West Asian Journal
Of Medical and Biological Sciences

Evaluation of the Efficacy of the modified Swim-Up Technique for sperm sex sorting

Saadi Shihab Dheyab Mustafa, Dr. Mowafak Khalil Hassan
Biology Department - College of Science- Mosul University - Iraq
Corresponding Author: Saadi Shihab Dheyab Mustafa - slivanisaadi@gmail.com

ARTICLE INFO

Received: 09 Jun
Accepted: 03 Aug
Volume: 1
Issue: 3

ABSTRACT

The development of non-invasive approaches for the sperm sex sorting remains an area of considerable interest in assisted reproductive technologies. Although X- and Y-bearing spermatozoa are genetically distinct, the subtle differences in their physical characteristics can be exploited for sperm sex enrichment remains controversial. The swim-up technique primarily selects spermatozoa depending on motility and migration capacity and may therefore provide a potential basis for differential enrichment when its conventional protocol is appropriately modified. By using this technique, the faster spermatozoa can be separated which are believed to carry a higher percentage of Y- chromosome based on hypotheses proposed first in the sixties of last century.This prospective study aimed to comparatively evaluate the modified swim-up based techniques to differentially enrich Y-chromosome-bearing spermatozoa in human semen samples and to determine whether modification of sperm migration conditions could induce a measurable shift in the Y bearing spermatozoa ratio .The study included 93 couples, undergoing ICSI/PGT-A protocol, involving 93 semen samples and a total of 1247 oocytes divided into 2 groups .Semen samples were obtained from 93 participants and analysed according to standard laboratory criteria. Each sample subjected to a modified swim-up protocol designed to enhance the selection of highly progressive motile spermatozoa. After ICSI procedure and embryo formation, the proportions of XY and XX yielded embryos were determined using NGS technique .As a result, the higher proportion of XY embryos was associated with swim-up techniques with a percentage of 39.34%, compared to the non-swim-up approaches (31.98%), and the difference was statistically significant with a p-value of 0.025. These findings indicate that sperm migration under the modified conditions was associated with differential enrichment rather than absolute separation of X- and Y-bearing sperm populations.

KEYWORDS: modified swim-up; sperm sex sorting; X-bearing spermatozoa; Y-bearing spermatozoa; sperm preparation; assisted reproduction.

Introduction

Interest in influencing the sex of future progeny has long existed across many human civilisations. Historically, many cultures and belief practices have sought possibilities to determine whether a male or female offspring could be conceived. Early records go back as early as 402 BC, documenting some Greek scholars such as Anaxagoras who had claimed that the right testicle is solely for male production whereas the left one is for conceiving female offspring (Kubiak et al., 2020).

As a result, the ancient methods practised by the many cultures such as Greece, China and Egypt for determining a particular sex have had no biological basis such as spiritual practices or dietary regimens (Farhud, 2022; Aynalem et al., 2023).

spermatozoa originally become completed germ cells in the testes, yet mature in the epididymis where they gain their motility and capacity for oocyte fertilisation. Genetically, they consist of sex chromosomes and are categorised into X spermatozoa and Y spermatozoa and proved to carry the genomic content responsible for sex determination. Although X and Y spermatozoa have the same origin and undergo the same maturation process; there are, nonetheless, some nuanced differences between the two types (Whitfield *et al*, 2024).

Some previous studies have claimed a significant difference between X and Y sperms. Recent studies, however, have refuted the former proposal and claim that there is a slight or no difference in terms of their shape, size, motility, strength, swimming patterns, electric charge and pH (He *et al*, 2022).

A host of other studies confirm the previous findings; however, they suggest that the X- and Y-bearing spermatozoa dissimilarity is in their genome content. In this respect, a set of proteins and genes show differential expression between X and Y cell types, as revealed by recent proteomic and genomic studies, and this difference, as a result, may contribute to the genomic or protein differential expression between the two cell populations (Rahman and Pang, 2020; Cheng *et al*, 2024; Bai *et al*., 2025).

By using the modified swim-up technique, the faster spermatozoa can be separated which are believed to carry a higher percentage of Y- chromosome based on hypotheses proposed first in the sixties (Shettles, 1960; Sarkar *et al*, 1984).

It has also been claimed that a statistically significant gender preselection was reported when a modified swim-up technique was supplemented with additional monitoring (Khatamee *et al*., 1999; Azizeddin *et al*., 2014; Asma-ul-Husna *et al*., 2017).

Some researchers regard the technique as still experimental which needs further development because it can be modified or adjusted, potentially resulting in variable outcomes (Naidu *et al*., 2025).

One significant factor that was oft-neglected in the studies demonstrated above is the incubation time. Put simply, extending the incubation time in the swim-up technique would allow both fast- and slow-moving sperm to reach the upper layers of the culture medium at the time of collection.

In a similar manner, another second significant factor is the depth of the culture medium. A shallow column of culture medium may allow slower spermatozoa to reach the upper layer within a shorter time, and eventually get mixed with fast-moving ones. Further, the last significant factor is the difficulty to deal with small volumes of semen and culture medium without mixing them during preparation with a pipette.

The issues above will be addressed in this study in order to carry out the technique in an optimized manner to achieve a more controlled separation of the fastest spermatozoa. This could be done by increasing the competitive swimming distance required for sperm to reach the upper layer of the culture medium between 10 and 12 cm. The arrival of the first batch of sperm is closely monitored, after which they are isolated and eventually analysed. In other words, the time for separating the samples varies and a fixed incubation time is not applied to all. Rather, each sample has its own time interval ranging from 5 to 10 minutes based on the sample's assessment as regards the sperm velocity, motility, and concentration.

The upper portion is then used for ICSI for further evaluation of the technique in terms of its efficiency in term of the potential deviation in Y bearing sperm ratio.

Methodology:

Study Design

This is a prospective experimental study carried out to examine the possibility of sperm sex sorting utilizing modified swim-up based techniques (pellet swim-up, density gradients and direct swim-up) comparing with non-swim-up based techniques (simple wash and microfluidics). Followed by ICSI/PGT-A to determine the ratio of XX and XY yielded embryos.

Study Population

The study was conducted primarily at Dr. Atyaf IVF Centre in Erbil-Iraq between September 2025 and April 2026. 93 couples, undergoing ICSI/PGT-A protocol involving 93 semen samples and a total of 1247 mature oocytes divided into 2 groups, swim-up based techniques (pellet swim-up, direct swim-up and PICSI technique) and non-swim-up based techniques (microfluidic, density gradients and simple wash)

The inclusion criteria comprised normozoospermic males according to WHO (2021). In addition, female participants aged less than 38 years with a good ovarian response were included.

Regarding the exclusion criteria, couples not undergoing PGT-A as well as azoospermic and oligozoospermic males were excluded. Selecting cases with homogenous, similar study-related variables contributes to obtaining reliable and accurate results.

Semen Collection and Analysis

Semen Samples were collected after 2-5 days of abstinence. Seminal fluid analysis was carried out following WHO 2021 guidelines. microscopical examination for motility, morphology, and count was performed using a CASA system (LensHooke R10 Plus analyser).

MESR
منار الشرق للدراسات والأبحاث
Manar Elsharq for Studies & Research

Pellet Swim-up

This technique is based on the sperm ability to swim upward from the pellet formed from centrifugation into the overlying sperm culture medium. (Mahadevan & Baker, 1984).

Methodology:

1. 1.0 mL of semen was placed in a conical bottom tube and mixed with 2.0 mL of Vitrolife G-MOPS™ PLUS medium.

2. The mixture centrifuged for 7 minutes at 1400 rpm.

3. The supernatant was then discarded, and the resulting pellet was dismantled.

4. Then 5.0 mL of the sperm medium was gently overlaid above the pellet without mixing, which was then placed in the incubator.

5. After 10 minutes, motile spermatozoa were observed reaching the upper layer of the culture medium, a small volume then taken for ICSI procedure

6. The technique was extended in order to obtain the most highly motile spermatozoa.
The sample was placed at the starting point of a 6.0 cm medium track in an ICSI dish, allowing the migration of the fastest sperm to the other end.

7. The highly motile sperm were then collected using an injection pipette and transferred into a droplet of polyvinylpyrrolidone (PVP) within the same ICSI dish, where the best morphologically sperm were collected for injection.

8. In this way, the selected spermatozoa were those that had traversed a total migration distance of 12 cm. The oocytes were then injected which will be later described.

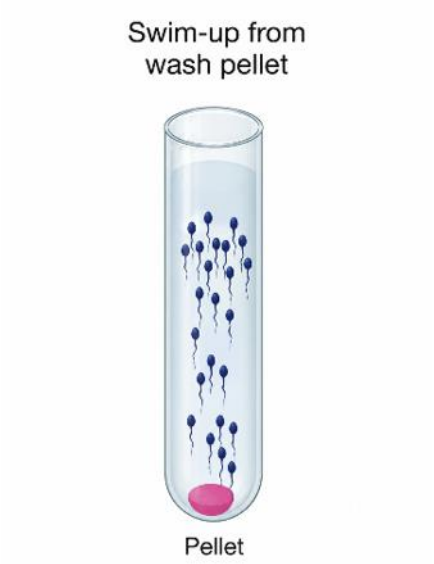

Figure (1) Pellet Swim-Up Technique (Christanto *et al*., 2024)

MESR
منار الشرق للدراسات والأبحاث
Manar Elsharq for Studies & Research

Direct Swim-up

Centrifugation is not used in this technique and the sperm directly migrate from the raw semen sample upward into the overlying culture medium. The technique is, therefore, similar to the microfluidic technique in this regard. This technique is considered the fastest, most cost-effective, and least-labour intensive; therefore, the decision of using it is based on the preliminary assessment of the sample as it requires normal motility and sperm count (Ješeta *et al*., 2025; Wen *et al*., 2025).

Procedure:

1. After liquefaction of the semen sample as previously described, 1.0 mL was placed in a round-bottom tube and gently overlaid with 5.0 mL of the sperm culture medium to avoid mixing it with the semen.
2. The tube was then placed vertically in a specialised incubator at 37 °C.
3. After 10 minutes, the arrival of the first batch of spermatozoa was observed.
4. The upper layer containing the most motile swimming spermatozoa was separated within 10-15 minutes.

5. The technique was extended in order to obtain the most highly motile spermatozoa.
6. The sample was placed at the starting point of a 6.0 cm medium track in an ICSI dish, allowing the migration of the fastest sperm to the other end.
7. The highly motile sperm were then collected using an injection pipette and transferred into a droplet of polyvinylpyrrolidone (PVP) within the same ICSI dish, where the best morphologically sperm were collected for injection.
8. In this way, the selected spermatozoa were those that had traversed a total migration distance of 12 cm. The oocytes were then injected which will be later described.

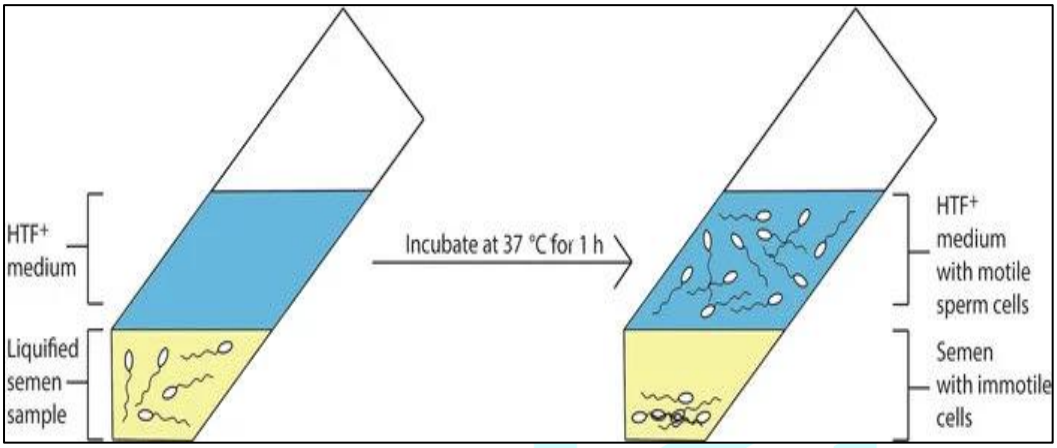

Figure (2) Direct Swim-Up Technique (Christanto *et al*., 2024)

Simple wash method:

Semen samples are prepared using simplified swim-up, or simple washing method, involving brief centrifugation followed by layering a small amount of culture media over the pellet to collect all motile spermatozoa, both fast and slow, within a short time. This method is widely adopted in IVF centres due to its simplicity, efficacy, speed, and cost-effectiveness (Fleming *et al*., 2025).

Procedure:

1. The sample was collected, liquefied, and analysed according to the 6th edition (2021) WHO guidelines.
2. 1.0 mL of the sample was mixed with 2 mL of sperm media in a 15 mL conical bottom tube.
3. Centrifugation was applied at 1400 rpm for five minutes.
4. The supernatant was discarded and the remaining pellet was gently resuspended.
5. 0.5 of sperm media was layered over the pellet or directly mixed, the tube was placed in an inclined position inside an incubator till the ICSI procedure was performed.

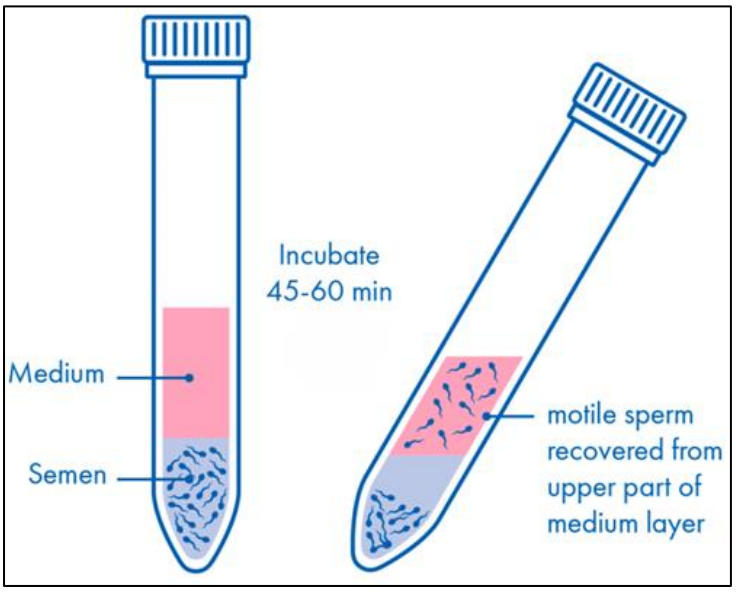

Figure (3) Swim-Up Technique (Fleming *et al*., 2025)

PICSI Technique:

This technique of sperm sorting depends mainly on the physiological of mature sperm to bind with hyaluronan or hyaluronic acid (HA). The method basically simulates conditions of natural fertilisation, where the selection occurs in vivo. The spermatozoa capable of binding with HA are the mature ones with lower rates of DNA fragmentation and reduced levels of chromosome abnormalities (Shivhare *et al*., 2024)

The technique includes swim-up step before applying ICSI dish; therefore, it included in swim-up based technique group in this study.

Procedure:

1. The semen sample was first prepared by swim-up method for 10 minutes to collect the upper layer enriched with high motile spermatozoa.
2. Under sterile conditions, the PICSI dish, which is pre-coated with hyaluronan microdots, was removed from its packaging, and each microdot was covered with 10 µL of G-MOPS™ PLUS sperm medium.
3. Droplets from the same medium in the dish were prepared for subsequent oocyte placement and injection.
4. A small droplet of PVP was added beside each culture medium droplet.
5. All the droplets were then overlaid with prewarmed mineral oil to avoid evaporation.
6. The dish was then placed in the incubator at 37°C.
7. The solid HA microdots were left to hydrate and swell for 10 minutes.
8. 1–2 µL of prepared sperm suspension was placed adjacent to the HA dots,
9. The dish was incubated for 10–15 minutes at 37°C.
10. Motile sperm that migrate toward and bind to the HA microdots were selectively aspirated using an ICSI micropipette, while free-swimming non-bound spermatozoa were overlooked.
11. The aspirated spermatozoa were transferred into a PVP droplet, leading to slowing sperm motility due to the resulting viscous medium. This will allow us to better handle the sample.
12. The ICSI needle was again used to handle the spermatozoon and inject the oocyte.

13. The injected Oocytes were transferred to their designated culture medium and placed in the incubator, where fertilisation, cleavage, and subsequent procedures were monitored in the following days, which will be described later.

Microfluidic Sperm Separation Procedure

this method is centrifugation-free and, therefore, no mechanical stress is exerted on the sperms. Instead, spermatozoa with high motility and better function are migrate through micro ducts, whereas immotile or poorly motile cells, leukocytes, and other debris remain in the lower chamber. (Meseguer et al., 2025).

Methodology:

1. the liquefied semen sample was mixed gently.

2. The microfluidic device (ZyMōt Multi) was opened under sterile conditions, and 850 µL of semen was gently loaded into the lower chamber via the inlet port using a sterile 1mL syringe, ensuring no formation of air bubbles.

3. the upper chamber was overlaid with 750 µL of sperm washing medium to cover the membrane surface.

4. The device was then placed in an incubator at 37°C for 20-30 minutes, allowing motile spermatozoa to move through the membrane and into the upper chamber.

5. Using a sterile syringe, the sperm suspension was gently aspirated from outlet port. The aspirated spermatozoa were then moved to a sterile tube to be ready for ICSI .

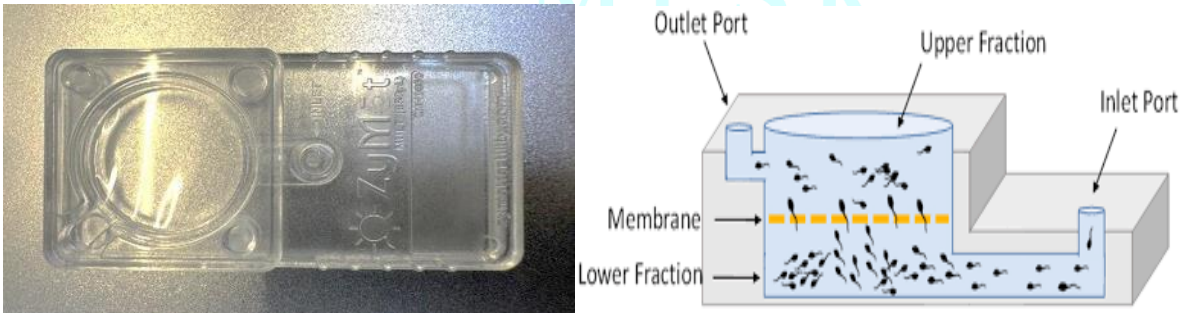

Figure (4) A- Microfluidic Device (Dr. Atyaf IVF Centre) B- Diagram of Microfluidic Device (Serrano-Albal *et al*., 2024)

Density Gradient Centrifugation

This method is used for sperm separation based on their density and motility. The spermatozoa with higher density are the ones which are motile and morphologically normal compared to the immature or damaged sperm, let alone the leukocytes, and other cellular debris which possess lower density (see figure below). Therefore, the highly motile spermatozoa migrate through the gradient layers and form a pellet at the bottom of the tube (Heidari *et al*., 2025).

Procedure:

1. Two concentrations of a silica-based colloidal solution (SpermGrad) were used: 45% for the upper layer and 90% for the lower layer, both of which are prepared in a conical centrifuge tube, each with a volume of 2.0 mL.
2. The layers were then warmed at 37 c for 15 minutes.
3. The liquefied semen sample of 1.0 mL was overlaid onto the gradients.
4. The tube was then centrifuged at 1400 rpm for 6 minutes.
5. The pellet remaining at the bottom and containing highly motile and morphologically normal sperm was carefully collected.
6. The pellet was then washed with a culture medium and centrifuged once again for 5 minutes so as to remove the residual gradient media.
7. A 20 µL of sperm medium was used to resuspend the final pellet for ICSI technique which will be detailed later.

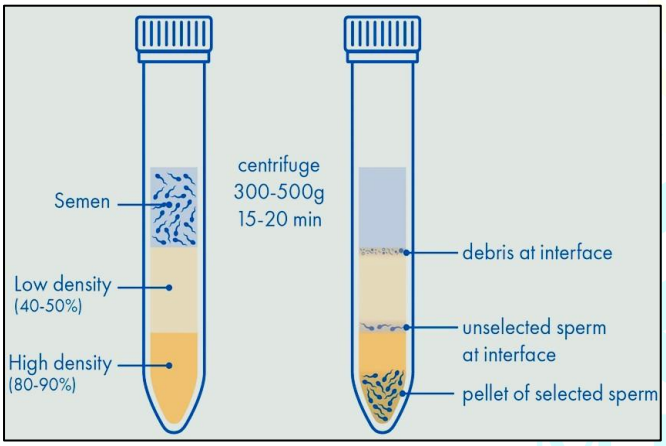

Figure (5) Density Gradient Technique (Tiptiri-Kourpeti *et al*., 2025)

MESR
منار الشرق للدراسات والأبحاث
Manar Elsharq for Studies & Research

Ovarian pick up an denudation:

following a controlled ovarian stimulation program mature Oocytes were aspirated at precisely calculated time under general anaesthesia (GA) and using specialised catheter connected to suction pump. using stereomicroscope aspirated oocyte were placed directly into culture medium and incubated until denudation. The cumulus cells surrounding the oocyte are removed using hyaluronidase enzyme and specialized pipettes and strippers to ensure the denuded Oocyte is visible for assessment and later ICSI procedure.

ICSI Procedure:

Direct injection of a spermatozoon into the oocyte cytoplasm in order to bypass barriers that may prevent fertilization in natural or conventional IVF. ICSI was performed following standard micromanipulation techniques using a special inverted microscope connected with holding and injection micro needles for Oocyte and sperm respectively.

Fertilisation assessment:

18-20 ours post-injection, fertilisation was assessed by observing the presence of 2 circular pronuclei (one from the spermatozoon and the other from the oocyte).

Cleavage assessment:

At 72 hours post-fertilisation, after the first, second, and third mitotic divisions of the fertilized oocyte embryo cleavage was assessed based on the number and symmetry of embryo blastomeres.

Biopsy procedure:

removal of one or two blastomere from the developing embryo, whose genetic content reflects that of the embryo itself. The embryo subsequently resumes normal growth again following this procedure, the biopsy procedure carried out using special biopsy medium, holding and biopsy needles connected to inverted ICSI station, laser beam applied to create a hole in zona pellucida, the 1-2 blastomeres aspirated under vacuumed pressure. blastomeres then transferred to SafeSeal PCR tubes and sent to the genetic laboratory for PGT-A analysis.

Preimplantation Genetic Testing for Aneuploidy (PGT-A)

Next-Generation Sequencing (NGS)is an advanced molecular technique used to evaluate the chromosomal constitution of preimplantation embryos. The primary aim of PGT-A is to detect whole chromosome and segmental aneuploidies affecting chromosomes 1–22 and the sex chromosomes (X and Y), thereby improving embryo selection and clinical outcomes in assisted reproductive technologies (ART).

Methodology:

1. Embryo biopsy: 1-2 cells aspirated from 8-10 cells of cleavage stage embryo.

2. cells were lysed to release genomic DNA, whole genome amplification (WGA) was performed using commercial kits supplied by Revvity. This step is critical to generate sufficient DNA while maintaining representative amplification across all chromosomes.

3. The amplified DNA underwent library preparation for NGS, which is included end-repair, A-tailing, adapter ligation using indexed (barcoded) adapters and PCR amplification of the library. Each sample was assigned a unique index sequence to allow multiplex sequencing within the same run.

4. Libraries were purified using magnetic bead-based cleanup to remove contaminants and short fragments. DNA concentration was measured, and libraries were normalized to ensure equal representation of each sample. Subsequently, libraries were pooled for sequencing.

5. The pooled libraries were loaded onto the Illumina MiSeq flow cell and subjected to sequencing-by-synthesis. During sequencing, fluorescently labeled nucleotides were incorporated into DNA strands, and signals were recorded at each cycle to generate millions of short sequencing reads.

6. Sequencing reads were aligned to a human reference genome using dedicated bioinformatics software. The read depth distribution across each chromosome was analysed to determine chromosomal copy number variations.

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software. Descriptive statistics, including the mean and standard deviation (SD), were calculated. The data were analyzed according to a Completely Randomized Design (CRD). Analysis of variance (ANOVA) was performed to determine significant differences among the treatment groups. Duncan's Multiple Range Test (DMRT) was subsequently applied to compare group means, and statistically significant differences were indicated by different alphabetical letters. In addition, *t*-test was used to compare the means between two groups where appropriate.

Results and discussion

The current results indicated that the semen samples were humogenous, the table (1) below showed no statistically significant differences in seminal fluid volume, sperm concentration, total motility and progressive motility.

	Husband age	**Abstinence period**	**sample volume**	**sperm concentration mil/ml**	**total motility**	**progressive motility**
Routine technique	37.72± 6.772	4.33±1.029	3.28 ± 1.152	37.22 ± 15.66	48.17 ±10.257	32.72±6.789
Pellet swim up	38.20± 2.833	4.40± 1.121	3.45±1.799	35.53± 11.87	50.67± 9.232	31.80± 6.327
Direct swim up	36.27± 6.135	4.53± 1.187	3.00± 1.325	43.53± 19.12	49.60±12.483	32.47± 6.058
Density gradient	37.27± 3.90	4.47± 1.246	3.08±0.941	34.40± 15.743	44.47± 9.877	30.07± 6.99
PICSI Dishes-hyaloronan	36.67± 6.956	4.47± 0.990	2.83±1.205	30.27± 9.917	47.67±13.478	31.00± 7.838
Microfluidics	37.33 ±5.65	4.73 ±1.39	3.25± 1.36	44.60 ± 11.89	46.07 ±11.20	30.13 ± 7.76
p-value	0.946	0.953	0.828	0.061	0.684	0.829

Table(1) different semen parameters of semen samples of study groups.

While the Table (2) shows the mean age of female participants between study groups, as one of the inclusion criteria was a female age of less than 37 years. The table also demonstrates the number of the retrieved oocytes, with a minimum of 7 Oocytes per case. These findings show the homogeneity of the study groups, aiding in more accurate comparisons between the study groups.

	Wife age	Oocyte harvested
Routine technique	32.39 ±4.245	19.06 ± 1.902
Pellet swim up	34.47 ±2.475	17.47 ± 1.810
Direct swim up	31.53± 4.015	18.40 ± 2.175
Density gradient	32.33± 3.697	18.40 ± 1.874
PICSI Dishes-hyaluronan	31.60± 5.054	13.80 ± 1.317
Microfluidics	32.53 ± 3.58	17.00± 7.95
p-value	0.369	0.419

Table (2) average age of wives and Oocyte mean numbers of the study groups .

Pre-implantation genetic testing for aneuploidies PGT-A

Embryonic chromosomal abnormalities in their early stages are considered one of the primary causes of pregnancy delay, embryo implantation failure, and early abortions. Therefore, it was necessary to have a method to examine them and detect these abnormalities. Chromosomal screening of embryos in IVF programmes using PGT-A represents a major breakthrough in the field. further this test generally utilized for embryo sex determination (Sichenze *et al*., 2026).

MESR
Manar Elsharq for Studies & Research

Embryo Sex Selection:

X-bearing spermatozoa are almost indistinguishably akin to Y-bearing spermatozoa due to their similarity in size and morphology, except that the former carries approximately 2.8% more DNA. Differences in their swimming behaviour are nonetheless, either inconsequential or have not yet been inconclusively proved. Therefore, techniques that rely on sperm motility, density, morphology, or maturity cannot totally separate X-bearing spermatozoa from Y-bearing spermatozoa (Munuera Puigvert *et al*., 2024).

The figure below illustrates (when comparing the groups together) that the differences in the percentages of XX embryos produced by ICSI using the different sperm preparation techniques did not reach a statistical significance, The percentages were 51.25%, 44.86%, 42.29%, 47.41%, 43.21%, 48.36% for the routine method, pellet swim-up, direct swim-up, density gradient, PICSI, and microfluidic technique, respectively.

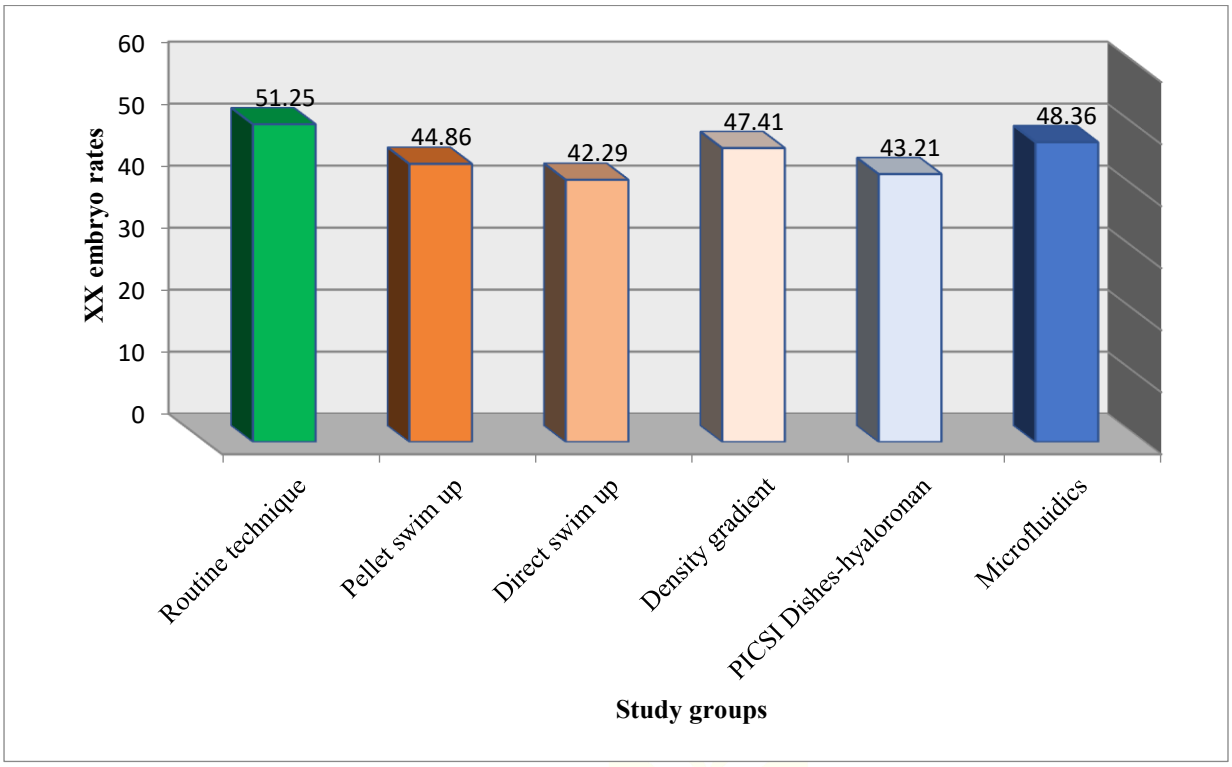

Figure (6): Percentage of female embryos produced by ICSI using the different sperm preparation techniques

On the other hand, the differences in XY ratios were favourable in swim-up based techniques which intended to enrich faster- and more motile spermatozoa that are presumed to carry the Y chromosome. When comparing all the groups together the percentages were 33.86%, 40.60%, 39.00%, 31.93%, 38.4%, 29.78% for the simple wash, pellet swim-up, direct swim-up, density gradient, PICSI, and microfluidic technique, respectively, and it was not reach significant difference as shown in figure () below.

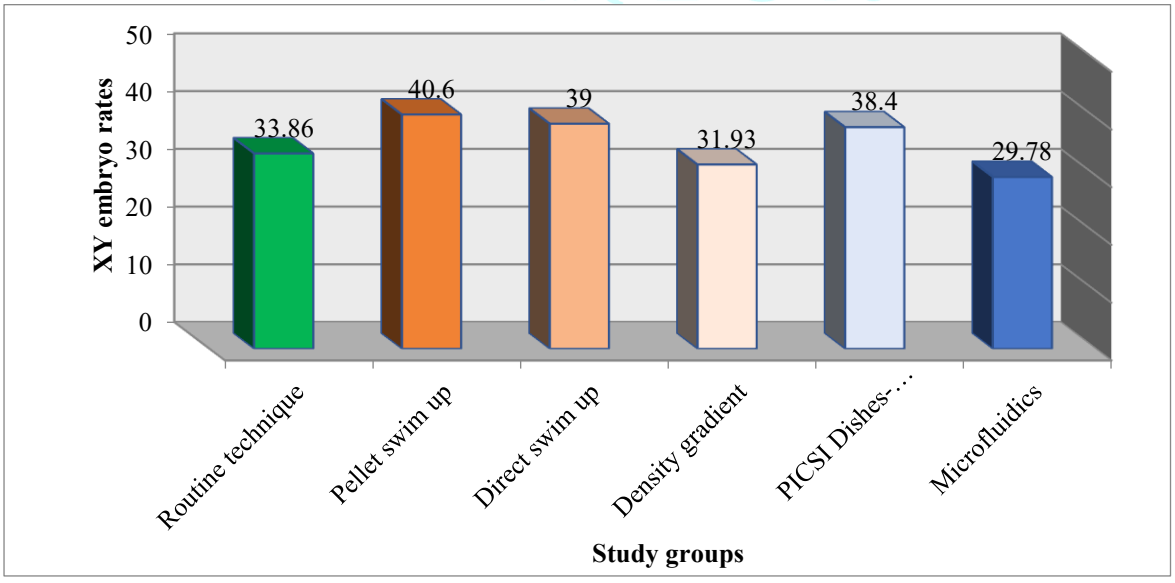

Figure (7): Percentage of male embryos across all groups.

However, when comparing the XY ratio of the cases of the three swim-up based groups which are (pellet swim up, direct swim up, and the PICSI technique which also incorporates a swim-up step) with the XY ratio of the cases of the three other groups (simple wash, density gradient, microfluidic technique) which are non-swim-up based techniques, we notice a significant difference worthy of attention. In the swim-up based

group, the XY rate as shown in the figure and table below was 39.34%, compared with 31.98% in the non-swim-up based group, with a *p*-value of 0.025.

The latter three non-swim-up based techniques, which yielded lower percentage of male embryos, are considered ineffective in separating the faster and more motile spermatozoa, because the competition distance is relatively short and inadequate to separate X-bearing spermatozoa from Y- bearing spermatozoa, allowing both types to mix-up more readily. This result is consistent with what the finding that a deviation in the percentage of Y-sperm was observed when using the modified swim-up techniques (Asma-ul-Husna et al., 2017).

	# of cases	Total # of embryos	Total # of XY embryo	XY embryo rate%	Std. Deviation	t-value	Std. Error Mean
Swim-up based separation	45	467	186	39.34	14.264	2.274	2.126
Non-swim-up based separation	48	507	164	31.98	16.732	2.285	2.415
P-value				0.025			

Table (3): Percentages of male embryos between swim-up based and non-swim-up-based groups.

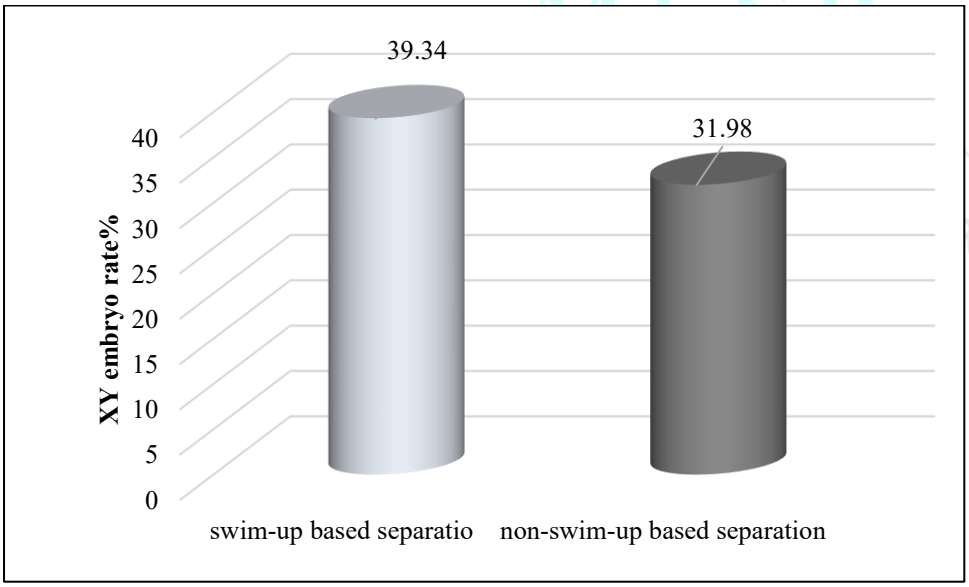

Figure (8): Percentages of male embryos between swim-up based and non-swim-up-based groups.

On the other hand, the swim-up-based group recorded a decreased rate in the percentage of XX embryos. compared to an increase in non-swim-up based group, with the difference being non-significant as shown in table

and figure below. This finding is consistent with the XY results above.

	# of cases	XX rate%	Std. Deviation	t-value	Std. Error Mean
Swim-up based separation	45	43.45	19.458	-1.640-	2.901
Non-swim-up based separation	48	49.15	13.725	-1.622-	1.981
P-value		0.109			

Table (4) Percentages of female embryos between swim-up based and non-swim-up-based groups.

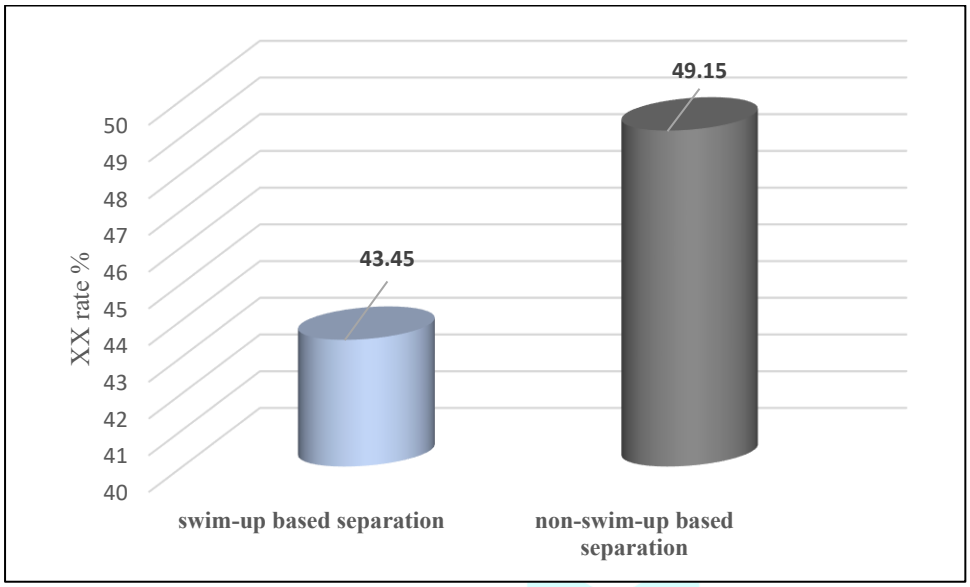

Figure (9): Percentages of female embryos between swim-up based and non-swim-up-based groups.

MESR
مجلة المنارة للدراسات والبحوث
Manar Elsharq for Studies & Research

Conclusion

Swim-up based techniques which enriches the semen prepared for ICSI with fastest spermatozoa resulted in significant increase in XY embryo rates after some modifications.

References

Kubiak, J. Z., Kloc, M., & Piprek, R. P. (2020). **History of the research on sex determination.** *Biomedical Journal of Scientific & Technical Research, 25*(3), 19104–19112. https://doi.org/10.26717/BJSTR.2020.25.004194

Farhud, D. (2022). Using non-invasive methods to choose gender. Iranian Journal of Public Health. https://doi.org/10.18502/ijph.v51i7.10275

Aynalem, B. Y., Melesse, M. F., & Bazezew, Y. B. (2023). Cultural beliefs and traditional practices during pregnancy. Women's Health Reports, 4(1), 415–422. https://doi.org/10.1089/whr.2023.0024

Whitfield, M., & colleagues. (2024). The annulus: composition, role and importance in sperm flagellum structure and function. *Basic and Clinical Andrology*, 34, 6. https://doi.org/10.1186/s12610-024-00241-2

He, Q., et al. (2022). Advancements in mammalian X and Y sperm differences and sex control technology. *Zygote, 30*(4), 423–430. https://doi.org/10.1017/S0967199421000939

Rahman, M. S., & Pang, M. G. (2020). New biological insights on X and Y chromosome-bearing spermatozoa. Frontiers in Cell and Developmental Biology, 7, 388

Cheng, J., et al. (2024). Proteomic and metabolomic profiling reveals alterations between X- and Y-chromosome-bearing spermatozoa. *Animals, 14*(24), 3672. https://doi.org/10.3390/ani14243672

Bai, L., et al. (2025). Advances in immunological sorting of X- and Y-bearing spermatozoa. *Frontiers in Veterinary Science, 12*, 1523491. https://doi.org/10.3389/fvets.2025.1523491

Shettles, L. B. (1960). Human spermatozoa in cervical mucus and their relation to the fertile period. *American Journal of Obstetrics and Gynecology*, 79(3), 531–535. https://doi.org/10.1016/0002-9378(60)90213-4

Sarkar, S., Jolly, D. J., Friedmann, T., & Jones, O. W. (1984). Swimming behavior of X and Y human sperm. *Differentiation*, *27*(1-3), 120-125. https://doi.org/10.1111/j.1432-0436.1984.tb01416.x

Khatamee, M. A., Horn, S. R., Weseley, A., Farooq, T., Jaffe, S. B., & Jewelewicz, R. (1999). A controlled study for gender selection using swim-up separation. Gynecologic and Obstetric Investigation, 48(1), 7–13. https://doi.org/10.1159/000010125

Azizeddin, A., Ashkar, F., King, W., & Revay, T. (2014). Enrichment of Y-chromosome-bearing bull spermatozoa by swim-up. Reproduction in Domestic Animals, 49(1), e1–e4. https://doi.org/10.1111/rda.12252

Asma-ul-Husna, A., Awan, M. A., Mehmood, A., Sultana, T., Shahzad, Q., Ansari, M. S., Rakha, B. A., Saqlan Naqvi, S. M., & Akhter, S. (2017). Sperm sexing in Nili-Ravi buffalo through modified swim-up. Animal Reproduction Science, 182, 69–76.

Naidu, S. J., Arangasamy, A., Selvaraju, S., Binsila, B. K., Ravindra, J. P., Reddy, I. J., & Bhatta, R. (2025). Spermatozoa sorting techniques for the sex pre-selection: A review. Indian Journal of Animal Research, 59(1), 1–10. https://doi.org/10.18805/IJAR.B-4530

Mahadevan, M., & Baker, G. (1984). Assessment and preparation of semen for in vitro fertilisation. In *Clinical in vitro fertilisation* (pp. 83-97). London: Springer London.

Ješeta, M., Doubravská, A., Antalíková, J., Mekiňová, L., Franzová, K., Remundová, K., ... & Hudeček, R. (2025). Comparison of Swim-Up and Microfluidic Sperm Sorting Methods in Selection of Sperm for Intracytoplasmic Sperm Injection. *International Journal of Molecular Sciences*, *26*(11), 5374.

Wen, Z. N., Duan, L., Chen, Y., Qiu, Q. H., Liu, G., Luo, N., ... & Ge, R. S. (2025). Comparative efficacy of swim-up, density-gradient centrifugation, and microfluidic sorting in sperm preparation, and the

impact on motility, morphology, and DNA integrity. *International Journal of General Medicine*, 2355-2366. https://doi.org/10.2147/IJGM.S517575

Christanto, R. B. I., Savira, M., & Birowo, P. (2024). *Sperm preparation and sperm selection techniques*. In P. Birowo (Ed.), *Human Semen Analysis* (pp. 331–351). Springer. https://doi.org/10.1007/978-3-031-55337-0_17

Fleming, S., Morroll, D., & Nijs, M. (2025). Sperm separation and selection techniques to mitigate sperm DNA damage. *Life, 15*(2), 302. https://doi.org/10.3390/life15020302

Fleming, S., Morroll, D., & Nijs, M. (2025). Sperm separation and selection techniques to mitigate sperm DNA damage. *Life, 15*(2), 302. https://doi.org/10.3390/life15020302

Shivhare, S., Karunakaran, S., Bose, A. S., Goel, R., & Ananthakrishnan, R. (2024). Does physiological intracytoplasmic sperm injection improve outcome in men with abnormal semen parameters: A retrospective cohort study. *Journal of Human Reproductive Sciences*, *17*(3), 200-206.

Meseguer, F., Carrión-Sisternas, L., Del Arco, A., Rivera-Egea, R., Vidal, C., Remohí, J. A., & Meseguer, M. (2025). Novel sperm selection device on the basis of microfluidics improves usable blastocyst rates, embryo morphology, and morphokinetic patterns: sibling cohort prospective study. *F&S Reports*, *6*(4), 436-445. https://doi.org/10.1016/j.xfre.2025.09.008

Serrano-Albal, M., Aquilina, M. C., Kiazim, L. G., Zak, L. J., Griffin, D. K., & Ellis, P. J. (2024). Effect of Two Different Sperm Selection Methods on Boar Sperm Parameters and In Vitro Fertilisation Outcomes. Animals, 14(17), 2544. https://doi.org/10.3390/ani14172544

Heidari, B., Nosrati, A., Pournourali, M., Seif, F., Akbari, N., & Shirazi, A. (2025). Sperm preparation techniques affect biological parameters, fertility indices, and DNA fragmentation in patients with oligo-astheno-teratozoospermia. *Reproduction and Fertility*, *6*(4).

Tiptiri-Kourpeti, A., Kourpetis, P., & colleagues. (2025). Advanced sperm selection techniques in assisted reproduction: Current perspectives and clinical implications. *Journal of Clinical Medicine, 14*(3), 1125.https://doi.org/10.3390/jcm14031125

Sichenze, L., Fiore, A., Casalechi, M., Somigliana, E., Viganò, P., Domenico, D. S. M. F. S., ... & Zurkirch, V. Z. (2026). Diverging guidelines and consensus statements on preimplantation genetic testing for aneuploidy: indications and strategies reviewed. *Reproductive Biology and Endocrinology: RB&E*, *24*, 70. https://doi.org/10.1186/s12958-026-01545-w

Munuera Puigvert, A., García-Faura Cirera, A., García José, F., Carella, C., Pujal Bravo, B., Montalvo Pallès, V., & Marquès López-Teijón, B. (2024). P-033 Microfluific sperm sorting does not impact the primary sex ratio in IVF-ICSI treatments. *Human Reproduction*, *39*(Supplement_1), deae108-410. https://doi.org/10.1093/humrep/deae108.410