Successful conventional IVF following density gradient preparation in patients with poor and borderline sperm morphology

AlShalian S, Nahas S, Al Rajeh L , AlFarraj A, AlBadran A, AlDossary J, AlBalawi R

IVF Department, Maternity and Children Hospital, Dammam, Eastern Province, Kingdom of Saudi Arabia

Abstract

Background
It is the norm to treat patients with ICSI if the sperm morphology (SM) according to Tygerberg Strict Criteria (TSC) is ≤3%.
Objective
To determine whether the proportion of normal SM can be increased post density gradient centrifugation (DGC) with concomitant improvement in sperm function to enable conventional IVF (cIVF) in patients with low and borderline SM (LBSM).
Subjects and methods
Sperm was separated by standard DGC. Improvement in SM following DGC was investigated retrospectively in 68 patients. The effect of higher SM in IUI was retrospectively determined in over 200 patients. cIVF was performed in 11 patients of which 5 had TSC ≤3% (2%=1; 3%=4). cIVF was performed in LBSM patients with informed consent. Inseminate contained 4000 sperm of normal morphology per egg. Patients received two day 2 embryos per embryo transfer. Embryos are graded in the range: 4=excellent to 1=poor. Statistical calculations were performed using software MedCalc®.
Results and discussion
The percentage (%) normal SM was always significantly ($p<0.006$) higher in all post-DGC specimens investigated. The IUI pregnancy rate was higher in patients with higher TSC (9.2% vs 15.0%; Two-tail Fisher's Exact Test = 0.0478; Pearson's Chi-Square, p= 0.0393 and comparison of proportions, p= 0.0454). Fertilization rate in cIVF patients with LBSM (n=5) and normal SM (n=6) was (96.7% [n=30 oocytes] vs n=77.1% [n=35 oocytes] respectively, p=0.0231). Quality of embryos generated in LBSM and normal SM groups were similar (Blastomere number: Mean ±1SD 4.0±1.1 vs 3.9±1.1; p=0.4364 and grade: 3.5±0.6 vs 3.2±0.7; p=0.1762) respectively. Pregnancies in LBSM (3/5; 66.7%; 2 singletons, 1 twin) and normal SM (2/6; 33.3%) were similar ($p>0.05$). In both groups, spare embryos proceeded to expanded/hatching blastocyst stages in vitro.
Conclusion
Morphologically normal sperms increased significantly and universally in almost all specimen after DGC. Fertilization and pregnancy were not compromised in LBSM following cIVF.

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Introduction

The outcome of semen analysis, in particular sperm morphology (SM), is the deciding factor in determining whether conventional IVF (cIVF) or intracytoplasmic sperm injection (ICSI) is performed. In the 5th edition of the WHO 2010 Semen Manual, the lower reference limit for SM is 4%. Most adherent clinics comply strictly with this cut-off point to decide on either cIVF, if the SM is ≥4%, or ICSI if below the reference limit.

It is a standard procedure to harvest motile sperm from semen prior to insemination. One of the earliest sperm harvesting techniques is the swim-up technique which owes its origins to Drevius (Drevius, 1971), originally called the "Sperm Rise" technique. An innovation to the methodology for sperm separation and harvesting technique was made possible by adaptation of the technique of density gradient centrifugation (DGC) described by Pertoft et al. (1978). This technique utilized colloidal silica particles called Percoll™ (Pertoft's colloids). Lessley & Garner (1983) and Bolton & Braude (1984) used Percoll™ gradients to harvest motile sperm but in subsequent years it was found to contain materials toxic to sperm. Colloidal particles currently used in sperm separation are supposedly less toxic or non-toxic to sperm. This is common knowledge.

It has long been established and well documented that sperm preparation, especially with density gradients, significantly increased the percentage of morphologically normal sperms. One of the earliest reports that demonstrated a significant improvement in SM following density gradient centrifugation was that of Dale-McClure and co-workers (Dale-McClure et al, 1986). In subsequent years a number of other workers have reported similar findings (Van Der Zwalmen et al., 1991; Liu et al., 1996: Claassens et al., 1996 and others). Higher SM had a positive impact on pregnancy rates (Oehninger et al.,1988; Van Der Zwalmen et al., 1991). The strict criteria (SC) for sperm morphology (SM) assessment also known as the Tygerberg Strict Criteria (TSC; Menkveld et al., 1990), suggested by Menkveld in the 1970's (Menkveld et al., 1987;1990) and its clinical application reported by the same group (Kruger et al., 1986; 1988; 1993; 1995) were based on the modification (Menkveld et al., 1990; also see review by Parastie, 2017) of the methods previously described by MacLeod and Gold (1951) and Eliasson (1971). However, it has also been documented that a number of studies do not agree on the clinical importance of measuring normal morphology by TSC (Check et al., 1989, 1992, 2002; Grow et al., 1994; Hammit et al., 1991; Kiefer et al., 1996; Host et al., 1999 and many more).

The present effort of performing cIVF for patients with male factor subfertility (MFS) was circumstantial. In the newly established authors' clinic the treatment of patients with MFS was delayed by a temporary ICSI service disruption due to disrepair of the ICSI equipment. The affected patients requested that their ART treatment be performed by cIVF instead. This prompted the authors to explore means to provide ART treatment with cIVF without compromising treatment outcome for MFS patients who had low and borderline SM (LBSM) but otherwise had normal sperm concentration and motility. The observation that many studies do not agree on the clinical importance of normal morphology or the TSC (Check et al., 1989, 1992, 2002; Grow et al., 1994; Hammit et al., 1991; Kiefer et al., 1996; Host et al., 1999) provided the impetus for the authors to undertake treatment with cIVF for LBSM patients.

In this report the authors present their findings with regard to their efforts to provide ART treatment with cIVF for patients with LBSM in the absence of an ICSI facility. The authors performed feasibility studies to obtain information that could indicate or predict a successful outcome after cIVF in LBSM patients prior to treatment with cIVF. Treatment with cIVF for the LBSM patients commenced after two feasibility studies provided data that supported the potential for a favourable outcome.

Subjects and Methods

Subjects

cIVF was performed for LBSM patients with informed consent due to disrepair of ICSI machine. The LBSM patients that opted for treatment by cIVF were counselled extensively of potential negative outcome. LBSM patients who decided to proceed with cIVF after counselling were treated with cIVF.

Semen analysis
Semen analysis was performed by standard methods (WHO, 2010). The slides for SM assessment were stained using SPERMAC (FertiPro, Belgium) according to the manufacturer's instructions. There were 3, and occasionally 4 operators, each examining and counting minimum 200 sperms. The final results were obtained by averaging the total numbers of sperms counted.

Sperm preparation for IUI treatment
For the IUI study motile sperm was prepared by density gradient centrifugation using density gradient kits (FertiPro, Belgium) according to the protocol provided by the manufacturer. About 0.5ml of prepared sperm specimen was used for IUI inseminations ensuring the total count in the inseminate does not exceed 20 million motile sperms. If the motile count is in excess of 20 million the specimen is diluted by equal volume of medium before insemination. Insemination was performed at 36hrs post hCG injection.

Sperm preparation for cIVF treatment
For the cIVF study motile sperm was prepared by density gradient centrifugation using density gradient kits (SupraSperm™ System; 55% + 80% gradients, Cooper Surgicals, Denmark or ALLGrad LifeGlobal™, USA) according to the protocol suggested by the manufacturer. The washed spermatozoa were re-suspended in fertilization media (Global Total™ Life Global, USA) or embryo culture media (Sage 1-Step Medium™, Cooper Surgicals, Denmark). The motile sperm count used was adjusted to 100,000 motile sperms per ml in normozoospermia but was increased by 1.34x, 2x and 4x for TSC morphologies of 3%, 2%, and 1% respectively using the method described by Ali (Personal Communication, 2018, IIUM, Kuantan, Malaysia). This method ensures the amount of morphologically normal sperms per inseminate always remains at around 4000 irrespective of the SM.

Fertilization and culture of embryos
Fertilization of oocytes and culture of resultant embryos were performed by standard procedures in droplet cultures. Fertilized oocytes were washed free of sperm and other debris, and cultured in droplet cultures. The embryo culture media used were Global Total obtained from LifeGlobal™, USA and/or Sage 1-Step Medium™ from Cooper Surgicals, Denmark.

Specific studies

1. Normal SM before and after density gradient centrifugation
A retrospective feasibility study was undertaken to determine whether % normal SM is increased following density gradient centrifugation in 68 patients undergoing IUI treatment.

2. Impact of SM on IUI pregnancies
Prior to 2018 the IUI service was the only infertility treatment available to the authors' patients. All patients were treated with IUI irrespective of their semen parameters. Consequently, patients who were not suitable for IUI also received treatment (Random group; RG). When ART treatment was made available in 2018, IUI was provided to patients who had minimal sperm characteristics (MSC) such as SM: ≥2% (TSC); progressive motility of ≥24% and normal sperm concentration (MSC group). This offered an opportunity to retrospectively investigate whether SM ≥2% in the latter group could result in a higher pregnancy rate? The numbers of patients in the RG group was n=262 while those that fulfilled criteria for MSC was n=226. Patients were treated by standard methods.

3. cIVF treatment in patients with LBSM
A retrospective study in 11 patients who underwent ART treatment by conventional IVF (cIVF) was undertaken to determine the fertilization rate, zygote arrest rate, quality of day 2 and 3 embryos, the pregnancy rate, and the proportion of leftover embryos that are fit for cryopreservation. Ovarian stimulations were by standard antagonist and long protocols. In most cases, two embryos were transferred on day 2 except for one patient who received 1 and another patient who received 3 embryos respectively (average 2 embryos per ET). Day 2 embryos are graded: range 4=excellent to 1=poor.

Statistical analysis

Statistical calculations were performed using the statistical software MedCalc®.

Results

1. SM before and after density gradient centrifugation

The pre-wash SM ranged from 1-6% with a mean ±1SD of 2.8±1.2 (n=68). The SM was always higher in all post-wash specimens except in one patient which ranged from 1-9% (Pearson's Correlation; r=+0.8845; p<0.00001) with a mean ±1SD of 4.4±1.7 (paired t-test; p<0.00001). The improvement in SM post-wash was seen in 67 of 68 patients (98.5%; p<0.00001; Table 1).

2.Impact of SM on IUI pregnancies

The pregnancy rate in the RG and MSC patients were significantly different (9.2% vs 15.0%; Two-tail Fisher's Exact Test = 0.0478; Pearson's Chi-Square, p = 0.0393 and comparison of proportions, p = 0.0454) which conclusively indicated the superiority of the MSC criteria over the RG protocol. The significant improvement in SM post density gradient sperm preparation appears to play a role in improved outcome.

3. cIVF treatment in patients with LBSM

i. Sperm morphology

The pre-wash SM ranged from 2-7% with a mean ±1SD of 4.0±1.4 (n=11). The SM was always higher (100.0%) in all post-wash specimen which ranged from 3-10% (Pearson's Correlation; r=+0.8136; p<0.0013) with a mean ±1SD of 5.6±2.0 (paired t-test; p<0.0006). The improvement in SM post-wash was 100.0% (p<0.0013; 11 of 11; Table 1).

ii. Fertilization Rates and embryo quality

A significantly higher fertilization rate was noted in cIVF patients with LBSM (n=5) compared to normal (n=6) SM (96.7% [n=30 oocytes] vs n=77.1% [n=35 oocytes] respectively, p=0.0231). The day 2 embryos generated in both the low and normal SM groups were of similar quality (day 2 blastomere number: Mean ±1SD 4.0±1.1 vs 3.9±1.1; p= 0.4364 and grade: 3.5±0.6 vs 3.2±0.7; p=0.1762) respectively. Following embryo transfer (ET) almost all of the leftover day 2 embryos in the LBSM patients were of quality (grade ≥3) suitable for cryopreservation. Embryos are graded: 4=excellent; 1= poor (Table 2).

iii. Pregnancy rates

Pregnancies were achieved in both groups. In the low SM group, 3 of 5 patients became pregnant (60%; n=3; singletons+2; Twins=1), and in the normal SM group, 2 of 6 patients became pregnant (33%; n=2; 2 singletons; p>0.05). In both groups spare embryos proceeded to expanded/hatching blastocyst stages in vitro (Table 2). All patients cIVF delivered.

Discussion

The feasibility studies provided data that supported the assumption that cIVF can be performed in LBSM patients. As anticipated, the present study has demonstrated it is possible to perform cIVF with acceptable outcome in patients with LBSM. As the sample size is small, the role of chance needed to be taken into consideration. Nevertheless, it is apparent the present findings provide convincing evidence for a role of cIVF in LBSM patients. Limitations of the study appear minimal and reasons for caution does not appear to be an issue. This significant finding suggests it is possible to achieve fertilization, generate viable and quality embryos, and pregnancies by cIVF in patients with LBSM if the %SM is increased by DGC. Sperm preparation by density gradients significantly and universally increased % normal SM and functionality which was in keeping with previous reports (Dale-McClure et al, 1986; Van Der Zwalmen et al., 1991; Liu et al., 1996: Claassens et al., 1996 and others). The proportion of increased normal SM following density gradient centrifugation had positive impact on cIVF fertilization in LBSM patients.

This study has shown that semen specimen with lower than normal characteristics could yield normal fertilization rates, generate quality viable embryos for ET and elicit a pregnancy rate that is comparable to that of Normozoo-spermic patients. This observation may appear to support the previous reports that do not agree on the clinical significance of the TSC (Check et al., 1989, 1992, 2002; Grow et al., 1994; Hammit et al., 1991; Kiefer et al., 1996; Host et al., 1999). It is worth noting a conference report by Bhattacharya et al. reported that the sperms of patients with less than 1 million motile forms

Table 1: Improvement in sperm morphology following post density gradient centrifugation in IUI and cIVF patients

Description	Intrauterine insemination (IUI)	Conventional IVF (cIVF)
<u>Pre-DGC</u> **%SM Mean ±1SD** **Range** **n**	 2.8±1.2 1-6 n=68	 4.0±1.4 2-7 n=11
<u>Post-DGC</u> **%SM Mean ±1SD** **Range; Paired t-test p-value** **n**	 4.4±1.7 1-9; p<0.00001 n=68	 5.6±2.0 3-10; p<0.0006 n=11
Post – DGC % % Improvement in SM **Pearson's correlation "r" coefficient & p- value**	98.5% (67/68) r = +0.8845 p<0.00001	100% (11/11) r = +0.8136 p<0.0013
DGC: Density gradient centrifugation		

Table 2: Fertilization and embryological characteristics in patients with normal and subnormal sperm morphology during cIVF treatment

Description	%Fertilization	Day 2 Blast No. Mean/SD	Day 2 Embryo grade Mean±1SD	Pregnancies
≤3%SM **Low morphology** **n=5**	96.7 (29/30)	4.0±1.1	3.5±0.6	3/5 2 singletons 1 twins
≥4%SM **Normal morphology** **n=6**	77.1 (27/35)	3.9±1.1	3.2±0.7	2/6 2 Singletons
p- value (Significance)	0.0231 (S)	0.4364 (NS)	0.1762 (NS)	>0.05 (NS)
Embryo grade: 4=excellent;3=Good;2=Average;1=Poor				

post sperm wash could fertilize oocytes by cIVF (59.01%) but significantly much lower than the ICSI procedure (64.94% p=0.0119) under similar conditions but the numbers of day 3 embryos were comparable in both groups. The total fertilization failure following cIVF and ICSI in this population was 5% and 2% respectively (Bhattacharya et al., 2018).

Almost all of the leftover day 2 embryos in LBSM patients were fit for cryopreservation. Likewise, this study also noted adherence to a stricter criteria based on sperm characteristics resulted in significantly higher pregnancy rate in IUI treatment cycles. In spite of the challenges faced by the ART team in a new facility, it was possible to obtain acceptable pregnancies with minimal wastage of embryos. The high output was achieved through careful control and management of all factors that can affect treatment outcome. Total quality management in this facility appears satisfactory, evident by the good outcome although the ART team was confronted with numerous constraints common to new ART facilities.

Conclusion

The percentage of morphologically normal sperms increased significantly and universally in almost all specimen after DGC that concomitantly improved sperm functionality. IUI pregnancy is higher in patients that had increased normal SM post DGC. Fertilization, generation of quality embryos and pregnancy were not compromised in LBSM during cIVF. It is concluded cIVF can be performed in LBSM if ICSI facility is not available.

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