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## Can inhibin-B predict the outcome of microsurgical epididymal sperm aspiration in patients with suspected primary obstructive azoospermia?

Marij Smit<sup>1</sup>, Gert R. Dohle<sup>1</sup>, Mark F. Wildhagen<sup>2</sup>, Rob F. A. Weber<sup>1</sup>

<sup>1</sup>Andrology Unit of the Department of Urology, Erasmus MC, 3000 CA Rotterdam, the Netherlands

<sup>2</sup>Department of Urology and Gynecology, Erasmus MC, 3000 CA Rotterdam, the Netherlands

### Abstract

**Aim:** To evaluate whether inhibin-B can predict the outcome of a microsurgical epididymal sperm aspiration (MESA) procedure in patients with suspected primary obstructive azoospermia (OA) and if inhibin-B can replace testicular biopsy in the diagnostic work-up of these patients. **Methods:** Inhibin-B levels and testicular biopsy scores were related to the outcome of MESA in 43 patients with suspected primary OA. MESA was considered to be successful when epididymal sperm could be identified during the procedure. **Results:** Spermatozoa were present in the epididymal aspirate in 28 out of the 43 patients (65%). Inhibin-B values were not significantly different in patients with successful or unsuccessful MESA. The modified Johnsen score, however, was significantly lower in patients with unsuccessful MESA ( $P = 0.003$ ). A rete testis obstruction or epididymal malfunctioning was found in 15% of patients with suspected primary OA, reflected by unsuccessful MESA despite normal inhibin-B levels and normal testicular histology. **Conclusion:** Inhibin-B cannot replace testicular biopsy as a diagnostic tool in the work-up of patients with suspected primary OA. Testicular biopsy is useful in identifying patients with spermatogenic arrest, who might have normal inhibin-B values. (*Asian J Androl* 2007 May; 9: 382–387)

**Keywords:** Inhibin B; male infertility; microsurgical epididymal sperm aspiration; primary obstructive azoospermia

### 1 Introduction

Obstructive azoospermia (OA) is present in 10–20% of infertile men and is characterized by normal testicular volume and normal hormonal parameters indicating normal spermatogenesis [1]. For a definitive diagnosis a

testicular biopsy confirming normal spermatogenesis can be performed [2]. Treatment options for OA are microsurgical reconstruction of the continuity of the genital ductal tract, sperm aspiration from the epididymis in a microsurgical operation (microsurgical epididymal sperm aspiration [MESA]) under local or general anesthesia, as well as a blind percutaneous procedure (PESA). Testicular sperm extraction (TESE) and testicular sperm aspiration are the alternative options for obtaining spermatozoa for intracytoplasmic sperm injection (ICSI). TESE can also be performed in men with non-obstructive azoospermia (NOA) with a residual, often focally developed, spermatogenesis [3, 4].

Correspondence to: Dr Gert R. Dohle, Department of Urology, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, the Netherlands.  
Tel: +31-10-4633-132 Fax: +31-10-4635-838  
E-mail: g.r.dohle@erasmusmc.nl  
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MESA is indicated in OA as a result of epididymal obstruction, congenital bilateral absence of the vas deferens (CBAVD), inoperable obstruction of the seminal ducts caused by infections or previous surgery, ejaculatory disorders if conservative therapy has failed, together with vaso-epididymostomy (VES) used for ICSI if surgery fails [5]. MESA offers the advantage of extensive sperm sampling under direct visualization that can be cryopreserved to be used in future assisted reproduction treatment.

Post-vasectomy or failed vasovasostomy represent the most prevalent causes of OA in Western Europe, followed by obstruction as a result of infections and CBAVD [6]. Primary idiopathic OA represents approximately 3%–6% of all men presenting with infertility [7].

Several recent studies have established inhibin-B as a sensitive marker for both normal and impaired spermatogenesis [2, 8–12]. In our clinic, patients with suspected primary OA are selected for surgical exploration if genital ductal obstruction are indicated by medical history, physical examination, hormonal analysis, including follicle stimulating hormone (FSH) and inhibin-B, and scrotal ultrasound. Testicular biopsy and microsurgical reconstruction are frequently performed in one setting to spare the patient multiple operations. Although spermatozoa retrieval rates for MESA in men with OA and the history of vasectomy or CBAVD are reported to be successful in more than 90% of patients [5], MESA appeared to be unsuccessful in a number of our primary obstructive patients, in spite of clinical suspicion of obstruction.

The objectives of the present study are to correlate the outcome of scrotal exploration and MESA with hormonal parameters in patients with suspected primary OA and to determine more specifically the diagnostic value of inhibin-B for a successful MESA procedure in these patients. We evaluate whether inhibin-B can replace testicular biopsy in the diagnostic work-up for patients with suspected primary OA.

## 2 Materials and methods

### 2.1 Subjects

We retrospectively analyzed the results of MESA procedures in 43 men with suspected primary OA. Patients with a history of vasectomy or CBAVD were excluded. The andrological work-up comprised medical history, physical examination, scrotal ultrasound, measurement of serum luteinizing hormone (LH), FSH, testosterone and inhibin-B, and semen analysis according to World

Health Organization (WHO) guidelines [13]. In all patients azoospermia was found during WHO semen analysis. Re-evaluation of the pellet after centrifugation confirmed the absence of spermatozoa.

### 2.2 Microsurgical epididymal sperm aspiration procedure

Surgery was conducted under general anesthesia. Both testicles and the funiculus were delivered through a bilateral scrotal incision and the epididymis was inspected under an operating microscope. A single epididymal tubule was opened under direct visualization, starting in the cauda of the epididymis. Seminal fluid was examined immediately by light microscope for motile spermatozoa. When no spermatozoa were seen, a more proximal tubule was opened, until motile spermatozoa were found. MESA was considered unsuccessful if no spermatozoa could be found in the tubules of the epididymal caput. If motile spermatozoa were found, a VES was performed if technically feasible, according to Berger [14, 15]. VES was only performed if the vas deferens could be easily flushed with saline, indicating that there was no distal vasal obstruction. Motile epididymal sperm were cryopreserved to be used for ICSI if surgery failed and whenever reconstruction was not possible. The outcome of bilateral MESA procedures was considered successful when sperm could be identified in at least one side. In five patients MESA was only performed unilaterally, and the outcome of the unilateral MESA was used in the analysis in these cases .

### 2.3 Hormone analyses

Serum FSH and LH were determined with the Immulite assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and were available in 40 and 36 patients, respectively. Total serum testosterone was determined in 38 patients by radioimmunoassay, as described previously [16]. Inhibin-B was measured in 41 patients, using kits purchased from Serotec (Oxford, UK) [17]. Using the above assays, mean ( $\pm$  SME) FSH, LH and testosterone levels in a control group of 72 normal men were  $2.5 \pm 0.2$  IU/L,  $3.6 \pm 0.2$  IU/L and  $17.6 \pm 0.8$  nmol/L, respectively [18]. Mean  $\pm$  SD values for inhibin-B, measured within the same assay, were  $220 \pm 91$  ng/L in 187 men selected from the general male Danish population [9]. In our clinic, inhibin-B levels above 150 ng/L are considered to be normal, based on a previous study indicating that this cut-off could identify patients with impaired spermatogenesis [2].

### 2.4 Testicular histology

Diagnostic testicular biopsies were taken in 37 patients, either as a separate diagnostic procedure or as the scrotal exploration. Biopsy specimens were scored using the method described by Johnsen [19], modified by Aafjes *et al.* [20]. Using the modified Johnsen score, seminiferous tubule cross-sections were rated with a score from 1 to 10, based on the most advanced stage of spermatogenesis observed. Testicular biopsies scored 10, complete spermatogenesis with at least five condensed spermatids; 9, when artefacts due to staining technique such as intra tubular sludge was observed in the presence complete spermatogenesis; 8, all stages of spermatogenesis were present but less than five condensed spermatids were seen; 7, no condensed spermatids but at least five round spermatids were seen; 6, less than five round spermatids were seen; 5, no spermatids but five or more spermatocytes were seen; 4, less than five spermatocytes were present; 3, only spermatogonia were seen; 2, Sertoli cell only; and 1, no cells in the tubules. The mean score of at least 50 tubules was calculated. The mean bilateral modified Johnsen score was calculated. In five patients a single biopsy was taken and this modified Johnsen score was used in the analysis.

### 2.5 Statistical analysis

SPSS version 11.5 (SPSS, Chicago, IL, USA) was used for statistical analysis. All results are expressed as mean  $\pm$  SD. Normal data distribution was tested using the one-sample Kolmogorov–Smirnov test. Statistical analyses were performed using Mann–Whitney *U*-test and correlations were assessed using Pearson’s correlation.  $P < 0.05$  was considered to be significant.

## 3 Results

MESA results and the mean values for gonadotrophins, testosterone, inhibin-B and modified Johnsen score are shown in Table 1. MESA was successful in 28 out of the 43 patients (65%) with suspected OA. FSH, LH, testosterone and inhibin-B values had no significant differences in patients with successful or unsuccessful MESA. The modified Johnsen score, however, was significantly lower if MESA was unsuccessful ( $P = 0.003$ ).

MESA was unsuccessful in 33% of patients with inhibin-B = 150 ng/L, whereas epididymal sperm was found in 63% of patients with an inhibin-B = 150 ng/L (Table 2). A normal modified Johnsen score had a sensi-

Table 1. Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), Testosterone, inhibin-B and modified Johnsen score in patients with suspected idiopathic obstructive azoospermia (OA) (mean  $\pm$  SD). Microsurgical epididymal sperm aspiration (MESA) was considered unsuccessful if no spermatozoa could be found in the tubules of the epididymal caput.  $^{\circ}P < 0.01$ , compared with the successful.

Idiopathic OA ( <i>n</i> = 43) <i>n</i> (%)	MESA	
	Successful	Unsuccessful
	28 (65)	15 (35)
FSH (U/L)	3.1 $\pm$ 2.2	3.9 $\pm$ 1.3
LH (U/L)	2.1 $\pm$ 1.0	3.1 $\pm$ 1.7
Testosterone (nmol/L)	17.7 $\pm$ 5.5	15.8 $\pm$ 4.2
Inhibin-B (ng/L)	203.7 $\pm$ 70.7	209.1 $\pm$ 66.0
Modified Johnsen score (1–10)	8.6 $\pm$ 0.9	6.2 $\pm$ 2.7 <sup>c</sup>

Table 2. Inhibin-B levels and microsurgical epididymal sperm aspiration (MESA) results in patients with suspected primary obstructive azoospermia (OA) (*n* = 43). MESA was considered unsuccessful if no spermatozoa could be found in the tubules of the epididymal caput.

Inhibin-B	MESA successful	MESA unsuccessful	Total
= 150 ng/L	22 (67%)	11 (33%)	33
< 150 ng/L	5 (63%)	3 (37%)	8
Missing	1 (50%)	1 (50%)	2

Table 3. Mean bilateral modified Johnson score and microsurgical epididymal sperm aspiration (MESA) results in patients with suspected primary OA (*n* = 43). MESA was considered unsuccessful if no spermatozoa could be found in the tubules of the epididymal caput.

Mean bilateral modified Johnson score	MESA successful	MESA unsuccessful	Total
= 7.5	22 (85%)	4 (15%)	26
< 7.5	2 (18%)	9 (82%)	11
Missing	4 (67%)	2 (33%)	6

tivity and specificity of 92% and 64%, respectively, to predict the outcome of MESA, compared with 81% and 21% for normal inhibin-B levels.

MESA was successful in 85% of patients with a mean bilateral modified Johnsen score  $> 7.5$ , while in only 18% of men with a mean bilateral modified Johnsen score  $< 7.5$  (Table 3).

VES was performed in 18 out of 43 patients (42%) and comprised bilateral VES in 6 patients and unilateral

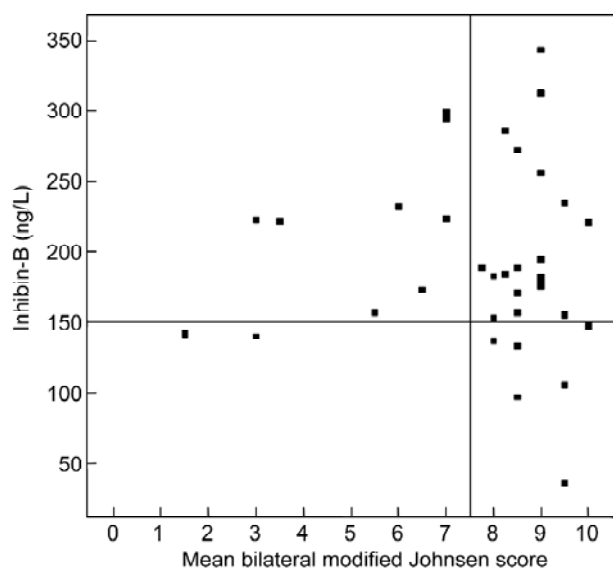


Figure 1. Pearson's correlation inhibin-B (ng/L) and mean bilateral modified Johnsen score,  $r = -0.00$ ,  $P = 0.983$ . Reference line represents mean bilateral modified Johnsen score of 7.5 and inhibin-B of 150 ng/L.

VES in 12 patients. Post-operative patency was achieved in 12 out of 18 patients (67%). In 3 patients, VES could not be performed due to a distal vasal obstruction. Of these patients, two were formerly treated with orchidopexy and scrotal surgery and one had suffered from urogenital tuberculosis. Inhibin-B was negatively correlated with FSH ( $r = -0.37$ ,  $P = 0.024$ ). In contrast, the testicular histology, expressed as the modified Johnsen score, did not correlate with inhibin-B levels ( $r = -0.00$ ,  $P = 0.983$ ) in the present study population (Figure 1).

#### 4 Discussion

The use of surgically retrieved sperm for ICSI has dramatically improved the chances for patients with azoospermia to obtain pregnancy [21]. Although patients with OA can now be treated with ICSI regardless of etiology, microsurgical reconstruction of the genital tract is the preferred approach for epididymal obstruction, with reported patency rates ranging from 67%–85% [22, 23] and spontaneous pregnancy rates ranging from 27%–49% [24, 25]. Scrotal exploration in patients with suspected primary OA offers the advantage of epididymal sperm sampling to be cryopreserved for later use in ICSI and concomitant reconstruction with VES. In the present

study, VES could be performed in 42% of patients with suspected primary OA and the postoperative patency rate was 67%. Whenever azoospermia persisted following VES, cryopreserved epididymal sperm obtained during MESA could be used for ICSI.

Bernardinucci *et al.* [7] reported a 65% epididymal sperm retrieval rate in men with idiopathic epididymal obstruction. In their study design, patients were included when azoospermia was found on semen analysis, testicular biopsy showed active spermatogenesis and there was no history of vasectomy or CBAVD. Although the objective of their study was to describe the anatomical abnormalities and outcome of surgical reconstruction, the results emphasize the limited success rate of MESA in patients with primary OA. Although we did not select patients with suspected primary OA on the basis of their testicular histology, with the combination of clinical findings, our success rate for identifying epididymal sperm during MESA was also 65%. Our results subscribe the limited success rate of MESA in patients with suspected primary OA, in whom the site of obstruction leading to azoospermia might be in the rete testis, epididymis or vas deferens.

In recent years, several studies have identified serum inhibin-B as a diagnostic marker of the quality of spermatogenesis. Pierik *et al.* [2] found inhibin-B levels positively correlated with quantitative histology assessment, whereas regression analysis indicated inhibin-B as the best predictor of the histological spermatogenesis. Von Eckardstein *et al.* [11] found that inhibin-B correlates positively with the percentage of tubules with elongated spermatids and negatively with the percentage of tubules with Sertoli cell only (SCO). In addition, several authors described a positive correlation with inhibin-B and sperm concentrations in the ejaculate and testicular volume, whereas a negative correlation is described between inhibin-B and serum FSH levels [2, 8–10, 26].

Our data show that in patients with suspected primary OA, inhibin-B values cannot predict the outcome of MESA. These results are in contrast to an observation by Ramos *et al.* [27] who concludes that inhibin-B has significant prognostic value to find sperm at PESA. It should be noted, however, that the discrepancy between the present study results and this study, might, in part, be explained by the selection of patients in the present study. Because the aetiology of the azoospermia might have led to unsuccessful MESA in patients with, for example, rete testis obstruction or spermatogenic arrest

despite normal inhibin B levels, we conclude that in patients with suspected OA inhibin-B levels cannot predict the outcome of MESA. Testicular histology, however, was significantly higher in patients with successful epididymal sperm aspiration and obviously remains the only predictor for successful MESA. Normal mean bilateral modified Johnsen score (= 7.5) had a sensitivity and specificity for predicting sperm during MESA of 92% and 64%, respectively, compared to 81% and 21% for normal inhibin-B levels (= 150 ng/L).

From these results we conclude that in patients with suspected primary OA, a discrepancy between serum inhibin-B levels and testicular histology is more prevalent than in the general infertile population. In testicular tissue with spermatogenic arrest, inhibin-B levels might remain normal or below normal, indicating normal Sertoli cell function [11, 28, 29]. Consequently, in some cases of NOA both inhibin-B and FSH might be in the normal range [11, 29] but inconsistent with testicular histology. In patients with suspected primary OA as indicated by clinical and endocrinological parameters, spermatogenic arrest is more prevalent, resulting in unsuccessful MESA because there is, in fact, NOA. In these patients, TESE procedure in combination with ICSI is the appropriate treatment option.

In our institution, we do not routinely use the determination of seminal alpha-glucosidase as a diagnostic tool for OA because reduced levels of alpha-glucosidase alone are not indicative for OA. Vesicula seminalis might also produce small amounts of alpha-glucosidase; consequently, even in patients with obstructive azoospermia, semen alpha-glucosidase levels might not be significantly reduced. Because of the inter-individual variation of seminal alpha-glucosidase levels and because clinically relevant cut-offs have not yet been established [30], we did not include this diagnostic tool in the evaluation of patients with suspected OA in the present study.

We can only speculate that the presence of epididymal sperm observed in 18% of patients with a mean bilateral modified Johnsen score below 7.5 can be explained by a sampling error of testicular tissue or inadequate pathological diagnosis.

Our results show that in 15% of patients with a modified Johnsen score above 7.5 no epididymal sperm could be identified during the MESA procedure. The absence of sperm during epididymal exploration can be the result of intratesticular blockage or nonfunctional seminiferous tubules that prevent normal testicular outflow

of spermatozoa to the epididymis. Also, epididymal dysfunction due to an extensive obstructive interval or infection might cause a negative MESA procedure. In these events, spermatogenesis is normal at testicular level, reflected by normal FSH, inhibin-B values and normal testicular histology, whereas the absence of spermatozoa outflow in the epididymis or a malfunctioning epididymis prevents successful MESA.

In summary, in the present study inhibin-B could not predict the outcome of MESA. Therefore, we conclude that inhibin-B cannot replace testicular biopsy as a diagnostic tool in the work-up of patients with suspected primary OA who are potential candidates for microsurgical reconstruction and MESA. Testicular biopsy is useful in identifying patients with spermatogenic arrest who might have normal inhibin-B values but a maturation defect causing MESA to fail. In 15% of our patients with suspected primary OA, rete testis obstruction or epididymal malfunctioning was diagnosed, reflected by unsuccessful MESA despite normal inhibin-B levels and normal testicular histology.

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