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Case Study: To Evaluate the Clinical Pregnancy Rate of iOAT Cases with Microfluidic Sperm Sorter

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***Abstract*: Background: To evaluate the effectiveness of MFSS in cases of iOATs. This study depicts the use of MFSS in cases of iOATs to increase the clinical pregnancy rate.**

Case Presentation: 10 patients were selected with iOATs with the history of one failed IVF cycle. MFSS was used to sort the sperms and clinical pregnancy rate was noted. Before and after sorting the sperms in MFSS, motility/morphology of the sperms were analysed. ICSI was performed from the sorted sperms. Frozen embryo transfer was done for the selected patients.

Conclusion: Significant change in the quality of the sperms was obtained after sperm sorting in MFSS. Better clinical rates were achieved for the iOATs patient with the use of MFSS. MFSS can be used in routine clinical practices.

***Keywords*: Assisted Reproduction Technology, Idiopathic Oligoasthenoteratozoospermia, Microfluidic Sperm Sorter, DNA fragmentation, Clinical pregnancy rate, and intra-cytoplasmic sperm injection.**

1. Introduction

Microfluidics has been an emerging technique in the field of bio medical sciences used for cell analysis, drug delivery systems and now increasing use in the field of Assisted Reproduction techniques. Male infertility accounts for about 40% of the infertility cases affecting 7% of the male population. About 32.5% of male infertility factors are not unknown [1]. The main reason for male infertility on semen examination is found to be low sperm count and morphologically abnormal sperms. Till date this problem was taken care by semen wash including Density Gradient Centrifugation (DGC), Swim up (SU) followed by intra cytoplasmic sperm injection (ICSI). It was found in the study of Arman Zini *et al.*, that the DNA integrity of the sperms was not maintained in DGC compared to the swim up. It was seen in multiple studies that DNA damage probability increases with these methods of semen preparation. Compared to the semen wash techniques, microfluidics has an upper hand in selection of motile sperms with significantly reduced DNA damage [2].

Microfluidic Sperm Sorter (MFSS) has been now used in multiple cases of severe male sperm factors like oligozoospermia, asthenozoospermia and oligoasthenoteratozoospermia to give better success rates to the patients. MFSS is used in selection or to isolate sperms with better morphology and higher motility sperms. It works on the variables like fluid density, viscosity, velocity and size of the MFSS. The principle lies in the design of multiple channels that control the formation of multiple laminar channels and motile sperms swim across the stream lines which are than collected by the dedicated reservoirs (3).

Fabrication of MFSS: MFSS is fabricated with polydimethylsioxane – PDMS which is used for its universal use of fabrication like flexibility, ease of soft-lithographic patterning and low auto- fluorescence. PDMA is surface modified by PEG-MA coating which helps in giving non-fouling surface and presents a hydrophilic nature to the MFSS [3].

In cases of OAT – Oligoasthenoteratozoospermia etiology is usually idiopathic (iOATs) in almost 30% of the infertile men. According to the WHO 2010 reference value, concentration less than 15 x 10^6 spermatozoa /ml is defined as oligozoospermia, sample with less than 40% of motile sperms is defined as asthenozoospermia and teratozoospermia is defined as the sample showing less than 4% of normal morphology (Kruger criteria). DGC followed by ICSI was considered as the most efficient and the best chosen method in cases of iOATs. Performing DGC and swim up along with other semen preparation techniques leads to the higher DNA damage in the cases of OAT, which basically does not solve the purpose of giving higher and better results [4]. In this study MFSS is used to isolate the higher motile sperms and morphologically better sperms. This paper discusses ten cases iOATs within the age group of 30- 40 years with previously failed one IVF cycle. For all the ten cases MFSS was used and was followed by ICSI. The fertilization rate and clinical pregnancy rate was noted for the use of MFSS in iOATs.

2. Case Presentation

A. *Materials and Method*

Our study presents 10 cases of iOATs with no familial

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Table 1
Patient details taken in the study showing the age range, sperm concentration (sperms/ml), total progressive motility (%) and morphology (%)

Sr. No.	Name	Age	Sperm Concentration (Sperms/ml)	Total Progressive Motility (%)	Morphology (%)
1	Patient 1001	35	2	20	2
2	Patient 2002	34	12	20	2
3	Patient 3003	33	5	30	2
4	Patient 4004	33	2	20	1
5	Patient 5005	40	12	30	2
6	Patient 6006	37	14	20	2
7	Patient 7007	40	5	30	3
8	Patient 8008	33	4	30	3
9	Patient 9009	32	4	30	3
10	Patient 10010	40	10	30	2
	Range	**30-40 Years**	**2-14/ml**	**20-30 %**	**1-3 %**

history of X-linked disorders, no smoking history and no history of professional hazards or any other known causes of the OATs. Hence the patients were classified under the title of iOATs. Patients were selected with at least one failed IVF cycle to compare the results with MFSS. Patient age group varies from 30-40 years; concentration varies from 2-14/ml, total progressive motility range from 20-30% and morphology ranging from 1-3%. Inclusive criteria for the patients with OAT were chosen according to the WHO 2010.

Semen sample was collected from ten patients with abstinence time of 3 days. After liquefaction the sample were analysed macroscopically and microscopically. Semen wash technique was not taken into consideration. The raw sample was directly used for the MFSS with dilution.

MFSS (Menicon, Kasugai, *Japan)* – was fitted in the 60-mm dish. The four chambers were first flushed with 100 µl of flushing media (Origio, Denmark: 1076 *with heparin*) to check and confirm the flow. Chamber C and chamber D was filled with 20µl and chamber B with 100 µl of flushing media. After adding flushing media in all the three chambers, chamber A was filled with 65µl of 1:1 sperm suspension with flushing media (5). After 30 minutes, initial 200 sperms were analysed from the thin laminar flow in the chamber C. Analysis of motility and morphology was done to assess the quality of the sperms achieved after the MFSS. Further the sperms from chamber C were taken up and used for the ICSI.

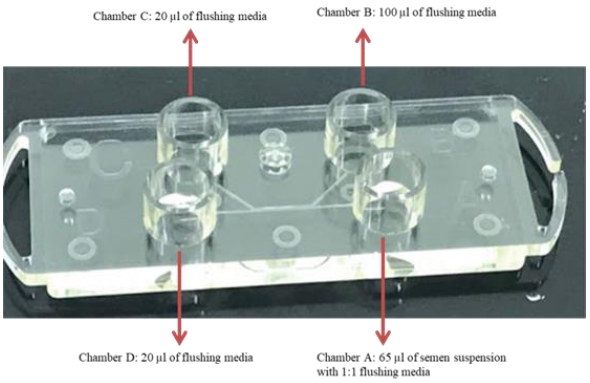
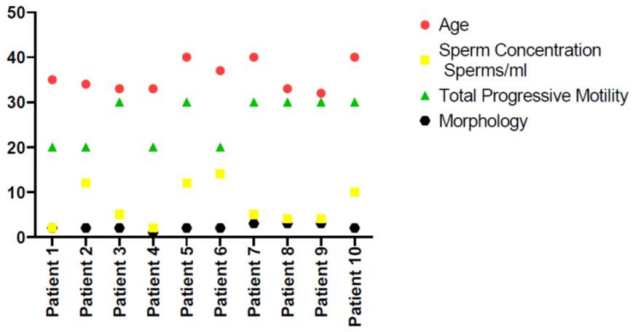
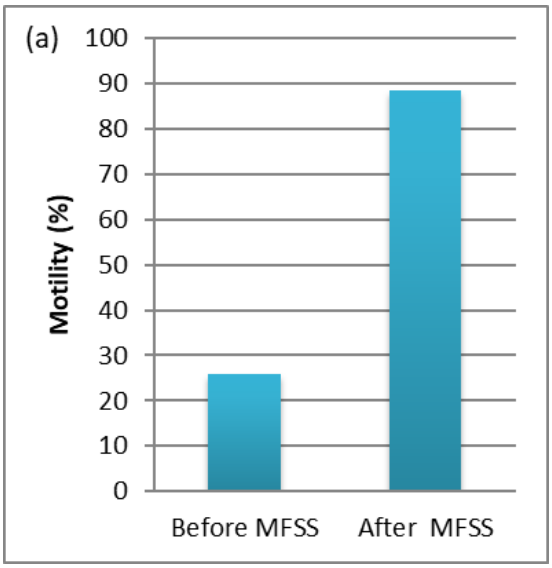


Fig. 1. MFSS – Qualis (Menicon, Kasugai, *Japan*) displaying its four champers and the media used to process the sperms further

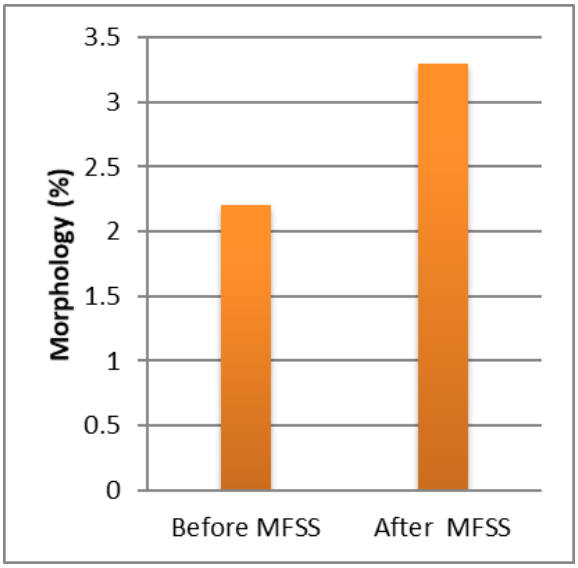
All the 10 patients had a history of one IVF failure cycle. ICSI was performed for the 10 patients after the sperms were sorted using the MFSS. Fertilization rate and blastocyst formation rate was assessed for all the patients. Correlation between the number of oocytes, fertilization ratio and blast formation ratio was noted. Out of 10 patients, transfers of eight patients were taken up. All the eight patients had frozen blast transfers.



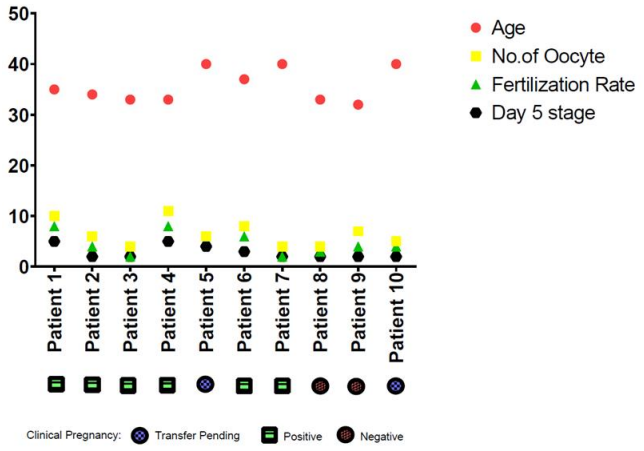
Graph 1: This scatter plot shows the distribution of all the 10 patients in terms of age, sperm concentration, total progressive motility and morphology



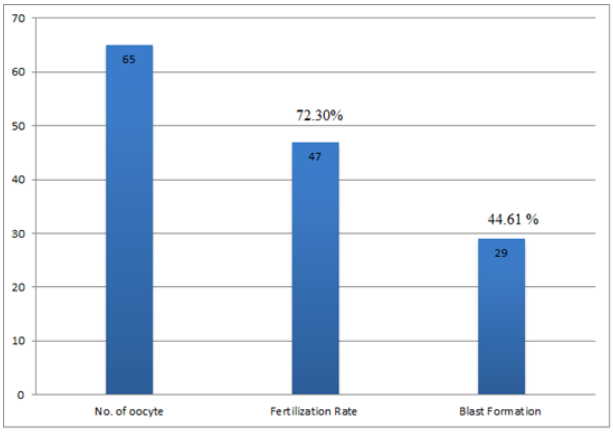
Graph 2 (a) Comparison of percentage sperm motility before and after sorting from MFSS was assessed. Initial 200 sperms were assessed to compare the sperm motility (%) from the wide outlet and the thin outlet (sorted column) of the MFSS. Total motility was taken into consideration



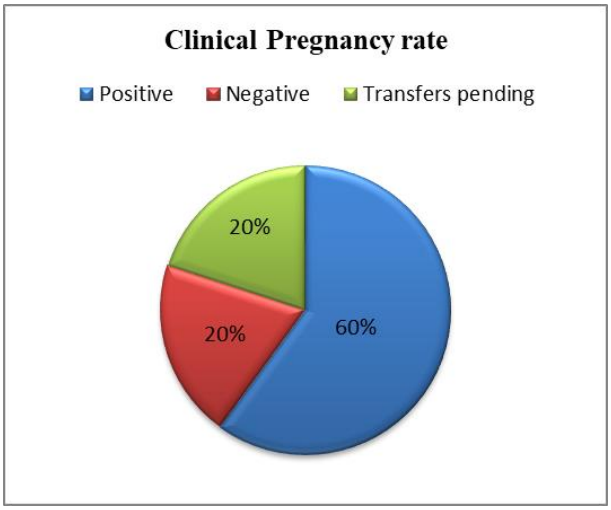
Graph 2(b) Comparison of percentage sperm morphology before and after sorting from MFSS was assessed. Initial 200 sperms were assessed from the wide outlet and the thin outlet (sorted column) of the MFSS



Graph 3: Scatter plot shows the distribution of number of oocytes, fertilization rate and blastocyst formation patient wise



Graph 4: Total numbers of oocytes were taken into consideration for all the 10 patients to understand the fertilization rate and blastocyst formation rate



Graph 5: Pie chart depicts the percentage of the total clinical pregnancy rate

3. Results

Patients were selected with age between 30-40 years with one previously failed IVF cycles (Table 1 & Graph 1). Semen parameters were analysed and diagnosed as

oligoasthenoteratozoospermia (iOATs). Mean initial motility of the patients was 26 ±1.6 % (mean ± SE), its mean initial morphology was 2.2 ± 0.2 (mean ± SE). After sperm sorting, sorted initial first 200 sperms were analysed and mean sorted motility was noted as 88.5 ± 1.9% (mean ± SE) and morphology was noted as 3.4 ± 0.16% (mean ± SE) (Graph 2). Sperms were collected from the chamber C to perform ICSI. Total of 10 patients 72.3% fertilization rate was obtained and 44.61% blastocyst formation rate was achieved (Graph 4). Frozen embryo transfer was performed for 8 patients out of 10, transfers of other two patients are still pending. 60% result was obtained with the frozen embryo transfer with sperms sorted by MFSS (Graph 5).

4. Discussion

Microfluidics has been an upcoming technique to sort the sperms in server sperm factors like oligozoospermia, teratozoospermia, asthenozoospermia or

oligoasthenoteratozoospermia. Sperm sorted by MFSS has been reported to have higher motility and better morphology as reported in the study of T.G Schuster *et al.*, 2003. Laminar flows of the fluids are maintained by the viscous force. Viscous force dominates the inertial forces and thus two separate flow is maintained (5). Hence two streams flowing parallel to one another will only allow diffusion and no mixing of the streams will take despite having no physical barriers between the two streams. (10). Microfluidics helps in sorting the motile sperms and morphologically better sperms. In this study, the initial motility assessed (before MFSS) was noted to be on an average 26% (collective data of 10 patients). The sample were run through MFSS, where after the sperm sorting initial 200 sperms were analysed from chamber C, which was found to be

increased to 88.5 % (mean) (collective data of 10 patients). (Graph 2 a, b).

Sperm DFI had multiple studies where in the clinical pregnancy rate was correlated. Mainly the studies noted that the pregnancy is difficult to achieve when the DFI value exceeds 27-30%. Usually, higher DFI does not have significant drawbacks at the stage of fertilization or early stages of embryo formation. DFI plays a significant role at the blastocyst development stage (11). Thus to reduce the miscarriage rate after ICSI, it is important to select the sperms which are genetically normal. DGC and other semen preparation method have been noted to increase the DFI in the sperms. Use of MFSS has noted to sort the sperms which are morphologically better and highly motile giving us more so genetically normal sperms. In our study it was found out that the sperms sorted with MFSS were of better quality and gave us about 72.3% of the fertilization rate. Overall, blastocyst formation rate was achieved to 44.61% in our cases of iOATs. Our 10 patients had history of one IVF failed cycle and noted iOATS. All the patients were at the age group of 30-40 years. Out of 10 patients, transfer of 8 patients was taken up of which 6 got positive results giving us the positive clinical pregnancy rate of 60%.

In this study Qualis MFSS (Menicon, Kasugai, *Japan*) is used which is relatively inexpensive to be used in regularly in the clinics. MFSS provides us with a very small number of sperms which is sufficient to perform ICSI. It helps in reducing the exposure of the sperms to reactive oxygen species and prevents DNA fragmentation. MFSS helps in reducing the steps of DGC or swim up and completes the sperm sorting in 30 minutes by giving us the sorted sperms having higher motility and better morphology. The detrimental effects of the centrifugation are not studied in this study.

Extensive research needs to be conducted to understand the effects of DFI in MFSS vs. DGC and clinical rate comparison.

5. Conclusion

Our study presents you 10 cases of iOATS with one previously failed IVF Cycle in which the use of Qualis MFSS was done to select the better quality of sperms. Through our cases we got 60% of clinical pregnancy rate. The MFSS gives an efficient means of sperm preparation especially in cases of iOATs.

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