Middle East Fertility Society Journal xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Middle East Fertility Society Journal

journal homepage: www.sciencedirect.com

Original Article

Clinical outcome of assisted reproductive technologies in advanced aged men ☆

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ARTICLE INFO

Article history:
Received 8 August 2016
Revised 21 December 2016
Accepted 7 January 2017
Available online xxxx

Keywords:
Paternal age
ART
Live birth

ABSTRACT

Objective: The purpose was to establish whether the advanced male age may influence the outcomes in a controlled assisted reproductive technology (ART) program.
Design: This is an experimental study.
Setting: Research and Clinical Center for Infertility of Yazd.
Sample: A total of 63 men undergoing ART cycles.
Material: In a retrospective study, the outcome of intracytoplasmic sperm injection (ICSI) in ART cycles in two groups of patients was studied. Patients regarding etiology of infertility were divided into two groups: (A) male factor infertility (n = 47), and (B) female factor infertility (n = 16). Sperm parameters, ART cycle characteristics and clinical outcomes were assessed between two groups.
Main outcome measures: Clinical outcomes of ART in advanced aged men.
Results: There were no significant differences in rates of sperm count and morphology between two groups, but the rate of spermatozoa with progressive motility were higher in group B in comparison with A ($P = 0.002$). The rates of high quality embryos, pregnancy and live birth showed no significant differences between the groups ($P = 0.4$, $P = 0.4$, $P = 0.2$, respectively).
Conclusion: It appears that the clinical outcome of ART in male and female factor infertility was not influenced by advanced paternal age.

1. Introduction

It seems that there is no controversy that increased maternal age has adverse influence on fertility potential [1]. Multiple reports have shown that advance maternal age resulted in decreased pregnancy and implantation rates, while increasing abortion rates in assisted reproductive technology (ART) cycles [2,3]. Additionally, decline in reproduction function in aged women is due to an increase in chromosomal abnormalities in oocytes and/or generated embryos [4,5]. Nevertheless, there is no certain consensus on male's age effect on reproductive outcomes, which is less described in the literature. Likewise, the relevance of advanced paternal age and success of ART cycles is still unclear [6]. Recently, data have shown that there was no adequate substantiation to prove the effects of paternal age on fertility outcomes [7]. Some studies stated that after adjusting for female age in general population, advanced male age became a significant factor in time of achieving pregnancy. So, they suggested that independent of maternal age, paternal age can influence reproductive success [8,9].

The reports in ART cycles noted that the fertilization rates were significantly decreased in men who were over 50 years of age [10,11]. Dain et al. did not notice correlation between advanced paternal age and the rates of fertilization, implantation, pregnancy, miscarriage, or even live birth. The authors, however, found a significant decrease in the blastocyst formation rate associated with increased paternal age being due to genomic changes within the embryos [7]. Meanwhile, Beguería and associates [6] mentioned that embryo quality were similar in different male age groups. In the oocyte donation model, male aging was not correlated with the pregnancy outcomes [12,13].

The first step in monitoring of the male reproductive potential is semen analysis. However, the effect of paternal age on semen quality is currently controversial [9]. Majority of studies suggested

Peer review under responsibility of Middle East Fertility Society.
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http://dx.doi.org/10.1016/j.mefs.2017.01.004
1110-5690/Production and hosting by Elsevier B.V. on behalf of Middle East Fertility Society.

Please cite this article in press as: Omidi M., et al. Clinical outcome of assisted reproductive technologies in advanced aged men. Middle East Fertil Soc J (2017), http://dx.doi.org/10.1016/j.mefs.2017.01.004

a significant decrease in semen quality in relation to advance male age [11,14]. There is a hypothesis that age-dependent alternations of semen quality can be associated with anatomical, histological and hormonal changes [7]. The increasing of serum FSH has been linked to reduced sertoli cell function, degeneration of germ cells and subsequently decreased sperm production in aged men [15]. It is well known that a physiologic loss of germ cells in germinal epithelium and decrease in spermatogenic efficiency will occur in aged men [16]. Furthermore, there would be age-related alternations in the hypothalamic-pituitary-testicular axis due to decrease serum steroid levels in aging men [17]. On the other hand, disorders in the offspring of older fathers apparently result in genetic mutations in germ and sperm cells [18,19]. Regarding to sperm DNA damage, it has been shown that the paternal age is related to an increase in the level of sperm with DNA damage [15]. Therefore, the purpose of the present study was to establish whether the advanced male age may influence the reproduction success in a controlled ART program in Iran.

2. Materials and methods

2.1. Study design

In this retrospective study, laboratory and clinical data from patients undergoing ICSI over 8 years from July 2006 to December 2014 were analyzed. The eligibility criteria for selecting study population were the age of $\geqslant$50 years for male partner. Patients regarding etiology of infertility were divided into two groups: (A) male factor infertility, and (B) female factor infertility. Sperm parameters, ART cycle characteristics and clinical outcomes were assessed between two groups. A total of 92 patients were selected for the study, but 29 cases were excluded, due to the rare sperm in their samples, embryo arresting, and loss of follow-up. Our study was approved by author's institute review board.

2.2. Semen analysis and sperm preparation

Semen samples were obtained by either masturbation or testicular biopsy. Routine macroscopic parameters were applied. Also, microscopic analysis of sperm concentration, motility, viability and morphology were done according to WHO guidelines [20]. The specimens were prepared using discontinuous density gradient technique. In this method, 1 mL of 80% Pure Sperm solution was overlaid with 1 mL of 40% solution. After that, 1 mL of semen was added to the top layer of gradient, then centrifuged at 300*g* for 20 min. The seminal plasma supernatant was discarded and the pellet resuspended in 5 mL human tubal fluid supplemented with 5% human serum albumin (HTF-HSA), and centrifuged at 300*g* for 7 min. The resultant pellet was resuspended in 0.5 mL of HTF-HAS, and stored at the 37C and 6% CO2 for insemination.

2.3. Ovarian stimulation and ICSI procedure

Ovarian stimulation was achieved by long pituitary down regulation using a combination of a gonadotrophin-releasing hormone (GnRH) agonist or antagonist and FSH (Gonal-F; Serono, Geneva, Switzerland). The recombinant hCG (rhCG; IBSA Co, Switzerland) was administered when the maximum diameter of leading follicles were exceeded 18 mm, followed 36 h later by oocyte retrieval. The retrieved cumulus oocyte complexes (COCs) were incubated at 37 °C in 6% CO2 and 95% air until denuding for ICSI. The COCs were denuded of their cells by 30–60 s exposure to HEPES buffered medium containing 80 IU mL^{-1} hyaluronidase (Irvine Sci, CA, USA) followed by pipetting the COCs with a pasture pipette. ICSI procedure was performed as described previously [21].

2.4. Fertilization and embryo assessments

Oocytes were checked for signs of fertilization 16–18 h after injection. Fertilization was approved by presence of two pronuclei (2PN) and two polar bodies. Two day embryos were graded as follow: Grade A: equal size blastomeres without fragmentation, Grade B: slightly unequal blastomere, up to 10% cytoplasmic fragments. Grade C: unequal sized blastomeres up to 50% fragments and large granules. Grade D: unequal blastomeres with significant fragmentation and large black granules [22]. Grades A and B were regarded as high quality embryos. Results of chemical pregnancy were determined 14 days after embryo transfer (ET) on day 2.

2.5. Statistical analysis

The data were presented as median (min–max) and percentage for quantitative and qualitative data, respectively. Man-Whitney *U* test and chi-square test was applied wherever appropriate to compare between two groups. All hypotheses were considered two tailed and significant level was defined at p less than 0.05.

3. Results

Table 1 summarizes the demographic and cycle characteristics of the male and female patients. There were no significant differences in the male age between two groups, but the female age was significantly higher in group B in comparison to group A ($P = 0.007$). In group B, the etiology of infertility was as follows: tubal factor, ovarian failure, PCOs, and endometriosis. Spermatozoa were retrieved from testis in 23.4% of the patients in group A; while, all semen samples were collected by ejaculation in group B. There were no significant differences for rates of sperm count and morphology between two groups, but percentage of spermatozoa with progressive motility were significantly higher in group B in comparison with A ($P = 0.002$). In addition, the number of retrieved oocytes had an increasing trend in group A, but the difference was insignificant. In total, 386 COCs were retrieved in group A, of which 341 were at metaphase II stage and 229 embryos were formed. In group B, however, 95 COCs were retrieved, 83 oocytes were mature and 54 embryos were developed in vitro (Table 1). The rates of high quality embryos (A + B), pregnancy and live birth showed no significant differences between the groups (Table 2).

Table 1
Comparison of patients demographic and cycle characteristics between two groups of A (male factor infertility) and B (female factor infertility).

Characteristics	Group A (n = 47)	Group B (n = 16)	P-value
Male age (year)	53 (50–69)	51 (50–70)	0.053
Female age (year)	32 (20–50)	40 (22–50)	0.007
COC number	7 (2–20)	5 (1–14)	0.054
MII oocyte	6 (2–16)	4 (1–12)	0.06
Formed embryos	4 (1–15)	3 (1–9)	0.057
Transferred embryos	3 (1–5)	2 (1–4)	0.5
Sperm source			
Ejaculate	36	16	0.02
Non-ejaculated	11	0	
Sperm count ($\times 10^6$/mL)	40 (2–400)	57.5 (35–170)	0.051
Progressive motility (%)	23 (0–79)	43.5 (2–75)	0.002
Non-progressive motility (%)	12 (0–45)	13.5 (7–22)	0.9
Normal morphology (%)	17 (1–60)	14 (5–45)	0.4

Data are presented as median (min-max).
COC: cumulus oocyte complex.
MII: metaphase II.

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