, Paola Iaconianni, Simona Gatti, Francesco Padula ............... 267

Review Article

► Aromatase Inhibitors for Endometriosis-Associated Infertility; Do We Have Sufficient Evidence?
Hatem Abu Hashim .................................................................................................................................................. 270

Original Articles

► Prevalence of Infertility Problems among Iranian Infertile Patients Referred to Royan Institute
Mahdi Sepidarkish, Amir Almasi-Hashiani, Fatemeh Shokri, Samira Vesali, Elaheh Karimi, Reza Omani Samani ...... 278

► Assisted Reproductive Technology in Iran: The First National Report on Centers, 2011
Mehrandokht Abedini, Azadeh Ghaheri, Reza Omani Samani .............................................................................. 283

► Comparison of Quality of Life, Sexual Satisfaction and Marital Satisfaction between Fertile and Infertile Couples
Seyedeh Zahra Masoumi, Maryam Garousian, Somayeh Khani, Seyedeh Reyhaneh Oliaei, Arezoo Shayan .......... 290

► Cord Blood Karyotyping: A Safe and Non-Invasive Method for Postnatal Testing of Assisted Reproductive Technology Children
Shabnam Zarei Moradi, Najmehsadat Masoudi, Anahita Mohseni Meybodi, Khadijeh Anisi Hemaseh, Ramin Mozaafari Kermani, Abolhasan Shahzadeh Fazeli, Hamid Gourabi ......................................................................................... 297

► Mitochondrial Genetic Variation in Iranian Infertile Men with Varicocele
Mohammad Mehdi Heidari, Mehri Khatami, Amirhossein Danafar, Tahere Dianat, Ghazaleh Farahmand, Ali Reza Talebi ..................................................................................................................................................... 303

► Expression Profile of Developmentally Important Genes in pre- and peri-Implantation Goat Embryos Produced In Vitro
Pouria HosseinNia, Mehdi Hajian, Mojtaba Tahmoorespur, Sayyed Morteza Hosseini, Somayyeh Ostadhosseini, Mohammad Reza Nasiri, Mohammad Hossein Nasr-Esfahani ........................................................................................................... 310
Male Infertility during Antihypertensive Therapy: Are We Addressing Correctly The Problem?

Antonio Simone Laganà, M.D.*, Salvatore Giovanni Vitale, M.D.1, Paola Iaconianni, M.D.2, Simona Gatti, M.D.2, Francesco Padula, M.D.3

1. Unit of Gynecology and Obstetrics, Department of Human Pathology in Adulthood and Childhood “G. Barresi”, University of Messina, Messina, Italy
2. Department of Reproductive Medicine, Altamedica Fetal Maternal Medical Centre, Rome, Italy
3. Department of Prenatal Diagnosis, Altamedica Fetal Maternal Medical Centre, Rome, Italy

Abstract
Male fertility significantly decreased in the last 50 years, as showed in several studies reporting a reduction of sperm counts per ml in the seminal fluid. Several “acute” pharmacological treatments, as antibiotics, could cause subclinical and temporary reduction of male fertility; conversely, long-term medical treatment may severely affect male fertility, although this effect could be considered transient in most of the cases. Thus, nowadays, several long-term pharmacological treatments may represent a clinical challenge. The association between several kind of antihypertensive drugs and reduction of male fertility has been showed in the mouse model, although the modification(s) which may alter this fine-regulated machinery are still far to be elucidated. Furthermore, well-designed observational studies and randomized controlled trials are needed to accurately define this association in human model, meaning a narrative overview synthesizing the findings of literature retrieved from searches of computerized databases. We strongly solicit future human studies (both observational and randomized clinical trials) on large cohorts with adequate statistical power which may clarify this possible association and the effects (reversible or permanent) of each drug. Furthermore, we suggest a close collaboration between general practitioners, cardiologists, and andrologists in order to choose the most appropriate antihypertensive therapy considering also patient’s reproductive desire and possible risk for his fertility.

Keywords: Andrology, Infertility, Antihypertensive

Citation: Laganà AS, Vitale SG, Iaconianni P, Gatti S, Padula F. Male infertility during antihypertensive therapy: are we addressing correctly the problem? Int J Fertil Steril. 2016; 10(3): 267-269.

Introduction
Male fertility significantly decreased in the last 50 years, as showed in several studies reporting a reduction of sperm counts per ml in the seminal fluid (1). To date, male factors account for almost 35% of couple infertility. As widely accepted, sperm counts may vary among different ejaculates according to several pathological conditions, lifestyle and exposure to pollutants (2). In this regard, although well-known diseases such as cryptorchidism, varicocele, hypospadias, testicular tumors, Y chromosome microdeletion and endocrine alterations can cause azoospermia and/or oligozoospermia, iatrogenic risk factors may also play a detrimental role in male fertility. Several “acute” pharmacological treatments, as antibiotics, could cause subclinical and temporary reduction of male fertility; conversely, long-term medical treatment may severely affect male fertility, although this effect could be considered transient in most of the cases. Thus, nowadays, several long-term pharmacological treatments may represent a clinical challenge. To the best of our knowledge, association between several kind of antihypertensive drugs and...
reduction of male fertility has been showed in the mouse model (3-5), although the modification(s)
which may alter this fine-regulated machinery are still far to be elucidated and human data are
still lacking. Indeed, data from the following animal studies are not robust: several studies (6-8)
showed that verapamil, nimodipine, and lisinopril worsen semen quality and testicular morphology,
while others (9, 10) have found that nifedipine and lisinopril improve these parameters.

In this regards, Bechara et al. (11) have studied the effects of an angiotensin-converting enzyme
(ACE) inhibitor (Enapril) on hypertension-induced morphological changes in the testis and sperma-
tozoid production in spontaneously hypertensive rats. According to their data analysis, sperm con-
centration was greater in the treated group than in the nontreated group, testicular vascular volumet-
ric density decreased in the nontreated group and, last but not least, volumetric density of the semi-
niferous epithelium in the treated group was higher than in the nontreated group. Although these
results could not be de facto translated in humans, they have suggested a possible pivotal role of ACE
inhibitors as first-line treatment when fertility is a relevant concern. Even if well-designed obser-
vational studies and randomized controlled trials are needed to accurately define this association in
human model, daily clinical experience seems to confirm that in case of antihypertensive therapy
with concomitant male infertility, the substitution of the drug with another one which does not affect
semen parameters may improve male fertility.

In particular, beta blockers and calcium-channel blockers (CCBs) seem to play a detrimental role
on male fertility, causing in several cases azoospermia and/or oligozoospermia. On the other
hand, inhibitors of the funny channel, such as oral ivabradine, seem not to be associated with reduc-
tion of male fertility. In this regard, it was already demonstrated in the mouse model that CCBs, like
amlodipine, can cause a reduction of testosterone, luteinizing hormone (LH) and follicular stimulat-
ing hormone (FSH), leading to affect spermatogenesis and sperm parameters (12, 13). However,
these data do not seem to be surprising, since accumulating evidence have already suggested that
Ca\(^{2+}\) plays a prominent role during fertilization in all animal species. On one hand, in mice, rats, pigs,
hamsters and bovines, extracellular Ca\(^{2+}\) is neces-

sary for epididymal acquisition of sperm motility (14-18).

Furthermore, it is known to regulate both activated and hyperactivated motility (19-21). In ad-
dition, flagellar motility is controlled by calcium through the regulation of dynein-driven microtu-
bule sliding and modulation of the sperm flagellar waveform (22, 23). Finally, calcium has a pivotal
role during the acrosome reaction in invertebrates, such as echinoderms, and superior vertebrates (24,
25). Also, in this case, although these data demonstrated that it does have a direct impact on humans,
they may underlie the possible detrimental effect of calcium antagonists administered for hyperten-
sion on male fertility.

**Conclusion**

The average life expectancy is increasing and even more older male patients refer to fertility
experts, the association between antihypertensive therapy and male infertility is a growing area of
interest. Based on this scenario, we strongly so-
licit future human studies (both observational and randomized clinical trials) on large cohorts and
with adequate statistical power which may show this possible association and the effects (reversible
or permanent) of each drug. Furthermore, we sug-
gest a close collaboration between general prac-
titioners, cardiologists, and andrologists in order
to choose the most appropriate antihypertensive
therapy considering also patient’s reproductive de-
sire and possible risk for his fertility.

**Acknowledgements**

The authors have no proprietary, financial, pro-
fessional or other personal interest of any nature
in any product, service or company. The authors
alone are responsible for the content and writing
of the paper. There is no conflict of interest in this
article.

**References**

1. Winters BR, Walsh TJ. The epidemiology of male infertil-
ity. Urol Clin North Am. 2014; 41(1): 195-204.
2. Nudell DM, Monoski MM, Lipshultz LI. Common medica-
tions and drugs: how they affect male fertility. Urol Clin
North Am. 2002; 29(4): 965-973.
3. Dantas da Silva Júnior E, Palmieri de Souza B, Rodrigues
JQD, Caricati-Neto A, Jurkiewicz A, Jurkiewicz NH. Ef-
fects of clonidine in the isolated rat testicular capsule. Eur
J Pharmacol. 2014; 726: 16-26.
4. Leal MC, Pinheiro SV, Ferreira AJ, Santos RA, Bordoni
Male Infertility during Antihypertensive Therapy

LS, Alenina N, et al. The role of angiotensin-(1-7) receptor Mas in spermatogenesis in mice and rats. J Anat. 2009; 214(5): 736-743.

5. Latif R, Lodhi GM, Aslam M. Effects of amloidine on serum testosterone, testicular weight and gonado-somatic index in adult rats. J Ayub Med Coll Abbottabad. 2008; 20(4): 8-10.

6. Juneja R, Gupta I, Wali A, Sanyal SN, Chakravarti RN, Majumdar S. Effect of verapamil on different spermatid functions in guinea pigs--a preliminary study. Contraception. 1990; 41(2): 179-187.

7. Saha L, Bhargava VK, Garg SK, Majumdar S. Effect of nimodipine on male reproductive functions in rats. Indian J Physiol Pharmacol. 2000; 44(4): 449-455.

8. Saha L, Garg SK, Bhargava VK, Mazumdar S. Role of angiotensin-converting enzyme inhibitor lisinopril, on spermatid functions in rats. Methods Find Exp Clin Pharmacol. 2000; 22(3): 159-162.

9. Akagashi K, Kumamoto Y, Itoh N, Tsukamoto T, Suzuki T, Ohta Y. Manidipine improves spermatogenesis in the stroke-prone spontaneously hypertensive rat. J Androl. 1997; 18(2): 210-216.

10. Okeahialam BN, Amadi K, Ameh AS. Effect of lisinopril, an angiotensin converting enzyme inhibitor on spermatogenesis in rats. Arch Androl. 2006; 52(3): 209-213.

11. Bechara GR, de Souza DB, Simoes M, Felix-Patricio B, Medeiros JL, Costa WS, et al. Testicular morphology and spermatid parameters in spontaneously hypertensive rats treated with enalapril. J Urol. 2015; 194(5): 1498-1503.

12. Almeida SA, Teófilo JM, Anselmo Franci JA, Brentegani LG, Lamano-Carvalho TL. Antireproductive effect of the calcium channel blocker amloidipine in male rats. Exp Toxicol Pathol. 2000; 52(4): 353-356.

13. Latif R, Aslam M, Mehrmood T. Spermatogenesis following discontinuation of calcium channel blocker amloidipine in rats. J Ayub Med Coll Abbottabad. 2009; 21(1): 25-27.

14. Morton B, Harrigan-Lum J, Albargi L, Jooss T. The activation of motility in quiescent hamster sperm from the epididymis by calcium and cyclic nucleotides. Biochem Biophys Res Commun. 1974; 56(2): 372-379.

15. Morton BE, Sagadracza R, Fraser C. Sperm motility within the mammalian epididymis: species variation and correlation with free calcium levels in epididymal plasma. Fertil Steril. 1978; 28(6): 695-698.

16. Davis BK. Effect of calcium on motility and fertilization by rat spermatozoa in vitro. Proc Soc Exp Biol Med. 1978; 157(1): 54-56.

17. Yanagimachi R. Acceleration of the acrosome reaction and activation of guinea pigs spermatozoa by detergents and other reagents. Biol Reprod. 1975; 13(5): 519-526.

18. Babcock DF, First NL, Lardy HA. Action of ionophore A23187 at the cellular level. Separation of effects at the plasma and mitochondrial membranes. J Biol Chem. 1976; 251(13): 3881-3886.

19. Tash JS, Means AR. Ca2+ regulation of sperm axonemal motility. Methods Enzymol. 1987; 139: 808-823.

20. Lindemann CB, Goltz JS. Calcium regulation of flagellar curvature and swimming pattern in triton X-100--extracted rat sperm. Cell Motil Cytoskeleton. 1988; 10(3): 420-431.

21. Ho HC, Granish KA, Suarez SS. Hyperactivated motility of bull sperm is triggered at the axoneme by Ca2+ and not cAMP. Dev Biol. 2002; 250(1): 208-217.

22. Suarez SS, Varosi SM, Dai X. Intracellular calcium increases with hyperactivation in intact, moving hamster sperm and oscillates with the flagellar beat cycle. Proc Natl Acad Sci USA. 1993; 90(10): 4660-4664.

23. Gross MK, Toscano DG, Toscano WA Jr. Calmodulin-mediated adenylyl cyclase from mammalian sperm. J Biol Chem. 1987; 262(18): 8672-8676.

24. Blackmore PF. Extranuclear actions of progesterone in human sperm and progesterone metabolites in human platelets. Steroids. 1999; 64(1-2): 149-156.

25. Florman HM, Corron ME, Kim TD, Babcock DF. Activation of voltage-dependent calcium channels of mammalian sperm is required for zona pellucida-induced acrosomal exocytosis. Dev Biol. 1992; 152(2): 304-314.
Aromatase Inhibitors for Endometriosis-Associated Infertility; Do We Have Sufficient Evidence?

Hatem Abu Hashim, M.D., M.R.C.O.G., Ph.D.*

Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Abstract

Orally active aromatase inhibitors (AIs) have gained attention for treatment of infertile women with endometriosis in whom aromatase p450 is aberrantly expressed. This review aimed to critically appraise and summarize the available evidence concerning the use of AIs for management of endometriosis-associated infertility. PubMed was searched to May 2015 with the following key words: endometriosis, infertility and aromatase. Priority was given for randomized controlled trials (RCTs) followed by other study designs. Main outcome measures were as follows: rates of clinical pregnancy, miscarriage and live birth as well as endocrine outcomes. Eighty-two abstracts were screened and six original articles were included. A RCT demonstrated that post-operative letrozole treatment did not improve spontaneous pregnancy rate. Another RCT reported no superiority of letrozole superovulation over clomiphene citrate (each combined with intrauterine insemination) in minimal–mild endometriosis and previous laparoscopic treatment. Anastrozole significantly inhibited the growth of endometriotic cells and their estrogen production in culture. In assisted reproductive technology (ART) cycles, dual suppression (Agonist/anastrozole) was tested in a pilot study with a pregnancy rate of 45% however, high pregnancy loss (30%) occurred. A retrospective study showed that letrozole may improve endometrial receptivity in endometriotic patients undergoing in vitro fertilization (IVF). An opposite view from an in vitro study showed lower estradiol production and aromatase expression in cultured granulosa cells from endometriotic women undergoing IVF and marked reduction under letrozole. In conclusion, current evidence is limited. More trials are warranted to enhance our knowledge and provide a clear and unequivocal evidence to guide our clinical management of infertile women with endometriosis using AIs.

Keywords: Endometriosis, Infertility, Aromatase, Assisted Reproductive Technology

Citation: Abu Hashim H. Aromatase inhibitors for endometriosis-associated infertility; do we have sufficient evidence? Int J Fertil Steril. 2016; 10(3): 270-277.

Introduction

Endometriosis is a complex clinical issue, in which the quote of Albert Einstein "The most incomprehensible thing of the world is that it is comprehensible" is merit worthy. In this context, endometriosis is not only classically defined as the presence of endometrial glands and stroma outside the uterine cavity (1), but also well known as three separate entities (peritoneal, ovarian, or deeply infiltrating) (2) as well as 4 distinct stages (from minimal or stage 1 to severe disease or stage 4) (3). Nevertheless, endometriosis remains enigmatic in its pathogenesis and controversial in therapy till now (4). It is estimated that 5 to 10% of reproductive age women have endometriosis with a higher prevalence rate up to 50% among infertile women (5). Importantly, endometriosis-associated infertility represents a therapeutic dilemma because of its illusive background including ovulatory dysfunction related to altered folliculogenesis as well as impaired steroidogenesis of granulosa cells, impaired fertilization, low quality embryos, defective implantation, sperm phagocytosis, the embryo toxic environment and pelvic adhesions in advanced stages (6). Aromatase p-450 enzyme is the key enzyme in the biosynthesis of estradiol (E2) in the ovarian granulose cells of premenopausal women. Notably, unlike women without endometriosis, high levels of aromatase P450 enzyme expression has been shown in eutopic endometrial tissue as well as in ectopic endometrial...
implants in endometriotic patients (7). This abnormal aromatase expression results in local estrogen production by endometriotic implants. Together with other mechanisms such as altered immune responses, angiogenesis and apoptosis, the autonomously produced estrogen leads to inflammation, proliferation and survival of endometriotic implants (8-10). In view of their ability to inhibit estrogen biosynthesis, third generation aromatase inhibitors (AIs) mainly letrozole and anastrozole have challenged clomiphene citrate (CC) in management of infertile patients with polycystic ovary syndrome as well as those with unexplained infertility (11). Additionally, the use of AIs to suppress the locally produced E$_2$ by endometriotic deposits has gained ground as a potential therapy for correcting abnormal endocrine and reproductive function of patients with endometriosis (9-11). Noteworthy, letrozole and anastrozole have favorable pharmacokinetics and pharmacodynamics facilitating their use in clinical practice due to the following reasons: being selective, completely absorbed orally with absolute bioavailability of 99.9%, reversible with mean half-life of 48 hours, greater potency with reduction in serum estrogen by 97 to 99% and clearance mainly by the liver (Fig. 1) (12). In that regard and given that this is a clinically important area to be addressed, this review was conducted to critically appraise and summarize the current evidence concerning the use of AIs for management of endometriosis-associated infertility in both non-assisted as well as assisted reproductive technology (ART) cycles.

Eighty-two abstracts were retrieved from an electronic search using the PubMed from inception to May 15, 2015 with the following search terms "endometriosis", "infertility" and "aromatase" without search filters. Studies in which reproductive outcome (rates of clinical pregnancy, miscarriage and live birth) as well as endocrine outcomes were included in the following order systematic reviews and meta-analyses of randomized controlled trials (RCTs), RCTs, prospective cohort studies and other observational studies. Manual screening of references of the retrieved articles was done to identify other pertinent studies. Finally, results of 6 original articles were included concerning the use of AIs for management of endometriosis-associated infertility in both non-assisted as well as ART cycles (Tables 1, 2) (13-18).

![Fig.1: Favorable pharmacokinetics and pharmacodynamics of letrozole and anastrozole.](image)

**Table 1: Aromatase inhibitors in endometriosis-associated infertility in non-ART cycles**

| References               | Study design/sample size | Concept                                                                 | Intervention                                                                 | Outcome                                                                 |
|--------------------------|--------------------------|-------------------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Alborzi et al. (13)      | RCT (n=144)              | Post-operative suppression with letrozole and pregnancy outcome as well as the disease recurrence rate | Letrozole 2.5 mg/day vs. triptorelin 3.75 mg IM every month vs. no medication for 2 months after laparoscopic surgery, with a 12 months follow up | No significant differences among the three groups with regard to the pregnancy rate (23.4 vs. 27.5 and 28.1% respectively) as well as the disease recurrence rate |
| Abu Hashim et al. (14)   | RCT (n=136)              | Superovulation with letrozole+IUI in stage I-II endometriosis with no pregnancy 6-12 months after laparoscopy | Letrozole/IUI vs. CC/IUI                                                      | No significant differences between both groups for clinical pregnancy rate per cycle, cumulative pregnancy rate, miscarriage, or live birth rates. |

ART: Assisted reproductive technology, CC; Clomiphene citrate, IM; Intramuscular injection, IUI; Intrauterine insemination, and RCT; Randomized controlled trial.
Table 2: Aromatase inhibitors in women with endometriosis-associated infertility undergoing ART

| References     | Study design/sample size                                                                 | Concept                                                                 | Intervention                                                                 | Outcome                                                                 |
|----------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Badawy et al. (15) | An *in vitro* study on cultured endometriotic cells                                        | To demonstrate the effect of anastrozole, on the growth and E₂ production of endometriotic cells in culture | First addition of testosterone (10 µg/mL) to the culture medium then addition of anastrozole, in a dose of 200 µg/mL and 300 µg/mL, Anastrozole produced significant decrease in endometriotic cell count as well as decrease in E₂ secretion and this effect was dose dependent. |
| Lossl et al. (16)       | A prospective pilot study [n=20 with endometriosis undergoing IVF (n=16)/ICSI (n=4)]    | Dual suppression                                                          | Prolonged down-regulation by combined 3-month GnRHa+1 mg anastrozole/day prior to IVF | Significant reduction of endometriomai volume (29%) and serum CA125 (61%). 45% clinical pregnancy rate and 15% live birth rate. |
| Miller et al. (17)      | A retrospective cohort study (n=97 with endometriosis undergoing IVF)                      | Letrozole co-treatment might improve the IVF success rates by improving endometrial receptivity | 29/79 women undergoing standard IVF lacked normal integrin expression. Other 18 integrin-negative women received letrozole early in IVF stimulation (5 mg, days 2-6). | Significantly higher clinical pregnancy and delivery rates observed in integrin-negative patients who received letrozole as compared to those who did not receive letrozole (61 vs. 14%, P<0.001 and 50 vs. 7%, P<0.001, respectively) |
| Lu et al. (18)          | An *in vitro* study on cultured LGC                                                       | Letrozole may compromise aromatase activity of LGC resulting in a poor reproductive outcome in patients with stage III/IV endometriosis undergoing ART | Effect of different concentrations of letrozole on E₂ production and P450 aromatase mRNA expression in cultured LGC from women with (n=23) and without endometriosis (n=19) | Significantly lower E₂ production and P450 aromatase mRNA expression occurred in women with endometriosis and further reduction of these parameters were demonstrated following letrozole in a concentration of 1 µmol/L. |

Discussion

The postoperative use of aromatase inhibitors in women who underwent laparoscopic surgery for endometriosis-associated infertility

The recent European Society of Human Reproduction and Embryology (ESHRE) Endometriosis Guideline demonstrated no evidence to support the use of postoperative hormonal therapy to improve spontaneous pregnancy rates in infertile women with endometriosis (19). This recommendation was based on the findings of a Cochrane meta-analysis by Furness et al. (20) including eight studies with 420 patients with endometriosis-associated infertility who were treated postoperatively by different modalities such as gonadotropin-releasing hormone agonist (GnRHa), medroxyprogesterone acetate, danazol and gestrinone [risk ratio (RR)=0.84, 95% confidence intervals (CI): 0.59-1.18]. Does the postoperative use of AIs increase the spontaneous pregnancy rate in women with endometriosis-associated infertility? This is a very relevant clinical question. Noteworthy, only one prospective RCT by Alborzi et al. (13) addressed this point among 144 patients who were diagnosed to have different stages of endometriosis ranging from minimal to severe disease by laparoscopy and histological confirmation. Patients were randomly allocated into the three following groups: group 1 who received letrozole 2.5 mg/day (n=47 cases), group 2 who had triptorelin (GnRHa) 3.75 mg intramuscular (IM) injection every 4 weeks (n=40 patients) and group 3 who received no medication for 2 months after laparoscopic surgery (n=57 patients) with a 12 months follow up period. The authors reported no significant differences among the three groups with regard to the pregnancy rate (23.4% in group 1 vs. 27.5% and 28.1% in groups 2 and 3 respectively) as well as the disease recurrence rate defined by recurrent symptoms in the form of dysmenorrhea, dyspareunia and pelvic pain (6.4% in group 1 vs. 5% and 5.3% in groups 2 and 3 respectively). Therefore, the authors did not recommend the post-operative use of letrozole or GnRHa in women undergoing surgery for endometriosis-associated infertility (13). The finding

ART; Assisted reproductive technology, CA; Cancer antigen, E₂; Estradiol, GnRHa; Gonadotropin-releasing hormone agonist, ICSI; Intracytoplasmic sperm injection, IVF; *In vitro* fertilization, and LGC; Luteinized granulosa cells.
from this RCT is in agreement with the aforementioned lack of beneficial effect of postoperative hormonal therapy on endometriosis-associated infertility (19).

Superovulation with aromatase inhibitors combined with intrauterine insemination for women with minimal or mild endometriosis-associated infertility

In view of the recent ESHRE Endometriosis Guideline, superovulation with intrauterine insemination (IUI) may be effective in increasing live birth rate, compared with expectant management in women with minimal to mild endometriosis (19, 21). In addition, superovulation with IUI may be more effective in increasing pregnancy rate than IUI alone and may be as effective in women with minimal or mild endometriosis within 6 months of surgical treatment as in women with unexplained infertility. In these women, there are potential benefits of superovulation including, improvement of the endocrine environment, correction of subtle ovulatory dysfunction as well as an increase in the number of mature oocytes. Thereby, it optimizes the chance per cycle of fertilization, embryo development and implantation (19, 22, 23). Unlike CC, letrozole has no downstream effects on the endometrium and cervical mucus. This could be attributed not only to its relatively shorter half-life (~2 days vs. ~2 weeks in CC), but also for its peculiar mechanism of action via down regulation of the $E_2$ synthesis rather than competitive inhibition of its action (11, 12). Furthermore, in ovulatory infertile patients, letrozole was reported to induce moderate ovarian hyperstimulation with $E_2$ levels similar to spontaneous cycles and higher midluteal progesterone, resulting in a normal endometrial histology and development of pinopodes, which could increase endometrial receptivity (24). Thereby, the biologic plausibility of the concept that superovulation with letrozole has the potential to increase pregnancy in women with minimal or mild endometriosis undergoing IUI is attractive, but is it clinically relevant?

In line with this concept, Abu Hashim et al. (14) in a RCT compared pregnancy rates following superovulation between letrozole and CC (each combined with IUI). The study included 136 women with primary infertility and no pregnancy following laparoscopic treatment for minimal to mild endometriosis over a 6-12-month follow-up period. Women were allocated to receive either 5 mg letrozole/day (69 women, 220 cycles) or 100 mg CC/day (67 women, 213 cycles) for 5 days, combined with IUI for up to 4 cycles. The authors demonstrated that the total number of follicles and serum $E_2$ on the day of HCG administration were significantly higher in the CC group; however, no significant differences were found with regard to the clinical pregnancy rate per cycle or the cumulative pregnancy rate after 4 cycles in both groups (15.9 vs. 14.5% and 64.7 vs. 57.2% in letrozole and CC groups, respectively). No significant differences were also found in miscarriage and live birth rates between both groups (11.4 vs. 12.9% and 44.9 vs. 40.3% in letrozole and CC groups, respectively). The authors concluded that superovulation with letrozole is not more effective than CC combined with IUI for women with minimal to mild endometriosis who did not achieve pregnancy after 6-12 months following laparoscopic treatment (14).

The effect of endometriosis on in vitro fertilization outcome

In a recent meta-analysis, Harb et al. (25) examined the effect of endometriosis on in vitro fertilization (IVF) outcome in twenty-seven observational studies that included 8984 women. The authors demonstrated that the presence of severe endometriosis (stage III/IV), but not minimal-mild endometriosis (stage I/II), was associated with reduced the implantation rate (RR=0.79, 95% CI: 0.67-0.93, P=0.006) and clinical pregnancy rate (RR=0.79, 95% CI: 0.69-0.91, P=0.0008) as compared to those of women with other causes of infertility. This finding may be attributed to defective embryo quality as well as defective endometrial receptivity (26, 27). High endometrial aromatase P450 mRNA expression in endometriotic women was also reported to be associated with poor IVF outcome (28). On the other hand, a reduction in fertilization rates was demonstrated in stage I/II endometriosis but not in stage III/IV disease as compared to controls (RR=0.93, 95% CI: 0.87-0.99, P=0.03). This interesting finding may be partially explained by impaired steroidogenesis of granulosa cells as well as poor oocyte quality in stage I/II disease (6). It is noteworthy that both of the third-generation AIs, letrozole and
anastrazole, have been used as adjuvant treatments in ART that is mainly for poor responders based on their ability to increase follicular sensitivity to follicle stimulating hormone (FSH) as a result of increased intrafollicular androgens (29). This point is outside the scope of this review.

The single and dual suppression concepts in women with endometriosis undergoing assisted reproductive technology

The merits of pituitary down-regulation for 3-6 months with a GnRHa in women with endometriosis before ART were previously demonstrated (30). Furthermore, this practice was recommended in the updated ESHRE Endometriosis Guideline (19). This recommendation was based on the findings of a Cochrane meta-analysis of three RCTs including 165 patients who had endometriosis-associated infertility (31). The authors concluded that this suppressive policy prior to ART increases the odds of clinical pregnancy by more than 4-fold (OR=4.28, 95% CI: 2.00-9.15, P=0.0001). This beneficial effect may be explained by increased activity of the immune system [increased natural killer (NK) cells, decreased auto-antibodies levels and reduction of elevated levels of peritoneal fluid interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α), stimulation of endometrial cell apoptosis as well as improved endometrial receptivity and implantation rates (30, 32). The latter action may be due to the suppression of endometrial aromatase expression by GnRHa (33). The concept of dual suppression (GnRHa+AI) prior to IVF has an appealing biological plausibility through the additional blockade of extraovarian aromatase enzyme aberrantly expressed in endometriotic implants as well as the eutopic endometrium of women with endometriosis (9-11). A more recent in vitro study demonstrated that anastrozole significantly suppressed endometriotic cells proliferation in culture as a result of marked reduction in the E$_2$ levels in these cells and this effect was dose dependent i.e. the suppression of growth and proliferation of the cultured endometriotic cells increased following increased anastrozole dosage (15). Thereby, dual suppression prior to IVF may result in a higher pregnancy rate rather than suppression with GnRHa alone. In a prospective pilot study, Lossl et al. (16) tested this concept among 20 infertile patients with endometriomas undergoing IVF/ Intra-cytoplasmic sperm injection (ICSI). A 3-month GnRHa course (3.6 mg goserelin administered through a subcutaneous injection on the treatment days 1, 28 and 56) combined with anastrozole (1 mg daily from the days 1 to 69) was used followed by controlled ovarian stimulation from the day 70. Interestingly, the authors demonstrated that prolonged dual suppression significantly reduces endometriomal volume by 29% (3-39%, P=0.007) and serum CA125 by 61% (21-74%, P=0.001). Nine of the 20 patients (45%) achieved pregnancy; however, only 3 patients (15%) delivered (two singletons and one twin). The authors speculated that the high pregnancy loss (6 patients) could be accidental or due to impaired quality of the oocytes and/or the endometrium (16). So far, there are no RCTs available in the literature comparing the use of goserelin+anastrozole suppression with single suppression by the GnRHa alone.

Aromatase inhibitors and endometrial receptivity in endometriotic women undergoing assisted reproductive technology

As endometrial aromatase expression was found to be associated with poor IVF outcome (28), it is theoretically plausible that the use of AIs in endometriotic women may be advantageous in improving their endometrial receptivity and the IVF outcome. Of note, reduced integrin expression, a marker of endometrial receptivity, has been shown in women with IVF failure (34). In a retrospective cohort study, Miller et al. (17) evaluated the effect of letrozole on endometriotic infertile women undergoing IVF and lacking normal integrin expression assessed by immunohistochemistry in mid-luteal endometrial biopsies. Out of 79 women undergoing standard IVF, 29 (36.7%) lacked normal integrin expression. Meanwhile other 18 integrin-negative women received letrozole early in IVF stimulation (5 mg, days 2-6). Notably, in women undergoing standard IVF, clinical pregnancy and delivery rates were significantly higher in women with normal integrin expression compared with integrin-negative women [20/50 (40%) vs. 4/29 (13.7%), P=0.02 and 19/50 (38%) vs. 2/29 (7%), P<0.01, respectively]. Additionally, among integrin-negative patients, significantly higher clinical pregnancy and delivery rates occurred in those who received letrozole as compared to women who did not receive letrozole [11/18 (61%]

Abu Hashim
vs. 4/29 (14%), P<0.001 and 9/18 (50%) vs. 2/29 (7%), P<0.001, respectively]. The authors concluded that letrozole co-treatment might improve the IVF success rates in a subset of women with endometriosis and implantation failure. However, they admitted the need for further prospective studies to confirm these findings (17).

The use of aromatase inhibitors in endometriotic women undergoing assisted reproductive technology; a counterintuitive view

Compared to women with tubal or unexplained infertility undergoing ARTs, reduced aromatase activity in granulosa cells as well as reduced expression of the cytochrome p450 family 19 subfamily A member 1 (CYP19A1) gene (that encodes aromatase) in cumulus cells has been shown in infertile women with endometriosis by in vitro studies (35, 36). Thereby, defective granulosa cell steroidogenesis could explain the impaired oocyte quality and the reduced fertilization rate associated with endometriosis. In that regard, an opposite view is that the use of AIs in patients with endometriosis undergoing ART may further compromise aromatase activity of granulose cells ending with a poor reproductive outcome. This possibility has been tested recently by Lu et al. (18) who compared E$_2$ production and P450 aromatase mRNA expression of cultured luteinized granulosa cells and the effect of letrozole on these parameters between women with (n=23) and without endometriosis (n=19). Of note, significantly lower E$_2$ production and P450 aromatase mRNA expression occurred in women with endometriosis and further reduction of these parameters were demonstrated following letrozole in a concentration of 1 µmol/L. Importantly, the results of this study should be interpreted with caution because it included women with advanced stage endometriosis (III-IV). So, whether early stage endometriosis (I-II) has a similar effect on in vitro E$_2$ production and P450 aromatase mRNA expression remains uncertain. The authors admitted the need for larger in vivo studies for further validation of these findings (18).

Safety of aromatase inhibitors

Data from the only abstract that did report increased rates of cardiac and bone malformations with letrozole treatment for infertility were subsequently unsubstantiated in larger trials (37, 38). More recently, in a large double-blind, multicenter RCT, Legro et al. (39) found no significant difference in the anomaly rates in infertile women with polycystic ovary syndrome who conceived with CC or letrozole (1.5 vs. 3.9%, respectively) and even these rates appear similar to that reported in healthy fertile women who conceived without undergoing ART (5.8%) (40).

Conclusion

In women with endometriosis, aromatase p450, the key enzyme in the biosynthesis of E$_2$ is not only present in the ovarian granulosa cells but also aberrantly expressed in the eutopic endometrium as well as in endometriotic deposits. Thereby, the orally active third-generation AIs letrozole and anastrozole have gained attention as a co-treatment for endometriosis associated infertility. In non-ART cycles, a RCT has demonstrated that post-operative treatment with letrozole or GnRHa for 2 months did not improve spontaneous pregnancy rates as compared to no treatment. Another RCT reported no superiority of superovulation with letrozole over CC followed by IUI in women with minimal-mild endometriosis who have not achieved pregnancy 6-12 months following laparoscopic treatment. Anastrozole significantly inhibited the growth of endometriotic cells and estrogen production in culture was also dose-dependent. In ART cycles, the concept of dual suppression (3-month GnRHa+1 mg anastrozole/day) was tested in a pilot study in 20 infertile women with endometriomas undergoing IVF. A significant reduction in endometrioma volume and serum CA-125 concentration was found. Of note, although the pregnancy rate was 45%, a high pregnancy loss was observed and the live-birth rate was 15%. So far, this concept has not been compared with single suppression by the agonist alone. Evidence from a retrospective cohort study has shown that letrozole could potentially improve endometrial receptivity in endometriosis patients undergoing IVF in whom aromatase is aberrantly expressed. On the other hand, an in vitro study reported lower E$_2$ production and aromatase expression of cultured granulosa cells in women with stage III-IV endometriosis undergoing IVF. Letrozole further reduced these parameters. Importantly, these findings should be interpreted with caution. No further validation has been made available so far in in vitro studies.
vivo studies. Overall, in view of the current limited body of evidence of 6 original studies, the research agenda for the future should address more questions concerning the use of AIs in patients with endometriosis such as the ideal timing for AIs co-treatment, optimum dosage, and definite effect on endometrial receptivity. In that respect, the quote of Albert Einstein "Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning" is merit worthy as evidence-based medicine is an evolving process. So, more trials are still needed to have a clear and unequivocal evidence to guide our clinical practice and to help achieve best outcomes.

Acknowledgements

There is no financial support and conflicts of interest in this study.

References

1. Giudice LC. Clinical practice. Endometriosis. N Engl J Med. 2010; 362(25): 2389-2398.
2. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. Fertil Steril. 1997; 68(4): 585-596.
3. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997; 67(5): 817-821.
4. Donnez J. Endometriosis: enigmatic in the pathogenesis and controversial in its therapy. Fertil Steril. 2012; 98(3): 509-510.
5. McLeod BS, Retzloff MG. Epidemiology of endometriosis: an assessment of risk factors. Clin Obstet Gynecol. 2010; 53(2): 389-396.
6. Gupta S, Goldberg JM, Aziz N, Goldberg E, Kraijer N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril. 2008; 90(2): 247-257.
7. Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. J Clin Endocrinol Metab. 1996; 81(1): 174-179.
8. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril. 2012; 98(3): 511-519.
9. Bulun SE, Zeitoun KM, Takayama K, Sasano H. Molecular basis for treating endometriosis with aromatase inhibitors. Hum Reprod Update. 2000; 6(5): 413-418.
10. Abu Hashim H. Potential role of aromatase inhibitors in the treatment of endometriosis. Int J Womens Health. 2014; 6: 671-680.
11. Pavone ME, Bulun SE. The use of aromatase inhibitors for ovulation induction and superovulation. J Clin Endocrinol Metab. 2013; 98(5): 1838-1844.
12. Lee VC, Ledger W. Aromatase inhibitors for ovulation induction and ovarian stimulation. Clin Endocrinol (Oxf). 2011; 74(5): 537-546.
13. Alborzi S, Hamedi B, Omidvar A, Dehbashi S, Alborzi S, Alborzi M. A comparison of the effect of short-term aromatase inhibitor (letrozole) and GnRH agonist (triprotolin) versus case control on pregnancy rate and symptom and sign recurrence after laparoscopic treatment of endometriosis.
14. Abu Hashim H, El Rakawy M, Abd Elaai I. Randomized comparison of superovulation with letrozole vs. clomiphene citrate in an IUI program for women with recently surgically treated minimal to mild endometriosis. Acta Obstet Gynecol Scand. 2012; 91(3): 338-345.
15. Badawy SZ, Brown S, Kaufman L, Woytowczy MA. Aromatase inhibitor (anastrozole) affects growth of endometrium cells in culture. Eur J Obstet Gynecol Reprod Biol. 2015; 188: 45-50.
16. Losel K, Loft A, Freiesleben NL, Bangsboll S, Andersen CY, Pedersen AT, et al. Combined down-regulation by aromatase inhibitor and GnRH-agonist in IVF patients with endometriomas-A pilot study. Eur J Obstet Gynecol Reprod Biol. 2009; 144(1): 48-53.
17. Miller PB, Parrell BA, Bushnell G, Tallman N, Forstein DA, Higdon HL 3rd, et al. Endometrial receptivity defects during IVF cycles with and without letrozole. Hum Reprod. 2012; 27(3): 881-888.
18. Lu X, Wu Y, Gao XY, Wang YW, Wang L, Sun X. Effect of letrozole on estradiol production and P450 aromatase messenger RNA expression of cultured luteinized granulosa cells from women with and without endometriosis. Fertil Steril. 2012; 98(1): 131-135.
19. Dunseltman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D’Hooghe T, De Bie B, et al.ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014; 29(3): 400-412.
20. Furness S, Yap C, Farquhar C, Cheong YC. Pre and post-operative medical therapy for endometriosis surgery. Cochrane Database Syst Rev. 2004; (3): CD003678.
21. Tummon IS, Asher LJ, Martin JS, Tulandi T. Randomized controlled trial of superovulation and insemination for infertility associated with minimal or mild endometriosis. Fertil Steril. 1997; 68(1): 8-12.
22. Nulsen JW, Walsh S, Dumetz S, Metzger DA, A randomized and longitudinal study of human menopausal gonadotropin with intrauterine insemination in the treatment of infertility. Obstet Gynecol. 1993; 82(5): 780-786.
23. Werbrouck E, Spiessens C, Meuleman C, D’Hooghe T. No difference in cycle pregnancy rate and in cumulative live birth rate between women with surgically treated minimal to mild endometriosis and women with unexplained infertility after controlled ovarian hyperstimulation and intrauterine insemination. Fertil Steril. 2006; 86(3): 566-575.
24. Cortinez A, De Carvalho I, Vantman D, Gabler F, Iniguez G, Vega M. Hormonal profile and endometrial morphology in letrozole-controlled ovarian hyperstimulation in ovolatory infertility patients. Fertil Steril. 2005; 83(1): 110-115.
25. Harb HM, Gallos ID, Chu J, Harb M, Coomarasamy A. The effect of endometriosis on in vitro fertilisation outcome: a systematic review and meta-analysis. BJOG. 2013; 120(11): 1308-1320.
26. Pellicer A, Navarro J, Bosch E, Garrido N, Garcia-Velasco JA, Remolli J, et al. Endometrial quality in infertile women with endometriosis. Ann N Y Acad Sci. 2001; 943: 122-130.
27. Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bikker W, et al. Aberrant integrin expression in the endometrium of women with endometriosis. J Clin Endocrinol Metab. 1994; 79(2): 643-649.
28. Brosens J, Verhoeven H, Campo R, Gianaroli L, Gordts S, Hazekamp J, et al. High endometrial aromatase P450 mRNA expression is associated with poor IVF outcome. Hum Reprod. 2004; 19(2): 352-356.
29. Papanikolaou EG, Polyzos NP, Humaidan P, Pados G, Bosch E, Tournaye H, et al. Aromatase inhibitors in stimulated IVF cycles. Reprod Biol Endocrinol. 2011; 9: 85.
logues and endometriosis: current strategies and new insights. Gynecol Endocrinol. 2012; 28(4): 314-321.
31. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst Rev. 2006; (1): CD004635.
32. Surrey ES. Gonadotropin-releasing hormone agonist and add-back therapy: what do the data show? Curr Opin Obstet Gynecol. 2010; 22(4): 283-288.
33. Ishihara H, Kitawaki J, Kado N, Koshiba H, Fushiki S, Horjo H. Gonadotropin-releasing hormone agonist and danazol normalize aromatase cytochrome P450 expression in eutopic endometrium from women with endometriosis, adenomyosis, or leiomyomas. Fertil Steril. 2003; 79(Suppl 1): 735-742.
34. Thomas K, Thomson A, Wood S, Kingsland C, Vince G, Lewis-Jones I. Endometrial integrin expression in women undergoing in vitro fertilization and the association with subsequent treatment outcome. Fertil Steril. 2003; 80(3): 502-507.
35. de Abreu LG, Romao GS, Dos Reis RM, Ferriani RA, De Sa MF, De Moura MD. Reduced aromatase activity in granulosa cells of women with endometriosis undergoing assisted reproduction techniques. Gynecol Endocrinol. 2006; 22(8): 432-436.
36. Barcelos ID, Donabella FC, Ribas CP, Meola J, Ferriani RA, de Paz CC, et al. Down-regulation of the CYP19A1 gene in cumulus cells of infertile women with endometriosis. Reprod Biomed Online. 2015; 30(5): 532-541.
37. Biljan MM, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. Fertil Steril. 2005; 84 Suppl 1: S95.
38. Tulandi T, Martin J, Al-Fadhili R, Kabil N, Forman R, Hitkari J, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril. 2006; 85(6): 1761-1765.
39. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med. 2014; 371(2): 119-129.
40. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012; 366(19): 1803-1813.
Prevalence of Infertility Problems among Iranian Infertile Patients Referred to Royan Institute

Mahdi Sepidarkish, M.Sc.1,2, Amir Almasi-Hashiani, M.Sc.1,2, Fatemeh Shokri, M.Sc.1, Samira Vesali, M.Sc.1, Elaheh Karimi, M.Sc.1, Reza Omani Samani, M.D.1

1. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Abstract—

Background: Few studies have been conducted on the infertility problems in Iran. This study aimed to investigate the prevalence of infertility problems and related factors in Iranian infertile patients.

Materials and Methods: In this cross sectional study, 405 infertile patients referred to Royan Institute, Tehran, Iran, between 2014 and 2015, were selected by simple random sampling. Participants completed the Fertility Problem Inventory (FPI) including 46 questions in five domains (social concern, sexual concern, relationship concern, rejection of parenthood, and need for parenthood). Mean difference between male and female was verified using independent-samples Student’s t test. A generalized linear model (GLM) was also used for testing the effect of variables on the fertility problems. Data was analyzed using Stata software version 13.

Results: The mean age (SD) of participants was 31.28 (5.42). Our results showed that 160 infertile men (95.23%) were classified as very high prevalence of infertility problems. Among infertile women, 83 patients (35.02%) were as very high prevalence of infertility problems, and 154 patients (64.98%) were as high prevalence. Age (P<0.001), sex (P<0.001), a history of abortion (P=0.009), failure of previous treatment (P<0.001), and education (P=0.014) had a significant relationship with FPI scores.

Conclusion: Bases on the results of current study, an younger male with lower education level, history of abortion and history of previous treatments failure experienced more infertility problems.

Keywords: Infertility, Assisted Reproductive Technology, Iran

Introduction

Infertility as a critical concept threatens individual stability and social relations (1). Although infertility is not considered as a medical issue, it critically affects the life of infertile couples in all aspects (2). Couples experiencing this crisis situation are more at risk for depression, anxiety, low self-esteem, and dissatisfaction (3). Infertile couples face both the physical and psychological problems during the diagnosis and treatment which can disrupt their lifestyle. Infertility can influence the relationship between the infertile patients with their spouse, friends, and colleagues, while it has a negative impact on their adherence performance (4, 5). Impulsive angry behaviors, feelings of helplessness and worthlessness, anxiety (particularly in the long-term treatments), concerns of sexual attraction, feeling of isolation, physical complaints, difficulty in sexual relationships, and sexual dissatisfaction are the problems reported by two recent studies (6, 7).
The prevalence of problems associated with infertility has also been reported in other studies. However, according to studies by Ashkani et al. (8) and Fassino et al. (9), only 20 to 60 percent of infertile couples experienced these types of problems. There is a remarkable infertility rate in Iran (10). In Iranian culture, fertility is considered as an important concept. Unfortunately, few studies have been carried out on the different infertility problems, and most of them have focused on the psychological aspects of the subject (11, 12). Assessment of the problems related to infertility with a comprehensive tool is necessary (13). Therefore, this study aimed to investigate the prevalence of infertility problems and related factors in Iranian infertile patients.

Materials and Methods

This cross-sectional study was performed on 405 infertile patients referred to Royan Institute, Tehran, Iran, between 2014 and 2015. Simple random sampling was applied using a random numbers table based on the medical records of patients in this center. Inclusion criteria were as follows: definitive confirmation of infertility (primary or secondary) by a reproductive specialist, male factor infertility/female factor infertility (or both), unexplained infertility, and willingness to participate in the study. The diagnosis of infertility, cause of infertility, and infertility were defined based on the recommendations of World Health Organization (WHO) as follows: clinical infertility is defined as the lack of clinical pregnancy after 12 months of regular intercourse to have children. No history of previous pregnancy as primary infertility and secondary infertility was defined as a couple who experienced successful fertility in the past, and face infertility after previous pregnancies. A phone call was made to all infertile patients using simple random sampling, as mentioned. In a meeting with the patients, purpose of the study and the confidentiality of the data were clearly explained. Eligible individuals were also assured that acceptance or refusal to participate in the research had no influence on their treatment procedures. Filling the questionnaire was considered as signing a written informed consent. The study was approved by the Ethics Committee of Royan Institute. Participants completed two questionnaires. Firstly, a questionnaire about the socio-demographic and clinical characteristics was filled out that includes age (years), sex (male or females), educational levels (primary, secondary, and university), duration of infertility (years), type of infertility (primary, secondary), duration of marriage (years), cause of infertility (male factor, female factor, both, or unexplained infertility), the number of previous abortion (gestational age <20 weeks), failure of previous treatment (0,1, 2, 3, ≥4), and a history of abortion (yes, no). Secondly, the Fertility Problem Inventory (FPI) was completed. This questionnaire, which was made by Newton in 1999 to detect problems associated with infertility, includes 46 questions and five domains (social concern, sexual concern, relationship concern, rejection of parenthood, and need for parenthood). It is a 6-point Likert scale, from strongly disagree=1 to strongly agree=6. In this tool, 19 questions are scored inversely. The scores are between 46 and 276, indicating that the highest numerical values represent very high prevalence of problems related to infertility. In men, the scores range from 0 to 87 as less prevalence, 88 to 113 as moderate prevalence, 114 to 146 as high prevalence, and more than 147 as very high prevalence. In women, scores 0 to 97 are classified as low prevalence, 98 to 132 as moderate prevalence, 133 to 167 as high prevalence, and over 168 as very high prevalence (13). In a study by Ramezanazadeh et al. (6), the validity and reliability of the Iranian version of FPI were evaluated in Iranian infertile patients and Cronbach’s alpha coefficient was greater than 70% for all domains.

Statistical analysis

Categorical data were presented as numbers (percent) and continuous data as mean (SD). Mean difference between male and female was verified by independent-samples Student’s t test. A generalized linear model (GLM, family: Gaussian, link: identity) was used to test the effect of age, sex, educational level, duration of infertility (years), cause of infertility, type of infertility, failure of previous treatment, and history of abortion on the fertility problem score. Results were presented as standardized coefficients with 95 percent confidence intervals (CIs). Values of P less than 0.05 were considered statistically significant. Data was analyzed using Stata software (StataCorp, USA).

Results

A group of 410 participants completed both questionnaires. Five questionnaires (1.21%) were excluded due to the completion rate of less than
70%. The mean (SD) age was 31.28 (5.42). Socio-demographic and clinical characteristics of the participants are shown in the Table 1.

Table 1: Socio-demographic and clinical characteristics of the participants

| Characteristic                        | Mean ± SD or Number (%) |
|---------------------------------------|-------------------------|
| Age (Y)                               | 31.28 ± 5.42            |
| Duration of infertility (Y)           | 4.93 ± 4.01             |
| Duration of marriage (Y)              | 7.07 ± 4.23             |
| Number of previous abortion           | 1.04 ± 0.87             |
| Sex                                   |                         |
| Male                                  | 168 (41.5)              |
| Female                                | 237 (58.5)              |
| Cause of infertility                  |                         |
| Male factor                           | 146 (36)                |
| Female factor                         | 88 (21.7)               |
| Both                                  | 71 (17.5)               |
| Unexplained                           | 100 (24.8)              |
| Type of infertility                   |                         |
| Primary                               | 287 (70.9)              |
| Secondary                             | 118 (29.1)              |
| Educational level                     |                         |
| Primary                               | 90 (22.2)               |
| Secondary                             | 150 (37)                |
| University                            | 165 (40.8)              |
| Failure of previous treatment         |                         |
| 0                                     | 208 (51.35)             |
| 1                                     | 81 (20)                 |
| 2                                     | 61 (15.06)              |
| 3                                     | 36 (8.88)               |
| ≥4                                    | 19 (4.69)               |
| History of abortion                   |                         |
| No                                    | 316 (78)                |
| Yes                                   | 89 (22)                 |

Values are given as mean ± SD or number (percentage) unless otherwise indicated.

Table 2 shows the mean scores of FPI domains compared between males and females. Our findings showed that a very high prevalence of fertility problems in infertile patients, meaning among infertile men, 160 individuals (95.23%) were classified as very high prevalence and the remaining 8 individuals (4.77%) as high prevalence. None of them were as less or moderate prevalence. Among infertile women, 83 patients (35.02%) were as very high prevalence and 154 (64.98%) as high prevalence. Like infertile men, no women were as less or moderate prevalence.

The GLM results showed that five out of eight predictors were statistically significant. The model was statistically significant [F (8, 397) =11.13, P<0.001] and accounted for approximately 16% of the variance of scores (R²=0.185, Adjusted R²=0.168). Basic descriptive statistics, crude and standardized regression coefficients are shown in Table 3. The strongest effect belongs to age followed by sex, a history of abortion, failure of previous treatment, and education. The results suggested that patients who had a history of abortion showed very high prevalence of fertility problem as compared to patients without a history of abortion (P<0.001). Similarly, patients with a history of failure in previous treatments had very high prevalence of fertility problems (P=0.001). As seen in Table 3, age was a significant predictor of prevalence of fertility problems. When the other predictors were ignored, age was negatively correlated with prevalence of fertility problem, meaning that an increase of one SD of age resulted in a decrease of 0.231 in mean scores of FPI. Like crude analysis, female had lower mean scores of FPI as compared to the related value of male. Also, mean scores of FPI decreased with an increase in the education level.

Table 2: Comparison of the mean scores of fertility problem inventory domains between males and females

| Scale                   | Total (n=405) | Male (n=168) | Female (n=237) | P value |
|-------------------------|--------------|--------------|----------------|---------|
| Social concern          | 34.36 ± 5.46 | 34.05 ± 5.64 | 34.58 ± 5.32   | 0.345   |
| Sexual concern          | 29.82 ± 4.35 | 31.11 ± 4.02 | 28.9 ± 4.35    | <0.001  |
| Relationship concern    | 36.9 ± 3.58  | 36.81 ± 3.54 | 36.96 ± 3.61   | 0.678   |
| Rejection of parenthood | 25.66 ± 5.3  | 27.46 ± 5.56 | 24.38 ± 4.71   | <0.001  |
| Need for parenthood     | 37.37 ± 5.09 | 36.37 ± 6    | 38.08 ± 4.2    | 0.002   |
| All scales              | 164.12 ± 10.42 | 165.82 ± 11.27 | 162.91 ± 9.61  | 0.007   |

Values are given as mean ± SD.
Discussion

Our findings revealed that the prevalence of infertility problems is very high in infertile patients. In our study, 95.23% of male patients and 35.02% of female patients showed very high prevalence of infertility problems, so none of them was classified as less or moderate prevalence. After adjusting confounder variables (including age, sex, educational level, failure of previous treatment, and a history of abortion), a significant increase in prevalence of infertility problems was seen in younger male patients with lower education level, a history of previous abortion, and failure of previous treatment.

Considering the cause of infertility, our findings showed that the male factor (36%) was more prevalent than the female factor (21.7%). In a study by Kamali et al. (14), they have found that 50.5% of participants suffered from the male factor, while 28.6% had the female factor. In another study by Noorbala et al. (15) they have reported prevalence values of 44 and 28% for psychiatric disorders in infertile and fertile women, respectively. Furthermore, the most important stress factors in infertile women were feedback from others (81.3%), loneliness (74%), treatment of infertility (60.7%), incomplete families (53%) and the identity disorder (50.7%).

In a study by Hussain et al. (16) on psychological disorders among women with polycystic ovary syndrome (PCOS), as one of the major causes of infertility, they have reported a prevalence of 23% for major depression in women with PCOS. They have also reported 15.4% suffering from panic disorder and 15.4% diagnosed with generalized anxiety disorder. The prevalence of suicide is also more among women with PCOS than others (16, 17). In a study by Ramazanzadeh et al. (6) on 370 infertile women, they have found that the prevalence values of depression and anxiety in infertile women were 40.8 and 86.8%, respectively. These findings were consistent with our results. Furthermore, the prevalence of psychiatric disorders in Iranian infertile women was higher as compared with the Western countries (15, 18). Odden et al. (19) have reported that the prevalence of 35.2% for psychiatric disorders in infertile women.

In our relationship between the age and total scores of FPI, suggesting, as age increased, the total scores decreased. It means that younger infertile patients experienced very high prevalence of problems related to infertility as competed to older infertile patients. Ramazanzadeh et al. (6) have also found that the prevalence of anxiety and depression is more common in 6-4 years after infertility. Another study has indicated that anxiety and depression improve with increasing age.

Various researches have showed different findings about the relationship between prevalence of infertility problems with age and education level. In a study by Beutel et al. (20), there was a significant correlation between prevalence of infertility problems with age and education level, while such a relationship was reported by two other studies (19, 21). In our study, the prevalence of infertility problems was significantly higher in the participants with a history of previous treatment failure. This may be due to the failure of fertility treatment, medical expenses, and the reaction of others. A history of abortion was known as one of the factors in prevalence of infertility problems. One of possible limitations of this study was the patients with more complex infertility problems who are mostly referred to Royan Institute from...
other centers. These patients are more likely to experience longer exposure to the problems related to infertility. Therefore, our findings can only be generalized to patients with more complex infertility problems referred to a fertility center. Finally, in all cross-sectional study, such as our study, there is no temporality. Therefore, determination of the exposure and the outcome is not easily performed and causality will be not detected.

Conclusion

The results showed that a very high prevalence of fertility problems in infertile couples. Bases on the results of current study, a younger male with lower education level, history of abortion and history of previous treatments failure experienced high prevalence of infertility problems.

Acknowledgements

We really appreciate the Royan Institute for financial support and the population participating in this study. The authors declare no conflict of interest.

References

1. Bolvin J, Bunting L, Collins JA, Ngren 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. Stellar C, Garcia-Moreno C, Temmerman M, van der Poel S. A systematic review and narrative report of the relationship between infertility, subfertility, and intimate partner violence. Int J Gynecol Obstet. 2016; 133(1): 3-8.
3. Nachigall RD. International disparities in access to infertility services. Fert Steril. 2006; 85(4): 871-875.
4. Begum BN, Hasan S. Psychological problems among women with infertility problem: a comparative study. J Pak Med Assoc. 2014; 64(11): 1287-1291.
5. Martins MV, Basto-Pereira M, Pedro J, Peterson B, Almeida V, Schmidt L, et al. Male psychological adaptation to unsuccessful medically assisted reproduction treatments: a systematic review. Hum Reprod Update. 2016; 22(4): 466-478.
6. Ramezanzadeh F, Aghssa MM, Abedinia N, Zayeri F, Khanafshar N, Shariat M, et al. A survey of relationship between anxiety, depression and duration of infertility. BMC Womens Health. 2004; 4(1): 9.
7. Slade P, O'Neil C, Simpson AJ, Lashen H. The relationship between perceived stigma, disclosure patterns, support and distress in new attendees at an infertility clinic. Hum Reprod. 2007; 22(8): 2309-2317.
8. Ashkani H, Akbari A, Heydari S. Epidemiology of depression among infertile and fertile couples in Shiraz, Southern Iran. Indian J Med Sci. 2006; 60(10): 399-406.
9. Fassino S, Piero A, Boggio S, Piccioni V, Garzaro L. Anxiety, depression and anger suppression in infertile couples: a controlled study. Hum Reprod. 2002; 17(11): 2986-2994.
10. Alieyeh G, Laya F. Qualitative life and its correlates among a group of infertile Iranian women. Med Sci Monit. 2007; 13(7): CR313-317.
11. Hermann D, Scherg H, Verres R, von Hagens C, Strowitzki T, Wischmann T. Resilience in infertile couples acts as a protective factor against infertility-specific distress and impaired quality of life. J Assist Reprod Genet. 2011; 28(11): 1111-1117.
12. Kazem M, Ali A. An overview of the epidemiology of primary infertility in Iran. J Reprod Infertil. 2009; 10(3): 213-216.
13. Newton CR, Sherrard W, Giacovici I. The fertility problem inventory: measuring perceived infertility-related stress. Fertil Steril. 1999; 72(1): 54-62.
14. Kamali M, Baghestani AR, Kashfi F, Kashani H, Tavajohi Sh, Amirchaghmaghi E. A survey on infertility in Royan Institute. Int J Fertil Steril. 2007; 1(1): 23-26.
15. Noorbalaa AA, Ramezanzadeh F, Abedi-Nia N, Naghizadeh MM, Haghighihi F. Prevalence of psychiatric disorders and types of personality in fertile and infertile women. J Reprod Infertil. 2009; 9(37): 350-360.
16. Hussain A, Chandel RK, Ganje MA, Dar MA, Rather YH, Wani ZA, et al. Prevalence of psychiatric disorders in patients with a diagnosis of polycystic ovary syndrome in Kashmir. Indian J Psychiatr. 2013; 57(1): 66-70.
17. Jedel E, Waem M, Gustafson D, Landen M, Eriksson E, Holm Gr, et al. Anxiety and depression symptoms in women with polycystic ovary syndrome compared with controls matched for body mass index. Hum Reprod. 2010; 25(2): 450-456.
18. Lu Y, Yang L, Lu G. [Mental status and personality of infertile women]. Zhonghua Fu Chan Ke Za Zhi. 1995; 30(1): 450-456.
19. Ashkani H, Akbari A, Heydari S. Epidemiology of depression among infertile and fertile couples in Shiraz, Southern Iran. Indian J Med Sci. 2006; 60(10): 399-406.
20. Beutel M, Kuper F, Kirchmeyer P, Kehde S, Köhn FM, Schroeder-Printzen I, et al. Treatment related stresses and depression in couples undergoing assisted reproductive treatment by IVF or ICSI. Andrologia. 1999; 31(1): 27-35.
21. Domar AD, Broome A, Zuttermeister PC, Seibert M, Friedman R. The prevalence and predictability of depression in infertile women. Fertil Steril. 1992; 58(6): 1158-1163.
Assisted Reproductive Technology in Iran: The First National Report on Centers, 2011

Mehrandokht Abedini, M.D.1, Azadeh Ghaheri, M.Sc.2, Reza Omani Samani, M.D.2

1. Deputy of Health, Department of Family Health, Ministry of Health and Medical Education, Tehran, Iran
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Background: Due to the worldwide increase in infertility, it is both necessary and important to have assisted reproductive technology (ART) registries. In Iran, donation and surrogacy programs are approved by decrees from religious scholars. ART has been used since 1984 in Iran and the first Iranian infant conceived by gamete intra-fallopian transfer (GIFT) was born in 1989. This report, however, is the first national report on Iranian ART centers.

Materials and Methods: This cross-sectional study, conducted under the supervision of the Iranian Ministry of Health, presented a summary of the numbers and percentages of centers that provided infertility services in Iran, as well as the status of ART in Iran during 2011.

Results: A total of 52 centers reported treatment cycles and performed approximately 29000 intrauterine insemination (IUI), in addition to 35000 in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) cycles.

Conclusion: Iran has considerable potential to provide IVF services for both Iranians as well as other nationalities throughout the region. This proves the need for a national center that will implement a registry system.

Keywords: Iran, Assisted Reproductive Technology, Monitoring

Introduction

With the expansion of infertility worldwide, the importance of assisted reproductive technology (ART) registries is critical. This registry information can assist health authorities, patients seeking medical assistance, the medical profession, and laboratory professionals in providing optimal patient care. A registry can provide the public a better understanding of ART procedures (1).

Reports of ART from European countries are presented annually with a four-year delay (2). The latest report of the European in vitro fertilization (IVF)-monitoring (EIM) has presented results of treatments initiated during 2010. Data is collected from existing national registries in the participating countries and directly entered by each national coordinator into the EIM database (3). ART data from the United States is also presented annually in surveillance papers (4). Data collected by the Latin American Registry of Assisted Reproduction (RLA) is also obtained from ART treatments performed in 155 institutions in 14 countries (5). Nevertheless, there is little information regarding the status of ART in Middle Eastern countries (6).

Iran is the only Islamic country in which donation and surrogacy programs are practiced. These
programs have been accepted and approved by decrees from clergy scholars (7, 8). ART was first used in 1984 in Iran and the first Iranian infant conceived by gamete intra-fallopian transfer (GIFT) was born in 1989. Since then, more than 50 centers have been established in Iran. However, scant attention has been paid to a national registry and this is the first report on Iranian IVF monitoring.

This paper aims to present data gathered from the Iranian infertility centers in 2011. It proceeds by tracing the accessibility, procedure, cost, and some challenges of IVF in Iran. While definitions used in medically assisted reproduction are different in various settings, this paper has used the definitions suggested by the International Committee for Monitoring Assisted Reproductive Technology (ICMART).

**Materials and Methods**

We gathered current registry information under the supervision of the Iranian Ministry of Health. This cross-sectional study dealt with the treatment outcomes during 2011 while the population of the country approximated 75.149 million. To collect data for this registry, we designed a form that used published literature and surveillance reports from American Society for Reproductive Medicine (ASRM), National Institutes of Health (NIH), and European Society of Human Reproduction and Embryology ESHRE (9, 10). The content of the questions was evaluated and approved by health and medical experts using the nominal group method and also by the Ethics Committee of the Iranian Ministry of Health.

In Iran, although infertility centers provide ART, clinics and hospitals also offer a portion of reproductive services. In Iran, all medical services provided in a hospital are under the supervision of a medical university. Thus, in order to obtain information from each center for completing the designed form, we sent there an expert familiar with basic concepts and definitions of ART from the medical university affiliated with the respective center. In this way, we contacted all centers and gathered data via the questionnaire.

In Iran, although infertility centers provide ART, clinics and hospitals also offer a portion of reproductive services. In Iran, all medical services provided in a hospital are under the supervision of a medical university. Thus, in order to obtain information from each center for completing the designed form, we sent there an expert familiar with basic concepts and definitions of ART from the medical university affiliated with the respective center. In this way, we contacted all centers and gathered data via the questionnaire.

Iran is divided into 31 provinces. This report provides data on the numbers of infertility centers, the numbers and types of provided services for ART, and the distribution of admitted clients according to the provinces of Iran in 2011. Specifically, this report provides summarized information of the numbers and percentages of centers that have provided infertility services based on the source of the egg (patient or donor) and the status of the embryos (fresh or thawed). Data also includes the number of treatments from standard IVF, intra-cytoplasmic sperm injection (ICSI), and intrauterine insemination (IUI) performed during 2011 in Iran.

Infertility clinics are divided according to their level of expertise: primary (level I), secondary (level II), and tertiary (level III). This report only focuses on level III infertility centers located throughout Iran. These centers have an ART laboratory which includes day care centers or centers in the hospitals that perform diagnostic and therapeutic functions at the highest level of specialization. In order to assess the level III criteria adjustment of these centers, data on the number of specialist staff or lack of specialists is provided in this report.

Hospitals and level III infertility clinics are integrated with medical universities in Iran. Iranian medical universities undergo an annual evaluation on the basis of research activities using criteria such as the numbers of faculty members and researchers, knowledge production and budget, and leadership and governance. Medical universities are then ranked according to three categories: type 1 with large numbers of academic staff and high levels of research funds, type 2 university with less staff and research funds compared to type 1, and type 3 with still fewer numbers of academic staff and lower research funds than type 2 universities (11). This report also presents the distribution of fertility centers based on the type of medical universities.

**Results**

In 2011 there were 52 infertility centers in Iran, with most located in major cities. The number of infertility centers that performed ART procedures varied by province and the type of university hospitals (Fig.1).

A total of 34 centers were affiliated with type 1 and 18 centers with type 2 university hospitals. Table 1 shows the distribution of infertility centers by the type of university hospital. Among all type 2 medical universities, Zahedan, Qazvin, Ardabil, and Gorgan did not have any level III infertility centers. From 46 medical universities in Iran, 18 had at least one level III infertility center (Table 1).
The infertility centers were divided into: private, government, and Academic Center of Education, Culture and Research [ACECR or the former Jihad Daneshgahi (non-government organizations)]. Among the 52 centers, there were 27 (52%) private, 21 (40%) government, and 4 (8%) ACECR.

Medical universities of Iran provide personal, educational, and research services. Thus infertility centers can be categorized as having one or all of the following: educational, clinical, and research departments. The percentage of centers with respect to their services is presented in Figure 2.

We have classified the centers according to the duration of their work. The oldest center is the government center of Yazd with 22 years of experience, followed by Shariati Hospital with 21 years, both of which are educational centers; after which are the private centers of Royan Institute with 20 years, Isfahan with 18 years, and Sarem and Navid centers, each with 17 years of experience (Fig.3).
Fig. 3: Frequency of clinics according to years of experience.

There were approximately 29000 IUI, as well as 35000 IVF and ICSI cycles in Iran during 2011. The sum of average number of visits in tertiary infertility centers for any type of diagnostic and therapeutic process per month was 39063; the mean number of new admissions among the centers was 641 per month. The minimum number of admissions in centers was three per month. The maximum numbers of admissions occurred in three private centers in Tehran and Isfahan. A total of 21 centers were located in Tehran, the capital of Iran, with a total number of 20786 clients per month, which was the highest proportion (53%) in the country (Fig. 4).

Fig. 4: Percentage of visited clients in infertility centers of Iran during 2011.

Table 2 shows the number of centers according to provided services. All centers had documented records of patients’ general information; however, 17 (33%) had electronic records. The monthly rate of ART services provided by centers is shown in Table 3.

Table 2: Numbers of centers that provide different ART services

| Type of ART service      | Number of centers (%) |
|--------------------------|-----------------------|
| IUI                      | 52 (100)              |
| IVF                      | 49 (94)               |
| ICSI                     | 50 (96)               |
| Surrogacy                | 35 (67)               |
| Egg donation             | 43 (83)               |
| Embryo donation          | 35 (67)               |
| Sperm donation           | 1 (2)                 |
| Sperm bank               | 6 (12)                |
| Embryo freezing          | 48 (92)               |
| Egg freezing             | 35 (67%)              |
| Sperm freezing           | 40 (77%)              |
| Diagnostic laparoscopy and hysteroscopy | 44 (85%) |
| Therapeutic laparoscopy and hysteroscopy | 42 (81%) |
| Male infertility surgeries | 43 (83%)           |

ART; Assisted reproductive technology, IUI; Intrauterine insemination, IVF; In vitro fertilization, and ICSI; Intra-cytoplasmic sperm injection.

In 2011, the mean cost of IVF ranged from $2250 to $3600 in government and private centers. In Iran, technical managers of all centers should be fellowship of infertility or qualified by having three years of experience in an infertility center. Therefore, having infertility fellowship was assessed in all centers as criteria. In 5 centers, an infertility fellowship did not exist, 3 had no urologists, and 2 did not have any embryologists. In numerous cases, one embryologist simultaneously cooperated with more than one center. In 16 centers, midwives were used as alternatives to nurses and 2 centers had no midwives.

Table 3: Monthly rate of assisted reproductive technology procedures in 2011

| Type of service               | Mean | Minimum | Maximum |
|------------------------------|------|---------|---------|
| Intrauterine insemination (IUI) | 47   | 2       | 300     |
| In vitro fertilization (IVF)   | 57   | 0       | 400     |
| Surrogacy                     | 165  | 1       | 61      |
| Egg donation                  | 997  | 1       | 150     |
| Embryo donation               | 545  | 2       | 300     |

*; Mar. 21st to Sept. 22nd, 2011.
A total of 36% of centers had neither an internal specialist nor an endocrinologist. Patients who needed specialized medical counseling (i.e., urology, endocrinology, and genetics) were referred to another clinic or hospital when there was no specialist located at that center. Some infertility centers presented their needs for specialists (Table 4).

| Type of required specialist | Number of centers (%) |
|-----------------------------|-----------------------|
| Gynecologists with infertility focus/infertility fellowship | 8 (15) |
| Urologist | 3 (6) |
| Embryologist | 11 (21) |
| Internal specialist or an endocrinologist | 19 (36) |
| Psychologist | 32 (62) |
| Genetics specialist | 30 (58) |
| Nutrition specialist | 25 (48) |

In order to assess level III criteria adjustment for diagnostic tests, we checked the sonohysterography and hysterosalpingography performances. In 31 centers, sonohysterography and hysterosalpingography were performed. All centers with the exception of one center, were equipped with a microinjection device.

Among 28 infertility research centers, 7 had scientific research journals: Vali Asr, Royan Institute, Avicenna Infertility Clinic, Family Hospital, Mehr Infertility Center, Sarem Hospital, and Mother Infertility Center.

Discussion

The increased use of reproductive science developments, as well as the least legal or religious barriers to ART in Iran as an Islamic country, have raised the hopes of infertile couples.

The availability of infertility services, as a product of public and private health policies, could determine the allocation of personnel, equipment, and facilities (12). According to our data, the number of infertility centers that performed ART varied considerably by province. Residents of Tehran with 21 infertility centers had the easiest access to infertility centers followed by East Azerbaijan, Khorasan Razavi, Gilan, and Mazandaran with 4 or 3 centers. The remainder of provinces had none to 2 centers. Lack of geographic access to level III infertility services has obviated the necessity for increasing the availability and utilization of these services in different areas of the country. Establishing at least one level III center in all provinces rather than multiple centers in specific areas could provide extensive facilities to cover the entire country.

Most medical universities that had an infertility center were type I. All centers provided clinical services, however 35% also had educational and research departments. Numerous centers solely focused on clinical activities (35%), which have suggested that stronger links among research, education, and practice are needed.

Although 12 centers had more than 15 years of experience, most had less than 5 years of experience in providing ART services. Compared with the most experienced infertility clinics in the US, which were established in the early 1980s, our first infertility center had almost a decade delay in establishment.

The estimated number of IVF per million population in 2011 showed that the national utilization of IVF was less than the equivalent reported from European countries in 2010 and the US in 2011 (10, 13). In 2011, most centers offered IUI, IVF, and ICSI. The number of centers that offered egg and embryo donation was almost half compared with clinics in the US. Only one center reported that they offered sperm donation to clients, which was entirely different from similar percentage of this service in the US (14).

It seems that due to lack of a supporting law in the country, some centers did not claim their donation practice. Due to legal and legislative approval of embryo donation in Iran, as well as the absence of laws regarding sperm donation, our data about embryo donation might be more realistic (8, 15). This could cause centers to perform embryo donation instead of sperm donation and as a result the portion of embryo donation cycles might increase compared with other donation programs. The low percentage of centers that provided sperm banks could be another consequence of a vague law and legislations on this program.

The mean number of new monthly admissions among centers was 641. As far as we know this has not been reported in other countries’ studies. Usually the number of cycles is reported for each infertility clinic. Lack of a referral system in Iran may lead to overestimation of the number of new admissions be-
cause a person as a new admission of a specific infertility center may refer to another center after a period of time and be counted in more than one center.

Worldwide, ethical issues in reproductive medicine and assisted reproduction are influenced by religions. In Iran, Islamic primary sharia. In Latin America, the Catholic Church applies considerable pressure to prevent access to IVF and third party assisted reproduction is banned (8). Studies have mostly focused on the view of Sunni Islam on ART, as they comprise 90% of the Muslim population. Shia Islam which is concentrated in Iran, Azerbaijan, Iraq, and Afghanistan relies on clergy scholars’ decrees on different new issues including ART. Although there are differences in the decrees, all are correct for the scholars’ followers. Some Shia scholars have approved ART using third party and the embryo donation law was passed by the Iranian parliament in 2003. In contrast, in 1986, the Islamic Fiqh Academy based in Jeddah (Majmaal-Fiqh al-Islami) considered all types of third party assisted reproduction forbidden (haram) (8).

Ethical conditions may lead to legislative rules that are adopted by legal experts, ethicists, religious scholars, and politicians. Therefore, some people may obtain their desired infertility treatment outside of their own countries. In this way, they travel from a restrictive country to a permissive one (16, 17). Infertility is highly stigmatized in developing countries and leads to profound social consequences for these couples. Hence, infertility is sometimes kept secret. Due to losing both social support and social capital, patients seek reproductive services in neighboring countries (18, 19). Since Iran is a Muslim country in which gamete and embryo donation are practiced as well as surrogacy, it can be the first choice for Muslims from other countries who seek infertility treatment. We have not gathered international patients’ data, so no conclusion could be made in this regard.

In most countries, cost is a serious concern for couples who receive infertility services. The cost of ART treatment is different worldwide due to the costliness of underlying health care systems and the level of patients’ subsidization. As all infertility centers in Iran operate outside of government-financed health facilities, they actually provide services only for patients that can pay out of pocket for ART treatments. Although ART is cost prohibitive in Iran, the cost is relatively lower than neighboring countries with better economic situations and stronger currencies. The relative cost differences encourage infertile couples to travel to Iran to undergo ART (20, 21).

Considering the rate of lifetime experience of infertility (6.4%), as well as the rate of primary (21.1%), and secondary infertility (7.8%) in Iran (22), it is essential to draw up a national guideline. This guideline can offer the most practical advice on assisting couples with infertility problems, and take into account individual needs and preferences (14). Lack of national auditing, supervision, and a registry are the major drawbacks of this system. A national center is required to implement a registry system that reports important national outcomes of infertility centers such as success rates, numbers of embryos transferred, numbers of frozen-thawed eggs, and the woman’s age at the time of retrieval, in addition to an introduction to the infertility centers and costs for ART cycles. A national registry and monitoring can lead to improvement in quality of aspects of the structures, processes, and outcomes of infertility centers. Establishing this registry system can be initially implemented by developing audit activity and outcome committees in the centers.

**Conclusion**

This paper has presented the status of ART in Iran during 2011. The most obvious finding to emerge from this study is that Iran has great potential to provide IVF services for both Iranians and other nationalities throughout the region. Therefore the implementation of a registry system seems to be vital.

**Acknowledgements**

This study was financially supported by the Iranian Ministry of Health and there was no conflict of interest.

**References**

1. Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O, Adhamoon GD, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) world report: assisted reproductive technology 2003. Fertil Steril. 2011; 95(7): 2209-2222.e17.
2. Ferraretti A, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA, et al. Assisted reproductive technology in Europe, 2009: results generated from European reg-
3. Ferraretti A, Goossens V, Bhattacharya S, Castilla JA, de Mouzon J, Korsak V, et al. European and Global ART Monitoring (presentation of the EIM and ICMART data reports). Hum Reprod. 2013; 28 Suppl 1: O-090.

4. Sunderam S, Kissin DM, Crawford SB, Anderson JE, Folger SG, Jamieson DJ, et al. Assisted Reproductive Technology Surveillance—United States, 2010. MMWR Surveill Summ. 2013; 62(9): 1-24.

5. Zegers-Hochschild F, Schwarze JE, Crosby JA, Musri C, de Souza MB. Assisted reproductive technologies (ART) in Latin America: the Latin American registry, 2012. JBRA Assist Reprod. 2014; 18(4): 127-135.

6. Mansour RT, Abou-Setta AM. Results of assisted reproductive technology in 2001 generated from the Middle East IVF registry. Middle East Fertil Soc J. 2006; 11(3): 145-151.

7. Serour GI. Ethical issues in human reproduction: Islamic perspectives. Gynecol Endocrinol. 2013; 29(11): 949-952.

8. Samani RO, Moalem MR, Merghati ST, Alizadeh L. Debate in embryo donation: embryo donation or both-gamete donation? Reprod Biomed Online. 2009; 19 Suppl 1: 29-33.

9. Abusief ME, Hornstein MD, Jain T; American Society for Reproductive Medicine; Society for Assisted Reproductive Technology: Assessment of United States fertility clinic websites according to the American Society for Reproductive Medicine (ASRM)/Society for Assisted Reproductive Technology (SART) guidelines. Fertil Steril. 2007; 87(1): 88-92.

10. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D’Hooghe T, Castilla JA, et al. Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. Hum Reprod. 2014; 29(10): 2099-2113.

11. Gholami J, Ahghari S, Motevalian A, Yousefnejad V, Moradi G, Keshtkar A, et al. Knowledge translation in Iranian universities: need for serious interventions. Health Res Policy Syst. 2013; 11: 43.

12. Nachtigall RD. International disparities in access to infertility services. Fertil Steril. 2006; 85(4): 871-875.

13. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Barfield WD, et al. Assisted reproductive technology surveillance—United States, 2011. MMWR Surveill Summ. 2014; 63(10): 1-28.

14. O’Flynn N. Assessment and treatment for people with fertility problems: NICE guideline. Br J Gen Pract. 2014; 64(618): 50-51.

15. embroidered donation law. Iran Judicature, Official Gazette of the Islamic Republic of Iran. Available from: http://www.dastour.ir/Brows/?id=245069 (16 June 2016).

16. Ron-El R. Reproductive tourism. Schlegel PN, Fauser BC, Carrell DT, Racowsky C, editors. Biennial review of infertility. 1st ed. New York: Springer; 2013; 163-169.

17. Van Hoof W, Pennings G. Cross-border reproductive care around the World: recent controversies. In: Botterill D, Pennings G, Mainil T, editors. Medical tourism and transnational health care. London: Palgrave Macmillan UK; 2013; 98-112.

18. Roucho B. Consequences of infertility in developing countries. Perspect Public Health. 2013; 133(3): 174-179.

19. Afshar L, Bagheri A. Embryo donation in Iran: an ethical review. Dev World Bioeth. 2013; 13(3): 119-124.

20. Jabbari A, Agharahimi Z, Hosseini Sh, Safari F. Capabilities of infertility tourism in Isfahan: a qualitative study. Int J Health Syst Disaster Manage. 2013; 1(2): 99-104.

21. Chambers GM, Adamson GD, Eijkemans MJ. Acceptable cost for the patient and society. Fertil Steril. 2013; 100(2): 319-327.

22. Rostami Dovom M, Ramezani Tehrani F, Abedini M, Amirshakari G, Mehrabi Y. Prevalence 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.
Comparison of Quality of Life, Sexual Satisfaction and Marital Satisfaction between Fertile and Infertile Couples

Seyedeh Zahra Masoumi, Ph.D.¹, Maryam Garousian, M.Sc.²*, Somayeh Khani, B.Sc.¹, Seyedeh Reyhaneh Oliaei, D.D.S.³, Arezoo Shayan, M.Sc.⁴

1. Students Research Center, School of Nursing and Midwifery, Hamadan University of Medical Sciences, Hamadan, Iran
2. Fatemieh Hospital, Hamadan University of Medical Sciences, Hamadan, Iran
3. Dentistry Faculty, Hamadan University of Medical Sciences, Hamadan, Iran
4. Department of Midwifery, School of Nursing and Midwifery, Hamadan University of Medical Sciences, Hamadan, Iran

Abstract

Background: Fertility plays an important role in sexual and psychological function in families. Infertility can result in major emotional, social, and mental disorders, including a reduction in satisfaction with marital life and quality of life. The present study aimed to compare the quality of life and marital satisfaction and sexual satisfaction between fertile and infertile couples.

Materials and Methods: This analytical cross-sectional study was conducted on 250 couples at the Fatemiyeh Educational Research Center affiliated to Hamadan University of Medical Sciences, Hamadan, Iran, from May to August in 2014. The subjects were randomly selected from the patients referred to this center using a table of random numbers. They were then allocated into two groups of infertile group (n=125) and fertile group (n=125). The study participants completed World Health Organization Quality of Life-BREF (WHOQOL-BREF) questionnaire, Linda Berg’s Sexual Satisfaction Scale, and Enrich Marital Satisfaction Scale. Then, the data were entered into the SPSS version 16 for statistical analysis. The Chi-square and Mann-Whitney tests were also applied to compare the data between the groups.

Results: The results revealed no significant difference between the two groups regarding demographic and general health variables. The mean scores of sexual satisfaction were 63.67 ± 13.13 and 46.37 ± 7.72 in the fertile and infertile couples, respectively. Furthermore, the mean scores of marital satisfaction were also 44.03 ± 9.36 and 36.20 ± 4.03 in the fertile and infertile groups, respectively. Our finding demonstrated that the fertile couples obtained significantly higher mean scores of quality of life as well as lower mean scores of sexual satisfaction and marital satisfaction as compared to the infertile ones (P<0.001).

Conclusion: According to the results, the fertile couples obtained significantly higher quality of life and lower sexual satisfaction and marital satisfaction as compared to the infertile ones. Therefore, holding consultation programs and conducting more studies are necessary for improving the quality of life and promoting sexual and marital satisfaction in infertile couples.

Keywords: Infertility, Quality of Life, Sexual Satisfaction, Marital Relationship

Citation: Masoumi SZ, Garousian M, Khani S, Oliaei SR, Shayan A. Comparison of quality of life, sexual satisfaction and marital satisfaction between fertile and infertile couples. Int J Fertil Steril. 2016; 10(3): 290-296.
Introduction

Quality of life is a complex concept that is related to physical health, psychological status, level of independence, social relations, personal beliefs, and environmental factors (1). It is also affected by age, culture, sex, education level, social status, disease, and social environment (2). Infertility is among the difficult conditions affecting quality of life. It is also among the major medical problems whose rate has increased by 50% since 1955. To date, 10-15% of couples suffer from infertility (3). Infertility status and its related factors affect the quality of life through creating psychosocial stress, reduction of life satisfaction, increase of marital conflicts, and decrease of sexual satisfaction and marital satisfaction (4, 5).

In fact, infertility is considered as a personal and social problem affecting couple’s life and family’s performance, so exposes couples to mental pressure and various psychological disorders (6, 7). Infertility, as an emotional shock, can even have an impact on couples’ communication, occupational, and sexual skills. Overall, infertility, as a serious medical problem, can have destructive effects on the quality of life (8). Evidence has indicated that infertility is a destructive or painful experience that leads to more disappointment, prostration, and anger in infertile couples as compared to their fertile peers. Besides, infertile couples have disturbed relationships with their spouses, families, and friends that make them more vulnerable to psycho-emotional disorders, depression, anxiety, low self-confidence, and dissatisfaction that subsequently lead to low quality of life (9). Nevertheless, some researches have demonstrated that in case of cooperation and sharing responsibilities between couples, treatment procedures can increase their intimacy and improve the quality of their marital life (10).

In a study by Nourani et al. (11) that was conducted at the Majidi Treatment Center, Tabriz, Iran, 12% of women reported low life quality, while more than half of them had desirable quality of life. In addition, familial and social pressure had a negative influence on infertile women’s quality of life. Marital and sexual satisfaction considerably affect couples’ physical and mental health. On the other hand, incompatibility in a marital relationship disturbs social relations, leads to tendency towards social deviations, and declines cultural values between couples. Thus, sexual satisfaction is necessary for solidity of marital life. Some researchers believe that sexual dissatisfaction accounts for 80% of marital conflicts (12). On the other hand, fertility status is one of the effective factors in sexual satisfaction. Based on various studies, infertility could result in several psychological disorders, including sexual dissatisfaction (13). However, some studies have shown no difference between the couples under infertility treatment and fertile couples in the context of marital satisfaction. In other words, marital satisfaction was not affected by infertility (14, 15). Tao in a systematic review has investigated marital relationship in infertility and reported that sexual satisfaction had impact on marital satisfaction (15). The problem of infertility has become deeper, especially in the Iranian culture in which there is extended family type. Because in this type of families having children is important. Infertility can be regarded as life crisis, identity crisis, chronic disease, or a combination of them (16).

Quality of life in infertile couple differs from one society/culture to another. Fertility is of utmost sociocultural importance, while controversial results have been obtained regarding the effect of infertility on quality of life in western countries. Besides, only limited studies have been performed in this regard in the Eastern Counties, including Iran. Due to inconsistencies in the available studies, this study aimed to compare the quality of life, sexual satisfaction and marital satisfaction between fertile and infertile couples.

Materials and Methods

The present analytical cross-sectional study was conducted on fertile couples and infertile couples referred to the Fatemieh Educational Research Center affiliated to Hamadan University of Medical Sciences, Hamadan, Iran, from May to August in 2014. The study protocol was approved by the Institutional Review Board and the Human Research Ethics Committee of the Hamadan University of Medical Sciences. Sample size was estimated using the following formula:

$$n = \frac{(Z_{1-2} + Z_{1-2})^2(\sigma_1^2 + \sigma_2^2)}{\left(\mu_2 - \mu_1\right)^2}$$

To achieve power of 90 and level of significance of 0.05, 125 couples were determined for each group.
At first, a list of eligible couples, referred to the Fertility Center of Hamadan University of Medical Sciences, was prepared, among whom 125 infertile couples were selected using a table of random numbers. Then, 125 fertile couples were selected from those referring to other clinics (like women’s health care, oncology, and children’s health care centers) using the same method, meaning 25 fertile couples were chosen from each clinic. The two groups in terms of age, socio-economic status and lack of acute or chronic diseases were matched.

The inclusion criteria of the infertile group were as follows: not conceiving after 5 years of trying, primary infertility, male factor infertility/female factor infertility (or both), unexplained infertility, and literacy skills in Farsi. The inclusion criteria for the fertile group were as follows: not suffering from infertility, having at least one child, willingness to cooperate in the study, and literacy skills in Farsi. On the other hand, the exclusion criteria of the study were as follows: use of medications other than those used for infertility treatment, physical or mental disorders, death of close relatives during the past two months, child adoption, and unwillingness to cooperate in the study. After the study objectives and procedures were explained to them, all participants signed a written informed consent.

Measurement tools

All participants completed demographic questionnaire, World Health Organization Quality of Life-BREF (WHOQOL-BREF) questionnaire, Linda Berg’s Sexual Satisfaction Scale, and Enrich Marital Satisfaction Scale.

WHOQOL-BREF questionnaire prepared by the WHO contains 24 items regarding physical health (7 items), mental health (6 items), social relationships (3 items), and environmental health (8 items) dimensions. This questionnaire also includes 2 other items to evaluate health status and quality of life generally. Thus, the questionnaire has 26 items. The items are responded through a 5-option Likert scale and a score between 0 and 100 is assigned to each dimension (17). Assessment of psychometric properties was done by the WHO (18). In Iran, the reliability and validity of this scale were approved by Nejat et al. (19). Besides, Keramat et al. (20) have evaluated the reliability of WHOQOL-BREF and reported the Cronbach’s alpha (reliability) of 0.78, 0.77, and 0.79 belonging to physical health, mental health, and environmental health dimensions, respectively.

Enrich Marital Satisfaction Scale designed by Olsun, Furnier, and Druckman contains 14 subscales. The reliability of this questionnaire was already confirmed (α=0.92), while Pazandeh and Sharghi (16) have reported a reliability of α=0.95. This scale is responded using a 5-option Likert scale and a score between 1 and 5 is allocated to each item. Accordingly, the scores are below 30 indicating severe dissatisfaction, between 30 and 40 indicating dissatisfaction, between 40 and 60 indicating relative and moderate satisfaction, between 60 and 70 indicating great satisfaction, and above 70 indicating very great satisfaction. Keramat et al. (20) have confirmed the reliability of this scale with Cronbach’s alpha of 0.91.

Linda Berg’s Sexual Satisfaction Scale designed by Linda Berg and Cresy in 1997 consists of 17 items with the following options: always, often, sometimes, rarely, and never receiving 5, 4, 3, 2, and 1 scores, respectively. Thus, the minimum and maximum scores of the scale are 17 and 85, respectively. Accordingly, the scores are 17-51 indicating weak, 52-67 indicating moderate, and 68-85 indicating good sexual satisfaction. The validity and the reliability (Cronbach’s alpha=0.94) of this scale were also approved by Keramat et al. (20). Assessment of psychometric properties of all questionnaires were done in many studies (18).

Statistical analysis

All data analyses were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., USA) version 16. Chi-square test and correlation analysis were used to assess the relationship between the study variables. Besides, student’s t test was employed for comparison of the study groups. A value of P<0.05 was considered as statistically significant.

Results

According to the results, the mean score of physical dimension of WHOQOL-BREF was significantly higher in the fertile group (15.46 ± 2.66) compared to the infertile group (14.86 ± 2.66, P<0.05). Also, in the environmental dimension, the fertile couples obtained a significantly higher mean score (13.90 ± 2.41) in comparison to the infertile ones (13.13 ± 2.49, P<0.05). In the mental
dimension, the fertile group gained a higher mean score (13.71 ± 2.73) compared to the infertile group (13.42 ± 2.64), indicating the difference was not statistically significant. Considering the social dimension, although no significant difference was observed between the two groups, the mean score of the infertile group (14.27 ± 2.85) was higher than that of the fertile group (13.97 ± 2.85).

The mean score of sexual satisfaction was significantly higher in the infertile group compared to the fertile group (63.67 ± 13.13 vs. 46.37 ± 7.72). The mean score of marital satisfaction was also significantly higher in the infertile couples compared to the fertile ones (44.03 ± 9.36 vs. 36.20 ± 4.03) (Table 1). The results showed weak sexual satisfaction in 21.6% of infertile women, 79.2% of fertile women, 15.2% of infertile men, and 62.4% of fertile men. In other words, weak sexual satisfaction was less common in infertile couples (Table 2). Moreover, relative and moderate marital satisfaction was observed among 48% of infertile women, 12% of fertile women, 52% of infertile men, and 9.6% of fertile men. Moreover, very great marital satisfaction was found neither in the infertile nor in the fertile group, and great satisfaction was also not observed in the fertile couples (0 vs. 32% in infertile women and 8.8% in fertile men). In other words, marital satisfaction was higher in the infertile couples compared to the fertile ones (Table 3).

### Table 1: Comparison the means scores of different dimensions belonging to WHOQOL-BREF questionnaire between two groups using t test

| Variables           | Group      | Frequency | Mean   | SD    | t     | P value |
|---------------------|------------|-----------|--------|-------|-------|---------|
| Physical dimension  | Fertile    | 250       | 15.46  | 2.66  | 2.56  | 0.01    |
|                     | Infertile  | 250       | 14.86  | 2.66  |       |         |
| Mental dimension    | Fertile    | 250       | 13.71  | 2.73  | 1.20  | 0.23    |
|                     | Infertile  | 250       | 13.42  | 2.64  |       |         |
| Social dimension    | Fertile    | 250       | 13.97  | 2.74  | -1.20 | 0.231   |
|                     | Infertile  | 250       | 14.27  | 2.85  |       |         |
| Environmental dimension | Fertile | 250       | 13.90  | 2.41  | 3.50  | <0.001  |
|                     | Infertile  | 250       | 13.13  | 2.49  |       |         |
| Sexual satisfaction | Fertile    | 250       | 46.37  | 7.72  | -17.96| <0.001  |
|                     | Infertile  | 250       | 63.67  | 13.13 |       |         |
| Marital satisfaction| Fertile    | 250       | 36.20  | 4.03  | -12.14| <0.001  |
|                     | Infertile  | 250       | 44.03  | 9.36  |       |         |

WHOQOL-BREF; World Health Organization Quality of Life-BREF questionnaire.

### Table 2: Comparison of frequency distribution of different sexual satisfaction categories between fertile and infertile couples

| Sexual satisfaction categories | Infertile couples n=125 | Fertile couples n=125 |
|-------------------------------|-------------------------|-----------------------|
|                               | Infertile women | Infertile men | Total | Fertile women | Fertile men | Total |
| Weak                          | n   | %     | n   | %     | n   | %     | n   | %     | n   | %     | n   | %     |
|                               | 27  | 21.6  | 19  | 15.2  | 46  | 36.8  | 99  | 79.2  | 78  | 62.4  | 177 | 141.6 |
| Moderate                      | 52  | 41.6  | 45  | 36.0  | 97  | 77.6  | 26  | 20.8  | 47  | 37.6  | 73  | 58.4  |
| Good                          | 46  | 36.8  | 61  | 48.8  | 107 | 85.6  | 0   | 0     | 0   | 0     | 0   | 0     |
| Total                         | 125 | 100   | 125 | 100   | 250 | 100   | 125 | 100   | 125 | 100   | 250 | 100   |

WHOQOL-BREF; World Health Organization Quality of Life-BREF questionnaire.
Discussion

Quality of life is the general well-being of individuals and societies, outlining negative and positive features of life. Sexual satisfaction is defined as an individual’s judgment about pleasure of one’s sexual behavior (21). The most important goal of sexual desire is reproduction and childbearing. Thus, sexual satisfaction is highly influenced by infertility (22). Compatibility and marital satisfaction are referred to a status in which a couple feels happy and satisfied that is created through mutual interest, caring, acceptance, understanding, and meeting each other’s needs, including sexual needs (23).

Throughout the recent two decades, quality of life has been considered as a major concern. In 1978, WHO indicated quality of life improves when an individual receives mental and physical care. Fertility has social, psychological and physiological aspects. In most cultures, reproduction is of great importance concept (24). The results of the present study showed that the fertile women obtained higher mean scores in all dimensions of quality of life compared to the infertile ones. This difference was statistically significant in physical and environmental dimensions, but not in mental and social dimensions. Infertility and other related issues, like treatment process, have a negative impact on physical and mental health of infertile couples.

Physical dimension (general health, physical role, and bodily pain) is a highly important aspect of life quality. Stress-related infertility leads to physiological stress that results in serious health problems (25). Infertile couples seeking treatment also experience a lot of physical problems (26). In current study, physical dimension of infertile couples is lower than fertile couples. In a study by Kamkary and Shokrzadeh (27), they have showed that control of the environmental factors, which is among psychological components, is higher in couples with higher mental functions. Mental pressures resulting from infertility affects couples’ attitude towards the environmental factors and reduces their determination to achieve their personal goals (28). According to the study by Direkvand Moghadam et al. (3), infertile women showed a lower mean score of physical role limitation due to physical problems as compared to the fertile ones. In addition, in a study by Hatamloye Saedabadi and Hashemi Nosratabad (29), he has indicated that control over the environment was lower among infertile women compared to fertile women. These results were consistent with those of the current study, showing that environment dimension of the quality of life in infertile couples was lower than fertile couples.

Evidence has demonstrated that psychological stress due to infertility treatment affect patients’ quality of life through disturbing their psychological, social, and welfare conditions (30). Infertility is a source of social pressure that is exerted by a traditional culture surrounding the infertile couples (31). A study has shown that infertile couples have more feelings of helplessness and disappointment (32). One study has revealed that almost one thirds of all women and their partners experienced a lack of social support (30). Nevertheless, some researchers have reported higher

| Table 3: Comparison of frequency distribution of marital satisfaction categories between fertile and infertile couples |
|---------------------------------------------------------------|
| Marital satisfaction categories | Infertile women | Infertile men | Total | Fertile women | Fertile men | Total |
|---------------------------------|-----------------|---------------|-------|---------------|-------------|-------|
| Severe dissatisfaction           | 7 5.6           | 5 4.0         | 12    | 9.6           | 14 11.2     | 18 14.4 |
| Dissatisfaction                  | 54 43.2         | 44 35.2       | 98    | 78.4          | 96 76.8     | 192 154 |
| Relative and moderate satisfaction | 60 48.0        | 65 52.0       | 125   | 100           | 15 12       | 27 21.6 |
| Great satisfaction              | 4 32            | 11 8.8        | 15    | 40.8          | 0 0         | 0 0    |
| Very great satisfaction         | 0 0             | 0 0           | 0     | 0 0           | 0 0         | 0 0    |
| Total                           | 125 100         | 125 100       | 250   | 100           | 125 100     | 250 100 |


Masoumi et al.
social support among infertile women. A study performed in Turkey has also showed that in spite of lower scores in mental dimension, infertile women had better social support (33). In our study, no significant difference was also found between fertile and infertile couples regarding mental and social dimensions. Unlike, another study in Iran has revealed statistically significant relationship between duration of infertility and mental disorders and marital conflicts (34). The findings of the current study demonstrated significantly higher sexual satisfaction among the infertile couples compared to the fertile ones, indicating the couples may be closer emotionally and psychologically to each other because of the conditions and continuation of treatment.

A previous study has indicated that infertile couples had lower sexual function in orgasm, arousal, and desire dimensions (23). On the other hand, a study by Jamali et al. (22) conducted in Iran has showed that infertility had no impact on couples’ sexual function. Also, another study has indicated that sexual dysfunction was only detected in 11% of infertile couples (35). Similarly, Monga et al. (36) has reported no significant difference between infertile and fertile couples with respect to sexual function, which was attributed to the need for large number of sexual relationships for treatment of infertility and getting pregnant. Yet, this can also be associated with an increase in intimacy of infertile couples (22). In the present study, the infertile couples showed higher marital satisfaction compared to the fertile ones. In a study by Lotfi Kashani and Vaziri (37), they have concluded that marital satisfaction was accompanied by sexual satisfaction, which in return, higher sexual satisfaction resulted in higher marital satisfaction. Up to now, a large number of researches have shown that infertility declined marital satisfaction (38). However, many studies have indicated that children play a major role in decreasing marital satisfaction (39). Although having children strengthens the marital relationship, it may decrease marital satisfaction with passage of time and growing number of children (40, 41). Overall, the findings of our study showed that despite a decrease in life quality, infertile couples had high sexual and marital satisfaction. There were a number of limitations in this study. Due to randomly selected samples, there was no choice to select all samples properly. Therefore, it is recommended that in future, a study should be conducted with a larger sample size to show the improvement of quality of life and marital satisfaction of infertile couples as the basis of family and society.

Conclusion

According to the results, the fertile couples obtained significantly higher quality of life and lower sexual satisfaction and marital satisfaction as compared to the infertile ones. This might have resulted from disturbance in couples’ marital relationships due to children, financial problems, etc. Therefore, holding consultation programs and conducting more studies are necessary for improving the quality of life and promoting sexual satisfaction and marital satisfaction in infertile couples.

Acknowledgements

This article was extracted from a student research proposal. Hereby, the authors would like to thank the Research and Treatment Vice-chancellors of Hamadan University of Medical Sciences for financial support, the authorities of the healthcare centers, and the personnel who cooperated in the research. The authors declared no conflict of interests.

References

1. Makvandi S, Zamani M. The survey of quality of life and its dimensions in Islamic Azad University Ahvaz branch students in 2010. Jentashapir Journal of Health Research. 2012; 2(4): 191-200.
2. King CR, Hinds PS. Quality of life: from nursing and patient perspectives. 3rd ed. Jones & Bartlett Learning, LLC; 2012; 32.
3. Direkvand-Moghadam A, Delpisheh A, Direkvand-Moghadam A. Compare the quality of life in fertile and infertile women attending public. Journal of Shahid Beheshti School of Nursing and Midwifery. 2014; 24(85): 39-44.
4. Chachamovich J, Chachamovich E, Fleck M, Cordova F, Knauth D, Passos E. Congruence of quality of life among infertile men and women: finding from a couple-based study. Hum Reprod. 2009; 24(9): 2151-2157.
5. Hassanin I, Abd-El-Raheem T, Shahin A. Primary infertility and health-related quality of life in Upper Egypt. Int J Gynaecol Obstet. 2010; 110(2): 118-121.
6. Dana Sh, Narimani M, Mikaeili N. Comparison of emotion regulation and emotion control in fertile and infertile women. Intl J Phys Beh Res. 2013; 2(8): 250-254.
7. Chachamovich JR, Chachamovich E, Ezer H, Fleck MP, Knauth D, Passos E. Investigating quality of life and health-related quality of life in infertility: a systematic review. J Psychosom Obstet Gynaecol. 2010; 31(2): 101-110.
Masoumi et al.

8. Carter J, Applegarth L, Josephs L, Grill E, Basar R, Rosenwaks Z. A cross-sectional cohort study of infertile women awaiting oocyte donation: the emotional, sexual, and quality-of-life impact. Fertil Steril. 2011; 95(2): 711-716.

9. Farrokh-Eslamlou H, Hajishafieh M, Sadat Kazemi E, Oshnouei S. Impact of primary infertility on life quality in Iran. Jurnal of Obstetrics & Gynecology. 2014; 25(7): 598-604.

10. Aliyeh G, Layla F. Quality of life and its correlated among a group of infertile Iranian women. Med Sci Monit. 2007; 13(7): CR313-317.

11. Nourani Sh, Joneidy E, Shakeri MT, Mohkber N. Comparison of quality of life in fertile and infertile women referred to the Public Clinics in Mashhad. Iranian Journal of Obstetrics, Gynecology and Infertility. 2012; 15(7): 24-31.

12. Rahmani A, Merghati Khoee E, Alah Gholi L. Sexual satisfaction and its relation to marital happiness in Iranians. Iran J Med Sci. 2014; 39(4): 1-12.

13. Jonaayd E, Sadodin SN, Mohkber N, Shakeri MT. Comparing the marital satisfaction in infertile and fertile women referred to the public clinics in mashhad in 2006-07. Iranian Journal of Obstetrics, Gynecology and Infertility. 2009; 12(7): 12-16.

14. Weaver SM, Clifford E, Hay DM, Robinson J. Psychosocial adjustment to unsuccessful IVF and ICSI treatment. Patient Educ Couns. 1997; 31(7): 7-18.

15. Tao P, Coates R, Maycock B. Investigating marital relationship in infertility: a systematic review of quantitative studies. J Reprod Infertil. 2012; 13(2): 71-80.

16. Pazandeh F, Sharghi N. Comparing wellbeing of fertile and infertile women referring to Health Centers at Sha- hidi Behesti University of Medical Sciences and Health Services and infertile centers at Tehran 1381. Journal of Shahid Behesti School of Nursing and Midwifery. 2004; 14(44): 4-10.

17. Saxena S, Carlson D, Billington R; WHQLQOL Group. World Health Organization Quality Of Life. The WHO quality of life assessment instrument (WHOQOL-Bref): the importance of its items for cross-cultural research. Qual Life Res. 2001; 10(8): 711-721.

18. Skevington SM, Lofty M, O’Connell KA; WHOQOL Group. The World Health Organization Quality of Life (WHOQOL) assessment: psychometric properties and results of the international field trial. A report from the WHOQOL group. Qual Life Res. 2004; 13(2): 299-310.

19. Nejat S, Montazeri A, Holakouie Naieni K, Mohammad k, Majztradel SR, The World Health Organization quality of Life (WHOQOL-BREF) questionnaire: translation and validation study of the iranian version. Journal of School of Public Health and Institute of Public Health Research. 2006; 4(4): 1-12.

20. Keramat A, Masoumi S, Mousavi SA, Poorolajal J, Shobeiri F, Hazavehie SM. Quality of life and its related factors in infertile couples. J Res Health Sci. 2014; 14(1): 57-63.

21. Jagadisha Kar N. Sexuality and psychopathology. In: Kar N, Kar GC, editors. Comprehensive textbook of sexual medicine, 1st ed. New Delhi: Jaypee Brothers Publishers; 2005; 429-438.

22. Jamali S, Rasekh Jahromi A, Javadvpor Sh. Sexual function in fertile and infertile women referring to the Jahrom Infertility in 2011. Jundishapur Journal of Chronic Disease Care. 2014; 3(1): 11-20.

23. Marci R, Graziano A, Piva I, Lo Monte G, Soave I, Giugliano E, et al. Procreative sex in infertile couples: the decay of pleasure? Health Qual Life Outcomes. 2012; 10: 140.

24. Chatmeh R, Kafi M. Investigation of relationship between irrational beliefs and worry in infertile women. J Appl Environ Biol Sci. 2015; 5(8): 341-346.

25. Peterson BD, Eftert GH. Using acceptance and commitment therapy to treat infertility stress. Cognitive and Behavioral Practice. 2011; 18(4): 57-67.

26. Beaurepaire J, Jones M, Thiering P, Saunders D, Tennant C. Psychological adjustment to infertility and its treatment: male and female responses and different stages of IVF/ET treatment. J Psychosom Res. 1994; 38(3): 229-240.

27. Kamkary K, Shokrzadeh Sh. The relationship between mental health and happiness feel in Tehran youth people. Euro J Exp Bio. 2012; 2(5): 1880-1886.

28. Alizadeh T, Farahani MN, Shahraray M, Alizadegan Sh. The relationship between self esteem and focus of control with infertility related status of infertile men and women. J Reprod Infertil. 2005; 6(22): 194-204.

29. Hatamlooye Saedabadi M, Hashemi Nosratabad T. The comparison of psychological well-being and marital satisfaction in the fertile and infertile women. Health Psychology. 2012; 1(1): 20-31.

30. Huppelschoten A, Van-Dongen A, Verhaak C, Smeenk J, Kremer J, Nelen W. Differences in quality of life and emotional status between infertile women and their partners. Hum Reprod. 2013; 28(8): 2168-2176.

31. Forsythe S. Social stigma and the medicalisation of infertility. Journal of the Manitoba Anthropology Students’ Association. 2009; 28: 22-36.

32. Greil AL. Infertility and psychological distress: a critical review of the literature. Soc Sci Med. 1997; 45(11): 1679-1707.

33. Bolsoy N, Taspinar A, Kavlak O, Sirin A. Differences in quality of life between infertile women and men in Turkey. J Obstet Gynecol Neonatal Nurs. 2010; 39(2): 191-198.

34. Haghhighatian M, Haghhigh F, Rostami Z. Studying social consequences of infertility on the family in the city of Isfa- han. J Health Syst Res. 2014; 10(2): 354-361.

35. Shakeri J, Hossieni M, Golshani S, Sadeghi Kh, Fizollahy V. Assessment of general health, stress coping and mar- tal satisfaction in infertile women undergoing IVF treat- ment. J Reprod Infertil. 2009; 5(7): 203-275.

36. Monga M, Alexanderuse B, Ekatz SE, Stein M, Ganiats T. Impact of infertility on quality of life, marital adjustment, and sexual function. Urology. 2004; 63(1): 126-130.

37. Lottf Kashani F, Vaziri Sh. The effect of sexual skills train- ing on marital satisfaction. Procedia -Social and Behavio- ral Sciences. 2011; 30: 2581-2585.

38. Litzinger S, Gordon KC. Exploring relationships among communication, sexual satisfaction and marital satisfac- tion. J Sex Marital Ther. 2005; 31(5): 409-425.

39. Ogletree SM. Gender role attitudes and expectations for standard and non standard, male and female responses and different stages of IVF/ET treatment. J Psychosom Res. 1994; 38(3): 229-240.

40. Wendorf CA, Lucas T, Imamoglu EO, Weisfeld C, Weisfeld GE. Marital satisfaction across three cultures: does the number of children have an impact after accounting for other marital demographics. J Cross-Cult Psychol. 2011; 42(3): 340-354.

41. Hirschberger G, Srivastava S, Marsh P, Cowan CP, Cowan PA. Attachment, marital satisfaction, and divorce during the first fifteen years of parenthood. Pers Relatsh. 2009; 16(3): 401-420.
Cord Blood Karyotyping: A Safe and Non-Invasive Method for Postnatal Testing of Assisted Reproductive Technology Children

Shabnam Zarei Moradi, M.Sc., Najmehsadat Masoudi, M.Sc., Anahita Mohseni Meybodi, Ph.D., Khadijeh Anisi Hemaseh, M.Sc., Ramin Mozafari Kermani, M.D., Abolhasan Shahzadeh Fazeli, M.D., Hamid Gourabi, Ph.D.*

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Child Health and Development Research Center, Iran Medical Science Branch of ACECR, Tehran, Iran

Abstract

Background: To verify the hypothesis that the incidence of chromosomal abnormalities increases in babies conceived by different assisted reproduction procedures. The availability of the umbilical cord blood encouraged us to study this hypothesis via this method.

Materials and Methods: This is a descriptive study, umbilical cord blood samples of assisted reproductive technology (ART) children were analyzed with standard cytogenetic techniques (G banding). Karyotyping was possible in 109 cases.

Results: The number of abnormal cases was four (3.7%), among which, three cases (2.8%) were inherited and only 1 case (0.9%) was a de novo translocation. In total, the incidence of de novo chromosomal abnormalities was in the range observed in all live births in the general population (0.7-1%).

Conclusion: No significant difference in the incidence of chromosomal abnormality was found between ART and naturally conceived babies. To date, several studies have examined the medical and developmental outcome of ART children and still have not reached a definite conclusion. Genetic counseling is recommended as an integral part of planning of treatment strategies for couples wishing to undergo ART.

Keywords: Assisted Reproductive Technics, Chromosomal Abnormality, Umbilical Cord Blood, karyotype

Citation: Zarei Moradi Sh, Masoudi N, Mohseni Meybodi A, Anisi Hemaseh Kh, Mozafari Kermani R, Shahzadeh Fazeli A, Gourabi H. Cord blood karyotyping: a safe and non-invasive method for postnatal testing of assisted reproductive technology children. Int J Fertil Steril. 2016; 10(3): 297-302.

Introduction

Assisted reproduction technology (ART) has provided great benefit for millions of couples who struggle with infertility. The definition of ART varies widely, but the US Center for Disease Control and Prevention (CDC) defines it as all fertility treatment in which both eggs and sperms are handled (1). Accordingly, in this study, we defined intrauterine insemination (IUI) babies along with in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) babies as one group. The growing use of ART has dramatically increased the possibility of conceiving babies from infertile couples. Since then, the safety of these methods alongside the associated long-term impacts on the health of children has been a major concern. There are evidences of greater risks of low birth weight, preterm delivery (2, 3), cerebral palsy (4), and major birth defects (5) after ART, although the causes remains unknown. Obviously the genetic problem plays a considerable role in these debates (6, 7). Some researchers have questioned the genetic implications for offspring of couples having ART and...
suggested higher incidences of fetal sex-chromosomal aberrations (8) and de novo chromosomal anomalies (9) after ICSI procedures.

The advent of IVF technique provided a unique opportunity to analyze human pre-implantation embryo (10), moreover, cytogenetic analysis of product of conception can be helpful to determine the cause of the pregnancy loss and brings valuable information in the setting of infertility and assisted reproduction (7). However, advanced maternal age, altered karyotype, multiple assisted reproductive technologies failure, repeated miscarriages and spermatozoa obtained by Simon et al. (11) and Magli et al. (12) are characteristics that expose the couples to an increased risk of generating chromosomally abnormal embryos (6).

The main purpose of this study was to evaluate the risks of chromosomal aberrations in ART offsprings of normal karyotype parents by karyotyping these children using their cord blood. Cord blood is widely usable and easy to access; collection is relatively non-invasive and painless. This means for all newborns conceived through ART procedures, cord blood karyotyping may be performed to ensure their normal chromosomal status. On the other hand, there could be some disadvantages such as maternal blood contamination and missing some genetic conditions. Although there are reports based on peripheral blood samples, no study has been reported on such children using their cord blood.

**Materials and Methods**

This is a descriptive study that was conducted in the Department of Genetics of Royan Institute, Iran, during January 2009 to January 2012. We considered preparation of umbilical cord blood to be a safe method and therefore, karyotyping was conducted on these samples.

From 88 participating infertile couple candidates for ART procedures, 109 umbilical cord blood samples (68 cases had a singleton, 19 cases had twins and 1 case had a triplet birth) were obtained and analyzed. Prior to commencing the study, ethical approval was received from the local institutional Ethics Committee. When pregnancy happens, a written informed consent was obtained from each couple who participated in the study. A genetic counselor visited all pregnant patients and a pedigree was recorded for each of them. Briefly, data on pregnancies and deliveries (information about ectopic pregnancies, miscarriages, preterm births, stillbirths, live births, multiple pregnancies and terminations) were obtained. Additional clinical findings including history of infertility and reported use of ARTs, maternal ages, date of delivery and presence of congenital abnormalities in the members of the family were also recorded.

At the time of delivery, the umbilical cord blood samples were collected during cesarean section in sterile heparinized containers and delivered to genetic laboratory within 2 hours. Because of high risks and emergencies in the field of infertility, all samples were obtained by cesarean section based on the gynecologist preference. General pediatric examinations were performed at birth to identify any apparent anatomical abnormalities of the children. In the cytogenetic lab, a trained technician prepared karyotype slides using the Giemsa (G-banding) technique (500-550 bands per karyotype). It should also be mentioned, a drawback of blood-based G-banding karyotyping is that it can miss extremely subtle chromosome abnormalities that are at the limit of resolution of light microscopy. In brief, approximately 0.5 ml of heparinized whole blood was placed/poured into a glass or plastic tube and inoculated with 10 ml of PB-MAX medium (Gibco, USA). The culture was then incubated at 37°C for 48 hours and thymidine was added at a final concentration of 0.22 μg/ml (0.92 mM) and further incubated for 16 hours in incubator. The culture was subsequently transferred to a centrifuge tube and at 500 xg for 10 minutes. Afterwards, 5 ml of fresh medium without Phytohemagglutinin (PHA) was added and the culture was incubated for an additional 5 hours. Next, the cells were centrifuged as before and washed in fixative for a second time and incubated for 10 minutes. 0.2 μg/ml of KaryoMAX™ Colcemid Solution (Gibco, USA) was added to each culture tube then the culture was incubated for an additional 15 minutes. Afterwards, the culture was transferred to a centrifuge tube and spun at 500 xg for 10 minutes, then the supernatant was removed and the cells were re-suspended in 10 ml of hypotonic 0.075 M KCl (Gibco, USA) and incubated at
37°C for 15 minutes and then spun at 500 x g for 10 minutes. Subsequently the supernatant was removed, the cellular sediment was agitated and 5-10 ml of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol was added drop-by-drop, and left in -20°C for 1 hour then spun at 500 x g for 10 minutes. The cell pellet was re-suspended in a small volume 0.5-1 ml of fresh fixative, dropped onto a clean slide and allowed to air dry. At this stage, the slide could be stained with Orecin or Giemsa. Giemsa banding has become the most widely used technique in cytogenetic analysis, and the most common method to obtain this staining is to treat slides with Trypsin-EDTA 10X (Gibco, USA). At least 15 metaphases were analyzed per baby and in the case of mosaicism or abnormal karyotype, 50 metaphases were analyzed. The chromosomal anomalies were reported in accordance with the current international standard nomenclature (13). From 109 newborns, in nine cases prenatal tests and amniocentesis eliminated the need for cord blood karyotyping.

**Statistical analysis**

This is a descriptive study and the reported rate is within the range reported in the literature. Abnormal karyotype rates were compared by Fisher’s exact test between ART children and P<0.05 was considered statistically significant.

**Results**

The age of females ranged from 26 to 42 years (mean age of 34 years). Table 1 summarizes family histories of all participating couples in this study (consanguinity, history of spontaneous abortions, ART failure, Intrauterine fetal death (IUFD) in each couple, cleft lip/club foot and mental retardation (MR) in 1st or 2nd cousins). As shown in Table 1, failed ART and IUFD were the most and least frequent features (40.4 and 1.83% respectively) observed in the patients and their extended family.

Descriptive characteristics of couples are summarized in Table 2.

About half of infertility factors were male-based (48.9%) and almost 8% were idiopathic infertiles. The most common infertility treatment was ICSI (78.41%), while IVF is the least used method (2%). And finally, the ratio of primary and secondary infertility was 84% and 4%, respectively. The most and least common sperm retrieval methods were masturbation (53.4%) and retrograde method (2.3%) respectively (Table 3).

### Table 1: Medical history of couples participating in this study

| Medical factors                              | n’ | Percentage |
|----------------------------------------------|----|------------|
| Spontaneous abortions                        | 8  | 9.1        |
| Consanguinity                                | 28 | 31.8       |
| ART failure                                  | 44 | 50         |
| IUFD                                         | 2  | 2.3        |
| Cleft lip/club foot in 1st or 2nd cousins    | 5  | 5.7        |
| MR in 1st or 2nd cousins                     | 12 | 13.6       |

ART: Assisted reproductive technology, MR: Mental retardation, IUFD; Intra Uterus Fetal Death, †: Some of the patients showed more than one medical factor, and ‡: Percentage of medical factors among 88 studied couples.

### Table 2: Descriptive characteristics of couples

| Characteristics        | n   | Percentage |
|------------------------|-----|------------|
| Infertility factor     |     |            |
| Female factor          | 24  | 27.2       |
| Male factor            | 43  | 48.9       |
| Male and female factor | 14  | 16         |
| Idiopathic             | 7   | 7.9        |
| Type of infertility    |     |            |
| Primary                | 84  | 95.45      |
| Secondary              | 4   | 4.55       |
| ART method             |     |            |
| IVF                    | 2   | 2.27       |
| ICSI                   | 69  | 78.41      |
| IUI                    | 17  | 19.32      |

ART: Assisted reproductive technology, IVF: In vitro fertilization, ICSI; Intra-cytoplasmic sperm injection, and IUI; Intrauterine insemination.

### Table 3: Type of sperm retrieval and their percentages

| Type of sperm retrieval | Masturbation | Coitus | PESA | TESE | R.G |
|-------------------------|--------------|--------|------|------|-----|
| Number                  | 47           | 27     | 7    | 5    | 2   |
| Percentage              | 53.4         | 30.7   | 7.9  | 5.7  | 2.3 |

PESA; Percutaneous epididymal sperm aspiration, TESE; Testicular sperm extraction, and R.G: Retro grade.
Chromosome analysis was successfully carried out for 109 ART children. As shown in Table 4, for 109 cord blood samples analyzed, the overall rate of abnormality was 3.7% (four cases), and among which, three cases (2.8%) were inherited (one marker chromosome and two inversions) and one case (0.9%) was a de novo chromosome abnormality (structural aberrations). In particular, the inherited cases were a non-identical twin who both showed inversion of chromosome 3 [(46, XX, inv (3) and 46, XY, inv (3)] and a baby who showed a marker chromosome with unknown source, inherited from the mother. In the case of the de novo abnormality, there was a non-identical twin of which the first one was normal (46, XX) and the second one showed translocation between chromosomes 18 and Y [(46, X, t(Y, 18) (q11.2; p11.3)]. The father had a normal karyotype (46, XY) and the baby’s external genitalia was normal. The observed translocation involved Yq11.2 which includes AZF genes, thought to be essential for normal spermatogenesis, thus investigation after puberty was recommended to the parents. Overall, our finding confirmed that there is no significant difference regarding de novo chromosomal abnormality rate in ART children in comparison with naturally conceived babies. Also, ICSI is shown to be applied more in ART babies with chromosomal abnormality.

Discussion

There is a growing belief that ART children are phenotypically and somehow genetically different from naturally conceived children. However, the mechanism(s) leading to these possible changes have not been elucidated and may include parental factors, maternal medications, culture media, as well as egg and embryo manipulation (14). The present study included 109 cord blood samples of pregnancies achieved by IVF, ICSI and IUI and was undertaken to examine whether the rate of chromosomal abnormalities is increased among ART conceived children. In our study, we found 0.9% de novo chromosomal abnormality in ART children. This rate compared to the prevalence of this kind of abnormality among naturally conceived newborns in the general population is within the range of 0.7-1% (15). This demonstrates that ART children do not show a higher cytogenetic risk compared to the natural conceived one or in comparison with data from literature in the normal population (7, 16, 17). There are some studies showing the conflicting conclusions in this area (7, 9, 10, 17). Although the incidence of genetic anomalies are high in countries with the higher rate of consanguineous marriage(18-20), our results do not show any increase in the rate of chromosomal abnormalities in babies conceived by consanguineous couples studied here. Also our results are limited to live-born infants and do not involve stillbirths and aborted fetuses.

Several studies suggest abnormal karyotypes in infertile patients and also meiotic aberrations in their germ cells may be considered as the origin of abnormal karyotypes in ART children. These studies reported that the rate of chromosomal abnormalities in infertile male population has risen above the population baseline and others found a higher incidence of sex chromosome aneuploidy in sperm of men that underwent ICSI (21-23). Ca-
sio supposed that an increased incidence of XY spermatozoa was noted in chromosomally normal infertile males, perhaps, due to testicular mosaicism not detected in peripheral blood (6). In 1989, a European survey showed that IVF, compared with natural conception, does not increase the incidence of abortions due to chromosomal abnormality (24). Some authors postulated that the presence of an unbalanced translocation in some gametes may predispose to pre or post implantation failure of embryo development and as a result, the risk of chromosomal abnormalities of ART treatment may be increased (25-28). Genetic chromosomal abnormalities may arise de novo or derive from a familial anomaly present in one of the parents (29).

Chromosomal aberrations of ART children have been most extensively studied by the Belgian group (9). The limited available data on ICSI fetal karyotypes in comparison with general neonatal population revealed that there is: i. A slight but significant increase in de novo sex chromosome aberrations and structural autosomal abnormalities and ii. An increased number of inherited (mostly from the infertile father) structural aberrations (30-33). A survey has provided data on the frequency of chromosomal anomalies in newborns after ICSI (23) and found two de novo chromosomal abnormalities (3.6%). They presumed that the other live born children were normal because they noticed no typical malformations consistent with chromosomal defects and that is a percentage of 1.2% (2 out of 167). This was compatible with the prevalence of de novo chromosome abnormalities after ICSI reported in Bonduelle’s study (9).

According to some other studies reporting increased risk of imprinting disorders (34, 35) and malignancies (36) in ART children, these kind of studies at least sound an alarm about the genetic alterations of ART offspring and these procedures should thus be used cautiously.

Conclusion

Comparing with some reports, our data showed that children born via ART were not subjected to a higher cytogenetic risk than naturally conceived babies in the general population. However, there is conflicting opinion on this area. Since the number of newborns conceived through ART procedures is growing, reports like this must be considered as a pilot study and prenatal tests must be performed for all pregnancies through ART. In lack of amniocentesis, cord blood karyotyping could be perform immediately after birth to find those aberrations which do not have phenotypic alterations such as sex chromosome aneuploidies. Further investigations by array based techniques and epigenetic tests are undergoing to evaluate possible subtle genetic alterations and different epigenetic modifications in ART conceived children.

Acknowledgements

The authors are thankful to the patients and many colleagues of the clinical, scientific, laboratory and nursing staff of the Royan Institute for participating and/or assisting in this study. This research was financially supported by a grant from the Royan Institute. The authors declare no conflict of interest.

References

1. Sunderam S, Chang J, Flowers L, Kul cardi A, Sentelle G, Geng G, et al. Assisted reproductive technology surveillance—United States, 2006. MMWR Surveill Summ. 2009; 58(5): 1-25.
2. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol. 2004; 103(3): 551-563.
3. Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ. 2004; 328(7434): 261.
4. Strömberg B, Dahlquist G, Ericson A, Finnström Ö, Köster M, Silbernagl K. Neurological sequelae in children born after in-vitro fertilization: a population-based study. Lancet. 2002; 359(9305): 461-465.
5. Hansen M, Bower C, Milne E, De Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects—a systematic review. Hum Reprod. 2005; 20(2): 328-338.
6. Causio F, Fischetto R, Sarcina E, Geusa S, Tartagni M. Chromosomal analysis of spontaneous abortions after in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Eur J Obstet Gynecol Reprod Biol. 2002; 105(1): 44-48.
7. Bettio D, Venci A, Levi Setti PE. Chromosomal abnormalities in miscarriages after different assisted reproduction procedures. Placenta. 2008; 29 Suppl B: 126-128.
8. Bonduelle M, Camous M, De Vos A, Staessen C, Tourmeye H, Van Assche E, et al. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. Hum Reprod. 1999; 14 Suppl 1: 243-264.
9. Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P, et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991-1999) and of 2995 infants born after IVF (1983-1999). Hum Reprod. 2002; 17(3): 671-694.
10. Fauzdar A, Sharma RK, Kumar A, Halder A. A preliminary study on chromosome aneuploidy & mosaicism in early ART Children Karyotyping

Int J Fertil Steril, Vol 10, No 3, Oct- Dec 2016 301
pre-implantation human embryo by fluorescence in situ hybridization. Indian J Med Res. 2008; 128(3): 287-293.

11. Simon C, Rubio C, Vidal F, Gimenez C, Moreno C, Parrilla JJ, et al. Increased chromosomal abnormalities in human pre-implantation embryos after in-vitro fertilization in patient with recurrent miscarriage. Reprod Fertil Dev. 1998; 10(1): 87-92.

12. Magli MC, Gianaroli L, Ferraretti AP. Chromosomal abnormalities in embryos. Mol Cell Endocrinol. 2001; 183 Suppl 1: S29-34.

13. Shaffer LG, Slovak ML, Campbell LJ. ISCN 2009: an international system for human cytogenetic nomenclature. Basel: Karger; 2009.

14. Savage T, Peek J, Hofman PL, Cutfield WS. Childhood outcomes of assisted reproductive technology. Hum Reprod. 2011; 26(9): 2392-2402.

15. Nussbaun RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 7th ed. Philadelphia: Saunders Elsevier Inc; 2007; 99-100.

16. Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. Prenatal testing among women pregnant after assisted reproductive techniques in Denmark 1995-2000: a national cohort study. Hum Reprod. 2008; 23(7): 1545-1552.

17. Belva F, De Schijver F, Tournaye H, Liebaers I, Devroey P, Haentjens P, et al. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod. 2011; 26(7): 1752-1758.

18. Tezel B, Dilli D, Bolat H, Sahman H, Ozbaj S, Accan D, et al. The development and organization of newborn screening programs in Turkey. J Clin Lab Anal. 2014; 28(1): 63-69.

19. Behjati F, Ghaseemi Firouzabadi S, Kahrizi K, Kariminejad R, Bagherizadeh I, Ansari J, et al. Chromosome abnormality rate among Iranian patients with idiopathic mental retardation from consanguineous marriages. Arch Med Sci. 2011; 7(2): 321-325.

20. Mosayebi Z, Movahedian AH. Pattern of congenital malformations in consanguineous versus nonconsanguineous marriages in Kashan, Islamic Republic of Iran. East Mediterr Health J. 2007; 13(4): 868-875.

21. Testart J, Gautier E, Brami C, Rolet F, Sedbon E, Thebault A. Intracytoplasmic sperm injection in infertile patients with structural chromosome abnormalities. Hum Reprod. 1996; 11(6): 2609-2612.

22. Burrello N, Vicari E, Calogero AE. Chromosome abnormalities in spermatozoa of patients with azoospermia and normal somatic karyotype. Cytogenet Genome Res. 2005; 111(3-4): 363-365.

23. Nagvenkar P, Zaveri K, Hinduja I. Comparison of the sperm aneuploidy rate in severe oligozoospermic and oligozoospermic men and its relation to intracytoplasmic sperm injection outcome. Fertil Steril. 2005; 84(4): 925-931.

24. Plachot M. Chromosome analysis of spontaneous abortions after IVF. A European Survey. Hum Reprod. 1989; 4(4): 425-429.

25. Joziwaik EA, Ulug U, Mesut A, Erden HF, Bahceci M. Prenatal karyotypes of fetuses conceived by intracytoplasmic sperm injection. Fertil Steril. 2004; 82(3): 628-633.

26. Feng C, Wang LQ, Dong MY, Huang HF. Assisted reproductive technology may increase clinical mutation detection in male offspring. Fertil Steril. 2008; 90(1): 92-96.

27. Hindruckx A, Peerera K, Debrock S, Legius E, de Zegher F, Francois I, et al. Has the prevalence of congenital abnormalities after intracytoplasmic sperm injection increased? The Leuven data 1994-2000 and a review of the literature. Gynecol Obstet Invest. 2010; 70(1): 11-22.

28. Stern C, Pertile M, Norris H, Hale L, Baker HW. Chromosome translocations in couples with in-vitro fertilization implantation failure. Hum Reprod. 1994; 14(8): 2097-2101.

29. Van Opsal D, Los FJ, Ramlakhan S, Van Hemel JO, Van Den Ouweland AM, Brandenburg H, et al. Determination of the parent of origin in nine cases of prenatally detected chromosome aberrations found after intracytoplasmic sperm injection. Hum Reprod. 1997; 12(4): 682-686.

30. Meschede D, Horst J. Sex chromosomal anomalies in pregnancies conceived through intracytoplasmic sperm injection: a case for genetic counseling. Hum Reprod. 1997; 12(6): 1125-1127.

31. Tarlatzis BC, Grimbizis G. Pregnancy and child outcome after assisted reproduction techniques. Hum Reprod. 1999; 14 Suppl 1: 231-242.

32. Abouighar H, Abouighar M, Mansour R, Serour G, Amin Y, Al-Iriny H. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. Fertil Steril. 2001; 76(2): 249-253.

33. Macas E, Inthurn B, Keller PJ. Increased incidence of numerical chromosome abnormalities in spermatozoa injected into human oocytes by ICSI. Hum Reprod. 2001; 16(1): 115-120.

34. Sutcliffe AG, Peters CJ, Bowdin S, Temple K, Reardon W, Wilson L, et al. Assisted reproductive therapies and imprinting disorders--a preliminary British survey. Hum Reprod. 2006; 21(4): 1009-1011.

35. Odom LN, Segars J. Imprinting disorders and assisted reproductive technology. Curr Opin Endocrinol Diabetes Obes. 2010; 17(6): 517-522.

36. Neelanjana M, Sabaratnam A. Malignant conditions in children born after assisted reproductive technology. Obstet Gynecol Surv. 2008; 63(10): 669-676.
Mitochondrial Genetic Variation in Iranian Infertile Men with Varicocele

Mohammad Mehdi Heidari, Ph.D. 1*, Mehri Khatami, Ph.D. 1, Amirhossein Danafar, M.Sc. 2, Tahere Dianat, M.Sc. 1, Ghazaleh Farahmand, M.Sc. 3, Ali Reza Talebi, Ph.D. 4

1. Department of Biology, Faculty of Science, Yazd University, Yazd, Iran
2. Department of Biology, Ashkezar Islamic Azad University, Ashkezar, Yazd, Iran
3. Department of Biology, Faculty of Science, Islamic Azad University Shahrekord, Shahrekord, Iran
4. Research and Clinical Center for Infertility and Department of Anatomy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstract

Background: Several recent studies have shown that mitochondrial DNA mutations lead to major disabilities and premature death in carriers. More than 150 mutations in human mitochondrial DNA (mtDNA) genes have been associated with a wide spectrum of disorders. Varicocele, one of the causes of infertility in men wherein abnormal inflexion and distension of veins of the pampiniform plexus is observed within spermatic cord, can increase reactive oxygen species (ROS) production in semen and cause oxidative stress and sperm dysfunction in patients. Given that mitochondria are the source of ROS production in cells, the aim of this study was to scan nine mitochondrial genes (MT-COX2, MT-tRNA Lys, MT-ATP8, MT-ATP6, MT-COX3, MT-tRNA Gly, MT-ND3, MT-tRNA Arg and MT-ND4L) for mutations in infertile patients with varicocele.

Materials and Methods: In this cross-sectional study, polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing were used to detect and identify point mutations respectively in 9 mitochondrial genes in 72 infertile men with varicocele and 159 fertile men. In brief, the samples showing altered electrophoretic patterns of DNA in the SSCP gel were sent for DNA sequencing to identify the exact nucleotide variation.

Results: Ten type nucleotide variants were detected exclusively in mitochondrial DNA of infertile men. These include six novel nucleotide changes and four variants previously reported for other disorders.

Conclusion: Mutations in mitochondrial genes may affect respiratory complexes in combination with environmental risk factors. Therefore these nucleotide variants probably lead to impaired ATP synthesis and mitochondrial function ultimately interfering with sperm motility and infertility.

Keywords: Infertility, Varicocele, Mutation, Mitochondrial Genes

Citation: Heidari MM, Khatami M, Danafar A, Dianat T, Farahmand Gh, Talebi AR. Mitochondrial genetic variation in Iranian infertile men with varicocele. Int J Fertil Steril. 2016; 10(3): 303-309.

Introduction

The major concern among married couples when they are unsuccessful to conceive after one year of regular unprotected intercourse is that they may be infertile. Male factors can be attributed to half of these cases (1, 2). The most common surgically reversible cause of infertility is varicocele. Its prevalence is about 4.4-22.6% in the general population, 21-41% in men with primary infertility and 75-80% in men with secondary infertility (3, 4). Despite the advances in molecular medicine, the pathophysiology of varicocele induced infertility remains unknown. Several proposed mechanisms include venous pressure changes and increased testicular temperature due to dilation and tortuosity of the pampiniform plexus of veins, oxidative stress, retrograde flow of
renal or adrenal products, Leydig cell dysfunction and hyperthermia (5, 6). In addition, a number of patients with varicocele have genetic abnormalities like Yq-microdeletions (7). Among them, oxidative stress-induced DNA damage appears to be a more likely cause which may severely affect sperm quality leading to infertility (8). This damage is one of the potential etiological factors in varicocele. A major source of partially reduced derivatives of molecular oxygen \( (O_2^-) \) is mitochondria (9). The variety of reactive oxygen species (ROS) that mitochondria produce principally include hydrogen peroxide \( (H_2O_2) \), superoxide anion \( (O_2^-) \) and the hydroxyl radical \( (OH^-) \) (10, 11). In normal physiology, ROS perform several roles in regulating cellular functions by interacting with cellular components (12). In fertile men, physiological levels of ROS play important roles in sperm function, acrosome reaction, capacitation, hyper-activation and the penetration of oocyte by spermatozoa. However, in varicocele patients ROS generation is abnormally enhanced (13, 14).

Specific point mutations and deletions of mitochondrial DNA (mtDNA) have been associated with poor sperm motility and semen quality in several studies. Sperm mtDNA is highly sensitive to mutations due to increased ROS by-products generated during oxidative respiration (15). When large amounts of mutant mtDNA accumulate in the testes, reduction in ATP production, mitochondrial respiratory dysfunction and meiotic arrest are induced in spermatogonia cells (16). Each mitochondrion has 2-10 mitochondrial genomes responsible for coding the subunits of the OXPHOS complex. The OXPHOS machinery is made up of over 80 different polypeptides, of which the mtDNA encodes 13 polypeptides including complex I, III, IV and V subunits (17).

In this study, for first time, we further analyzed nine genes \( (MT-COX2, MT-tRNA^{Lys}, MT-ATP8, MT-ATP6, MT-COX3, MT-tRNA^{Arg}, MT-ND3, MT-tRNA^{Glu}, MT-ND4L) \) in the mitochondrial genome by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) assay and direct sequencing techniques to identify the possible association between mtDNA variation with varicocele in the Iranian population.

Materials and Methods

Patients

This study was a cross sectional study. Seventy-two Iranian infertile men with clinical varicocele were recruited in the study. The varicocele diagnosis was made by the urologists for the patients by physical examination in standing position and via scrotal palpation in a temperature controlled room (23°C). Semen analysis was performed according to the WHO laboratory manual (18). Patients with varicocele were in 3 grades: i. Grade I \( (n=12) \), ii. Grade II \( (n=27) \) and iii. Grade III \( (n=33) \). The control group (healthy volunteers) consisted of 159 fertile and normospermic men from the Yazd Infertility Center who fathered at least one child. The ethnic and geographical origin of all patients and controls was the same. All participants were fully informed of the objectives of the study and those that signed the consent form were assigned to the study. All infertile men in the age group ranging from 22 to 36 years (mean, 29 years) were referred for evaluation of their infertility (1 year of unprotected intercourse and not leading to conception). The Yazd University Ethics Committee approved recruitment of patients and laboratory protocols in this study.

DNA extraction and mutation analysis

Peripheral blood samples were obtained from varicocele patients and the DNA was extracted using a standard salting-out procedure. Purified DNA samples from leukocytes were used for the PCR reactions. To amplify \( MT-COX2, MT-tRNA^{Lys}, MT-ATP8, MT-ATP6, MT-COX3, MT-tRNA^{Arg}, MT-ND3, MT-tRNA^{Glu}, MT-ND4L \) mitochondrial genes, four pairs of PCR primers were designed, which were located in the flanking regions of each gene (Table 1). Primer Design was based on the human mitochondrial sequence by primer design software (Primer Premier 5.0; Premier Biosoft Inc., Canada), and their secondary structure was examined with Gene Runner version 3.05 (Hastings Software Inc., Hastings, NY, USA, http://www.generunner.com). Each reaction was prepared to a final volume of 25 µl containing 1X MasterMix PCR (Yekta Tajhiz Azma Co., Iran), 0.2 mM of each primer and 0.5 µg DNA template. The PCR conditions were an initial denaturation of 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, the annealing temperature (Table 1) for 30 seconds and extension at 72°C for 30 seconds, which was extended for 5 minutes in the final cycle. The PCR products were electrophoresed on an ethidium bromide-stained 2% agarose gel.
For the SSCP assay, PCR products were heat-denatured at 95 °C for 5 minutes and chilled on ice for 5 minutes, and then loaded onto an 8% non-denaturing polyacrylamide/TBE 0.5x gel. Gels were stained with silver nitrate to reveal the bands of single strand DNA. Various band patterns of the amplified PCR products were marked and scored. The typical gene variants got sequenced using a commercial company (Macrogen, South Korea). All the data obtained from automated sequencing was checked with Sequencher. The online multiple sequence alignment software ClustalW2 (http://www.ebi.ac.uk/tools/msa/clustalw2/) and BLAST analysis were used to determine the nature of mutations and percent homology of the sequences that have been obtained in the study with all other sequences of five other species (chimpanzee, monkey, cattle, zebrafish and drosophila).

Software and databases

We used the tool PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) for prediction of the functional consequences of mutations and damaging effect of missense mutations on protein structure. The sequence alignment was performed using the blastp program available at the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov/Blastp) and the ClustalW program (http://bioinfo.hku.hk/services/analyseq/cgi-bin/clustalw_in.pl). For detection of structural features of mammalian mitochondrial tRNAs and human diseases linked to point mutations in mitochondrial tRNA genes, we used Mamit-tRNA (http://mamit-trna.u-strasbg.fr).

Statistical analysis

The GraphPad Prism software (GraphPad Software, Inc. USA) was used for statistical analysis. Distributions of continuous variables in groups were expressed as mean ± SD, and compared with unpaired Student’s t test. P<0.05 were regarded as statistically significant.

Results

The age difference between the 72 Iranian infertile men with varicocele (mean age of 30.76 ± 6.47) and 159 normal controls (mean age: 28.8 ± 6.01) was not significant (P=0.785). Mutation analyses for the mitochondrial MT-COX2, MT-tRNA\textsubscript{Lys}, MT-ATP8, MT-ATP6, MT-COX3, MT-tRNA\textsubscript{Gly}, MT-ND3, MT-tRNA\textsubscript{Arg} and MT-ND4L genes were carried out in all of patients and healthy controls by PCR-SSCP. Mobility shift of single strand DNA on polyacrylamide gel electrophoresis was the criterion for sequencing and the identification of DNA variation (Fig.1). We found ten different nucleotide substitutions of which 4 caused an amino acid change, of which one occurred in tRNA\textsubscript{Arg}. None of the ten mutations were found in healthy controls. All the mutations identified are summarized in Table 2. In addition, 6 were novel mutations of which four were silent mutations. Four reported polymorphisms, including m.8258T>C, m.9911C>A, m.9932G>A and m.10463T>C were found in six patients. The m.9911C>A variant in MT-COX3 was heteroplasmic. The novel 9 bp heteroplasmic insertion was found in the non-coding MT-NC7 locus in one patient.

Table 1: Primers used for mitochondrial genes

| Segment | Primer sequence (5’-3’) | Primer position | Tm (°C) | Size (bp) | Gene |
|---------|------------------------|-----------------|---------|-----------|------|
| Seg. 1  | F: CTACGGGTCAATGCTCTGAAA R: TAGGTGGTAGTTTGTGTTTA | 8161-8180 | 56.5 | 309 | MT-COX2, MT-tRNA\textsubscript{Lys}, MT-ATP8, MT-ATP6 |
| Seg. 2  | F: AGCCCACTTCTCACCACAAG R: TACTATATGATAGGCATGTA | 8901-8920 | 56 | 338 | MT-ATP6 |
| Seg. 3  | F: CACTATCGCTTCATCCGCCC R: ATGTAGCCGTTGAGTTGTTGA | 9851-9870 | 57 | | MT-COX3, MT-ND3 |
| Seg. 4  | F: TCTGGGCTATGAGTGACTAC R: AGTAATATTCCCTCAGGGCA | 10361-10380 | 57 | 221 | MT-ND4L, MT-tRNA\textsubscript{Arg} |

Tm; Temperature melting.
The m.9911C>A mutation, an aromatic amino acid phenylalanine codon (TTC), changes to leucine codon (TTA), a hydrophobic amino acid at position 235 (designated F235L) in 1 patient (Fig.2) and the novel 9929C>A mutation changes a polar tyrosine to threonine.

Two novel mutations were detected in 5 patients with one (9929C>A) being a nonsense mutation and changes tyrosine to stop codon (Y241X) and the other (10141C>G) being a missense mutation that changes Asparagine to Lysine (N27K). Also, three synonymous polymorphisms were found that were not reported previously (Table 2). The other variation was the m.10463T>C substitution (homoplasmic state) in the tRNA<sup>Arg</sup> gene that was found in 3 patients.

![Fig.1: Silver staining SSCP analysis of fragment 3. A. Polyacrylamide gel electrophoresis. Lanes 1, 3 and 5 show 3 patients who did not have mutations, B. Lane 6 shows a patient with the m.9929 C>A mutation, C. Lanes 2, 4 and 8 show 2 patients with the m.10141A>C mutation, and, D. Lane 7 shows a patient with m.9911C>A and lanes 9, 10 and 11 are men without varicocele.](image)

![Fig.2: Protein alignment of m.9911C>A missense mutation MT-COX3 and the arrow indicate the site of the F235L mutation.](image)
Discussion

One of the most frequent causes of male infertility is varicocele, however, the pathogenic mechanisms by which it leads to changes in spermatogenesis are not clear (25). Some of these mechanisms may be related to mutations in mitochondrial complexes that affect flagellar movement and cause sperm dysmotility.

DNA alterations including point mutations and deletions of mtDNA have been reported in infertile patients with low sperm motility (26). The effect of mtDNA mutations on male infertility has also been studied. Shamsi et al. (27) reported that generation of ROS and mtDNA mutations are associated with pathogenic molecular mechanisms. Agarwal et al. (28) showed an increased oxidative stress in varicocele patients. Thangaraj et al. (29) demonstrated that sperm mitochondrial mutations is one of the causes of low sperm motility which is strongly dependent on ATP biosynthesis which is carried out by the mitochondrial OXPHOS system. Furthermore, it has been demonstrated that cells with some base substitutions in mtDNA can greatly influence semen quality (9, 30, 31).

It has been established that mitochondrial dysfunction caused by mtDNA mutations and oxidative damages is one of the important reasons for most types of infertility such as Varicocele (32). The mtDNAs alterations may accumulate in the spermatids or during gametogenesis and thereby impair the respiratory function and motility of spermatozoa (33).

We observed three heteroplasmic variations in 4 patients. A nine base pair heteroplasmic insertion in the non-coding MT-NC7 locus were found in 1 patient. Although this insertion (5’-CCCCCTCTA-3’) has been found in a non-coding region, it may cause mitochondrial rearrangements and DNA strand break affected by topoisomerases or DNA recombinase (34).

The heteroplasmic m.9911C>A and m.9929C>A transversions in MT-COX3 alter two conserved codons. Given that these variants change highly conserved amino acids and were not identified in normal controls, they may be considered as pathogenic mutations for the following reasons. First, these missense mutations are found in several patients. Second, these mutations are not reported as polymorphisms in the general population and are not detected in the control individuals from the same ethnic background. Third, the mutations are heteroplasmic in the lymphocyte cells. Fourth, we propose that these mutations may affect the polarity of the protein due to the replacement of a natural amino acid with a polar amino acid. Using PolyPhen2, we found that these mutations are expected to change protein function.

Here, we describe seven homoplasmic variants in 25 patients: two missense mutations in MT-COX2 (8258T>C) and MT-ND3 (10141C>A), 4 synonymous polymorphisms in MT-COX3, MT-ATPase6, MT-ND3 and MT-ND4L and one mutation (10463T>C) in MT-tRNA Arg. This mutation is located at a moderate conserved region of the acceptor stem of tRNA arginine. This mutation was

| Locus       | Position | Nucleotide change | Amino acid position | No. of individuals | Hetero/Homo | Previously reported |
|-------------|----------|-------------------|---------------------|--------------------|-------------|-------------------|
| MT-COX2     | 8258     | T→C               | F225L               | 1                  | Homo        | Yes (19)          |
| MT-NC7      | Ins8288  | 9 bp              | Non-coding          | 1                  | Hetero      | No                |
| MT-COX3     | 9911     | C→A               | F235L               | 1                  | Hetero      | Yes (20)          |
| MT-COX3     | 9929     | C→A               | Y241X               | 2                  | Hetero      | No                |
| MT-ATP6     | 9063     | A→G               | L179L               | 1                  | Homo        | No                |
| MT-ND3      | 10103    | A→G               | L15L                | 1                  | Homo        | No                |
| MT-TR       | 10141    | C→A               | N27K                | 3                  | Homo        | No                |
| MT-ND3      | 10463    | T→C               | tRNA Arg            | 6                  | Homo        | Yes (23, 24)      |
| MT-ND4L     | 10550    | A→G               | M27M                | 12                 | Homo        | No                |
not observed in healthy control subjects but was previously reported as a polymorphism in mitochondrial encephalomyopathy (35) and may be one of the several predisposing factors for varicocele.

**Conclusion**

Because sperms require an optimal energy to reach the oviduct during fertilization, the appropriate bioenergetic function of mitochondria is critical for male infertility. Therefore, any changes in mitochondrial genome can cause improper functioning of respiratory chain that in combination with environmental risk factors lead to infertility in men. This first Iranian study revealed that some Iranian infertile men carry variants in the nine mitochondrial genes and suggests that variants in these genes may be associated with varicocele.

**Acknowledgements**

This work was financially supported by Yazd University. We thank all the patients for providing blood samples for this study. The authors hereby declare that there is no conflict of interests.

**References**

1. Raman JD, Walmsey K, Goldstein M. Inheritance of varicoceles. Urology. 2005; 65(6): 1186-1189.
2. Evans CJ, Prowkes FG, Hajivassiliou CA, Harper DR, Ruckley CV. Epidemiology of varicose veins. A review. Int Angiol. 1994; 13(3): 263-720.
3. Canales BK, Zapzalka DM, Ercole CJ, Carey P, Haus E, Appell D, et al. Prevalence and effect of varicoceles in an elderly population. Urology. 2005; 66(3): 627-631.
4. Niederberger C. Inheritance of varicoceles. J Urol. 2005; 174(5): 1942.
5. Benoff S, Gilbert BR. Varicocele and male infertility: part I. Preface. Hum Reprod Update. 2001; 7(1): 47-54.
6. Talebi AR, Moein MR, Tabibnejad N, Ghasemzadeh J. Effect of varicocele on chromatin condensation and DNA integrity of ejaculated spermatozoa using cytchemical tests. Andrologia. 2006; 40(4): 245-251.
7. Güney AI, Javadova D, Kirac D, Ulucan K, Koc G, Ergec C, et al. Detection of Y chromosome microdeletions and mitochondrial DNA mutations in male infertility patients. Hum Reprod. 2004; 19(3): 616-627.
8. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male infertility: an update. Am J Reprod Immunol. 2008; 59(1): 2-11.
9. Baklouti-Gargouri S, Ghorbel M, Ben Mahmoud A, Mkaouar-Rebai E, Cherif M, Chakroun N, et al. Mitochondrial DNA mutations and polymorphisms in asthenospermic infertile men. Mol Biol Rep. 2013; 40(8): 4705-4712.
10. Tuppen HA, Blakeley EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutations and human disease. Biochim Biophys Acta. 2010; 1797(2): 113-128.
11. Achilli A, Rengo C, Magri C, Battaglia V, Olivieri A, Scozzari R, et al. The molecular dissection of mtDNA haplogroup H confirms that the Franco-Cantabrian glacial refuge was a major source for the European gene pool. Am J Hum Genet. 2004; 75(5): 910-918.
12. Tang S, Wang J, Zhang W, Li F, Landsverk M, Cui H, et al. Transition to next generation analysis of the whole mitochondrial genome: a summary of molecular defects. Hum Mutat. 2013; 34(6): 882-893.
13. Ozawa T, Tanaka M, Ino H, Ohno K, Sano T, Wada Y, et al. Distinct clustering of point mutations in mitochondrial DNA among patients with mitochondrial encephalomyopathies and with Parkinson’s disease. Biochim Biophys Res Commun. 1991; 176(2): 938-946.
14. Yeh JJ, Lunetta KL, van Oorsouw NJ, Moore FD Jr, Mutter GL, Vigg J, et al. Somatic mitochondrial DNA (mtDNA) mutations in papillary thyroid carcinomas and differential mtDNA sequence variants in cases with thyroid tumours. Oncogene. 2000; 19(16): 2060-2066.
15. Houshmand M, Larsson NG, Holme E, Oldfors A, Tulinius H, Andersson O. Automatic sequencing of mitochondrial tRNA genes in patients with mitochondrial encephalomyopathy. Biochim Biophys Acta. 1994; 1226(1): 49-55.
16. Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey D. Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. Genetics. 1995; 140(1): 285-302.
17. Dada R, Shamsi MB, Venkatesh S, Gupta NP, Kumar R. Attenuation of oxidative stress & DNA damage in varicocele-ectomized: implications in infertility management. Indian J Med Res. 2010; 132: 680-730.
18. Benoff S, Marmar JL, Hurley JR. Molecular and other predictors for infertility in patients with varicoceles. Front Biosci (Landmark Ed). 2009; 14: 3641-3672.
19. Shamsi MB, Kumar R, Bhatt A, Bamezai RN, Kumar R, Gupta NP, et al. Mitochondrial DNA mutations in idiopathic male infertility. Indian J Urol. 2008; 24(2): 150-154.
rez JG, Sikka SC. Reactive oxygen species as an independent marker of male factor infertility. Fertil Steril. 2006; 86(4): 878-885.
29. Thangaraj K, Joshi MB, Reddy AG, Rasalkar AA, Singh L. Sperm mitochondrial mutations as a cause of low sperm motility. J Androl. 2003; 24(3): 388-392.
30. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update. 2003; 9(4): 331-345.
31. Schoor RA, Elhanbly SM, Niederberger C. The pathophysiology of varicocele-associated male infertility. Curr Urol Rep. 2001; 2(6): 432-436.
32. Palanichamy MG, Zhang YP. Identifying potential pitfalls in interpreting mitochondrial DNA mutations of male infertility cases. Indian J Med Res. 2011; 134: 447-451.
33. Venkatesh S, Deecaraman M, Kumar R, Shamsi MB, Dada R. Role of reactive oxygen species in the pathogenesis of mitochondrial DNA (mtDNA) mutations in male infertility. Indian J Med Res. 2009; 129(2): 127-137.
34. Fritsch ES, Chabbert CD, Klaus B, Steinmetz LM. A genome-wide map of mitochondrial DNA recombination in yeast. Genetics. 2014; 198(2): 755-771.
35. Uusimaa J, Finnilä S, Remes AM, Rantala H, Vainionpää L, Hassinen IE, et al. Molecular epidemiology of childhood mitochondrial encephalomyopathies in a Finnish population: sequence analysis of entire mtDNA of 17 children reveals heteroplasmic mutations in tRNAArg, tRNAGlu, and tRNALeu(UUR) genes. Pediatrics. 2004; 114(2): 443-450.
Expression Profile of Developmentally Important Genes in pre- and peri-Implantation Goat Embryos Produced In Vitro

Pouria HosseinNia, Ph.D.,*1, 2 #, Mehdi Hajian, M.Sc.2 #, Mojtaba Tahmoorespur, Ph.D.1, Sayyed Morteza Hosseini, Ph.D.2, Somayyeh Ostadhosseini, D.V.M.2, Mohammad Reza Nasiri, Ph.D.1, Mohammad Hossein Nasr-Esfahani, Ph.D.2 *

1. Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Abstract

Background: Little is understood about the regulation of gene expression during early goat embryo development. This study investigated the expression profile of 19 genes, known to be critical for early embryo development in mouse and human, at five different stages of goat in vitro embryo development (oocyte, 8-16 cell, morula, day-7 blastocyst, and day 14 blastocyst).

Materials and Methods: In this experimental study, stage-specific profiling using real-time quantitative polymerase chain reaction (RT-qPCR) revealed robust and dynamic patterns of stage-specific gene activity that fall into four major clusters depending on their respective mRNA profiles.

Results: The gradual pattern of reduction in the maternally stored transcripts without renewal thereafter (cluster-1: Lifr1, Bmpr1, Alk4, Id3, Ctnnb, Akt, Oct4, Rex1, Erk1, Smad1 and 5) implies that their protein products are essential during early cleavages when the goat embryo is silent and reliant to the maternal legacy of mRNA. The potential importance of transcription augment at day-3 (cluster-2: Fzd, c-Myc, Cdc25a, Sox2) or day-14 (cluster-3: Fgfr4, Nanog) suggests that they are nascent embryonic mRNAs which intimately involved in the overriding of MET or regulation of blastocyst formation, respectively. The observation of two expression peaks at both day-3 and day-14 (cluster-4: Gata4, Cdx2) would imply their potential importance during these two critical stages of pre- and peri-implantation development.

Conclusion: Evolutionary comparison revealed that the selected subset of genes has been rewired in goat and human/goat similarity is greater than the mouse/goat or bovine/goat similarities. The developed profiles provide a resource for comprehensive understanding of goat preimplantation development and pluripotent stem cell engineering as well.

Keywords: Goat, Developmental Stage, Gene Expression, Preimplantation

Introduction

Mammalian preimplantation embryonic development encompasses the period from fertilization to implantation. During this period, the embryonic stages and critical developmental events assessed are transition from germinal vesicle stage (GV) to metaphase-II (MII) oocyte, maternal-to-embryonic transition (MET), and the first lineage dif-
Differentiation to the inner cell mass (ICM) and trophectoderm (TE) during blastocyst formation (1). Notably, implantation in ungulates, unlike human and mice, occurs with a delay of around 7 days. During this “peri-implantation” period, the rapid development of TE dramatically alters the blastocyst morphology from a sphere to a day 14 hatched blastocyst (2).

An improved understanding of gene activity that regulates preimplantation development is crucially important for assisted reproduction techniques and for derivation of embryonic stem cells (3). This goal has been largely achieved in mouse and human (4, 5). For example, it has been shown that embryonic developmental program is regulated by intricate cooperation of several important genes in the context of cell-signaling pathways. It was initially presumed that the developmental genes regulating early embryonic events are conserved across all mammalian species. Researchers attempted to extrapolate mice and human knowledge databases to the embryonic development of other species as well. However, further comparative studies revealed that species-specific differences exist between gene regulatory networks regulating embryo development in mammals (3, 5-7), which will provide a roadmap for differentiating definitive species-specific differences.

The goat is a valuable livestock animal with promising importance in agriculture, biomedicine and transgenesis (8, 9). However, the molecular basis of goat early embryonic development is poorly understood. Yan et al. (10) for the first time demonstrated that the expression of Oct4 and Nanog proteins were not restricted to the ICM of goat blastocysts. To date, four studies have reported derivation of goat “putative” embryonic stem cells (ESCs) from embryos produced either in vivo or in vitro (11-14). However, chimera production and germ line transmission of ESC have yet remained to be established in the goat (15).

An improved understanding of expression profiles of developmentally important genes in pre- and peri-implantation goat embryos would improve current attempts to establish ESC in this valuable farm species. Therefore, this study for the time investigated the expression profile of 19 genes, known to be critical for early embryo development in mouse and human, at five different stages of goat in vitro embryo development (oocyte, 8-16 cell, morula, day-7 and 14 blastocysts).

**Materials and Methods**

In this experimental study, unless otherwise stated, all chemicals and media were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Gibco (Grand Island, NY, USA), respectively.

**Selection of genes set**

Nineteen candidate genes for the investigation were selected from the human and mouse data bases if i. They were only present in ESCs and either in the oocyte or blastocyst and ii. Their gene ontology applications indicated a critical role in transcription regulation, pluripotency and differentiation. This gene set included Lifr1, Bmpr1, Alk4, Id3, Ctnnb, Akt, Oct4, Rex1, Smad1, 5, Fzd, c-Myc, Cdc25a, Sox2, Fgfr4, Nanog, Erk1, Gata4, and Cdx2. Since sequence data of some genes was not available in the goat, we designed specific primers based on ortholog conserved regions in other studies. The registered cDNA for Erk1, Alk4, Bmpr1, Fgfr4 and Lifr1 were deposited into NCBI database under accession numbers KC687077, KF039752, KF039753, KF039754, and KF356183, respectively). Sequences and characteristics of successful polymerase chain reaction (PCR) primers can be found in Table 1.

**In vitro production of goat embryos**

The procedure used for in vitro production of goat embryos was as described previously (16). In brief, cumulus-oocyte complexes (COCs) were obtained from abattoir-derived goat ovaries. COCs were cultured in maturation medium comprised of tissue culture medium-199 (TCM199) supplemented with 10% fetal calf serum (FCS), 2.5 mM sodium pyruvate, 100 IU/mL penicillin, 100 µg/mL streptomycin, 10 µg/mL follicle-stimulating hormone (FSH), 10 µg/mL luteinizing hormone (LH), 1 µg/mL estradiol-17β, and 0.1 mM cysteamine under mineral oil for 20-22 hours at 39°C, 6% CO₂, and maximum humidity. Matured oocytes were used for in vitro fertilization (IVF) and presumptive zygotes were cultured in groups...
of six in 20 µl droplets of modified formulation of synthetic oviductal fluid (mSOF) at 39°C, 6% CO₂, 5% O₂, and maximum humidity (16, 17).

For real time-quantitative PCR (RT-qPCR) experiments, oocytes and embryos at five different stages of goat in vitro embryo development (MII-oocyte, 8-16 cell, morula, expanded blastocyst, and day 14 blastocyst) were used. MII oocytes were collected at 20-22 hours post maturation. The 8-16-cell embryos, expanded blastocysts and day 14 blastocysts were collected during different days post embryo culture (days 3, 7 and 14 post embryo culture). Therefore, variation effect was removed from samples. After through washing in phosphate buffer saline (PBS), oocytes and embryos in pools of 60 (oocyte), 35-40 (D3), and 20 (D7) were collected in 500 µL microtubes containing 75 µl RLT buffer, frozen and stored at -70°C until RNA extraction.

Derivation of in vitro D14 embryos

For extended in vitro culture of goat day-7 blastocysts until day-14, we prepared a co-culture system using a feeder layer of caprine fetal fibroblasts (CFF) as described by Behboodi et al. (12). Accordingly, CFF cell-line was prepared using fetal tissues of three 40-day goat fetuses. Single-cell suspension was prepared by mincing fetal tissues and culturing them in Dulbecco’s modified eagle medium (DMEM) supplemented with 10% FCS, 0.25% amphotericin-B, 1% penicillin-streptomycin, 1% gentamycin in 25 cm² culture flasks and cultured at 37°C, 6% CO₂. Confluent monolayer was appeared from day 4 of culture onwards. The monolayer was trypsinized and passaged for proliferation of CFF cell-line. Each round of cell proliferation took around 3-4 days. The CFF cell-line at passages 2-to-4 was treated with mitomycin (10 mg/mL) for 2 hours. Treated cells were seeded at 1×10⁵ cells/mL in drops of 100 µl DMEM which was placed in close proximity to a feeder-free 100µl droplet of DMEM supplemented with 10% FCS, 1% L-glutamine, 1% non-essential amino acid, and 0.1% β-mercaptoethanol under mineral oil. Five to six D7-blastocysts were transferred to each 100 µl droplet of feeder-free DMEM. Then, the DMEM drops containing blastocysts were gently connected to their adjacent DMEM containing CFF monolayer using a mouse pipette. This culture system provided beneficial effects of feeder layer for extended in vitro embryo culture while preventing attachment the day 14 blastocysts to the feeder layer (Fig.1). The culture medium was refreshed every other day until D14 of embryo development. Then, groups of 7-10 well-developed D14 embryos were pooled for RNA extraction as described above. A range of 50-65% of the developed blastocysts could progress to day 14.

RNA extraction and real time-quantitative polymerase chaine reaction

The procedure for RT-qPCR was as described previously (18). In brief, total RNA of oocytes and embryos was extracted suing RNeasy Micro kit (Qiagen, Mississauga, ON, Canada) followed by treatment with DNase I (Ambion, Streetsville, ON, Canada) according to the manufacturer’s protocol. The RNA quality and quantity was determined using WPA Biowave spectrophotometer (Cambridge, United Kingdom). For reverse transcription, 10 µl of total RNA was used in a final volume of 20 µl reaction containing 1 µl of Random Hexamer, 4 µl RT buffer (10 x), 2 µl of dNTP, 1 µl of RNase inhibitor (20 IU), and 1µl of reverse transcriptase (Fermentas, Glen Burnie, Ontario, Canada). Reverse transcription was carried out at 25°C for 10 minutes, 42°C for 1 hour and 70°C for 10 minutes.

The selection of appropriate reference gene is of crucial importance in the accuracy and fidelity of the data of RT-qPCR results. Accordingly, we searched the literature to find the best suitable candidate reference gene for the goat. We observed that in almost infield studies, ACTB has been used as the choice reference gene in several similar studies in the goat (19), bovine (3, 9, 20). Moreover, ACTB was selected as a suitable internal control for study of gene expression in cryopreserved egg and embryo because it efficiently withstands cryoshocks and oocyte manipulation (9, 17, 21). After ascertaining that the expression of ACTB was stable among different development stages of embryos (data not shown), relative quantification of the target genes was undertaken with ACTB as the reference gene. For RT-qPCR, total RNAs of oocytes and embryos were extracted and used for cDNA
RT-qPCR was carried out using 1 µl of cDNA (50 ng), 5 µl of the SYBR Green/0.2 µl ROX qPCR Master Mix (2X) (Fermentas, Germany) and 1 µl of forward and reverse primers (5 pM) adjusted to a total volume of 10 µl using water nuclease-free. Three technical replicates of RT-qPCR were conducted for each primer. CT samples of each target gene were normalized to the CT of ACTB and represented as 2^ΔΔCT (22).

The primer sequences, annealing temperatures and the size of amplified products are shown in Table 1.

### Table 1: Specific real-time primers were designed for gene sequences

| Gene | Primer sequences (5’-3’) | Length of PCR product | TM |
|------|--------------------------|-----------------------|-----|
| Lifr1 | F: ATTTTTCGGTGTAATGGGTGC  
R: CAGATGATTCCTCAAACGGTA | 117 | 56 |
| Bmpr1 | F: CCTTGTCGGTGTTCTCAT  
R: CTGTTGCTAAAGTTACTCC | 116 | 58 |
| Alk4 | F: TCTCCAAGGACAGACGCTC  
R: ACGCCACACTTCTCAAACC | 152 | 62 |
| Smad1 | F: TCACACCTCTGGCTCCTCT  
R: AAACCTGCACTTCAAACGG | 140 | 60 |
| Smad5 | F: ACAGCAACCTCTGGTCTTC  
R: GGGGTTAGGGACTATTTTGG | 136 | 60 |
| Id3 | F: CCGTCTAGGGAACCTGTA  
R: CCTTTGCTGTGGAGAGTG | 198 | 58 |
| Ctnnb | F: AGTTGGTGCGCATAGAGG  
R: CACAGGAGCCCGTAG | 160 | 54 |
| Akt | F: TTCAGCAGCATTGGTGGA  
R: TCATCAAAATACCTGGTCCG | 98 | 60 |
| Oct4 | F: GCCAGAAAGGCAAACGAT  
R: GAGGAAGGAGATACGGGTC | 96 | 56 |
| Rex1 | F: GCAGCGAGCCTTCAGTACAC  
R: ACAACAGCTCATCCTCGG | 94 | 61 |
| Fzd | F: CATCGGCACCTCTTTATCC  
R: GCTTTGCTGTTTCTCCC | 89 | 59 |
| c-Myc | F: CAACACCCAGGACACC  
R: GCCGTATTTTTCACTACC | 160 | 61 |
| Sox2 | F: ATGGGCTGGTTGGA  
R: CTCTGGTAGTGCTGGA | 182 | 54 |
| Fgfr4 | F: GCTGACTGGTAGGGAAGG  
R: AGTGGCTGAAGCACATCG | 193 | 56 |
| Nanog | F: GATCTTGCCAAGCCCT  
R: TCAATGAGCACAACACC | 137 | 54 |
| Erk1 | F: TCAAGCGCTCCAAATCTCAT  
R: CGACACGCATATCAACC | 204 | 58 |
| Gata4 | F: TCCCCTTCGGGTAGGCATG  
R: GTGTCCAGGTGGCTGGGTG | 128 | 64 |
| Cdx2 | F: CCCCAAGTGAAAACAC  
R: TGAGCATGACACATTAG | 144 | 53 |
| Cdc25a | F: TGCCAGGCGGTATCGTGTG  
R: GGTAGTGGATGTTGGGTA | 119 | 58 |
| Actb | F: CCACTGGCAATAGGCGGT  
R: CGTGTGCTGGAGGTC | 146 | 60 |

PCR; Polymerase chain reaction and TM; Melting temperature.
Statistical analysis

Statistical analysis were carried out using SPSS software. For the analysis of developmental data and real-time PCR data a two-tailed Students t test with equal variance was used to determine significance data. Statistical significance was accepted at P<0.05.

Results

All the 19 genes examined were expressed throughout embryo development, from MII-oocyte to D14 developing blastocysts (23, 24). Even though, the levels of expression of all genes varied during different developmental stages as no gene was found to be stably expressed throughout embryo development. Moreover, different genes had different levels of expression with respect to a certain stage of development. Stage profiling revealed robust and dynamic patterns of stage-specific gene activity that fall into four major clusters depending on the respective mRNA profile (Figs.2-6).

Fig.1. Extended in vitro culture system for goat embryos. A. The modified system for culture of expanded goat embryos in medium conditioned by feeder cells and B. Comparison between D7 expanded blastocyst (arrow) with D14 blastocysts developed in the modified culture system.

Fig.2. Schematic design for classification of profiles of gene expressions.
The first gene cluster (Figs. 2, 3) exhibited highest levels of mRNA in MII-oocyte which gradually and consistently decreased during subsequent stages of embryo development. This cluster encompassed 11 genes including Lifr1, Bmpr1, Alk4, Smad1, Smad5, Id3, Ctnnb, Akt, Oct4, Erk1 and Rex1. The speed and extent of the stepwise decreases in the transcripts varied between the genes.
The second gene cluster (Figs. 2, 4) showed low levels of the transcripts in MII-oocytes, reached their highest relative mRNA levels in D3 embryos and significantly decreased thereafter with no sign of regain in transcription in D7 and D14 blastocysts. This gene set encompassed 4 genes including Fzd, Sox2, c-Myc, and Cdc25a which showed 14-, 22-, 60-, and 3- fold increase in their D3 transcripts compared to MII-oocytes.

In the third gene cluster (Figs.2, 5), the original levels of transcripts of MII-oocyte gradually decreased in developing embryos with a significant reduction in D7 blastocysts. However, these genes initiated transcription from D7 onwards which resulted in a peak of expression in D14 blastocysts. This gene set composed of 2 genes, Gata4 and Cdx2. Despite similar pattern of expression, the magnitude of D14 gain in transcription was different between Gata4 and Cdx2 (4- and 2-fold, respectively) compared to the relative mRNA levels initially found in MII-oocytes.

The fourth gene cluster (Figs.2, 6), showed low levels of the transcripts in MII-oocytes, reached their first the peak of expression in D3 embryos and followed by a significant decrease in transcription in D7 blastocysts. However, this group of genes reinitiated transcription from D7 onwards which resulted in the second peak of expression in D14 blastocysts. This gene set encompassed 2 genes including Fgfr4 and Nanog.

**Fig.4.** Profiles of expression of genes categorized in the second class based on Figure 2. a, b, and c; Significant difference at P<0.05%.

**Fig.5.** Profiles of expression of genes categorized in the third class based on Figure 2. a, b, and c; Significant difference at P<0.05%.
Discussion

This study demonstrated that all developmental genes assessed are present throughout the pre and peri implantation stages of goat in vitro embryo development. Even though, none of these genes could exhibit significantly stable and ubiquitous expression patterns throughout these five developmental stages. Instead, all genes showed fluctuations in expression levels, and if we exclude Oct4 and Rex1, the source of the greatest variations in relative mRNA expression was between MII-oocyte and D3 stages of embryo development. To better explain the quantitative results, schematic patterns of gene expression were categorized in the context of four groups based on the actual patterns of gene expression observed.

The first set of genes, which importantly comprised two-third of the examined genes, revealed a consistent trend of gradual mRNA reduction as the highest and lowest levels of transcripts were observed in MII-oocyte and D14 blastocysts, respectively. This may suggest that the transcripts of these set of genes have been transcribed and accumulated during oocyte growth phase. Because MII-oocyte and early embryo are considered transcriptionally silent (25) and since there is no evidence of active transcription during meiosis resumption, these maternal transcripts should be produced during earlier stages of oocyte growth preceding germinal vesicle breakdown stage.

Theoretically, the steady state of mRNA reduction without renewal (gene cluster 1) may suggest the potential importance of these mRNA for production of proteins that are required during early stages of embryo development, especially to support maternal embryonic transition (MET), when the goat embryos are self-reliant in their transcription. Mechanistically, the distinctive processes have been associated with the declines of maternal mRNA in the eukaryotes including deadenylation, degradation, and protein translation or synthesis (26). Moreover, it has been suggested that the oocyte unlikely would keep useless products (27). Therefore, the quick reduction in relative mRNA abundance could be associated with the protein production.

The second group of genes revealed a significant elevation in their transcripts at D3 compared to MII-oocyte. But, this elevation in the transcripts did not continue and gradually declined and reached the lowest levels in D14 blastocysts. The potential importance of D3 burst-in-the-transcription is indicative of their crucial importance at the MET stage. Accordingly, the lower abundances of these transcripts during post-MET period may not play an important role in the regulation of blastocyst formation and further stages.

The third group of genes revealed a gradual decrease in the maternal mRNA abundances similar to the first group, but their transcripts increased from D7 and reached their highest levels at D14. This would imply that this set of genes is of critical importance during pre- and post- MET phases and underscores the facts that: i. The exact time windows that the second and third sets of genes are in demand for the embryo development are different, and ii. The maternal stockpiles of the third, but not second, set of genes are quite enough to support MET without any need for the additional source of embryo-specific transcripts.

The fourth group of genes was those showing two peaks in embryo-specific mRNA transcription at two distinctive time points of MET and day 14 blastocysts. This may suggest that these transcripts
are crucially needed during both stages. This group can be considered as the combined model of the second and third groups, and correspondingly, the genes in this final group may be of theoretical alternative capacity to cover the duties of genes in both second and third groups.

One of the shortcomings of this study was usage of in vitro derived embryos while in vivo -derived embryos provide the best source of samples for gene expression studies. However, this was not possible due to technical limitations. A wide range of infield studies have used in vitro -derived embryos for similar analyses in the goat (9), equine (28), and bovine (3, 20). In ungulates, embryos are migrating within the uterus for about 7 days before implantation (12). This delay in implantation has provided a unique opportunity to extended in vitro culture of ungulates (28-30). At this stage, we expected to see day 14 blastocysts but a search in filed studies revealed that at this time window, embryos look similar to the blastocysts observed on day 14 in this study. For better clarifying this issue and also confirm the quality of these embryos to continue to development, we had previously transferred a number of in vitro derived day-7 blastocysts into uterine horn of synchronized goat recipients as routine. The transferred embryos were then flushed from the uteruses at day 20-21. Surprisingly enough, we observed that the embryos at day 21 start to form ovoid or tubular like structures with sizes ranged 0.5 to 5 mm in diameter. One may suggest that the elongation process of goat embryos possibly begins on day 14 onwards.

Conclusion

The results obtained through this work highlight the fact that transcription factors involved in the regulation of early events of pluripotency and differentiation are present through pre- and peri-implantation in the goat embryos. Since the blastocysts of ungulates, unlike human and mice, implant with a delay of around 7 days, the obtained results in D7 and 14 blastocysts may provide useful information to figure out the expression profiles of developmentally important genes between these two stages. The profiles obtained may be useful for derivation of ESC in this valuable farm species.

Acknowledgements

This study was performed by the grant from Royan Institute. Authors would like to thank Dr. Farnoosh Jafarpour and Nima TanhaieVash for technical helps. There is no conflict of interest in this study.

References

1. Adjaye J, Huntriss J, Henwig R, BenKahia A, Brink TC, Wierling C, et al. Primary differentiation in the human blastocyst: comparative molecular portraits of inner cell mass and trophectoderm cells. Stem Cells. 2005; 23(1): 1514-1525.
2. Cauffman G, De Rycke M, Sermon K, Liebaers I, Van de Velde H. Markers that define stemness in ESC are unable to identify the totipotent cells in human preimplantation embryos. Hum Reprod. 2009; 24(1): 63-70.
3. Berg DK, Smith CS, Pearson DJ, Wells DN, Broadhurst R, Donnison M, et al. Trophectoderm lineage determination in cattle. Dev Cell. 2011; 20(2): 244-255.
4. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282(5391): 1145-1147.
5. Xie D, Chen CC, Plaszek LM, Xiao S, Cao X, Fang F, et al. Rewritable gene regulatory networks in the preimplantation embryonic development of three mammalian species. Genome Res. 2010; 20(6): 804-815.
6. Rossant J. Developmental biology: a mouse is not a cow. Nature. 2011; 471(7339): 457-458.
7. Sylwestre EL, Robert C, Pennetier S, Labrecque R, Gilbert I, Dufort I, et al. Evolutionary conservation of the oocyte transcriptome among vertebrates and its implications for understanding human reproductive function. Mol Hum Reprod. 2013; 19(6): 369-379.
8. Hosseini SM, Hajian M, Forouzanfar S, Ostadhosseini S, Mouavi F, Ghanaei HR, et al. chemically assisted somatic cell nuclear transfer without micromanipulator in the goat: effects of demecolcine, cytochalasin-B, and MG-132 on the efficiency of a manual method of oocyte enucleation using a pulled Pasteur pipette. Anim Reprod Sci. 2015; 158: 11-18.
9. Hosseini SM, Hajian M, Ostadhosseini S, Forouzanfar M, Abedi P, Jafarfour P, et al. Contrastng effects of G1.2/G2.2 and SOF1/SOF2 embryo culture media on pre- and post-implantation development of non-transgenic and transgenic cloned goat embryos. Reprod Biomed Online. 2015; 31(3): 372-383.
10. Yan L, Lei L, Yang C, Gao Z, Lei A, Ma X, et al. Isolation and cultivation of goat embryo stem cells. Sheng Wu Gong Cheng Xue Bao. 2008; 24(9): 1670-1676.
11. De AK, Garg S, Singhal DK, Malik HN, Mukherjee A, Jena MK, et al. Derivation of goat embryonic stem cell-like cell lines from in vitro produced parthenogenetic blastocysts. Small Rumin Res. 2013; 113: 145-153.
12. Behboodi E, Bondareva A, Begin I, Rao K, Neveu N, Pierson JT, et al. Establishment of goat embryonic stem cells from in vivo produced blastocyst-stage embryos. Mol Reprod Dev. 2011; 78(3): 202-211.
13. Keefer CL, Pant D, Blomberg L, Talbot NC. Challenges and prospects for the establishment of embryonic stem cell lines of domesticated ungulates. Anim Reprod Sci. 2007; 98(1-2): 147-168.
14. Kimber SJ, Sneddon SF, Bloor DJ, El-Bareg AM, Hawk...
head JA, Metcalfe AD, et al. Expression of genes involved in early cell fate decisions in human embryos and their regulation by growth factors. Reproduction. 2008; 135(5): 635-647.

15. Mishina Y, Starbuck MW, Gentile MA, Fukuda T, Kasparcova V, Seedor JG, et al. Bone morphogenetic protein type IA receptor signaling regulates postnatal osteoblast function and bone remodeling. J Biol Chem. 2004; 279(26): 27560-27566.

16. Nasr-Esfahani MH, Hosseini SM, Hajian M, Forouzanfar M, Ostadhosseini S, Abedi P, et al. Development of an optimized zona-free method of somatic cell nuclear transfer in the goat. Cell Reprogram. 2011; 13(2): 157-1570.

17. Hosseini SM, Asgari V, Ostadhosseini S, Hajian M, Piryaei A, Najarsi M, et al. Potential applications of sheep oocytes as affected by vitrification and in vitro aging. Theriogenology. 2013; 80(8): 923-932.

18. Abbasi H, Tahmoorespur M, Hosseini SM, Nasiri Z, Bahadorani M, Hajian M, et al. THY1 as a reliable marker for enrichment of undifferentiated spermatozoa in the goat. Theriogenology. 2013; 80(8): 1741-1753.

19. Degrelle SA, Campion E, Cabau C, Piumi F, Reinaud P, Richard C, et al. Molecular evidence for a critical period in mural trophoblast development in bovine blastocysts. Dev Biol. 2005; 288(2): 448-460.

20. Moulavi F, Hosseini SM, Hajian M, Forouzanfar M, Abedi P, Ostadhosseini S, et al. Nuclear transfer technique affects mRNA abundance and cell fate of the reconstituted sheep oocytes. Reproduction. 2013; 145(4): 345-355.

22. Schmitgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008; 3(6): 1101-1108.

23. Bar-Or C, Czosnek H, Koltai H. Cross-species microarray hybridizations: a developing tool for studying species diversity. Trends Genet. 2007; 23(4): 200-207.

24. Hamatani T, Carter MG, Sharov AA, Ko MS. Dynamics of global gene expression changes during mouse preimplantation development. Dev Cell. 2004; 6(1): 117-131.

25. Vigneault C, McGraw S, Sirard MA. Spatiotemporal expression of transcriptional regulators in concert with the maternal-to-embryonic transition during bovine in vitro embryogenesis. Reproduction. 2009; 137(1): 13-21.

26. De Moor CH, Meijer H, Lissenden S. Mechanisms of translational control by the 3' UTR in development and differentiation. Semin Cell Dev Biol. 2005; 16(1): 49-58.

27. McGraw S, Vigneault C, Sirard MA. Temporal expression of factors involved in chromatin remodeling and in gene regulation during early bovine in vitro embryo development. Reproduction. 2007; 133(3): 597-608.

28. Lagares MA, Castanheira PN, Amaral DC, Vasconcelos AB, Vead JC, Arantes RM, et al. Addition of ficoll and disaccharides to vitrification solutions improve in vitro viability of vitrified equine embryos. Cryo Letters. 2009; 30(6): 408-413.

29. Xie D, Chen CC, Ptaszek LM, Xiao S, Cao X, Fang F, et al. Rewirable gene regulatory network in the embryonic development of three mammalian species. Genome Res. 2010; 20(6): 804-815.

30. Zhang Y, Yang Z, Wu J. Signaling pathways and preimplantation development of mammalian embryos. FEBS J. 2007; 274(17): 4349-4359.
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. It has been certified by Ministry of Culture and Islamic Guidance since 2007. If not, only write the first letter in capital and italic (e.g., Int. J. Fertil. Steril.).

Guide for Authors

1. Types of articles

The articles in the field of Fertility and Sterility can be considered for publications in International Journal of Fertility and Sterility (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.

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.

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 International Journal of Fertility and Sterility. 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). 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 (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 mobile 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), eds. 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: Eftekhar Yazdi P. Comparison of fragment removal and co-culture with Vero cell monolayer’s on development of humanfragmented embryos. Presented for the Ph.D., Tehran. Tarbiyat Modarres University. 2004.

Conferences:
Surname(s) of editor(s). Conference title; Holding date; Holding place. Publication place; Publisher name; Publication date (year).
Example: Harnsen R, 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: Nabavi SM. 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).

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