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ARTICLE

The obstructive interval predicts pregnancy rates in post-vasectomy patients undergoing ICSI with surgical sperm retrieval

BIOGRAPHY

Edson Borges Jr obtained his MD degree in 1984 (University of Campinas), PhDs in urology in 2005 (Federal University of São Paulo) and in gynaecology in 2007 (São Paulo State University). He is founder and managing partner of Fertility Medical Group and director at Sapientiae Institute, São Paulo, Brazil.

Edson BorgesJr[1,2,*], Daniela Paes de Almeida Ferreira Braga[1,2], Assumpto IaconelliJr[1,2], Amanda Souza Setti[1,2]

KEY MESSAGE

This study suggests that the obstructive interval negatively influences the SSR and ICSI outcomes in vasectomized men. These findings are useful for counselling (i) men undertaking vasectomy regarding the long-term effects and their implications for reproductive treatment, and (ii) vasectomized men planning for treatment with assisted reproductive technologies regarding the most appropriate treatment.

ABSTRACT

Research question: Are the outcomes of (i) surgical sperm retrieval (SSR) and (ii) intracytoplasmic sperm injection (ICSI) influenced by the obstructive interval (time elapsed since vasectomy)?

Design: Medical records from 148 patients (194 cycles) with secondary azoospermia due to vasectomy, who presented for percutaneous epididymal sperm aspiration (PESA) and ICSI in a private university-affiliated IVF centre, from January 2012 to February 2017, were analysed in this historical cohort study. The obstructive interval was recorded for each couple, and its influences on the outcomes of SSR and ICSI treatment were investigated using general mixed models with adjustment for potential confounders. Clinical pregnancy rate was the main outcome measure.

Results: The obstructive interval was negatively correlated with the presence of spermatozoa ($\beta = -0.032$, $P = 0.009$) and motile spermatozoa ($\beta = -0.031$, $P = 0.010$) during PESA. The need to convert to testicular sperm aspiration was significantly influenced by the obstructive interval ($\beta = 0.012$, $P = 0.003$). The blastocyst development rate on day 5 was inversely correlated with the obstructive interval ($\beta = -0.011$, $P = 0.014$). Implantation and clinical pregnancy rates were negatively influenced by the obstructive interval ($\beta = -1.107$, $P = 0.039$ and $\beta = -0.016$, $P = 0.031$, respectively). The receiver operating characteristic curve analysis demonstrated that the obstructive interval has a predictive value on the achievement of clinical pregnancy (area under the curve = 0.667, $P = 0.001$, Youden index 0.3385, associated criterion >17 years).

Conclusions: Men undertaking vasectomy should be made aware of the long-term effects and their implications for future reproductive treatment.

[1] Fertility Medical Group, Av. Brigadeiro Luis Antônio, 4545, Jardim Paulista, São Paulo, SP 01401-002, Brazil
[2] Instituto Sapientiae – Centro de Estudos e Pesquisa em Reprodução Assistida, Rua Vieira Maciel, 62, São Paulo, SP 04203-040, Brazil

*Corresponding author. E-mail address: edson@fertility.com.br (E. Borges Jr). https://doi.org/10.1016/j.rbmo.2019.02.002
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INTRODUCTION

Vasectomy is a simple, safe and highly effective procedure for permanent male sterilization, chosen by up to 8% of couples worldwide (Pile and Barone, 2009), with an estimated prevalence of 12% in North America and 11% in Oceania and Northern Europe (Jacobstein, 2015). Nearly 6% of couples with obstructive azoospermia due to prior vasectomy often seek medical care for vasectomy reversal, due to remarriage, a desire to father again or other changes in life circumstances (Patel and Smith, 2016).

Vasectomy reversal is much more challenging than vasectomy itself, and so many urologists do not perform the procedure. The reversal is achieved by vasovasostomy or vasoepididymostomy, and because most urologists consider the latter to be the most technically challenging, some only offer vasovasostomy to their patients. It has been suggested that many patients with previous failed vasovasostomy would have benefitted from a vasoepididymostomy instead (Chawla et al., 2004).

The pregnancy rate after vasectomy reversal relies on many factors such as the urologist's experience, the quality of the vasal fluid observed intra-operatively, whether or not secondary sites of epididymal obstruction are present, and associated female factors (Gerrard et al., 2007). Additionally, the obstructive interval (time elapsed since vasectomy) is also an important factor, because negative effects on semen quality, such as a higher incidence of anti-sperm antibodies and sperm clumping with decreased motility, have been reported to occur over time in vasectomized men (Weiske, 2001).

Vasectomy reversal is typically more cost-effective than intracytoplasmic sperm injection (ICSI) with surgical sperm retrieval (SSR) (Vieira, 2015). Garceau et al. (2002) found a cost per delivery nearly three-fold higher in ICSI with SSR compared with vasectomy reversal (£42,163.50 versus £16,134.00, respectively). Regarding effectiveness, the overall live delivery rates after either vasectomy reversal or ICSI with SSR correspond to 44% (Lee et al., 2008). Although recent reports have recommended vasectomy reversal as first choice of treatment (Kapadia et al., 2018), some patients still struggle to achieve pregnancy after reversal or even opt to undergo ICSI with SSR, namely, percutaneous epididymal sperm aspiration (PESA) and testicular sperm aspiration (TESA) (Abdelmassih et al., 2002).

There are thought to be few studies addressing the influence of obstructive interval on the outcomes of ICSI with SSR. Sukcharoen et al. (2000) found that the interval between vasectomy and SSR with ICSI treatment has no effect on the outcome; however, only 21 cycles performed in 17 patients were analysed in this study. On the other hand, another study observed inverse correlations between obstructive interval and implantation and pregnancy rates (Abdelmassih et al., 2002). This study aims to investigate the influence of obstructive interval on the outcomes of (i) SSR and (ii) ICSI, in couples undergoing ICSI with SSR due to previous vasectomy.

MATERIALS AND METHODS

Experimental design, patients, and inclusion and exclusion criteria

This historical cohort study analysed the medical records of 148 patients (194 cycles) with secondary azoospermia due to vasectomy, who presented for PESA and ICSI from January 2012 to February 2017, in a private university-affiliated IVF centre. The obstructive interval (time elapsed since vasectomy) was recorded for each couple, and its influences on the outcomes of SSR and ICSI treatment were investigated.

Only couples undergoing ICSI with fresh embryo transfer performed on day 5 of development were included in the analysis. Couples were included only in the presence of isolated secondary azoospermia due to vasectomy. Couples with associated female factors of infertility, those presenting with abnormal karyotype, using vitrified/warmed or donated oocytes, vitrified/warmed embryo transfer, donated embryos, or preimplantation genetic testing were excluded from the analysis.

All patients signed a written informed consent form, and the study was approved by the local Institutional Review Board on 19 December 2012 (reference number 411/2012).

Physical examination of the male partner

Hormonal profile and karyotyping were requested for every patient. All male partners underwent physical examination of their testis, which was performed by the same consultant urological surgeon. The testis size (normal volume ≥15 ml) and consistency, presence and consistency of the vasa deferentia, consistency of the epididymis, and the presence of varicoceles were evaluated during the examination. All patients had a testicular ultrasound evaluation. Additionally, patients were asked in which year they had a vasectomy, whether they had children prior to vasectomy, and whether vasectomy reversal had been performed. Vasectomy reversal was offered to all patients as primary treatment. Both patients that had failed vasectomy reversal or had elected SSR were included in the study. The obstructive interval was calculated and rounded off to the nearest completed year from the date of vasectomy until the time of SSR for ICSI.

Ovarian stimulation

Ovarian stimulation was achieved by the administration of recombinant FSH (rFSH, Gonal-F®; Serono, Geneva, Switzerland) and gonadotrophin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide; Serono). Ovulation was triggered with recombinant human chorionic gonadotrophin (Ovidrel™; Serono).

Epididymal sperm aspiration

On the day of oocyte retrieval, PESA was performed by a consultant urological surgeon and a senior clinical embryologist. All procedures were conducted under local anaesthetic. A 27.5-gauge needle was introduced into the head of the epididymis, followed by a delicate aspiration using a 1 ml syringe containing buffered supplemented culture medium (global® w/HEPES, LifeGlobal). The aspirate was transferred to a dish and examined for spermatozoa immediately by the embryologist. When no spermatozoa, immotile spermatozoa or insufficient spermatozoa were retrieved after three or four aspiration attempts, percutaneous TESA was carried out. TESA was performed using a 19-gauge needle, using syringe with positive pressure. Four testicular fragments were aspirated from different directions through the testis. The samples were transferred to the embryologist and assessed to determine their suitability for ICSI.

The samples were then prepared for ICSI using simple washing. Briefly, aspirates were diluted with buffered supplemented culture medium to a final volume of 1.5 ml, and then centrifuged at 300*g* for 10 min. Finally, the supernatant was discharged and the pellet was resuspended in 0.2 ml of the same medium.

ICSI

Mature oocytes were used for ICSI. Sperm motility status (motile or immotile) was recorded for each oocyte. Fertilization was confirmed approximately 16 h after ICSI. Embryos were morphologically evaluated on days 1, 2, 3 and 5 of development. The high-quality cleavage-stage embryos were defined as those with all of the following characteristics: four cells on day 2 or 8–10 cells on day 3, <15% fragmentation, symmetric blastomeres, the absence of multinucleation, colourless cytoplasm with moderate granulation and no inclusions, the absence of perivitelline space granularity and the absence of zona pellucida dimorphisms. Embryos lacking any of these characteristics were of low quality. On day 5 of development, embryos that reached the blastocyst stage were considered when: (i) the blastocoel was greater than half the volume of the embryo; (ii) the blastocoel completely filled the embryo; (iii) the blastocyst was expanded; (iv) blastocyst hatching occurred; and (v) blastocyst hatched.

The luteal phase was supported by intravaginal progesterone 200 mg (Utrogestan®, Farmoquímica, Rio de Janeiro, Brazil) twice a day. Embryo transfers were performed on day 5 of embryo development. Up to three embryos were transferred per patient, depending on maternal age and embryo quality. A pregnancy test was performed 10 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed when the fetal heartbeat was detected. Clinical pregnancy rates were calculated per transfer. Implantation rate was calculated by dividing the number of gestational sacs with fetal heartbeat by the number of transferred embryos. Miscarriage was defined as clinical pregnancy loss before 20 weeks.

Data analysis and statistics

The sample size calculation revealed that a sample of at least 132 treatment cycles had 95% power to detect a 10% effect with a significance level (α) of 5% (two-tailed). The calculation was performed using G*Power 3.1.7. Data are expressed as the mean ± SD for continuous variables, while percentages are used for categorical variables. General mixed models fit by restricted maximum likelihood were used to investigate the associations between the obstructive interval and the following.

1. SSR outcomes (presence or absence of spermatozoa in the aspirates, motility status of spermatozoa, and need to convert to TESA) – adjusted for paternal age, smoking habit, previous vasectomy reversal attempt, hormonal profile and abnormalities found in the male partner physical examination.
2. Laboratory ICSI outcomes (fertilization rate, high-quality embryo rate on days 2 and 3, and blastocyst development rate on day 5) – adjusted for maternal and paternal ages, smoking habits, previous vasectomy reversal attempt, hormonal profile and abnormalities found in the male partner physical examination, and number of retrieved oocytes.
3. Clinical ICSI outcomes (implantation rate, clinical pregnancy rate and miscarriage rate) – adjusted for the same variables cited in (2), as well as for the number of transferred embryos.

Potential confounders were selected when a strong association between the variable and the dependent variable was noted.

Linear mixed effects models were generated using covariates as fixed effects and individuals and treatment cycles as random effects, with unstructured covariance structure. A Gaussian distribution was assumed and the normal distribution of model residuals was checked to confirm goodness of fit. Final model selection was decided using the Akaike Information Criterion and Schwarz's Bayesian Criterion.

In a further step, receiver operating characteristic (ROC) curve analysis (*Borges Junior et al., 2003*) was performed to assess the predictive value of time of vasectomy on the achievement of clinical pregnancy. For each couple, only the first attempt of ICSI with SSR was included in this analysis. The best cut-off value was defined by Youden's index (*J*), according to the maximized sensitivity and specificity. The results are expressed as standardized regression coefficients (β), standard errors, 95% confidence intervals (CI) and *P*-values. The ROC curve results are expressed as area under the curve (AUC) with 95% CI. $P < 0.05$ was considered statistically significant. Data analyses were conducted using SPSS Statistics 21 (IBM, New York, NY, USA) and MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016).

RESULTS

SSR outcomes

The mean male age was 48.6 ± 6.8 years. From 148 males with secondary azoospermia following vasectomy, 33 (22.3%) had attempted vasectomy reversal. Motile spermatozoa were successfully retrieved in 136 (70.1%) of 194 cycles using PESA. Twelve males underwent TESA due to low or no spermatozoa being retrieved with PESA. All TESA procedures yielded spermatozoa. Baseline characteristics of males and SSR outcomes are shown in TABLE 1.

Mean obstructive interval was 15.4 ± 6.4 years. The obstructive interval was negatively correlated with the presence of spermatozoa ($\beta = -0.032$, $P = 0.009$) and motile spermatozoa ($\beta = 0.031$, $P = 0.010$) during PESA. The need to convert to TESA was significantly influenced by the obstructive interval ($\beta = 0.012$, $P = 0.003$) (TABLE 2).

ICSI outcomes

The mean age of the female partner was 35.4 ± 4.8 (range 24–42) years. A total of 194 ICSI treatment cycles were performed in 148 couples. A hundred and eleven couples (75%) underwent one ICSI cycle, and the remainder (25%) up to four cycles. Embryo transfer occurred in 165 cycles (122 couples) (85.1%), and there were 49 clinical pregnancies per transfer cycle (29.7%), three of which resulted in miscarriage (6.1%). Baseline characteristics of cycles and ICSI outcomes are shown in TABLE 3.

The longest obstructive interval associated with clinical pregnancy was 25 years. Motile sperm were exclusively used for ICSI in 135 embryo transfers (94 couples), resulting in 42 clinical pregnancies (31.1%). In the absence of adequate numbers of motile spermatozoa (according to the number of oocytes

TABLE 1 BASELINE CHARACTERISTICS OF MALES (*N* = 148) AND SSR OUTCOMES (*N* = 194)

Baseline and SSR characteristics	Value
Male age (years)	48.6 ± 6.8 (30–69)
Period of vasectomy (years)	15.4 ± 6.4 (2–31)
Endocrine profiling	
FSH (IU/ml)	5.3 ± 4.6
LH (mIU/ml)	4.1 ± 1.8
Total testosterone (ng/dl)	362.2 ± 141.1
Prolactin (ng/ml)	7.3 ± 3.3
Physical examination	
LT volume (ml)	22.4 ± 5.4 (10–33)
RT volume (ml)	23.0 ± 5.2 (12–33)
SSR	
PESA (%)	182/194 (93.8)
PESA + TESA (%)	12/194 (6.2)
LT PESA (%)	36/194 (18.6)
RT PESA (%)	85/194 (43.8)
LT + RT PESA (%)	73/194 (37.6)
Presence of spermatozoa in LT PESA (%)	101/109 (92.7)
Presence of spermatozoa in RT PESA (%)	141/158 (89.2)
Presence of motile spermatozoa in LT PESA (%)	92/101 (91.1)
Presence of motile spermatozoa in RT PESA (%)	123/141 (87.2)

Values are mean ± SD (range), unless otherwise noted.

LT = left testis; PESA = percutaneous epididymal sperm aspiration; RT = right testis; SSR = surgical sperm retrieval; TESA = testicular sperm aspiration.

suitable for injection), both motile and immotile spermatozoa were used for ICSI in 28 embryo transfers (26 couples), resulting in seven clinical pregnancies (25.0%). Immotile spermatozoa were exclusively used for ICSI in two embryo transfers (two couples), which resulted in negative pregnancy results.

The obstructive interval did not influence the fertilization rate (β = –0.098, NS) and the high-quality embryo rates on days 2 (β = –0.001, NS) and 3 (β = 0.001, NS). The blastocyst development rate on day 5 was inversely correlated with the obstructive interval (β = –0.011, *P* = 0.014). Implantation and clinical pregnancy rates were negatively influenced by the obstructive interval (β = –1.107, *P* = 0.039 and β = –0.016, *P* = 0.031, respectively). The miscarriage rate was not significantly associated with the obstructive interval (β = 0.006, NS) (TABLE 4).

The ROC curve analysis demonstrated that the obstructive interval has a predictive value on the achievement of clinical pregnancy (AUC = 0.667, 95% CI: 0.573–0.752, *P* = 0.001) (FIGURE 1).

TABLE 2 INFLUENCE OF THE OBSTRUCTIVE INTERVAL ON SSR OUTCOMES

SSR parameter[a]	Estimate (β)	SE	*P*-value	95% CI Lower bound	 Upper bound
Presence of spermatozoa during PESA	–0.032	0.012	0.009	–0.056	–0.009
Presence of motile spermatozoa during PESA	–0.031	0.012	0.010	–0.054	–0.008
Need to convert to TESA	0.012	0.004	0.003	0.004	0.019

CI = confidence interval; PESA = percutaneous epididymal sperm aspiration; SE = standard error; SSR = surgical sperm retrieval; TESA = testicular sperm aspiration.

[a] Adjusted for paternal age, smoking habit, previous vasectomy reversal attempt, hormonal profile and abnormalities found in the male partner physical examination.

The cut-off value defined by Youden's index demonstrated a negative predictive value on the chance of clinical pregnancy with >17 years of vasectomy (*J* = 0.3385, sensitivity= 90.32, specificity = 43.53).

DISCUSSION

The results of this study demonstrate that the higher the obstructive interval, the lower the chance of finding spermatozoa and motile spermatozoa with PESA, and consequently, the higher the necessity of converting to TESA. Additionally, increasing obstructive interval was inversely correlated with the blastocyst development and the implantation rates, and was also determinant to the reduced odds of clinical pregnancy. Because fertility is a couples phenomenon, the impact on ICSI outcomes were adjusted for potential confounders, such as maternal and paternal ages, paternal smoking habit, previous vasectomy reversal attempt, paternal hormonal profile and abnormalities found in the male partner physical examination, number of retrieved oocytes, and number of transferred embryos.

It is thought that only three studies have analysed the impact of the obstructive interval on ICSI cycles with SSR outcomes. *Sukcharoen et al. (2000)* studied the influence of the obstructive interval on the outcomes of 21 ICSI cycles with SSR performed in 17 patients divided into three groups according to obstructive time (I: 0–10 years, II: 11–20 years and III: >20 years). Pregnancy rates per transfer were lower in groups II and III, but did not reach statistical significance. The authors themselves stated that their study had limited power to detect a statistically significant difference because of the small number of analysed patients, which could explain the lack of agreement with the present study. On the other hand, the findings of this study are corroborated by a previous study from *Abdelmassih et al. (2002)*, which analysed the impact of the obstructive interval on 151 ICSI cycles with SSR, split into three groups according to obstructive time (I: 0–10 years, II: 11–19 years and III: ≥20 years). Pregnancy, ongoing pregnancy and implantation rates significantly decreased from group I to III. The differences

TABLE 3 OVARIAN STIMULATION AND ICSI WITH SSR TREATMENT OUTCOMES (*N* = 194)

Variables	Value
Female age (years)	35.4 ± 4.8
Number of follicles	19.1 ± 13.2
Number of retrieved oocytes	15.0 ± 10.1
Mature oocytes	9.6 ± 6.9
Fertilization rate (%)	68.0 (1273/1872)
Number of embryos	7.6 ± 5.3
Day 2 high-quality embryos (%)	53.0 (629/1187)
Day 3 high-quality embryos (%)	34.8 (413/1187)
Blastocyst development (%)	48.4 (574/1187)
High-quality blastocysts (%)	76.0 (436/574)
Number of transferred embryos	1.8 ± 1.1
Cycles with embryo transfer (%)	165/194 (85.1)
Clinical pregnancy rate (%)	49/165(29.7)
Implantation rate (%)	28.0 ± 27.5
Miscarriage rate (%)	3/49 (6.1)

Values are mean ± SD, unless otherwise noted.

ICSI = intracytoplasmic sperm injection; SSR = surgical sperm retrieval.

remained even when only cycles with females ≤35 years old were analysed.

A previous study (Borges Jr et al., 2003) evaluated the relationship between the obstructive interval and the outcomes of 77 ICSI cycles with SSR divided into four groups according to the obstructive interval (I: 0–5 years, II: 6–8 years, III: 9–14 years and IV: >15 years). The outcomes of ICSI were similar between groups I, II and III. Couples with >15 years of obstructive interval had significantly lower pregnancy, ongoing pregnancy and implantation rates, and higher miscarriage rate, compared with couples with shorter obstructive intervals. These previous results are somewhat in agreement with the present findings, because the mean obstructive interval in the present study population was >15 years.

ICSI with SSR has been offered as a surrogate method for allowing vasectomized men to have children again. In the present study, only 33 out of 148 males had attempted vasectomy reversal. Although vasectomy reversal is recommended in our centre to all couples with isolated secondary azoospermia due to vasectomy, the majority of the couples opted to proceed with IVF treatment. We could speculate that three factors might have influenced the striking dominance of IVF treatment over vasectomy reversal. The first does not have much basis in medicine and may lie in deeply engrained cultural ideas. The conception per se is considered a women's issue, which also explains why female sterilization is more popular than male sterilization, despite the unquestionable simplicity of vasectomy over tubal ligation. The male partner might believe that once their spermatozoa are surgically retrieved, the burden of pregnancy success is on the woman. On the other hand, if vasectomy reversal was performed, that burden would rely on both partners. The second factor is time to pregnancy. The mean time to pregnancy is about one year following vasectomy reversal, while IVF generally offers the fastest way to achieve pregnancy (Valerie et al., 2018), even though most couples will have to undergo more than one IVF cycle. The last factor is maternal age. In the present study, although the mean age of the female partner was 35.4 years, it ranged from 24 to 42 years. We believe that couples in which women were of advanced maternal age preferred to undergo IVF than wait for male fertility to be restored and natural pregnancy to occur.

The likelihoods of fertility restoration and pregnancy achievement after vasectomy reversal were reported to be inversely related to the length of the obstructive interval. A study from the Vasovasostomy Study Group showed a gradual downward trend in patency rates (Belker et al., 1991), while a precipitous decrease in success 10 years after vasectomy was found by Silber (1989). Dohle and Smit (2005) found higher patency rates with obstructive interval up to 5 years compared with >10 years. Boorjian and colleagues (2004) found no influence of obstructive interval on patency rates; however, a 50% decline in pregnancy rate was noted after 15 years of vasectomy. A more recent study demonstrated consistent patency over the first 15 years of vasectomy (Magheli et al., 2010). Nevertheless, longer obstructive interval is associated with a higher incidence of epididymal obstruction and the subsequent need for vasoepididymostomy, thus vasectomy reversal becomes more challenging as the obstructive interval lengthens

TABLE 4 INFLUENCE OF THE OBSTRUCTIVE INTERVAL ON THE OUTCOMES OF ICSI WITH SSR

				95% CI	
ICSI outcome	Estimate (β)	SE	*P*-value	Lower bound	Upper bound
Fertilization rate[a]	–0.098	0.302	NS	–0.696	0.500
Day 2 high-quality embryos rate[a]	–0.001	0.003	NS	–0.007	0.005
Day 3 high-quality embryos rate[a]	0.001	0.003	NS	–0.003	0.007
Blastocyst development rate[a]	–0.011	0.004	0.014	–0.019	–0.002
Clinical pregnancy rate[b]	–0.016	0.007	0.031	–0.031	–0.001
Implantation rate[b]	–1.107	0.530	0.039	–2.157	–0.056
Miscarriage rate[b]	0.006	0.009	NS	–0.012	0.025

[a] Adjusted for maternal and paternal ages, paternal smoking habit, previous vasectomy reversal attempt, paternal hormonal profile and abnormalities found in the male partner physical examination, and number of retrieved oocytes.
[b] Adjusted for the same variables cited above plus number of transferred embryos.CI = confidence interval; ICSI = intracytoplasmic sperm injection; NS = not significant; SE = standard error; SSR = surgical sperm retrieval.

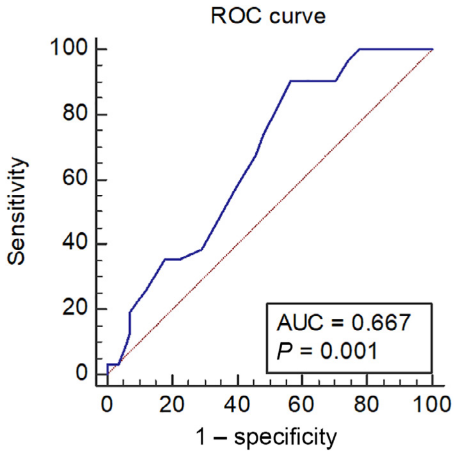

FIGURE 1 Receiver operating characteristic (ROC) curve for predicting clinical pregnancy using obstruction interval as test variable.

(*Patel and Smith, 2016*). Similarly, in the present study, we demonstrated that obstructive interval >17 years was associated with lower clinical pregnancy chance, suggesting that vasectomy reversal compares to ICSI with SSR in the setting of a prolonged obstructive interval.

Fertility may not be restored even after vasectomy reversal had been performed, due to secondary epididymal blockage, and anti-spermatozoa antibody formation. The risks of developing these two phenomena increase with increasing obstructive interval (*Sukcharoen et al., 2000*). The use of epididymal or testicular spermatozoa for ICSI can bypass those factors that interfere with fertility in vasectomized men. However, two earlier studies demonstrated that spermatozoa suffer detrimental changes related to stagnation in the epididymis post-vasectomy (*Moore, 1998; Silber et al., 1995*). One might presume that the vasectomized male is fertile and, once the obstruction is circumvented, a high quantity of spermatozoa will be retrieved in the epididymal aspirate, but this study found that increasing obstructive interval is negatively associated with the odds of finding spermatozoa and motile spermatozoa with PESA, and increases the odds of converting to TESA.

Several mechanisms have been proposed to explain the reduced sperm yield in vasectomized men. Some of those mechanisms were reported to be irreversible even after vasectomy reversal. Sertoli cell vacuolation and dysfunction have been reported by *Kubota (1969)* and may result from endocrine disruption post-vasectomy (*Mo et al., 1995; Smith et al., 1976*,). A study conducted in rabbits showed that vasectomy leads to damage to the testis related to electrolyte and transmembrane gradients, and a permanent injury in androgen–protein binding and nuclear androgen receptor levels was noted (*Wang et al., 1994*). High levels of reactive oxygen species were also found in sperm cells after vasectomy reversal (*Kolettis et al., 1999; Shapiro et al., 1998*), suggesting that seminal oxidative stress is associated with vasectomy reversal. *Shiraishi et al. (2003)* found that increased interstitial fibrosis was responsible for the irreversible damage of vasectomized testes. Finally, increased apoptosis in the seminiferous tubules, Sertoli cells, primary spermatocytes and round spermatids of vasectomized men was demonstrated by *O'Neill et al. (2007)*.

Our findings concur with previous studies that demonstrated detrimental effects of vasectomy on spermatogenesis. *Raleigh et al. (2004)* observed a significant decrease in germ cells in the later stages of spermatogenesis in vasectomized males compared with control biopsies from normal males. Moreover, the obstructive interval showed a significant relationship with the loss of spermatids. *McVicar et al. (2005)* found that sperm retrieval, and early and mature spermatid numbers, were significantly reduced in vasectomized men compared with fertile men. Additionally, clinical pregnancy rates in vasectomized couples were also significantly reduced compared with those in couples with non-obstructive azoospermia. *O'Neill et al. (2007)* showed increased testicular apoptosis in vasectomized men. They also reported that an increasing obstructive interval was associated with higher testicular sperm DNA fragmentation.

There is only so much that can be done to minimize the negative effects of obstructive interval on reproductive outcomes. For patients planning on undergoing a vasectomy, we recommend pre-vasectomy sperm banking, especially for those young males who are more likely to have a change of heart about fathering once more, thus providing sperm availability at a low cost, avoiding SSR or vasectomy reversal. For patients who have already had a vasectomy, we suggest that (i) they seek reproductive medical help as soon as the desire for fatherhood is felt, and do not delay treatment any further; (ii) vasectomy reversal is offered prior to assisted reproductive treatment (*Kapadia et al., 2018*), (iii) ICSI with PESA should be considered when fertility is not restored with vasectomy reversal, and (iv) ICSI with TESA should be considered when pregnancy has not been previously achieved with PESA.

The strengths of our study are the reasonable number of analysed subjects (supported by sample size calculation), the adjustment of statistical analyses for potential confounders, and the establishment of a cut-off value for the obstructive interval over which clinical pregnancy chance is negatively affected. The main limitation of this study is its retrospective design. Despite being the largest cohort series, our study is still limited in size when compared with the large cohort of couples undertaking ICSI.

In conclusion, our findings suggest that the obstructive interval negatively influences the SSR and reproductive outcomes in vasectomized men undergoing ICSI. Obstructive intervals >17 years showed detrimental effects on clinical pregnancy chance. The study findings are useful for counselling (i) men undertaking vasectomy, who should be made aware of the long-term effects and their implications for future reproductive treatment, and therefore be given the chance of cryopreserving spermatozoa prior to vasectomy, and (ii) vasectomized men planning for IVF regarding the most appropriate treatment, whether vasectomy reversal or ICSI with PESA or TESA.

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