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Research Article

The Outcome of Intracytoplasmic Sperm Injection with Fresh and Cryopreserved Ejaculated Sperm: A Comparative Study at the Yaounde Gynecological Endoscopic Surgery and Human Reproductive Teaching Hospital

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ABSTRACT

Objective: To evaluate fertilization rate, embryo quality and pregnancy rate in patients undergoing Intracytoplasmic Sperm Injection (ICSI) using fresh and cryopreserved ejaculated sperm.

Methods: We carried out an analytical cross-sectional study with retrospective data collection from December 1st, 2017 to May 1st, 2018. We compared two groups of patients undergoing ICSI using ejaculated sperm: group I comprised of those in whom fresh semen was used and group II was comprised of those in whom cryopreserved semen was used. We collected the fertilization rate, embryo quality, and pregnancy rate.

The Odds Ratio was calculated to determine the association between the variables. A p-value of <0.05 was considered significant. A logistic regression analysis was done.

Results: when OATS sperm was used, we had higher fertilization rate 67, 5% vs. 46, 9%, p=0,042 and better embryo quality on day 3, 95% vs. 5%, p=0.008 with freshly ejaculated sperm against the cryopreserved semen.

After logistic regression, the fertilization rate, embryo quality and pregnancy rate in the fresh and cryopreserved ejaculated semen were similar.

Conclusion: The semen with OATS may be more susceptible to damages caused by cryopreservation, resulting in lower fertilization rate and embryo quality but similar pregnancy rates.

Keywords: Fresh ejaculated sperm; Cryopreserved ejaculated sperm; Infertility; Pregnancy

INTRODUCTION

Since the introduction in 1953 and 1954 of a simple "Dry Ice" sperm cryopreservation method which maintained fertilizing capacity and resulted in normal progeny, the need of developing sperm banking has become paramount [1-10].

The role of this method of preserving fertility is to overcome the difficulty of having sperm available the day of oocyte retrieval in patients undergoing Assisted Reproductive Technique (ART) to treat infertility.

More than in only medical indications like azoospermia, the ability to cryopreserve sperm before undergoing ICSI allows more flexibility to the male partner. Also, it increases the range and number of therapeutic options available [3].

However, cryopreservation of sperm may decrease sperm parameters on motility and fertilizing capacity. It is responsible for some membrane distortion and possible DNA abnormalities [7,8].

Thus, using this process may impact on results of ART. Many studies throughout literature have compared ICSI outcome with the use of frozen-thawed and fresh sperm have shown a similar clinical outcome.

However, the spermatozoa used were surgically retrieved from the testis or from the epididymis in many of these studies [1].

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In our center, we use ejaculated fresh or frozen-thawed sperm, irrespective of the spermogram being normal or having any abnormality. The choice of freezing the sperm is made in the event of difficulty in the timing of collection or availability of the male partner.

From the opening of our fertility center three years ago till date, no data is available on the effects and impact of cryopreserved ejaculated semen and ICSI. This study is the very first-ever, evaluating the outcome and impact of cryopreserved sperm in ICSI in the Yaoundé Gynecological Endoscopic Surgery and Human Reproductive Teaching hospital, in a sub-Saharan setting

MATERIALS AND METHODS

We carried out an analytical cross-sectional study with retrospective data collection at the Yaounde Gynecological Endoscopic Surgery and Human Reproductive Teaching Hospital.

Our study extended over a period of 5 months, from December 1st, 2017 to May 1st, 2018.

The sampling was consecutive and exhaustive after institutional authorization, and ethical committee approval to carry out the study.

We included all patients who underwent an intracytoplasmic sperm injection after oocytes retrieval.

Patients who received fresh oocytes from a donor were also included. All the women were stimulated using an agonist protocol.

ICSI was carried out after egg decolonization. Evaluation of the injection was done eighteen hours later (Day 1) and zygotes could be observed and classified as 2PN when having 2 pronuclei, 3PN when having 3 pronuclei and non-cleaved for those which remained undivided.

The embryo was qualified based on the symmetry of blastomeres, the number, and the fragmentation rate.

Thus, high-quality embryos had 4 to 6 symmetrical blastomeres with less than 20% fragmentation rate. On day 3, high-quality embryos had 8 to 12 blastomeres with less than 20% fragmentation rate.

Lower quality Embryos were classified as intermediate quality or degenerated. The pregnancy rate was assessed 12 days after the transfer of the embryos.

We excluded patients who had incomplete clinical records and those in whom frozen oocytes were used during ICSI.

DATA COLLECTION

The data collection started by examining the records of all patients who had undergone Assisted Reproduction Therapy (ART) with Intracytoplasmic Sperm Injection during the study period.

Then, the partners' sperm and clinical data were collected.

Also, we recorded all data on the stimulation process, fertilization results, embryos quality at day 1, 2 and 3, the number transferred and the pregnancy test on day 12 after transfer or transvaginal ultrasound.

STATISTICAL ANALYSIS

The variables were compared using Chi-Square and Fischer's exact tests. The error threshold was set at 5% as statistically significant for each variable studied.

The association between the variables was made using the Odds Ratio expressed with its confidence interval of 95%.

A logistic regression analysis was carried out to eliminate confounding factors. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 67 patients were included in the study with freshly ejaculated sperm being used in 42 patients using and cryopreserved ejaculated semen in 25 patients.

The mean ages were similar amongst women of groups I and II being 36.81 years ± 7.99years vs. 39.60 years ± 8.68 years respectively with p: 0.185 and for men being 42.61 years ± 7.95 years vs. 44.92 years ± 8.69 years; respectively with p: 0.273.

Table 1: Distribution of the study population by fertilization rates.

Fertilization Rates	Fresh Ejaculated Sperm (%)	Cryopreserved Ejaculated Sperm (%)	0R CI 95%	p-value
Total sperm	67,9	56,1	1,2 (0,9-1,5)	0,099
Normal Sperm	68,3	64,7	1,1 (0,8-1,4)	0,833
OATS Sperm	67,5	46,9	1.4 (1.01-2.1)	0,042

a) Comparison of fertilization rate between the fresh and the cryopreserved ejaculated sperm

When oligo-astheno-teratozoospermia (OATS) was present, the fertilization rate was higher in the fresh ejaculated sperm group 67.5% vs. 46,9%, than in the cryopreserved ejaculated group p=0,042 (Table 1).

b) Embryo quality in the fresh and the frozen-thawed ejaculated sperm

The quality of embryo was higher when freshly ejaculated sperm was used more specifically with total sperm and OATS.

Table 2: Quality of the embryos on day 1, day 2 and day 3.

Quality of Embryos	Fresh Ejaculated Sperm (n%)	Frozen Ejaculated Sperm n (%)	OR (CI 95%)	P value
Day 1				
Total sperm				
2PN	127 (77,5)	37 (22,5)	1.3 (1.02-1.6)	0.017
Normal sperm				
2PN	71 (76.34)	22 (23.66)	1.2 (0.8-1.6)	0.207
OATS Sperm				
2PN	56 (78.88)	15 (21.12)	1.5 (1.01-2.2)	0.034
DAY 2				
Total sperm				
Top quality	81 (81.41)	25 (36.8)	1.2 (0.8-1.7)	0.318
Intermediate quality	66 (35.9)	14 (20.6)	1.7 (1.1-2.9)	0.022
Normal sperm				
Top quality	42 (77.7)	12 (22.3)	1.2 (0.7-2.1)	0.551
Intermediate quality	37 (80.44)	9 (19.56)	1.4 (0.8-2.7)	0.304
OATS sperm				
Top quality	39 (75)	13 (25)	1.2 (0.7-1.9)	0.533
Intermediate quality	29 (85.29)	5 (14.71)	2.3 (1.1-5.4)	0.041
DAY 3				
Total sperm				
Top quality	34 (91.8)	3 (08.2)	1.7 (0.6-4.6)	0.311
Intermediate quality	30 (81)	7 (19)	0.6 (0.4-1.1)	0.185
Normal sperm				
Top quality	15 (88.23)	2 (11.77)	0.6 (0.2-1.4)	0.552
Intermediate	23 (95.83)	1 (04.17)	1.7 (0.3-8.7)	0.575
OATS sperm				
Top quality	19 (95)	1 (05)	5.1 (1.1-31.9)	0.008

Intermediate quality	7 (53.84)	6 (46.16)	0.3 (0.2-0.6)	0.008

Table 3: Distribution of the study population by the pregnancy rates.

Pregnancy rate	Fresh ejaculated sperm (%)	Frozen ejaculated sperm (%)	OR CI 95%	P Value
Total sperm	35	26,1	1,3 (0,6-3)	0,980
Normal Sperm	40	23,1	1,7 (0,6-5,4)	0,455
OATS Sperm	30	30	1 (0,5-2,1)	1,000

As far as pregnancy rate was concerned, the freshly ejaculated sperm was shown to have a similar pregnancy rate with the frozen-thawed ejaculated sperm regardless of the quality of Sperm used.

c) Pregnancy rates in the fresh and the frozen-thawed ejaculated sperm groups after ICSI

The pregnancy rate was similar in the fresh and the frozen-thawed ejaculated sperm as highlighted in Table 3, in total sperm as well as in the normal parameters and OATS subgroups of sperm.

DISCUSSION

Our objective was to evaluate the effect of sperm cryopreservation on fertilization rate, embryo quality and pregnancy rate in patients undergoing ICSI using either fresh or cryopreserved ejaculated sperm. Prior studies showed higher fertilization rates in ICSI after using freshly ejaculated sperm compared with cryopreserved sperm [6]. When evaluating the pregnancy rate, no significant difference was observed [1,9]. Recent studies showed no difference in terms of fertilization rate and embryo quality after ICSI with either fresh or cryopreserved ejaculated sperm used [1].

However, some of the studies published evaluated the effect of cryopreservation on sperm surgically retrieved from testis or epididymis compared with ejaculated sperm. The cryopreservation procedure has been shown to decrease sperm quality without affecting fertilization capacity and thus qualified as a reliable and favorable method with the same outcome as fresh semen.

Our results highlight that when the sperm sample had normal characteristics; the fertilization rate was similar to freshly ejaculated sperm and cryopreserved sperm. However, the fertilization rate was higher in a fresh ejaculated group when OATS was present. Our study also demonstrated higher quality embryo on day 3 in the fresh ejaculated group when OATS was present. In spite of these differences, the pregnancy rates were similar in the freshly ejaculated sperm and the frozen-thawed ejaculated sperm groups.

Our findings correlate with the idea that the cryopreservation procedure worsens damages present in OATS patients than in patients with fresh semen. Thus, we postulate that the frozen-thawed procedure worsens sperm parameters when OATS is already present before the procedure. But in the case of fertilization, this effect persists, impacting the quality of the embryo but doesn't affect the pregnancy rate. Probably because the embryos that were transferred had already survived under the deleterious effect of cryopreservation.

CONCLUSION

In conclusion, semen with OATS may be more susceptible to sperm cryopreservation damage, resulting in lower fertilization rate and embryo quality. However, the pregnancy rate was similar in the fresh and frozen-thawed ejaculated sperm group is it with the use of total sperm or in patients with normal or OATS sperm.

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