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ORIGINAL ARTICLE

Effect of injecting slowly motile sperms in ICSI cases with sex selection: a randomized controlled trial

Short title: PGD sex selection

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ABSTRACT

Objective. To compare injecting slowly motile sperms versus rapidly motile ones in PGD cases requesting sex selection for family balance.

Materials and Methods. A prospective, randomized controlled clinical trial was done from January 2020 to July 2022. 132 patients seeking ICSI for sex selection in Engab private infertility center, Alexandria, Egypt, were included. Spermatozoa with slowly progressive motility were selected and used for ICSI (group A=94), and spermatozoa with rapidly progressive motility were selected and used for ICSI (group B=38). Day 3 after fertilization, pre-implantation genetic diagnosis (PGD) regarding the gender of the resulted embryos were performed, recorded and compared.

Results. As regards the number of resulted embryos, there was no statistical significant difference between both groups (mean ± SD = 5.26 ± 2.17 for group A, 6.16 ± 2.76 for group B, P= 0.052). The percentage of male embryos to the total embryos was calculated and compared, but there were no statistical differences between groups (% mean ± SD = 36.29 ± 20.82 for group A, 33.99 ± 15.30 for group B, P = 0.442). On the other hand, there was a statistically significant difference when female embryo percentages were compared between groups, in favor of group B (% mean ± SD = 22.78 ± 22.73 for group A, 30.25 ± 20.45, P= 0.031).

Conclusions. Using slowly motile sperms in ICSI cases requiring sex selection, yielded more male embryos, but did not reach statistically significance. On the contrary, using rapidly motile sperms yielded more female embryos and achieved a statistical significant difference.

Key words

Intra-cytoplasmic Sperm Injection; family balancing; sex selection; pre-implantation genetic testing; ethics.

Introduction

The biologically normal sex ratio at birth ranges from 102 to 106 males per 100 females. Because of the greater natural vulnerability of boys, male mortality below 5 years of age is usually 10–20% higher than female mortality. As a result the child sex ratio is normally lower than the sex ratio at birth and this decline continues as the cohort ages, often resulting in a sex ratio below 100 (i.e. fewer men than women) in the older population [1].

Throughout history, people have attempted to control the sex of their children. In the beginning, sex selection was done for medical purposes. One reason is to avoid the risk of having a child with a sex-linked genetic disease such as Duchenne's muscular dystrophy or haemophilia. Sex selection for 'family balancing' was practiced afterwards. Couples that already had a child or more of one sex could use Microsort or pre-implantation genetic diagnosis (PGD) in attempt to get a child of the sex they do not already have. In a study done in the United States, the data suggest a strong sex selection towards males was confined to some ethnic groups of Chinese, Middle Eastern/Muslim and Indian origin and that no bias or a slight preference for females was observed among couples of Western origin [2].

Sex selection for IUI

MicroSort is a distinctive technique compared to PGD, it aims to separate X-carrying sperms from Y- carrying sperms, to use the desired type for intra-uterine insemination (IUI). It works by exposing sperms to a fluorescent dye. Then sperms are passed through a flow cytometer, which is able to sort the sperms on the basis of cell fluorescence. Sperms with an X chromosome glow more brightly as they contain more DNA. This technology has proven to be 93% effective for selecting girls and 82% effective for selecting boys [3].

The sorting procedure might cause variations in cleavage (0 to 89%) and blastocyst rates (3.5 to 28.8%) [4]. Sperm sexed by flow cytometer may have some damage due to alteration of mRNA expression patterns, semen fertility reduction [5], and ongoing pregnancy failure [6]. Therefore, other sperm sex selection methods with the purpose of preserving sperm viability have been introduced, such as the swim-up method (Madrid-Burry et al., 2003) [7], and Percoll™ density gradient centrifugation [8].

Sex selection for ET

Blastomere biopsy protocols (PGD) provided another even more precise means of determining gender, whereby human embryos could be classified before embryo transfer [9]. In PGD, a blastomere from a four- to eight-cell embryo is biopsied and subjected to DNA analysis either by fluorescent in-situ hybridization (FISH) for chromosome labeling, or by fluorochrome PCR based assay for specific gene identification. This does not negatively affect the viability of the embryo, which will be transferred if it has the desired sex [10]. In all cases, patients must be carefully counseled prior to this procedure since it is associated with a higher than anticipated failure rate related to lack of desired embryos for transfer. Table (1) compares PGD and sperm sorting for sex selection [11].

Based on the difference in swimming speed between X- and Y- bearing sperm [12], we hypothesized that Y- bearing sperms swim more slowly than X-bearing sperms. In this study, we aimed to compare injecting slowly motile sperms versus rapidly motile ones in PGD cases requesting sex selection for family balance.

Materials and Methods

A prospective randomized controlled clinical trial was done from January 2020 to July 2022. It was followed by data entry and data analysis that took two months ending in September 2022.The research got the approval of the Ethics Committee of Faculty of Medicine, Alexandria University for conducting this study and complied with the international research ethics guidelines. The study was registered at the Pan African Clinical Trial Registry (PACTR), trial number PACTR202304900872465. The sample size was calculated based on a previous study [13], using a margin of error 5% and alpha error of 0.05.
The minimum required sample size was 35 women in each group.

132 patients seeking ICSI for sex selection in Engab private infertility center, Alexandria, Egypt, were included in the study. A written consent was obtained from the participants after an explanation of the purpose of the research. Simple randomization was done by flipping a coin. For example, with 2 treatment groups, the side of the coin with the king determined the assignment to group A and the other side determined the assignment to group B.

Inclusion criteria: couples seeking ICSI for sex selection (males only) because of non-medical causes (family balancing), age of female partners: from 20–40 years old. Exclusion criteria: seeking ICSI PGD for female embryos, female factor infertility (ovarian reserve, tubal factor, endometriosis, hormonal disturbances or karyotype alterations); or male factor infertility (varicocele, hormonal disturbances, and genitourinary infection or karyotype alterations). The rationale for including only couples who seek sex selection for males, is mainly based on day by day clinical practice in Egypt, where the main desired child sex is a male, due to cultural beliefs and civil benefits.

Induction and oocyte retrieval: females underwent controlled ovarian hyper-stimulation with gonadotropins using GnRH-antagonist or GnRH-agonist protocols. Serial monitoring by serum estradiol measurements and ultrasound examinations. When at least two follicles measured 18 mm in diameter, Human Chorionic Gonadotropin (HCG) (5000-10,000 IU intramuscularly) was administered, and trans-vaginal ultrasound guided oocyte retrieval was performed 36 hours later.

Seminal sample preparation and sperm selection: Samples were obtained after 3 days of sexual abstinence, by masturbation at the laboratory and prepared by swim-up procedure in HAM's medium. For sperm microselection, 1 µL of the swim-up sample was placed in a 5-µL drop of 10% polyvinylpyrrolidone (PVP; Origio, Denmark). Sperms with an apparent normal morphology and slowly progressive motility were selected and used for ICSI (group A= 94), and spermatozoa with an apparent normal morphology and rapidly progressive motility were selected and used for ICSI (group B= 38).

In all cases, intra-cytoplasmic sperm injection (ICSI) was performed, and injected oocytes were incubated in (Global Total)® culture media which contains gentamycin sulfate and human serum albumin, under carbon dioxide and nitrogen conditions adjusted at PH= 7.3. All patients had an embryo biopsy performed on Day 3 after oocyte retrieval, by direct aspiration of a single blastomere through the zona pellucida. Pre-implantation genetic diagnosis (PGD) regarding the gender of the embryos was performed, and nuclear DNA was analyzed by Fluorescent In Situ Hybridization (FISH) using a 2-chromosome (X, Y) probe. Patients were counseled about the results prior to embryo transfer and/or cryopreservation on Day 5 of embryonic development. Embryo transfer was done for cases whom had one, two, or three embryos of the desired sex. For cases that had four embryos and more of the desired sex; two embryos were transferred and the remaining were cryopreserved for a future trial.

Data were collected and compared between the two groups; 1. Female patient age 2. The number of mature oocytes injected, 3. Number of biopsied embryos, 4. Number of male, female, or non-conclusive embryos according to PGD, 5. Number of embryos transferred or cryopreserved. 6.

Biochemical pregnancy rate in cases whom had embryo transfer. Our primary outcome was the percentage of male embryos and our secondary outcome was the pregnancy rate in both groups.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** **(**Armonk, NY: IBM Corp**)**.Qualitative data were described using numbers and percents. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Chi-square test for categorical variables, to compare between different groups. Mann Whitney test for abnormally distributed quantitative variables, to compare between two studied groups. The significance of the obtained results was judged at the 5% level.

Results

132 patients underwent 132 ICSI cycles, all participants had attempted one cycle only, and all of them desired male embryos for transfer. In all cases, patients had at least one child prior to the IVF/PGD cycle for sex selection. The two groups were comparable in age; (Mean ± SD. = 31.35 ± 3.70 for group A, and 28.50 ± 5.28 for group B, P= 0.055). There was a statistically significant difference in the number of meiosis 2 oocytes (M2), with higher oocyte numbers in group B than group A (mean ± SD. = 13.11 ± 6.01 and 10.57 ± 6.23, P= 0.012) respectively, but there was no statistical difference between groups in total number of fertilized embryos or fertilization rate, (P =0.052 and 0.251, respectively) as shown in table (2).

A total of 728 embryos (494 of group A + 234 of group B) were biopsied for PGD. The biopsy results included 260 male embryos (35.7%), 190 female embryos (26.1%), and 278 (38.1%) embryos that were non-conclusive for genetic gender. There was no statistically significant difference between the two studied groups as regards the total numbers of male or non-conclusive embryos, (P =0.210 and 0.382, respectively) but the total numbers of female embryos were significantly higher in group B (P = 0.019), as shown in figures (1), (2) and (3). All embryo transfers (ET) occurred at the blastocyst stage of development on Day 5 after fertilization. A total of 224 embryos were transferred; 152 in group A, 72 in group B, and 36 embryos were cryopreserved for later transfers. There were no statistically significant differences in transfer rate between groups, but cryopreservation was significantly higher in group A, (P =1.000 and 0.006, respectively) as shown in table (3).

When we compared both groups as regards the mean and median of male and female embryos, the absolute numbers and the percentages of male embryos / total embryos did not give a significant difference in both groups (P = 0.434 and 0.442, respectively) but again, there was a significant difference in the absolute number and percentages of females / total embryos between the two groups, with higher female embryos and percentages in group B, (P = 0.31and 0.31, respectively). When we subtracted the number of males from the number of females for each woman, there were more male embryos than females for women of group A (Median = 1.0 (0.0 - 2.0)). Although the difference did not reach statistical significance, it was almost approaching (P= 0.069), as shown in table (4) and figure (4).

When we considered "slow sperm motility" as a subjective test to predict male embryos, we found its positive predictive value = 61.33%, negative predictive value = 49,33%, sensitivity = 61,33%, specificity = 49,33%, and the test accuracy = 57,33%.

Embryo transfer was done in 122 cases (92.4%); 84 cases (89.4%) in group A and 38 cases (100%) in group B. In 10 cases (10.6%) of group A the embryo transfer was cancelled due to the lack of normal embryos of the desired male sex for transfer. There were no statistical differences between groups in ET rate or cancellation rate, (P =0.062 and 0.062 respectively). The total biochemical pregnancy rate per transfer was 39.3% (48/122), as evidenced by B HCG testing done

14 days later. For the 84 cycles of group A, 36 had a biochemical pregnancy (42.9%) and for the 38 cases of group B, 12 had a biochemical pregnancy (31.6%). Again, there were no statistically significant differences in pregnancy rates between groups (P =0.238), as shown in Table (5) and Figure (5).

Discussion

While sex selection of embryos for medical indications is well accepted, controversy arises regarding sex selection for gender preference purposes. General speaking, sex selection for nonmedical indications should not be encouraged [14].

Cultures in which male children are expected to provide continued familial loyalty, heritage and support to elderly parents, we can understand such a strong preference for male children. In Egypt and all similar Islamic countries, giving a baby up for adoption as well as voluntary abortion are prohibited by religion and criminalized by law, so the only legal way to get more boys is to perform ICSI followed by PGD. The International Islamic Fiqh Academy and the Holy Azhar [15] have allowed non-medical sex selection in restricted circumstances, such as family balancing. If sex selection is used only with such intention, there would be no big effects on the equilibrium of both sexes in the population. Also, this may lead to a smaller family size and, therefore, a reduction in overall births with all its beneficial economic effects for the country.

In Egypt, some families may continue to have children until they finally get a son. Such preference frequently elicits emotional reactions concerning issues of valuing all children – not only boys- as a gift of God. Sex selection in favor of boys is a feature of pervasive social, cultural, political and economic injustices against women, and a manifest violation of women's human rights [16]. At the same time, it is still a human right to choose to get a child or not and to choose to get that child as a boy or a girl, such a wish that we cannot allege being harmful to others by any means!! In "On Liberty", John Stuart Mill argued that the only purpose for which someone's freedom can be constrained is to protect other individuals [17].

In our study, we included 132 cases wishing sex selection for non-medical family balancing proposes, all requiring boys only. Noteworthy, during the recruitment phase, only 5 cases were excluded because of their desire for females not males! Those strong determinations and great hopes, have made patient counseling a critical phase of utmost importance, to describe the plan of action, steps, and what shall we expect after all those complex procedures and high costs?

Nature has developed many mechanisms to make genetically different sperm phenotypically identical within a male to avoid a fertilization advantage of one allele over another. The only de novo difference identified between X and Y sperm to date is in their DNA content. Studies have shown that the X and Y sperm DNA content differ by about 2.7–2.8% [18], which might be responsible for the differential expression of some genes and proteins. However, it is still not proven whether this difference may result in other physical, chemical, or functional differences between the two types of sperms.

Despite these functional similarities, differences in X- and Y-sperm mobility have been extensively studied, with conflicting results. Some researchers reported that the mobility of human sperm in the stationary fluid was not different between X-sperm and Y-sperm, the movement of X-sperm, but not Y-sperm, shifted to the nearly straight path in a flow-stream protocol [19]. Others studied the effects of specific in vitro conditions, such as low pH, high temperature, and high oxidative stress on the motility of X-sperm and Y-sperm. The motility of Y-sperm rapidly decreased in these conditions compared with that of X-sperm [20].

We hypothesized that Y-sperms move slower in sperm micro-selection media (a 5-µL drop of 10% poly vinyl pyrrolidone (PVP; Origio, Denmark)). Spermatozoa with normal morphology and slowly progressive motility were selected and used for ICSI (group A =94) with the intention of getting

more males, and Spermatozoa with normal morphology and rapidly progressive motility were selected and used for ICSI (group B =38) for comparison. We did not observe any statistical difference between groups in fertilization rate, total number of fertilized embryos, embryo transfer rate, cancellation rate and biochemical pregnancy rate.

A total of 728 embryos (494 of group A + 234 of group B) were biopsied for PGD. The biopsy results included 260 male embryos (35.7%), 190 female embryos (26.1%), and 278 (38.1%) embryos that were non-conclusive for genetic gender. Again, there was no statistically significant difference between the two studied groups as regards the total numbers of male or non-conclusive embryos, (P =0.210 and 0.382, respectively), but the total numbers of female embryos were significantly higher in group B (P = 0.019), which indicates that X-sperms actually move faster and can be correlated with more female embryos after ICSI PGD. This simple subjective test has a sensitivity equals 61,33%, specificity equals 49,33%, and the test accuracy equals 57,33%.

On the other hand, the cryopreservation percentage was higher in group A, with statistical significance (P= 0.006). Also by subtracting the number of males from the number of females for each case, there were more male embryos than females for the cases of group A (median = 1.0 (0.0 - 2.0)). Although the difference did not reach statistical significance between groups, it was almost approaching it (P = 0.069). These results indicate that using the slowly moving sperms gave us more male embryos for cases of group A, which were available for fresh transfer and cryopreservation, with a statistically significant difference more than group B.

Our results agreed with Balli K. S. et al. 2004 [21], who observed the sperm curvilinear velocity in seminal plasma parameter, with a cut-off value set at less than 49μ/ sec can correctly predict male off-springs with 75% accuracy. Faster sperm were associated with female offspring. Similar results were reported by Samura O. et al 1997 [22], who confirmed higher sperm velocities in X-sperm enriched fractions, this can vary according to sperm separation method, culture media and flow conditions. On the contrary, Sarkar et al. 1984 [19] reported that human X sperm move slower (angular velocity decrease) than Y sperm in the flow stream. However, the movement of both cells is similar in the stationary fluid.

A completely different theory was studied, it hypothesized that the Y-bearing chromosome is faster than the X- bearing chromosome because the X sperm has more DNA than the Y sperm which results in a different migration velocity [12]. Based on this theory, many researchers studied the different proportions of Y-sperms and X-sperms in the supernatant of the swim-up procedure with conflicting results. While some stated that the supernatant of the swim-up procedure contained more Y sperm (Check and Katsoff, 1993 [23], Check et al., 1994 [24]). Bottcher-Luiz *et al*. 1997 [25], and Lucio A.C. et al. 2012 [26], observed 69.6% and 61.80% in favor of X-bearing sperm in the supernatant, respectively.

Rawlings et al. (for review refer to De Jonge et al., [28]) reported that after swim-up in the supernatant two fractions could be observed: the upper fraction enriched with X- bearing spermatozoa (64%) and the lower fraction enriched with Y- bearing spermatozoa (60%). Others; (De Jonge et al. 1997 [27], Madrid- Bury et al. 2003 [7] and Yan et al. 2006 [12]) have failed to demonstrate any differences in X- and Y- bearing sperm proportion after swim up [7,12,27].

In an interesting study, Umehara et al. 2019 [28] reported that ligand activation of Toll-like receptors 7/8 (TLR7/8), selectively encoded by the X chromosome, obviously suppresses the motility of X-bearing sperms without altering their fertilization capacity. This procedure allows producing over 90% of the male embryos following in vitro fertilization using ligand-selected highly motile sperms [28].

The ambiguity in the available findings may be a result of the use of less-specific methods for distinguishing between X and Y bearing sperms. Therefore, Intra-cytoplasmic Sperm Injection followed by pre-implantation genetic diagnosis is considered the most efficacious procedure for

sex selection, compared to sperm sorting, swim-up method, and Percoll™ density gradient centrifugation [29]. Couples seeking PGD for sex selection have reduced success rates when compared to the traditional ICSI cycles with a higher cancellation rate due to a lack of embryos of the desired sex, arrested embryonic development, or abnormal genotyping.

ICSI-PGD sex selection for family balancing will always remain controversial for many people, who deny the use of science in such ways that may alter nature as we know it. Although it is more invasive and leads to discarding normal embryos of the un-desired sex, it is still more ethical than a prenatal diagnosis of fetal sex followed by the induction of abortion, infanticide or putting the baby up for adoption. The strong male preference observed in our sample of the Egyptian community, reflects an obvious violation of women's human rights, that cannot be resolved just by prohibiting sex selection procedures, but by improving the status and value of women in the community and encouraging their complete autonomy.

Study strength

To the best of our knowledge, this study was the first of a kind to test a physical characteristic; "sperm motility" for distinguishing Y and X bearing sperms used for ICSI-PGD cycles in Egypt. Most of the cases recruited were requesting male embryos, which minimized the exclusion of cases.

Study limitations

All patients sought a procedure that is very expensive and not covered by health insurance services. The procedure is associated with a higher than anticipated failure rate related to a lack of desired embryos for transfer, so counseling was of utmost importance. We recorded and calculated the biochemical pregnancy rate only because we were unable to trace most of the patients to detect the clinical pregnancy rate afterwards, mostly due to remote residency areas and a lack of contact with the center after completing the procedure.

Future study perspectives and recommendations

We need to design a study in which the cut value of sperm velocity can be precisely measured and test different physiological sperm motility characteristics. Larger studies with a larger number of patients are needed to properly evaluate this simple procedure, which may be of help in cases where non-medical sex selection is requested.

Conclusion

Our technique was able to simply distinguish between the Y and the X bearing sperms by a physical characteristic; "motility". We found a statistically significant difference in the number of female embryos produced by using the rapidly motile sperms for ICSI. Also, there was an increase in the number of male embryos produced by using slowly motile sperms for ICSI, that were available for transfer and cryopreservation for cases requiring male siblings. Although the procedure is not 100 percent accurate, it is simple, non-invasive, and adds no cost to the already expensive ICSI-PGD procedures. Further studies using more specific, non-invasive methods to distinguish between the Y and X-bearing sperms are warranted.

Compliance with Ethical Standards

Author Contributions: M. H. K.: Conceptualization. Methodology and Data collection: M. H. K., W. S. E. Statistical Analysis: S. E. Writing–Original Draft Preparation: S. E. Reviewing & Editing: S. E., W. S. E. All authors have read and agreed to the published version of the manuscript.

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Disclosure of interest: The authors have no conflicts of interest to declare.

Ethical Approval: The study was approved by the ethical committee of faculty of medicine Alexandria University, Egypt.

Trial registration: the trial was registered at the Pan African Clinical Trial Registry (PACTR), trial number PACTR202304900872465.

Informed consent: was taken from each candidate in the study

Data sharing: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table (1): comparison between PGD and sperm sorting for sex selection [11].

	PGD	**Sperm sorting**
Invasiveness	Highly invasive - requires women to undergo IVF treatment involving intensive hormone treatment and extraction of eggs.	Requires only the collection of a sperm sample from the man, followed by artificial insemination.
Ethical issues	Ethical issues arise over the fate of unneeded embryos. .	Only sperm are manipulated in the laboratory
Pregnancy rate	~20% per cycle.	~16-25% per cycle
Reliability	Nearly all pregnancies are with a child of the desired sex.	A child of the desired sex is produced in 70- 90% of pregnancies.
Safety	Insufficient number of births to draw statistically significant conclusions on safety.	Insufficient number of births to draw statistically significant conclusions on safety
Cost	From £4000.	UK clinics charge £4000. Fees in the US are much lower – starting from ~£360

Table (2) comparison between the two studied groups as regards; Age, total number of embryos and fertilization rate.

	Group A (n = 94)	**Group B (n = 38)**	**U**	**P**
Age				
Min. – Max.	26.0 – 37.0	21.0 – 39.0		
Mean ± SD.	31.35 ± 3.70	28.50 ± 5.28	t=1.977	0.055
Median	31.50	28.0		
M2 Oocytes				
Min. – Max.	2.0 – 31.0	3.0 – 25.0		
Mean ± SD.	10.57 ± 6.23	13.11 ± 6.01	1290.0*	0.012*
Median (IQR)	9.0 (6.0 – 14.0)	11.0 (8.0 – 19.0)		
Embryo				
Min. – Max.	2.0 – 10.0	2.0 – 14.0		
Mean ± SD.	5.26 ± 2.17	6.16 ± 2.76	1404.0	0.052
Median (IQR)	5.0 (4.0 – 7.0)	6.0 (5.0 – 7.0)		
Fertilization rate%				
Min. – Max.	18.75 – 100.0	11.76 – 100.0		
Mean ± SD.	57.45 ± 20.26	52.46 ± 19.28	1558.0	0.251
Median (IQR)	57.14 (42.11 – 66.7)	52.63 (40.0 – 62.5)		

U: Mann Whitney test, IQR: **Inter quartile range,** SD: **Standard deviation** p: p value for comparing between Group **A** and **B**

*: Statistically significant at $p \leq 0.05$

Table (3) comparison between the two studied groups as regards embryonic parameters.

Embryos	**Group A (n=494)**	**Group B (n=234)**	**Total (n=728)**	**□²**	**P value**	**χ^2: Chi square test**
Males	184 (37.2%)	76 (32.5%)	260 (35.7%)	1.572	0.210	
Females	116 (23.5%)	74 (31.6%)	190 (26.1%)	5.458*	0.019*	p: p value for comparing between the studied groups
Non-conclusive	194 (39.3%)	84 (35.9%)	278 (38.1%)	0.766	0.382	
Transfer	152 (30.8%)	72 (30.8%)	224 (30.8%)	0.000	1.000	*: Statistically significant at p ≤ 0.05
Cryopreservation	32 (6.5%)	4 (1.7)	36 (4.9%)	7.681*	0.006*	

Table (4): Comparison between the two studied groups according to number and percentage of embryos for the two studied group

All cases	Group A (slow) (n = 94)	Group B (progressive) (n = 38)	U	p
Male				
Min. – Max.	0.0 – 6.0	0.0 – 4.0		
Mean ± SD.	1.96 ± 1.39	2.0 ± 0.93	1636.0	0.434
Median (IQR)	2.0 (1.0 – 3.0)	2.0 (1.0 – 3.0)		
% Males/embryo				
Min. – Max.	0.0 – 75.0	0.0 – 66.70		
Mean ± SD.	36.29 ± 20.82	33.99 ± 15.30	1634.0	0.442
Median (IQR)	37.50 (25.0 – 50.0)	33.30 (21.4 – 40.0)		
Female				
Min. – Max.	0.0 – 5.0	0.0 – 8.0		
Mean ± SD.	1.23 ± 1.30	1.95 ± 1.90	1372.0*	0.031*
Median (IQR)	1.0 (0.0 – 2.0)	2.0 (1.0 – 2.0)		
% Females/embryo				
Min. – Max.	0.0 – 80.0	0.0 – 66.70		
Mean ± SD.	22.78 ± 22.73	30.25 ± 20.45	1372.0*	0.031*
Median (IQR)	20.0 (0.0 – 37.5)	33.30 (14.3 – 50.0)		
Difference between males and females				
Min. – Max.	-5.0 – 5.0	-5.0 – 3.0		
Mean ± SD.	0.72 ± 2.03	0.05 ± 1.84	1430.0	0.069
Median (IQR)	1.0 (0.0 - 2.0)	0.0 (-1.0 – 1.0)		

U: Mann Whitney test, IQR: **Inter quartile range,** SD: **Standard deviation** p: p value for comparing between Group **A** and **B**, *: Statistically significant at p ≤ 0.05

Table (5) comparison between the two studied groups as regards: transfer rate, cancellation rate, cryopreservation and pregnancy rate.

case	**Group A (n=94)**	**Group B (n=38)**	**Total (n=132)**	**□²**	**P value**	**χ^2: Chi square test**
Transfer	84/94 (89.4%)	38/38 (100%)	122/132 (92.4%)	4.374	^{FE}p=0.062	FE: **Fisher Exact**
Cancellation	10/94 (10.6%)	0/38 (0.0%)	10/132 (7.6%)	4.374	^{FE}p=0.062	p: p value for comparing between the studied groups
Cryopreservation	12/94 (12.8%)	2/38 (5.3%)	14/132 (10.6%)	1.607	^{FE}p=0.349	*:
Pregnancy	36/84 (42.9%)	12/38 (31.6%)	48/122 (39.3%)	1.395	0.238	

Statistically significant at p ≤ 0.05

Figure (1) distribution of male /female /non-conclusive embryos in group A.

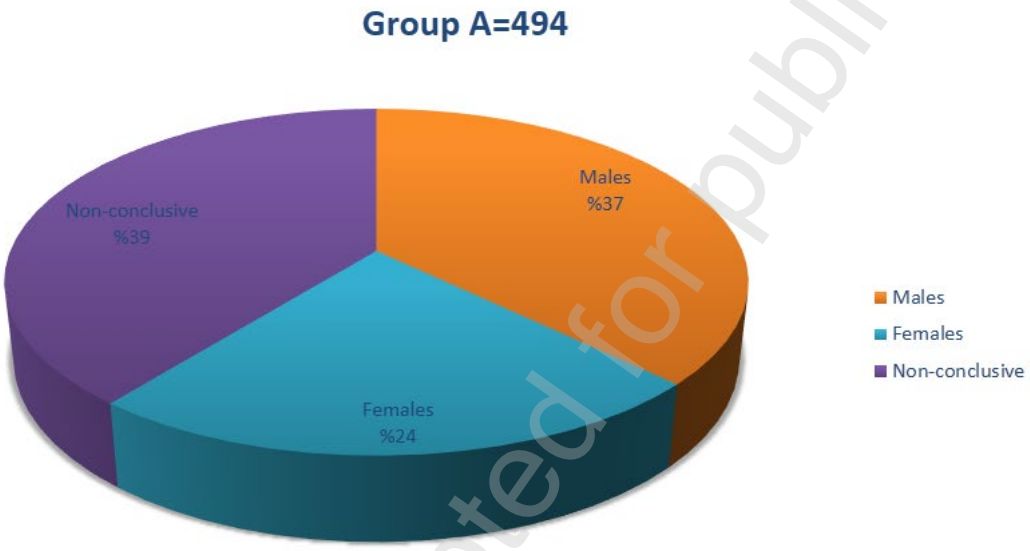

Figure (2) distribution of male /female /non-conclusive embryos in group B.

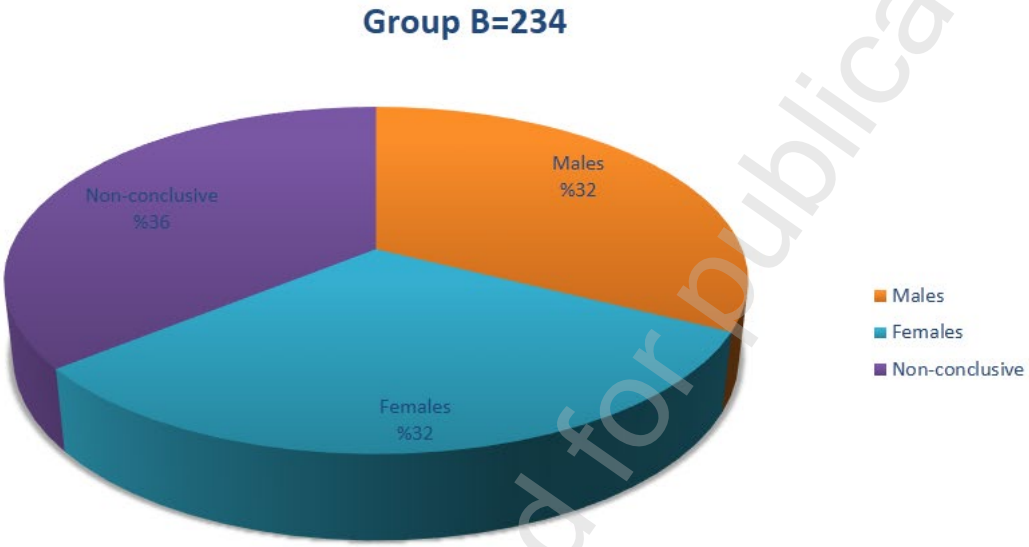

Figure (3) diagrammatic distribution of male /female /non-conclusive embryos in both groups.

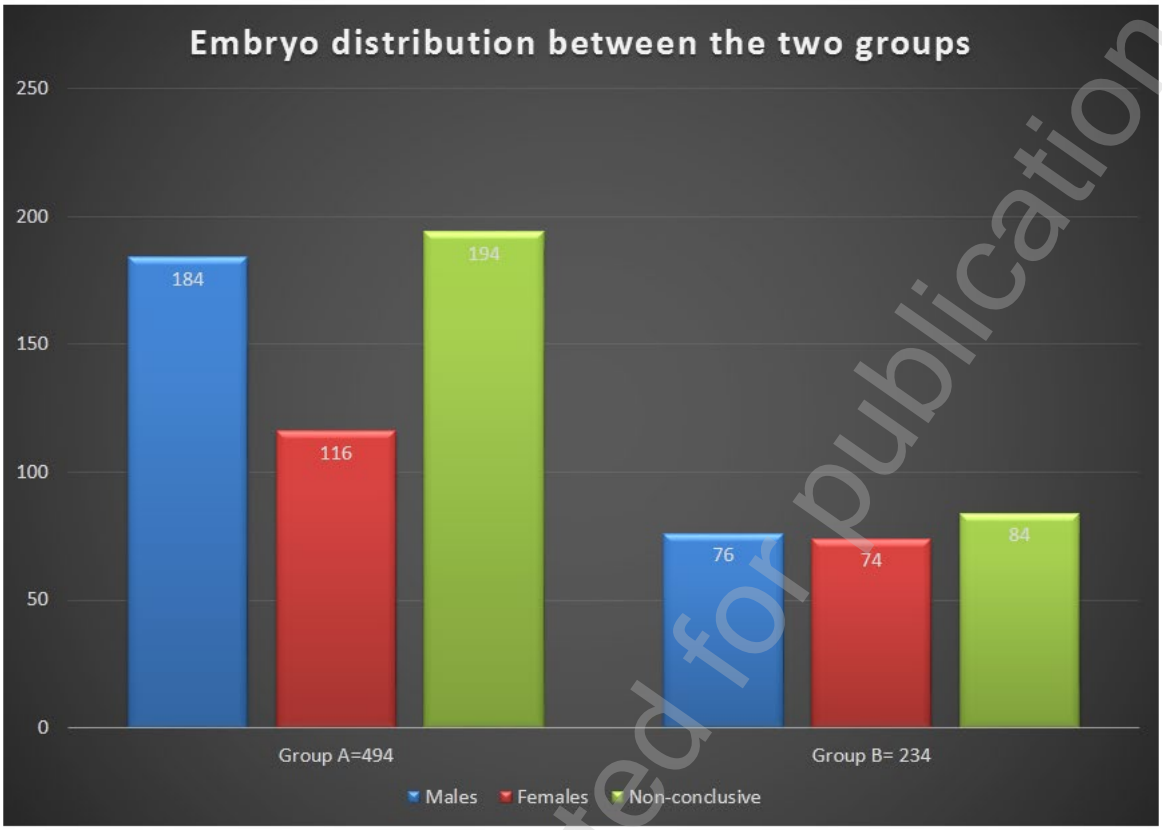

Figure (4): Comparison between the two studied groups according to difference between males and females for all cases.

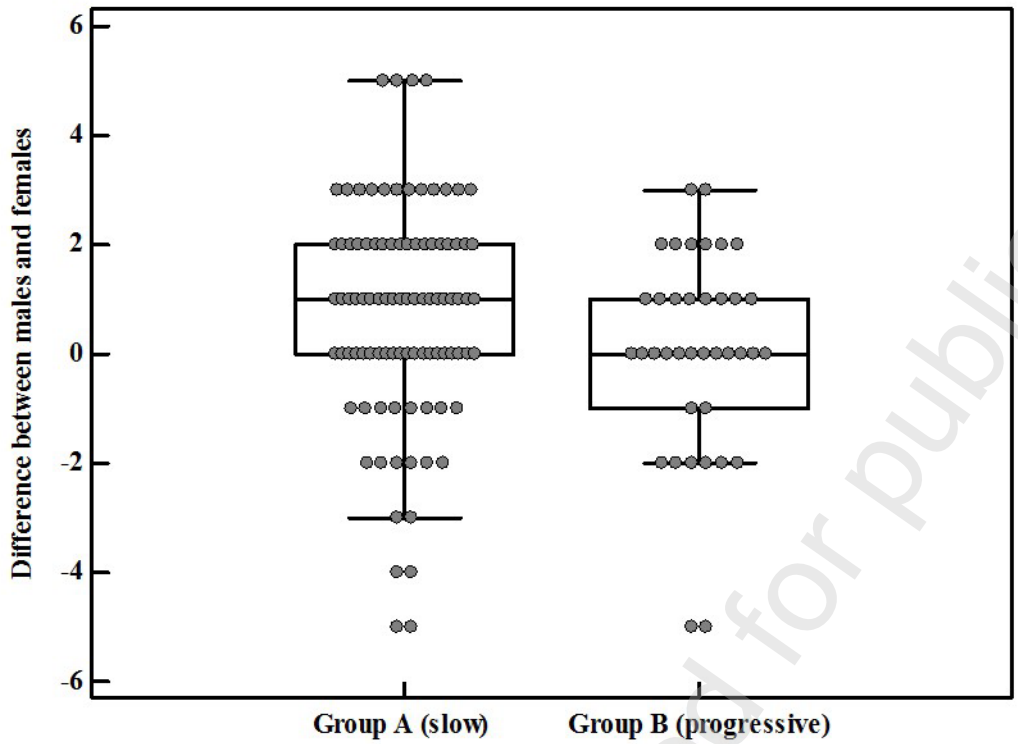

Figure (5) schematic representation of results and fate of biopsied embryos.