Original paper

Outcomes of Intracytoplasmic Sperm Injection among Obstructive Azoospermia Patients in Kerbala, Iraq.

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Abstract

Background: Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technology (ART) used to treat sperm-related infertility problems. There is heterogeneity in the success rates of ICSI among center, thus it would be useful to describe the data of different centers.
Aim of the study: to evaluate the rate of ICSI success among Obstructive azoospermia treated in AL-Kafeel IVF center, Kerbala, Iraq, with some emphasis on female factors that may predict that ICSI outcome.
Study design: A retrospective study
Materials and Methods: Data of all couples who underwent ICSI with fresh sperm retrieval using testicular sperm aspiration (TESA) and reached the stage of embryo transfer during the period from 1st January 2015 to 1st January 2019 were included. Demographic data of couples and hormonal assays results were recorded. Data included such as number of oocytes collected, quality of oocytes, fertilization rate and clinical pregnancy rate. Clinical pregnancy was identified by measuring HCG hormone level in day 12-14 after embryo transfer.
Results and Discussion: The total couple underwent ICSI was 42. The rate of fertilization was 69.55% whereas the clinical pregnancy rate was 33.3% which is similar to pregnancy rates in normospermic individuals. No effect of age of couples on the outcome of ICSI. TSH level in females was significantly higher in females with ICSI positive compared to females with failed ICSI (p=0.047) with average TSH concentration of 3.4 in ICSI successor and 2.038 in failed ICSI. Number of Grade 1 embryos was higher in ICSI successor than in ICSI failed couples. In addition, the levels of LH and AMH were shown to be increased I ICSI successors, however, this increase did not reach statistical significance.
Conclusion: The rate of ICSI success in azospermic patients is similar to normospermic patients. TSH level in females could serve as a predictor for the outcome of ICSI.
Keywords: ICSI, pregnancy rate, Fertilization rate, Oocytes, IVF

Introduction

Azoospermia is the absence of spermatozoa in the ejaculate. It is observed in 1% of the general population and in 10% of infertile men and comprise 2% of human infertility [(1)]. The diagnosis of azoospermia requires two or more centrifuged semen samples 2-4 weeks apart to confirm that sperms are totally absent [(2)].

The causes of azoospermia are classified within 3 categories. Pretesticular (also known as secondary testicular failure) causes include (a) hypothalamic gonadotrophin deficiency such as hypogonadotrophic hypogonadesa (Kallmann Syndrome), creniopharyngioma and hemochromatosis [(3)], (b) pituitary

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insults such as trauma, adenoma and meningitis, (c) drug anabolic steroids such as testosterone injections [4]. Testicular causes (also known as primary testicular failure) include, (a) congenital; cryptorchidism, aneuploidy, Y-deletion, anorchia and others, (b) acquired; mumps orchitis, tortions..etc, (c) Iatrogenic; radiotherapy and chemotherapy [5]. Post-testicular causes include, (a) congenital; cystic fibrosis, (b) Acquired; gonorrhea, chlamydia and Tuberculosis, (c) Iatrogenic; vasectomy, renal transplantation, hydrocele repair.etc [5, 6]. It is important to identify hypogonadotrophic hypogondism as gonadotrophin therapy is usually effective achieving natural pregnancy [7]. For obstructive azoospermia reconstructive surgery can be enough treatment as in reversal of vasectomy. If surgery is ineffective, as in bilateral absence of deference, sperm recovery and ICSI is the treatment of choice [8].

There are many methods for surgical sperm recovery utilized in men with azoospermia such as PESA (percutaneous epididymal sperm aspiration), TESA (testicular sperm aspiration, and TESE (testicular sperm extraction –from biopsy. The procedure should be minimally invasive as possible [9]. Indeed, no test can be used to confirm no sperm within the testis, therefore, sperm recovery should be offered to all cases of azoospermia even if testis are atrophic or the FSH level is very high [10, 11].

In this study, the aim is to present the findings of a newly established IVF and assisted reproduction center in Kerbala, Iraq too shed light on the current outcomes of ICSI and some factors that may affect those outcomes.

Materials and Methods

Patients

This retrospective study included 42 couples with apparent male factor infertility presenting to IVF center at Al-kafeel Super-specialty Hospital, Kerbala, Iraq for the period from 1st January 2015 to 1st January 2019. All men had normal testicular volume and normal serum FSH concentration. Most (92.86%) suffered from primary infertility for 1 to 18 years duration. The following data were obtained:

1. Age of the males and females
2. Type of infertility (primary or secondary)
3. Period of infertility
4. Hormonal assays result of the females
5. Educational status

Ovarian stimulation:

Controlled ovarian stimulation was done for all females using either short agonist protocol with Decopeptyl (0.1mg/day), or antagonist protocol with Cetrotide (0.25 mg/day). Ovarian stimulation done using rFSH hormone in form of follitropin, Gonal F or HMG. Follicle growth was followed using hormonal assay and transvaginal ultrasonography. If minimum 2-3 follicle reached the size of 18mm, a trigger was given by human chorionic gonadotrphin (HCG) in form of Pregnyl; 5000-10000 unit. Trigger was given 36 hours prior to oocyte pick up.

Oocyte Pick Up

The oocyte picks up was done under general anesthesia using trans-vaginal ultrasound guided needle. The oocytes were collected in aspiration media (Fertipro) and after a washing step, were transferred into fertilization media and incubated in 5% CO_2 atmosphere at 37°C. denudation (the process of removing the cumulus mass from oocyte) was done 2 hours later with use of both hyaluronidase enzyme and mechanical methods. Thereafter, the oocyte transferred into Cleavage Media and incubated in 5% CO_2 atmosphere at 37°C. Evaluation of the oocytes was done. Normal oocytes were described as round clear Zona pellucida with single polar body

which was not fragmented and with small perivittaline space. In addition, the cytoplasm was clear with no inclusion cyst.

Surgical Sperm Extraction

Males underwent testicular sperm aspiration (TESA) under general anesthesia. The obtained testicular tissue was crashed and cutted by small guage needle. And sperm collection is done using inverted microscope.

Intracytoplasmic Sperm Injection (ICSI) and embryo transfer

One viable sperm was injected in each oocyte. ICSI was done 3-4 hours after oocyte collection. Results of fertilization usually checked 16-18 hours after ICSI. Any abnormal oocyte removed and only 2 pronuclei oocytes were collected. embryo transfer was done on day 3 after oocyte pick up in all patients. Two to 3 embryos were transferred into each female. Endometrium was well-prepared with thickness between 8-10 mm on the day of transfer. Embryo transfer was done under abdominal ultrasound guide with embryo transfer catheter.

Detection of Pregnancy

Pregnancy rate was defined as positive for pregnancy test by measuring HCG hormone level in day 12-14 after embryo transfer. Miscarriage rate was defined as pregnancy loss before 20 weeks of gestation.

Results

Table 1 presents data on ICSI cycles in this study. The total couples included in this study was 42 couples. All of the males were diagnosed to have obstructive azoospermia. The average of oocyte collected were 12.29 oocyte. The denuded oocytes were examined by light microscopy; only oocytes in metaphase II were suitable for ICSI. The average number of oocytes in metaphase II was 9.90 oocyte. All oocytes in metaphase II were injected with viable sperms. All fertilized oocytes possessed two pronuclei in the ooplasma with two clear polar bodies. Fertilization rate (FR) was calculated as percentage transformation of micro injected oocytes into two pronuclei. The fertilization rate was 69.55%. The averages of good quality embryos (grade 1 and grade 2) were 2.89 and 1.17 respectively. The rate of clinical pregnancy was 33.3%.

As shown in table (2), the pregnancies have occurred in age slightly younger than those couple who failed to get pregnancies, however these differences in mean age were not statistically significant ($p>0.05$). The hormone levels of females were measured before commencing the IVF programs. These levels were compared between couples with ICSI success and ICSI failed to see which hormone(s) would predict the ICSI outcomes. Surprisingly, TSH level was significantly higher in females with ICSI positive compared to females with failed ICSI. Both AMH and LH were also shown to be higher in females ICSI successor compared to females with failed ICSI, however, these differences is not significant. In contrast, mean level of serum prolactin was lower in ICSI successor females.

Embryos were evaluated and classified into grades based on different parameters that are mentioned in the materials and methods. The mean number of Grade 1 embryos in ICSI successors was higher than number in ICSI failed couples, however, this increase did not reach a statistical significance (p= 0.063), whereas other grades had no infleunce on the outcomes of ICSI (as shown in table 4).

Table 1. The results of ovarian stimulation and intracytoplasmic sperm injection.

parameter	Lowest	Highest	Average (± STD)
Number of oocyte collected	1	24	12.29 (± 5.93)
Oocytes in metaphase II	1	20	9.90 (± 4.68)
Fertilization	14.29%	100%	69.55% (± 21.52%)
number of grade 1 zygotes	2	15	4.76 (± 2.89)
number of embryos produced	1	15	
clinical pregnancy rate			33.3%

Table 2. Distribution of the mean ages of the couples according to ICSI outcomes.

	ICSI positive Mean± SE (SD)	ICSI negative Mean± SE (SD)	*P-Value*
Age F	30.36 +1.696 (SD=6.344)	31.25 +1.261 (SD=6.670)	0.680
Age Male	38.57 +1.440 (SD=5.388)	41.64+2.201 (SD=11.647)	0.356

Table 3. comparison of hormones levels between ICSI positive and negative outcomes

	ICSI positive Mean± SE	ICSI negative Mean± SE	*P-Value*
FSH (mIU/ml	5.49±0.35 (SD=1.31)	5.55±0.38 (SD=2.02)	0.926
LH (mIU/ml)	5.03±0.71 (SD=2.66)	3.78±0.30 (SD= 1.56)	0.062
Serum Prolactin (ng/ml)	16.87± 2.91 (SD=10.87)	20.53±1.89 (SD=9.99)	0.284
AMH	4.946±1.184 (SD=4.43)	3.15±0.32 (SD=1.71)	0.065
TSH (µIU/ml)	3.4±0.83 (SD=3.12	2.04±0.22 (SD=1.18)	0.047*

* Statistically significant at $p< 0.05$

Table 4. Distribution of the qualities of embryos after ICSI according to ICSI outcomes

	ICSI positive Mean± SE	ICSI negative Mean± SE	*P-Value*
Grade 1	5.93±0.81 (SD=3.025)	4.11±0.54 (SD=2.85)	0.063
Grade 2	1.36±0.33 (SD=1.216)	1.43±0.22 (SD=1.17)	0.855
Grade 3	0.57±0.33 (SD=1.22)	0.64+0.20 (SD=1.06)	0.846

Discussion

In this study, the rates of fertilization and clinical pregnancies were comparable to those reported in other studies [12]. In addition, the those rates reported in this study are similar to ICSI used ejaculated sperms [13]and normo-spermic individual [14]. This makes this fertilization techniques is superior to other pregnancy-assisted techniques. This could explain the global increase in the use of this technique even in patients without severe male factor infertility without clear evidence of a benefit over conventional in vitro fertilization (IVF) [15]

Indeed, among fresh IVF cycles in the United States, ICSI use increased from 36.4% in 1996 to 76.2% in 2012, with the largest relative increase among cycles without male factor infertility. Compared with conventional IVF, ICSI use was not associated with improved post fertilization reproductive outcomes, irrespective of male factor infertility diagnosis[15].

In this study the average of oocyte collected were around 12 oocyte and average oocyte in metaphase II was 10 all the oocyte in metaphase II were injected with viable sperm [16, 17]. Several studies released by the European Society of Human Reproduction and Embryology (ESHRE) summarized that "15 oocytes is the perfect number" and suggested an optimal chance for achieving a pregnancy in one cycle with the percentage going up to 37% when 15 oocytes had been collected [18].

Fertilization rate was calculated as percentage transformation of micro injected oocytes into two pronuclei. In this study, the fertilization rate was high (average 69.55%). This is may be due most of the females in this study are of young age. Indeed, studies reported that with increasing age, there was a trend toward a lower fertilization rate [19].

The current study revealed a significant difference between levels of TSH in ICSI successors and failed ICSI cycles. Indeed, there is ongoing debate on the association of thyroid disorders and outcomes of IVF/ICSI. Where many studies were able to show a direct link, whereas other studies failed to confirm this link [20]. In this study, the TSH levels itself, apart from thyroid function, was shown to be associated with certain ICSI out comes where the range of TSH was less than 3.5 µIU/ml in all failed ICSI, whereas, the TSH level in ICSI successor distributed over a wider range, nevertheless, the average in ICSI successor was significantly higher. These findings my pinpoint another level of importance for TSH in outcomes of the assisted reproductive technologies.

Anti-Müllerian hormone (AMH), a dimeric glycoprotein belonging to the transforming growth factor-β (TGF-β) family, is produced by fetal Sertoli cells at the time of testicular differentiation, and induces regression of the Mullerian ducts. In women, it is secreted by granulosa cells within preantral and early antral follicles, < 6 mm in diameter [21].

Several studies have revealed significant positive correlation between AMH concentrations and pregnancy rate [22], ongoing pregnancy rate[23] and live birth rate [24]. However the results from the other studies indicated that the predictive value for serum AMH in relation to clinical pregnancy rate, ongoing pregnancy rate and live birth rate is controversial [25, 26].

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