IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Yahya, et al., Vol. 12, Issue 2, Pp. 81-91 (2022) Original Research
DOI: http://doi.org/10.28969/IJEIR.v12.i2.r6.22 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Comparison in Pregnancy Rates After ICSI Using Fresh Sperm Versus Cryopreserved

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Received: 01-Oct-2022
Accepted: 31-Oct-2022
Published: 06-Nov-2022

ABSTRACT

For couples with a variety of male factor disorders, intracytoplasmic sperm injection has made pregnancy and motherhood possible. Two hundred-one males who had non-obstructive azoospermia or severe oligospermia were included in this study. One hundred forty trials of ICSI were done by using fresh sperms (fresh group n=140), while 61 trials of ICSI were done by using cryopreserved sperms (cryopreserved group n=61) to compare the conception rates between fresh and cryopreserved sperms used in intracytoplasmic sperm injection (ICSI). Twenty-five samples from each group were collected by doing a testicular biopsy. Pregnancy rates were higher using cryopreserved sperms as compared to fresh spermatozoa, 39.3% compared to 24.3% in the fresh group (*P* .030), respectively. Binary logistic regression analysis showed that the odds ratio of achieving pregnancy with cryopreserved sperms was 2.022 higher than with fresh (*P* .030). Cryopreservation has no negative effect on the pregnancy rate. Instead, it might improve the results of ICSI in comparison with a fresh sperm sample.

How to Cite

Yahya F. Zamil; Ula Al-Kawaz; Relation of serum and follicular level of GDF9 to oocyte quality, embryo quality and pregnancy rate; Iraqi Journal ofEmbryos and InfertilityResearches (IJEIR), (2022); 12 (2): 81-91.
Doi: http://doi.org/10.28969/IJEIR.v12.i2.r6.22

KEYWORDS

GDF9, folliculogenesis, oocyte quality, ICSI.

1. Introduction

Infertility Fertility refers to the ability to establish a clinical pregnancy. Some medical professionals tend to use both the terms infertility and sub-fertility. However, for the optimal management of reproductive problems, uniform definitions are crucial. According to the most recent international glossary on infertility and fertility care, Infertility is when a clinical pregnancy cannot be established after a year of regular, frequent intercourse or in which a person's ability to reproduce, either alone or with a partner, is defective. (Zegers-Hochschild F, et al. 2017 [1]). There are four main areas in which causes of male infertility can be categorized into. The predominance of these categories is based on shaky evidence with numerous confounding factors such as varying definitions of male infertility and its causes, selection bias, and assessment variations: Primary testicular abnormalities in spermatogenesis, endocrine and systemic illnesses with hypogonadotropic hypogonadism, acquired testicular diseases (e.g., mumps orchitis), Sperm Transport Disorder, idiopathic male infertility and primary testicular abnormalities in spermatogenesis (Aktan G et al. 2013 [2]). In research on untreated couples looking for pregnancy, 50 percent conceived within three months, 70 percent within six months, and 85 percent within a year.

Up to 50 percent of young, healthy couples who fail to accomplish chemical or clinical pregnancy in the first year will conceive in the next one. As a result, in couples where the female partner has normal menstrual cycles, the male partner has normal sperm analyses, and neither partner has a specific cause of infertility, it may be appropriate to postpone invasive reproductive techniques (Jungwirth A et al. 2012 [3]). The advancement of ART has enabled many couples suffering from male factor infertility to conceive. Because ART may be beneficial in any man with evidence of spermatogenesis (even microscopic nests of sperm visible

only on testicular histology, as in men with Klinefelter syndrome), there are a variety of causes of male infertility that can be treated with ART. (Tournaye H, et, al. 2012 [4]). In the United States and other wealthy or industrialized countries, (ART) is widely used. In 2014, ART was used in approximately 1.6 percent of live births in the United States. (Sunderam S, et ,al. 2018 [5]).

Some infertile men have been successful in conceiving with ART using an (ICSI) that has been obtained from the ejaculate, epididymis, or testis (e.g., men with azoospermia and men with immotile sperm). A meta-analysis of 10 cycles of in vitro fertilization (IVF), ICSI, and intrauterine insemination for couples with male infertility found no differences in pregnancy or live birth rates between ART and expectant management. Therefore, ART does not improve rates of live births for all types of male infertility (abnormal sperm parameters) (Cissen M et al., 2016 [6]). Male factor infertility treatment is still understudied and mostly based on professional opinion and personal experience. Injection of intracytoplasmic sperm ICSI involves only one sperm being inserted into the ovum, skipping all the natural fertilization processes of gamete fusion and egg coat penetration. (Palermo, G. D., et, al. 2019 [7]). Only in Belgium were the first ICSI-based human pregnancies carried out in 1992, and thousands of ICSI babies have been born ever since. For many types of male factor infertility, ICSI has made pregnancy and motherhood achievable for couples and at rates comparable to those in patients receiving standard IVF with gametes that appear to be normal. (Palermo G. et, al. 1992 [8]), The word cryopreservation is derived from the Geek word κρύος (krýos, “icy cold, chill, frost”). Using extremely low temperatures, this method preserves living tissues and cells that are structurally intact. Spallanzani first used cryopreservation in 1776 when he froze sperm in snow and kept their motility after rewarming. Mantegazza revealed the survival of human sperm frozen at 17 °C

for more than four days a century later (Neri QV et al. 2008 [9]). Some treatments for malignancy, medical disorders, or gender affirmation can indelibly end reproductive function. Patients who need to go through such therapies should be rapidly referred for counseling regarding options for fertility preservation.

Established cryopreservation procedures to preserve fertility include freezing of embryos and gametes. With appropriate pretreatment planning and intervention, future biologic parenthood is possible (Curry MR. 1995 [10]). Freezing that relies on cell dehydration and uses small amounts of a cryoprotectant in combination with gradual cooling to reach equilibrium. In this cryopreservation technique, ice crystals are created at a specific temperature as the temperature steadily drops. Either a programmed freezer or manually dropping the samples into liquid nitrogen vapor are used to achieve slow cooling. Samples are first frozen by being kept at 20 °C for about 20 minutes and then at -86 °C for an additional 60 minutes. The samples will then be kept in liquid nitrogen at -196 degrees Celsius (Kuczynski W et al. 2001 [11]). With fresh, complete ejaculates or washed spermatozoa, slow freezing can be done. It is the method of choice for preserving human spermatozoa because testicular tissue and medically obtained spermatozoa can both be used in its execution (PerssonPS et al., 1971 [12]). It is the method of choice for preserving human spermatozoa because testicular tissue and medically obtained spermatozoa can both be used in its execution. Although the traditional slow-freezing technique has been crucial for sperm cryopreservation, current research indicates that vitrifying human spermatozoa results in greater recovery rates after warming (Sharma R et al. 2015 [13]).

2. Patients, Materials and Methods

In a retrospective comparative study, a total of 201 men were selected from those

who visited the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies. Hormonal assay (FSH, LH, E2, PRL, Testosterone, and TSH) was done for all of them. One hundred forty trials of ICSI were done by using fresh sperms (fresh group n=140), while 61 trials of ICSI were done by using cryopreserved sperms (cryopreserved group n=61). Twenty-five samples from each group were collected by doing a testicular biopsy.

All patients who received (ART) with (ICSI) throughout the study period had their records checked to start the data-gathering process. After that, the partner's sperm and medical information were gathered. As long as patient identity and the confidentiality of their medical records are respected, all of the patients completed an informed consent form allowing us to study their medical data for research reasons.

All men who visited **the** High Institute of Infertility Diagnosis and Assisted Reproductive Technologies aged ≥ 18 years, with a history of infertility (more than one year) with severe oligospermia or azoospermia (non-obstructive) were included regardless of female age or etiology.

1- Testicular Tissue Retrieval:

Testicular tissue is taken under anesthesia using an open technique. Incision of the tunica albuginea is performed in a specific area to avoid injuries to testicular blood vessels, and testicular tissue is collected from one upper and one lower site in each testis. A sample from each part is sent for histopathological evaluation. In order to confirm the viability of spermatozoa, testicular specimens were put to Ham's F10 medium and then viewed under contrast microscopy. Spermatozoa were then cryopreserved in up to 10 fractions using the streamlined freezing process.

2- **Freezing technique of testicular tissue/ ejaculated:**

The testicular samples are preserved in 2 ml containers (Greiner, Frickenhausen, Germany) containing 0.5 ml HEPES-buffered medium consisting of modified

Earle's balanced salt solution with 0.4% human serum albumin and 15% glycerol as a cryoprotectant (Sperm Freeze; Medicult, Hamburg, Germany). After incubation, the specimens with cryoprotectant are placed in canes of a Styrofoam box, which is loaded with liquid nitrogen for 20–30 min. After that, the specimens will be soaked in liquid nitrogen and kept till the time of ICSI.

3- Ovarian stimulation:

Follicular development is stimulated by giving a follicle-stimulating agent (Gonal-F); the initial dose was on the 2nd or 3rd cycle day (150 IU S/C every day) until we had sufficient follicular development. The target was to obtain 12-14 oocytes of about 17mm in size, then be given recombinant human chorionic

gonadotropin (HCG) to enhance ovulation. The retrieval was performed under ultrasound guidance by using a vaginal probe to pierce the follicles 36 hours after HCG injection. Testicular tissue specimens containing spermatozoa are prepared for ICSI utilizing the mechanical approach by melting them in a 37°C water bath for 3-5 minutes after obtaining the oocytes. To be more precise, the testicular tissue is separated with a surgical knife (1 minute of mincing is adequate) in a center-well dish after being bathed in two drops of Ham's F-10 medium to remove the cryoprotectant, then 0.5–1 ml of Ham's F-10 medium is added to the divided tissue, for about 5 hours before the ICSI procedure is performed. All patients included had undergone one trial of ICSI during their enrollment the supernatant medium in the Eppendorf tube is centrifuged for 1-2 min. Then, from each pellet, one microliter will be added to one or two drops of the medium under oil to be used for injection. In a condition where no motile spermatozoa could be selected, normal shape ones are injected directly

4- Statistical Analysis:

Fischer's exact tests and Chi-Square testing were used to compare the variables. For each variable under investigation, the error threshold was established at 5% as being statistically significant. The odds ratio,

presented with a 95% confidence interval, was used to determine the relationship between the variables. To remove confounding variables, a logistic regression analysis was performed. A 0.05 p-value was regarded as statistically significant.

3. Results

The mean age of women in the fresh group was 30.9 ±5.23 years, which was not statistically different from the mean age of women in the cryopreservation domain (31.1 ±5.84 years). There was a statistically significant association between the pregnancy rate and the cryopreservation group, as the pregnancy rate among this group was 39.3% compared to 24.3% in the fresh group (*P* .030). Using binary logistic regression, the odds for successful pregnancy was two times (95% CI= 1.06 up to 3.85 times) higher in the cryopreservation group compared to the fresh group (*P* .032).

The mean age of women in the fresh group who had successful pregnancy was 29.9 ± 4.02 years, which was not statistically different from the mean age of women in who did not have pregnancy (31.2 ±5.54 years). The same applied to the cryopreservation group, with no pregnant women with a mean age of 31.1 ±5.32 years compared to 31.2 ±6.69 years for pregnant women.

Table (1): Distribution of the sample groups according to pregnancy rate

Variables	Fresh Group	Cryopreservation Group	Total	P-value
	No. (%)	No. (%)	No. (%)	
Not pregnant	106 (75.7)	37 (60.7)	143 (71.1)	
Pregnant	34 (24.3)	24 (39.3)	58 (28.9)	
Total	140 (100)	61 (100)	201 (100)	
	Chi-square test			

Table (2): Cross-Sectional model for predicting pregnancy for women in cryopreservation

Variable	Mean Odd's ratio	95% Confidence interval		P-value
	No. (%)	Lower	Upper	
Cryopreservation	2.022	1.06	3.85	.032
Cross-Sectional Model				

Table (3): Distribution of the sample population according to mean age

Age in years	Pregnancy		P-value
	Not pregnant	Pregnant	
	Mean ±SD	Mean ±SD	
Fresh group	31.2± 5.54	29.9± 4.02	0.190
Cryopreservation group	31.1± 5.32	31.2± 6.69	0.935
Independent samples T-test			

4. Discussion

Our objective was to evaluate the outcome of ICSI, in terms of clinical pregnancy rates, using either fresh or cryopreserved sperms. To our best knowledge, this is the first study in Iraq to address this issue. Our results show that the cryopreserved sperms group (testicular biopsy or ejaculation) had a higher pregnancy rate compared with the fresh sperms group (testicular biopsy or ejaculation) (*P=0.03*, odds ratio 2.02). Although prior studies showed there is no significant difference in the pregnancy rates between cryopreserved and fresh sperms, for example, Cayan et al. show that there is no statistically significant difference in outcome between the fresh sperm and cryopreserved one, in their study, they conclude that motile, cryopreserved and thawed epididymal spermatozoa are equal to freshly retrieved spermatozoa for ICSI (Sharma R, et, al. 2015 [13]).

Also, M. Falah conclude that in terms of pregnancy rate, there was no difference between the fresh sperm and cryopreserved one (Tao Y et al. 2020 [14] and Falah, K. 2020 [16]). While *Belinga et al.* conclude that cryopreservation might have a lower pregnancy rate (Cayan S et al. 2001 [15]).

Advantages of this research include a relatively large number of patients, none of who were lost to follow-up, and that the study includes patients with oligo- and non-obstructive azoospermia using both normal ejaculate and testicular biopsy. So that our findings can be applicable to a wide range of couples with infertility planning for ICSI, limitations of this research include the retrospective nature of the study in a single center, lack of randomization, and lack of karyotype and genetic analysis prior to enrollment.

5. Conclusions

Cryopreservation has no negative effect on the pregnancy rate. Instead, it might improve the results of ICSI in comparison with a fresh sperm sample.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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