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ORIGINAL PAPER

# Factors predicting the outcome of percutaneous epididymal sperm aspiration in men with obstructive azoospermia

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**Summary**

***Objectives:*** *To report on the live birth rates (LBRs) following percutaneous epididymal sperm aspiration (PESA) in men with obstructive azoospermia (OA) and factors affecting treatment outcome which is under reported in the literature.*

***Methods:*** *This is a multicenter study that was conducted in Egypt including all couples undergoing intra cytoplasmic sperm injection (ICSI) for OA using PESA-derived sperms. Men were subdivided according to aetiology into congenital, iatrogenic and idiopathic groups. Fertilization, pregnancy and LBRs were determined and compared in each group. The longitudinal LBR, crude and expected cumulative delivery rates (CCDR, ECDR) were calculated. Multiple logistic regression analysis was used to determine significant associations between maternal, paternal and ICSI factors with successful live births.*

***Results:*** *Ninety couples were included in the study. Viable sperm for ICSI was retrieved in 89 men (98.9%). A total of 155 ICSI cycles with 17 frozen embryo transfers resulted in 81 pregnancies and 55 live births. After 5 cycles, the longitudinal LBR, CCDR and ECDR were 30%, 57.3% and 88.6% respectively. Maternal age and number of fertilized eggs were the only factors significantly affecting LBRs.*

***Conclusions:*** *PESA is a minimally invasive procedure for securing viable sperm for ICSI in OA men, with high cumulative delivery rates. Maternal age and number of fertilized eggs are the only factors that significantly affecting LBR. The contemporary longitudinal and cumulative LBRs provide objective outcome data to counsel OA patients undergoing fertility treatments.*

KEY WORDS: *Live birth rates; Obstructive azoospermia; Percutaneous epididymal sperm aspiration.*

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## INTRODUCTION

Azoospermia is defined as the complete absence of sperm in the ejaculate and accounts for 10% of all male factor infertility, with an obstructive aetiology constituting up to 40% of azoospermia cases.

*Obstructive azoospermia* (OA) may be congenital, as *congenital bilateral absence of vas deferens* (CBAVD), or acquired as following genitourinary infection, vasectomy or idiopathic (1). The therapeutic treatment options include either reconstructive surgery, or sperm retrieval with *intracytoplasmic sperm injection* (ICSI). Surgical sperm retrieval with ICSI can be used as a primary treatment modality in patients who do not wish to proceed with surgical reconstruction, or in patients who have failed reconstructive surgery, or in cases of CBAVD.

*Tournaye et al*. in 1994 reported successful fertilization and pregnancy with spermatozoa retrieved with *Microsurgical Epididymal Sperm Aspiration* (MESA) in patients with CBAVD. Following this; *percutaneous epididymal sperm aspiration* (PESA) was described as an alternative to MESA for patients with OA (2, 3). Whilst the technique of PESA is well established, the *live birth rates* (LBRs) and factors affecting outcomes following PESA-ICSI are under-reported in the literature (4). In this context, the sperm retrieval, fertilization and pregnancy rates following PESA-ICSI vary from 69 to 100%, 58-77.7% and 40-50% respectively, although longitudinal, cumulative LBRs and the impact of male/female and ICSI variables on LBR have not been systematically analyzed (3-5). Furthermore, the effect of using fresh versus frozen sperm and embryos on LBRs, remains controversial with very limited contemporary data in the literature.

The aim of this study was to analyze fertilization, pregnancy and LBR in couples undergoing PESA-ICSI and factors affecting outcomes, including etiology of obstruction, the use of fresh versus frozen sperm and fresh versus frozen embryo transfer.

## MATERIALS AND METHODS

This is a multicenter study that was conducted in Egypt in *Tanta, 6th of October* and *Al-Mansoura universities*. The medical records of patients with OA who underwent PESA-ICSI between 2011 and 2021 were analyzed. Men were subdivided according to the aetiology of OA into congenital (eg vasal aplasia), iatrogenic (eg inguinoscrotal surgeries or vasectomy) and idiopathic (epididymal/ejaculatory duct obstruction). The age of the male/female partner, fertilization, pregnancy and LBRs were determined in each group. All men in the vasal aplasia and the idiopathic groups underwent genetic analysis (including karyotyping, Y chromosome micro-deletions and CF gene analysis).

No conflict of interest declared.

### *Technique of PESA*

PESA was performed under local anaesthetic cord block or general anesthesia on the same day as oocyte retrieval and used immediately for ICSI or cryopreserved for elective use. After taking written informed consent, the head of the epididymis was grasped between thumb and index finger and a 26-gauge needle attached to 3-ml syringe containing sperm buffer medium was inserted through scrotal skin into the epididymal head. Suction with gradual withdrawal of the needle was performed. The aspirate was flushed with sperm buffering medium and immediately examined microscopically for presence of viable sperm by an embryologist.

### *ICSI protocol*

The unit ICSI protocol including ovarian stimulation, oocyte retrieval and embryo transfer protocol has been previously described (6).

### *Primary outcome measures*

Primary outcome measures were *sperm retrieval rate* (SRR), *fertilization rate* (FR), *pregnancy rate* (PR), longitudinal LBR and two measures of cumulative delivery rates:

- *Crude cumulative delivery rate* (CCDR) is the observed number of live births following a specific, pre-determined number of ICSI cycles, divided by the total number of participating couples who had ICSI treatment (7).
- *Expected cumulative delivery rate* (ECDR) is the delivery rate assuming that couples who did not return for treatment had the same chance of a live birth over a specific number of cycles as those who continued their treatment (7).

Longitudinal LBR is the LBR after a single PESA-ICSI cycle in all patients undergoing PESA (7).
Miscarriage was defined as the spontaneous loss of a clinical pregnancy before 12 weeks of gestation.

### *Secondary outcome measures*

Secondary outcome measures included potential variables affecting LBRs including male/female age, etiology of obstruction; time elapsed since iatrogenic obstruction, the use of fresh versus frozen sperm and fresh versus frozen embryo transfer.

### *Ethics statement*

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the *Institutional Review Board* of *Human Fertilization and Embryology Authority* (HFEA) and was approved by our institutional review board (IRB approval No. 35472/5/22) and individual consent for this retrospective analysis was waived.

### *Statistical analysis*

SPSS (IBM, version 22 (13.8.2013) software package was used to analyse data. Descriptive statistics were expressed as median (IQR). Differences in outcomes between fresh and frozen sperm were analysed using Chi Square test. CCDR and ECDR were calculated using a Kaplan Meier Table & Plot. Multiple linear regression was used to determine whether secondary outcome measures were associated with LBR and p-values of 0·05 or less were deemed significant.

## RESULTS

Ninety couples with OA underwent PESA-ICSI with a median male and female age of 44 (IQR 38-49) and 36 (IQR 32-39) years respectively. In the bilateral vasal aplasia group, 7/18 (38.9%) patients were CFTR-gene positive. All patients had normal karyotype and no Y microdeletions.

### *Sperm retrieval data*

Sperm was successfully retrieved in 89 of 90 patients (98.9%). In one patient, PESA was unsuccessful and a conventional TESE was alternatively performed. In 2 out of 35 cryopreserved sperm samples (5.7%), sperm was not viable on the day of ICSI and a simultaneous fresh PESA

***Table 1.***
*Treatment characteristics and ICSI outcomes.*

| | Values |
|---|---|
| Demographics: | |
| Male age, median (IQR) | 44 (IQR 38-49) |
| Female age, median (IQR) | 36 (IQR 32-39) |
| Total number of ICSI cycles | 155 |
| Total number of frozen ET | 27 |
| ICSI cycles/couple, median (IQR) | 2 (IQR 1-2) |
| Fertilization: | |
| Ova retrieved, median (IQR) | 7 (5-11) |
| Sperm retrieved | 89/90 (98.9%) |
| Cycles using fresh sperm | 120 (77.4%) |
| Cycles using Frozen sperm | 35 (22.6%) |
| Fertilized ova, median (IQR) | 4 (3-8) |
| Fertilization rate | 95.5% (148/155) |
| Embryo transfer: | |
| Per cycle, median (IQR) | 2(1-2) |
| Frozen embryo transfer | 17 |
| Pregnancy rate: | |
| Miscarriage | 15/81 (18.5%) |
| Clinical pregnancy | 81/155 (52.3%) |
| Livebirth rate: | |
| Longitudinal | 30% (27/90) |
| of primary cycle | 32.6% |
| CCDR after 5 cycles | 57.3% |
| ECDR after 5 cycles | 88.6% |

*PET: Embryo transfer; ICSI: Intra Cytoplasmic Sperm Injection; IQR: Interquartile range; CCDR: Crude Cumulative Delivery Rates; ECDR: Expected Cumulative Delivery Rates.*

***Table 2.***
*CCDR and ECDR following PESA-ICSI.*

| Cycle | CCDR (%) | ECDR (%) |
|---|---|---|
| 1 | 32.6 | 32.6 |
| 2 | 49.4 | 58.5 |
| 3 | 53.9 | 69.6 |
| 4 | 55.1 | 77.2 |
| 5 | 57.3 | 88.6 |

*CCDR: Crude Cumulative Delivery Rates; ECDR: Expected Cumulative Delivery Rates.*

was performed. One patient required a prolonged course of antibiotics to treat epididymitis post-operatively.

### *ICSI outcomes*

The total number of ICSI cycles was 155, with a further 17 frozen embryo transfers. 89 couples underwent a primary cycle of ICSI and 39 couples had repeated cycles (a total of 66 repeated cycles), ranging from 1-5 cycles per couple. Detailed treatment characteristics and ICSI outcomes are shown in Table 1.

Following 5 cycles of ICSI, the CCDR and ECDR were 57.3% and 88.6% respectively (Table 2, Figure 1), with a longitudinal LBR of 30% (27/90). In total, PESA-ICSI resulted in the birth of 6 twins and 49 singletons.

Fifty-five (61.1%) couples used fresh, while 35 (38.9%) couples used cryopreserved sperm on the day of oocyte retrieval. There was no significant difference in pregnancy (p = 0.731) or live birth rates (p = 0.553) on comparing the use of fresh and frozen-thawed sperm (Table 3).

Multiple logistic regression analysis demonstrated that PR significantly correlated to the number of fertilized ova (p = 0.018), while LBR correlated to the number of fertilized ova and maternal age (p = 0.037 and 0.0067 respectively). All other factors including male age, aetiology of obstruction, time elapsed since iatrogenic obstruction, the use of fresh versus frozen sperm or fresh versus frozen embryos were not significantly associated with PR or LBR (Table 3).

***Table 3.***
*Factors affecting PR and LBR (multiple regression analysis).*

| | Association with pregnancy (p value) | Association with live birth (p value) |
|---|---|---|
| Male age | 0.672 | 0.800 |
| Female age | 0.146 | 0.067* |
| Number of eggs collected | 0.400 | 0.443 |
| Number of eggs injected | 0.129 | 0.606 |
| Number of eggs fertilised | 0.018* | 0.037* |
| Number of embryo transfers | 0.528 | 0.484 |
| Fresh/frozen sperm | 0.972 | 0.798 |
| Fresh/frozen embryo transfer | 0.166 | 0.482 |
| Aetiology-iatrogenic | 0.325 | 0.542 |
| Aetiology-congenital | 0.165 | 0.411 |
| Aetiology-ildiopathic | 0.618 | 0.881 |
| Presence of gene mutation (male) | 0.221 | 0.192 |

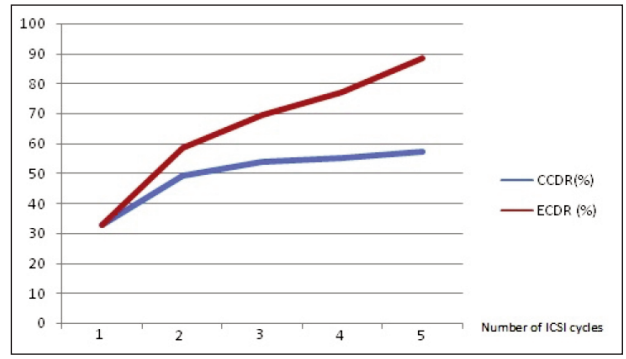
* *Statistical significance (p < 0.05). CF: Cystic fibrosis.*

***Table 4.***
*Reported PESA and ICSI outcomes in the literature.*

| Study | Number of patients or PESA attempts | Pregnancy rate (/cycle or/patients) | Miscarriage (Number or %) | Livebirth rate |
|---|---|---|---|---|
| *Pasqualotto et al*, 2002 | 130 patients | /cycle: 34.6%/patient: 54.5% | Abortion: 11.1% | NA |
| *Pasqualotto et al*, 2003 | 23 PESA for 20 patients | /cycle: 37.5% | 0 | NA |
| *Glina*, 2003 | 79 PESA for 58 patients | /cycle: 21/55 (38%) | 9 | 9 livebirths |
| *Naru et al*, 2008 | 53 patients | /cycle: 30 (43.5%) | 5 | NA |
| *Kovac et al*, 2014 | 51 (40 fresh, 11 frozen) | /cycle: 17/35 (48.6%) | NA | NA |
| *Elhanbly et al*, 2015 | 85 patients | /patient: 35 (41.1%) | NA | 29 livebirths |
| Current study | 90 patients | /cycle: 81/155 (52.3%) | 15 | Longitudinal = 27/90 (30%)<br>CCDR = 57.3%<br>ECDR = 88.6% |

*NA: not assessed. CCDR: Crude Cumulative Delivery Rate; ECDR: Expected Cumulative Delivery Rates.*

***Figure 1.***
*CCDR and ECDR following PESA-ICSI.*




## Discussion

In OA patients, microsurgical reconstruction can be done to restore patency of the seminal tract. Alternatively, surgical sperm retrieval combined with ICSI can be utilized as a primary therapeutic option or in men who do not wish to proceed with surgical reconstruction or in whom reconstruction has failed.

The advancement in the surgical sperm retrieval methods and introduction of ICSI has been a landmark achievement in the treatment of OA, although there is very limited contemporary data in the literature reporting on LBRs and factors affecting LBRs in this particular cohort of patients (Table 4) (4, 8-12).

In the current study, viable sperm was obtained in almost all men (98.9% SRR) using PESA which proved to be a minimally invasive and effective means of sperm acquisition in men with OA. Similarly, *Kovac et al.* reported that in men with OA viable sperm was obtained in all 51 patients using PESA (100% retrieval rate) (4). In contrast, *Yafiet al.* reported a much lower retrieval rate in similar patients (75.3%) (13).

*Esteve et al.* 2013 analyzed the outcome of PESA and TESA in various aetiologies of OA and found that sperm retrieval using PESA was higher in men with CBAVD compared to post-vasectomy and post-infection groups (96.8, 69.5 and 76.4% respectively) and recommended the use of PESA for CBAVD and TESA for other aetiologies (5). These findings were not substantiated in the present study, in which we demonstrate a universally high sperm retrieval rate regardless of the aetiology of obstruction, a finding also observed in other series (4). It could be speculated that improvement in surgical and laboratory techniques may explain the improved outcomes in this series of patients. More importantly, there was no difference in LBRs, irrespective of the aetiology of obstruction.

Whilst we have demonstrated no difference in LBR comparing different aetiologies, an interesting further study would be to determine the LBR from ICSI in men with OA comparing epididymal versus testicular sperm in the context of increased DNA fragmenta-

tion and examine the potential for improving outcomes using testicular sperm in this cohort of men.

An increased association of CFTR mutations has been described in patients with OA "*other than CBAVD*" and OAT syndrome, and it has been suggested that CFTR mutation screening should be limited to men with vasal aplasia on at least one side or in those with idiopathic epididymal obstruction (14). Interestingly, we did not find any CFTR mutations, other than those found in the vasal aplasia group.

Within the literature, the overall patency and pregnancy rates following vasectomy reversal are 86 and 58% respectively, with pregnancy rates inversely correlated with the duration of obstruction (15). In the present study, iatrogenic obstruction was attributed to bilateral inguinoscrotal surgeries mainly with vasectomy in only 2 patients done abroad. Vasectomy as a means of male contraception is not allowed in the Arab and Islamic countries. However, LBRs were not affected by the duration of obstruction and thus patients can be reassured that the time elapsed since obstruction does not negatively affect LBRs in couples undergoing PESA/ICSI. This is in agreement with a previous study showing no association between the time since vasectomy and clinical pregnancy after PESA (16).

Pregnancy rates following vasectomy reversal have been reported to be between 37-60% with a meantime elapsed since vasectomy of 5.7-10 years (17, 18), Similarly, PESA coupled with ICSI has been reported to result in clinical PR of 40-50 % in OA, although the number of studies reporting on LBRs in the literature is sparse (Table 4) (2-4).

Whilst it is difficult to make direct comparisons, the overall CCDR and ECDR in this study are comparable and consistent with the reported natural PR following vasectomy reversal. However, whilst this study demonstrates that PESA/ICSI patients have high CCDR (57.3%) and ECDR (88.6%), ICSI may not be as cost effective as surgical reconstruction, as up to 5 cycles of ICSI will be required to achieve comparable paternity rates to surgical reconstruction. Nevertheless, this study does provide important and contemporary comparative data on LBRs, which can be utilized when counseling patients prior to intervention for OA and therefore impact upon their decision to proceed with ICSI or surgical repair.

Some studies have suggested that cryopreservation of sperm can negatively affect the vitality; motility of sperm and their fertilizing capacity (13). However, in this study, there were no differences in miscarriage, pregnancy or LBRs using fresh and frozen-thawed epididymal sperm. Similarly, *Kovac* reported no statistically significant difference between fresh and frozen-thawed PESA-derived sperm in fertilization, overall pregnancy and multiple gestation rates (4). Thus cryopreservation, of sperm does not appear to have any negative impact on LBRs compared to fresh sperm, although a small number of patients (5.7%) did require a further fresh PESA backup due to non-viable sperm found on thawing cryopreserved sperm on the day of oocyte retrieval. Similarly, the LBRs from frozen ET were comparable to fresh ET, which may not only improve the cost effectiveness of treatment for couples undergoing repeated ICSI cycles, but also reduce repeated interventions in the female partner.

In the present study, pregnancy and LBRs were only affected by maternal age and number of fertilized ova. Similarly, *Kumtepe et al.* have reported that male age did not affect outcome of ICSI, whereas female age did (18). In contrast, *Elhanbly et al.* reported that male age negatively affected the sperm characteristics obtained by PESA, clinical pregnancy and LBRs, yet the age of the female partner did not (12).

This study has the limitation of being retrospective and including a relatively small number of patients in each aetiological subgroup and further well-designed prospective studies are needed to consolidate these current findings.

PESA is not well-adopted in Egypt or Arab countries because vasectomy is rarely done there. The population of OA in these countries is different from Europe and US. Most cases are congenital or idiopathic while iatrogenic cases are rare. However, the outcome of the current study shows that the cumulative LBRs in the form of CCDR and ECDR, in OA patients undergoing PESA-ICSI are high (75.3% and 88.6% after 5 ICSI cycles) whilst the longitudinal LBR of 30% is consistent with the LBR following other assisted reproductive technologies.

Based on this study, PESA should be more widely adopted in Egypt and Arab countries as a simple noninvasive method of sperm retrieval in OA patients. Also, it provides contemporary data that should be useful for counseling patients embarking on PESA/ICSI treatment.

## Conclusions

PESA is a simple and effective method of obtaining sperm for ICSI in OA patients, with the opportunity for sperm cryopreservation. Only maternal age and number of fertilized eggs affect pregnancy and LBR. There was no difference in outcomes using fresh or frozen epididymal sperm or fresh or frozen embryos. The longitudinal and cumulative LBRs from PESA/ICSI reported in this study provide contemporary outcome data to counsel patients with OA undergoing fertility interventions.

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