CLINICS

REVIEW

Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: case series and systematic review

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We compared pregnancy outcomes following intracytoplasmic sperm injection for the treatment of male infertility according to the type of azoospermia. First, we analyzed our data from 370 couples who underwent intracytoplasmic sperm injection using sperm from men with obstructive azoospermia and nonobstructive azoospermia, and the outcomes were compared to a group of 465 non-azoospermic infertile males. Then, we performed a systematic review of the published data on pregnancy and neonatal outcomes of children born after sperm injection using sperm from men with obstructive and nonobstructive azoospermia. Live birth rates were significantly lower in the nonobstructive azoospermia group (21.4%) compared with the obstructive azoospermia (37.5%) and ejaculated sperm (32.3%) groups. A total of 326 live births resulted in 427 babies born. Differences were not observed between the groups in gestational age, preterm birth, birth weight and low birth weight, although we noted a tendency towards poorer neonatal outcomes in the azoospermia categories. The overall perinatal death and malformation rates were 2.8% and 1.6%, respectively, and the results did not differ between the groups. We identified 20 published studies that directly compared pregnancy outcomes between obstructive azoospermia and nonobstructive azoospermia. Most of these studies were not designed to detect differences in live birth rates and had lower power to detect differences in less frequent outcomes, and the reporting of neonatal outcomes was unusual. The included studies reported either a decrease or no difference in pregnancy outcomes with intracytoplasmic sperm injection in cases of nonobstructive azoospermia and obstructive azoospermia. In general, no major differences were noted in short-term neonatal outcomes and congenital malformation rates between children from fathers with nonobstructive azoospermia and obstructive azoospermia.

KEYWORDS: Male Infertility; Azoospermia; Intracytoplasmic Sperm Injection; Pregnancy Outcomes; Systematic Review.

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INTRODUCTION

Worldwide, an estimated 9% of couples meet the definition of infertility, with 50% to 60% of these couples seeking care (1). From a global perspective, these figures indicate that approximately 140 million individuals at reproductive age are unintentionally childless or have undergone treatment to reproduce (2). With regards to the male population, it has been estimated that 8% of men at reproductive ages seek medical assistance for infertility-related problems, and 1-10% of them have a condition that affects their reproductive potential (3).

One of these conditions is azoospermia, defined as the complete absence of spermatozoa in the ejaculate after centrifugation, which occurs in 1-3% of the male population and approximately 10% of infertile males (4). Despite being associated with infertility, azoospermia does not necessarily imply sterility because many azoospermic men maintain sperm production at varying levels within the testes (5). In fact, two distinct clinical presentations are usually seen in men with azoospermia. In obstructive azoospermia (OA), spermatogenesis is normal, but either a mechanical blockage exists in the genital tract between the epididymis and the ejaculatory duct or the vasa deferentia are absent. Causes of

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OA may be either acquired or congenital and include vasectomy, failure of vasectomy reversal, post-infectious diseases, surgical procedures in the scrotal, inguinal, pelvic or abdominal regions, cystic fibrosis, congenital absence of the vas deferens (CAVD), ejaculatory duct or prostatic cysts and Young's syndrome. Unlike OA, men presenting with nonobstructive azoospermia (NOA) either lack or have severely impaired spermatogenesis. NOA comprises a spectrum of testicular histopathology resulting from various causes that include genetic and congenital abnormalities, infection, exposure to gonadotoxins, medications, varicocele, trauma, endocrine disorders, and idiopathic disorders (5). In a group of 2,383 infertile men attending the tertiary center for male reproduction of one of the authors (SE), 835 (35%) were identified as having azoospermia; approximately 36% of those cases resulted from obstruction in the ductal system, whereas over 60% were associated with testicular failure caused by different conditions (Table 1).

Although selected cases of OA may be surgically correctable, treatment options for most couples with azoospermia-related infertility will ultimately include assisted reproductive techniques (ART), which is a broad term used to define any procedure that involves handling of both sperm and oocytes outside the body, such as in vitro fertilization (IVF) and its variant, intracytoplasmic sperm injection (ICSI) (6). To this end, several sperm retrieval methods have been developed to collect epididymal and testicular sperm to be used in conjunction with ART for men with azoospermia. Briefly, either percutaneous (PESA) or microsurgical epididymal sperm aspiration (MESA) are used to retrieve sperm from the epididymis in men with obstructive azoospermia, and testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) are used to retrieve sperm from the testes both in men with OA who fail PESA and those with NOA (7).

The clinical application of ART has increased significantly over time. According to the International Committee for Monitoring Assisted Reproductive Technology (ICMART), an international non-profit organization that collects data on ART and monitors approximately 2/3 of ART treatments performed worldwide, the number of treatments has steadily increased since its first report in 1998 (8,9). Along the same trend, the number of babies born from such treatments rose from 84,594 in 1998 to approximately 180,000 in 2003 (an increase of 103%). Similarly, the proportion of total births resulting from ART increased from 0.37% in 1996 to slightly more than 1% in 2009 in industrialized countries such as the United States (10). Intracytoplasmic sperm injection, which is mainly intended to bypass severe male factor infertility, including azoospermia, has become the most used form of ART treatment (8). Although these treatments improve the chances that a couple become parents, they also carry risks, including multiple gestations and preterm delivery, which carries an increased risk of short- and long-term post-natal complications. Nevertheless, there has been a large number of babies born after ICSI in cases of severe male infertility, including azoospermia, and concerns still exist regarding whether the use of spermatozoa from such individuals might affect the health of offspring (11).

The purpose of this study was to compare the pregnancy results due to intracytoplasmic sperm injection and neonatal outcomes of children born after ICSI using surgically-retrieved sperm from men with obstructive and nonobstructive azoospermia with the results from non-azoospermic infertile males treated with sperm injection. In addition, we present a systematic review of published data comparing the pregnancy outcomes after ICSI for the treatment of male infertility due to obstructive and nonobstructive azoospermia and the short- and long-term safety of such interventions.

METHODS

Study Group

Consecutive ICSI cycles involving fresh embryo transfers performed at Androfert from January 2004 to December 2010 were initially screened. A total of 471 ICSI cycles using fresh surgically-extracted sperm from men with azoospermia and 621 cycles using fresh ejaculated sperm from men with male factor infertility were reviewed in detail and included in the analysis. A complete male and female workup was conducted in all couples before enrollment in our ART program to both determine the cause of infertility and the treatment strategy as previously described (5). Semen analyses were performed on at least two different occasions according to the World Health Organization criteria (12). Azoospermic individuals were seen by the

Table 1 - Distribution of Diagnostic Categories and Frequency of Azoospermia in a Group of Infertile Men Attending a Tertiary Center for Male Reproduction.

Category	Number and Absolute Frequencies; N (%)	Men Presenting with Azoospermia; Number and Relative Frequencies, N (%)
Varicocele	629 (26.4)	32 (5.1)
Post-infectious[§]	161 (6.9)	57 (35.4)
Endocrine	54 (2.3)	26 (48.1)
Ejaculatory dysfunction	28 (1.1)	NA
Systemic disease	11 (0.4)	2 (18.1)
Idiopathic[‖]	645 (27.1)	178 (27.6)
Immunologic	54 (2.2)	None
Obstruction[¶]	259 (10.9)	244 (94.2)
Cancer	11 (0.4)	4 (36.3)
Cryptorchidism	342 (14.4)	174 (50.8)
Genetic[‡]	189 (7.9)	118 (62.4)
TOTAL	2,383 (100.0)	835 (35.0)
Obstructive Azoospermia; N (%)		302 (36.1)
Nonobstructive Azoospermia; N (%)		507 (60.7)

[§] include orchitis and sexually transmitted diseases; [‖]include testicular failure and idiopathic obstruction; [¶] include vasectomy and ejaculatory duct obstruction; [‡]include congenital absence of vas deferens, Yq microdeletion and Klinefelter syndrome.

urological team to diagnose whether the azoospermia was obstructive or nonobstructive. Of the 370 azoospermic men, 182 and 188 had OA and NOA, respectively. The distinction between obstructive and nonobstructive azoospermia took into account the history, physical examination, endocrine analysis, and genetic testing as appropriate (5). In addition, diagnostic sperm retrieval and testis biopsy for histopathology analysis were conducted in selected cases. The type of azoospermia was further confirmed by testicular histopathology in all testicular sperm retrievals performed at the time of sperm injections. Indications for ICSI were in accordance with the guidelines of the II Brazilian Consensus of Male Infertility (13).

Laboratory and clinical protocols remained practically unchanged during these time periods. Ovarian stimulation, oocyte and sperm retrieval, sperm processing, and IVF were conducted as previously reported (14-17). Briefly, percutaneous epididymal (PESA) and/or testicular sperm aspiration (TESA) were performed in cases of obstructive azoospermia, and microsurgical testicular sperm extraction (micro-TESE) or testicular sperm aspiration (TESA) was used for sperm collection in nonobstructive azoospermia. Ejaculated spermatozoa were processed by discontinuous colloidal gradient. Oocytes were retrieved after ovarian stimulation with gonadotropins in association with either pituitary down-regulation with gonadotropin-releasing hormone (GnRH) agonists or luteinizing hormone (LH) surge suppression with GnRH antagonists. The cumulus-corona-oocytes complexes were stripped, classified according to nuclear maturity, and maintained in culture until sperm microinjection. Sperm injections were conducted under 400× magnification using epididymal or testicular sperm in OA, testicular sperm in NOA and ejaculated sperm in non-azoospermic cases. The injected oocytes were transferred to a closed culture system and incubated for 16-18 hours at 37℃ and 5.5% CO2 until fertilization was confirmed. Fertilized oocytes were maintained in culture, and embryo quality was scored daily according to the criteria described by Veeck for cleaving embryos (18) and by Gardner for blastocysts (19). The embryos were classified as top quality when they had 3-4 and 7-8 symmetrical blastomeres on the second and third days of culture, respectively, with no multinucleation, 0-20% of the perivitelline space occupied with fragments, and exhibited no abnormalities in the zona pellucida. Embryos kept in extended culture were considered top quality upon exhibiting full blastocyst formation and onwards. Selected embryos were transferred to the uterine cavity on the third or fifth day of embryo culture. Luteal support with a once-daily transvaginal application of progesterone gel was initiated in the day of oocyte retrieval and continued up to the 12th gestational week. Oocyte and sperm retrieval, micromanipulation of gametes, embryo culture, and the transfer of embryos to the uterine cavity were conducted in clean room environments (20).

Pregnancy was first detected using quantitative serum beta-hCG testing and further confirmed clinically by observing the presence of gestational sac on the seventh-week ultrasound scan. In our program, clinical and laboratory ART data are systematically and continually entered into a database, and pregnancy follow-up is conducted by telephone interviews on a monthly basis. With confirmation of live birth, follow-ups continue with the registration of gestational ages, birth weights, neonatal disorders, and eventual malformations for a period of 30 days post-delivery. Pregnancy outcomes of all ART cycles are annually reported to the Latin America ART Registry (REDLARA). Signed informed consent was obtained from patients to use both clinical and laboratory data for analysis with guarantees of confidentiality.

For the statistical analysis, sperm injection cycles were grouped according to the source of sperm used for ICSI, i.e., ejaculated or surgically-retrieved. In ICSI cycles with non-ejaculated sperm, a distinction was made between OA and NOA. The main pregnancy outcomes analyzed were clinical pregnancy, ectopic pregnancy, miscarriage, live birth, and the perinatal outcomes of babies born (parity, weight and gestational age at the time of delivery, mortality and birth defects). The qualitative variables were expressed as both absolute (n) and relative (%) frequencies. The relationship between the variables among groups was evaluated by the Chi-square test and Fisher's exact test as appropriate. The quantitative variables were expressed as means and standard deviations. An analysis of variance for one factor (one-way ANOVA) was used to compare these variables when there was a normal distribution, and differences were analyzed by the Tukey multiple comparisons test. For the variables without normal distribution, comparisons were performed with the Kruskal–Wallis test, and the differences were compared using the Dunn multiple comparisons test. A p-value below 0.05 was considered significant. All analyzes were processed using SPSS®, version 13.0 (SPSS Inc., Chicago, IL, USA). Multiple pregnancies were considered as a single event, and when one gestation produced one live birth and one abortion, the result was registered as a live birth.

Systematic Review

The review was structured around one key question involving short-term (including clinical pregnancy, spontaneous abortion, ectopic pregnancy, live birth and multiple pregnancy) and long-term (preterm delivery, low birth weight, neonatal and infant complications and longer-term physical and developmental problems) pregnancy outcomes for the fetus/child following sperm injections using fresh surgically-retrieved sperm from men with OA and NOA. Specifically, we intended to investigate if the pregnancy outcomes after ICSI differ according to the classification of azoospermia as obstructive or nonobstructive.

We searched Pubmed, Scielo and Open J-gate for English-, Portuguese-, or Spanish-language studies published from January 1995 through March 2012. The search was supplemented by a hand search of reviews published by the Cochrane Library.

The search strategy used the National Library of Medicine's Medical Subject Headings (MeSH) keyword nomenclature. The keywords were ''reproductive techniques, assisted;'' ''infertility, male;'' ''sperm injections, intracytoplasmic;'' and ''pregnancy'' or ''child.'' Case reports and reviews were excluded. Articles were also excluded if the type of azoospermia (obstructive or nonobstructive) was not clearly stated or if only one azoospermia category was studied, regardless of whether or not a control group of sperm injections using ejaculated sperm had been included. If more than one paper presented data from the same group of patients, we selected the most recent paper.

Nomenclature description

For the purpose of this study, pregnancy outcome nomenclature was defined as follows: i) The pregnancy was considered clinical if a gestational sac was visualized by ultrasonography on the seventh week of gestation. The

clinical pregnancy rate was the ratio between the number of clinical pregnancies after embryo transfers and the number of initiated ICSI cycles (per cycle) or number of embryo transfers (per transfer). ii) Ectopic pregnancies were registered as clinical pregnancies occurring outside the uterine cavity. iii) Spontaneous abortion was defined as pregnancy loss anytime from the establishment of pregnancy to the completion of 20 gestational weeks. The miscarriage rate was the ratio of the number of spontaneous abortions and number of clinical pregnancies. iv) Live birth included deliveries that resulted in at least one live birth. The live birth rate was the ratio between the number of live birth deliveries and the number of initiated ICSI cycles (per cycle) or number of embryo transfers (per transfer). v) Multiple births were pregnancies resulting in more than one birth. vi) Preterm births were those taking place after 20 but before 37 gestational weeks. vii) Low birth weight and very low birth weight were defined as babies weighing less than 2,500 g and 1,500 g, respectively, at birth. viii) Perinatal mortality included stillbirths after 20 weeks of gestation and neonatal deaths (deaths within the first 28 days). ix) Birth defects were defined as structural, functional or developmental abnormalities presented at birth or later due to genetic or non-genetic factors acting before birth.

RESULTS

Study Group

A total of 1,092 ICSI cycles were performed in 835 patients. Patient characteristics and a comparison of laboratory and clinical ICSI outcomes are shown in Table 2. Groups were homogeneous in terms of the mean age of the female partners, female serum hormones, and the proportion of females with an associated fertility problem. There were no statistically significant differences in the numbers of retrieved oocytes and transferred embryos to the uterine cavity among the groups. The percentages of normal fertilization and high-quality embryos available for transfer were significantly lower in the group of men with NOA, but they did not differ if sperm injections were performed using non-ejaculated sperm from men with OA or ejaculated sperm from patients with male infertility.

The rates of clinical pregnancy and live birth were lowest in the NOA group. Live birth rates differed between the NOA group (21.4%) compared with OA (37.5%) and ejaculated sperm (32.3%; $p = 0.003$). Miscarriage, ectopic pregnancy and multiple pregnancy rates did not differ among the groups.

Three hundred and twenty-six live births resulted from 1,041 fresh embryo transfers (live birth rate of 31.3%). The distribution of live births in relation to parity, gestational age, and birth weight is shown in Table 3. A total of 427 babies was delivered and assessed. Overall, differences were not observed among groups for gestational age and birth weight in the three parity stratifications. Similarly, the rates of preterm birth, low birth weight and very low birth weight did not differ among groups in the studied parity stratifications. The preterm birth rates were greatest for singletons in OA (17.9%) and for twins in both OA (47.1%) and NOA (44.5%) compared with the ejaculated group (9.7% and 27%, respectively), but these differences were not significant ($p = 0.15$). We noted a tendency towards lower gestational age for twins in the OA group (35.6 ± 2.8) compared with the NOA (36.2 ± 2.4) and ejaculated groups (37.0 ± 2.3), but the numbers were relatively small to reach statistical significance ($p = 0.06$). The overall perinatal death and malformation rates were 2.8% and 1.6%, respectively, and the results did not differ among groups. The frequency of babies of the male gender was higher in the OA group (56.4%) compared with the NOA (41.4%) and ejaculated (39.7%) groups ($p = 0.02$).

Systematic Review

We reviewed 373 abstracts relevant to ICSI, male infertility and pregnancy. To address the key question discussed in this

Table 2 - Patient Characteristics and Comparison of Laboratory and Clinical Outcomes after Sperm Injections in Azoospermic (Obstructive and Nonobstructive) and Non-azoospermic Infertile Males.

	Obstructive Azoospermia	Nonobstructive Azoospermia	Ejaculated Sperm	*p*-value
Patient Characteristics				
No. of patients	182	188	465	
Mean ± SD male age; years	42.6 ± 9.0^{a}	37.0 ± 7.6^{b}	36.3 ± 8.9^{c}	<0.001(^{a} *vs.* ^{b,c})
Mean ± SD female age; years	32.6 ± 5.8	32.4 ± 4.7	33.0 ± 6.8	0.26
Laboratory Outcomes				
No. of cycles	243	228	621	
Mean ± SD no. oocytes retrieved	11.8 ± 7.7	12.7 ± 6.9	11.7 ± 7.0	0.07
Mean ± SD% 2PN fertilization*	62.9 ± 22.3^{a}	43.7 ± 27.9^{b}	64.5 ± 35.8^{c}	<0.001(^{b} *vs.* ^{a,c})
Mean ± SD% high-quality embryo§	52.50 ± 30.2^{a}	45.3 ± 33.6^{b}	47.8 ± 32.4^{c}	$=0.01$(^{b} *vs.* ^{a,c})
No. embryo transfers	237	210	594	
Mean ± SD no. embryos transferred	2.7 ± 1.3	2.7 ± 1.6	2.7 ± 1.4	0.99
Clinical Outcomes				
No. clinical pregnancies (%)	116 (48.9)^{a}	60 (28.6)^{b}	248 (41.7)^{c}	<0.001(^{b} *vs.* ^{a,c})
No. ectopic pregnancies (%)	2 (1.7)	3 (5.0)	8 (3.2)	0.22
No. miscarriages (%)	24 (21.0)	11 (19.2)	47 (19.6)	0.75
No. live births (%)	89 (37.5)^{a}	45 (21.4)^{b}	192 (32.3)^{c}	$=0.003$(^{b} *vs.* ^{a,c})
No. multiple births (%)	22 (24.4)	13 (28.3)	46 (23.8)	0.52

*PN = pronuclei (normal fertilization after sperm injections was defined as the presence of two pronuclei and a second polar body); 2PN fertilization rate defined as the ratio of the number of normal 2PN fertilized oocytes and number of mature injected oocytes. §Embryo quality was assessed according to the arrangement and number of blastomeres, presence or absence of multinucleation, and degree of cytoplasmic fragmentation. Embryos were considered high quality when 3-4 and 7-8 symmetrical blastomeres were seen on the second and third days of culture, respectively, with no multinucleation and no more than 20% of the perivitelline space occupied with cytoplasmic fragments. The high-quality embryo rate is the ratio of the number of high-quality embryos and number of embryos developed. Multiple births include live births resulting in more than one baby delivered. Each live birth is considered a single event.

Table 3 - Neonatal Outcomes of Children Born Following Sperm Injection in Azoospermic and Non-azoospermic Infertile Males.

	Obstructive Azoospermia	Nonobstructive Azoospermia	Ejaculated Sperm	*p*-value
No. live birth singletons	67	32	145	
Mean ± SD gestational weeks at birth	37.5±2.2	37.8±2.1	38.0±2.1	0.11
No. preterm births (%)	12 (17.9)	3 (9.4)	14 (9.7)	0.10
Mean ± SD birth weight (grams)	2,963±480	2,957±667	3,092±579	0.24
No. low birth weights (%)	7 (10.5)	3 (9.4)	10 (6.9)	0.37
No. very low birth weights (%)	2 (2.9)	2 (6.2)	4 (2.8)	0.38
No. live birth twins	17	9	37	
Mean ± SD gestational weeks at birth	35.6±2.8	36.2±2.4	37.0±2.3	0.06
No. preterm births (%)	8 (47.1)	4 (44.5)	10 (27.0)	0.15
Mean ± SD birth weight (grams)	2,261±594	2,357±403	2,461±672	0.30
No. low birth weights (%)	11 (64.7)	6 (66.6)	18 (48.7)	0.28
No. very low birth weights (%)	2 (11.7)	1 (11.1)	4 (10.8)	0.92
No. live birth triplets	5	4	9	
Mean ± SD gestational weeks at birth	32.6±3.1	32.3±5.9	32.6±4.5	0.93
No. preterm births (%)	4 (80.0)	2 (50.0)	2 (22.2)	0.37
Mean ± SD birth weight (grams)	1,660±624	1,311±471	1,600±642	0.35
No. low birth weights (%)	4 (80.0)	3 (75.0)	8 (77.8)	0.87
No. very low birth weight (%)	2 (40.0)	3 (75.0)	3 (33.3)	0.32
Total No. children born	117	63	247	
No. perinatal deaths*	3 (2.5)	4 (6.3)	5 (2.0)	0.10
Gender				
No. boys (%)	66 (56.4)a	26 (41.4)[b]	98 (39.7)[c]	0.02 [a] *vs.* [b,c]
No. girls (%)	43 (36.8)	35 (55.5)	122 (49.4)	0.02 [a] *vs.* [b,c]
No. unknown (%)	8 (6.8)	2 (3.1)	27 (10.9)	0.06
No. birth defects (%)	2 (1.7)	2 (3.2)	3 (1.2)	0.26

*Perinatal deaths included stillbirths (birth of fetuses with no sign of life that occur after 20 weeks of gestation) and neonatal deaths (deaths within the first 28 days). One stillbirth occurred in each group. Birth defects were defined as structural, functional or developmental abnormalities presented at birth or later due to genetic or non-genetic factors acting before birth.

study, 20 manuscripts that directly compared pregnancy outcomes between men with OA and NOA were included in our analysis. Most studies were retrospective in nature. Twelve studies provided data on pregnancy rates and/or live birth only (Table 4). Four studies reported on neonatal outcomes (Table 5). All studies were published in English.

Short-term pregnancy outcomes

We identified 12 studies that compared pregnancy outcomes with ICSI using surgically-retrieved spermatozoa of men with OA and NOA (Table 4). Six of these reported decreased pregnancy rates (clinical or live birth) with sperm from men with NOA compared with OA, and the other half showed no difference in outcomes, regardless of the type of azoospermia. A control group of sperm injections using ejaculated sperm was available in six studies, and again, conflicting results were noted. Three studies found lower outcomes in the NOA group compared with either the OA or ejaculated sperm groups, whereas three reported similar pregnancy rates among the groups. One consistent finding was that pregnancy outcomes did not differ if ejaculated sperm or non-ejaculated sperm obtained from men with OA were used for ICSI. Only three studies compared miscarriage rates; two reported no difference in risk of miscarriage among the groups, whereas one showed a significant increase in loss rates with ICSI using sperm from NOA men. Ectopic pregnancy was occasionally reported but not compared. None reported multiple pregnancy rates.

Long-term pregnancy outcomes

Four studies compared neonatal outcomes of children born after ICSI using surgically-retrieved spermatozoa in men with OA and NOA (Table 5). In general, the data showed no major differences between children from fathers with NOA and OA. Of note, lower gestational age in singletons and increased frequency of premature twins were reported in one study in the NOA group. Similar congenital malformation rates, ranging from 1.3% to 5.2% and 0% to 4.2%, were observed in the OA and NOA groups, respectively. However, the data were based on a very limited population. We did not identify any study comparing long-term physical, neurological and developmental outcomes in these categories of male infertility.

Expert Commentary

Currently, ICSI is widely used for patients with azoospermia, and several publications have reported the sperm injection outcomes with non-ejaculated sperm. It has been shown that neonatal outcomes, including karyotype results and malformation rates, of children conceived with non-ejaculated sperm are comparable to those of counterparts conceived with the use of ejaculated sperm (35,36). Bonduelle et al. and Jozwiak et al. analyzed karyotype results of fetuses and newborns for which ejaculated and testicular sperm were used for ICSI. Abnormal karyotypes were found in 3.1% and 1.9% in the respective aforementioned studies when ICSI was performed with ejaculated sperm, and those results did not differ from those obtained by using testicular sperm (4.8% and 1.5%). However, Bonduelle et al. called attention to the fact that the frequency of *de novo* chromosomal anomalies was higher in the testicular sperm group, but the numbers were too small to allow definitive conclusions. Several investigators have also studied congenital anomalies in such groups (37-40). Ludwig et al. reported major malformation rates of 9.2%

Table 4 - Studies Comparing Pregnancy Outcomes after Intracytoplasmic Sperm Injection using Surgically-retrieved Spermatozoa in Men with Obstructive and Nonobstructed Azoospermia.

Authors; Year (reference)	Design	Region	OA *vs.* NOA; number of cycles	Control group of ejaculated sperm; yes/no; No. cycles	Most relevant pregnancy outcome assessed	Main findings	Other findings
Aboulghar et al.; 1997 (21)	Retrospective	Egypt	126 *vs.* 80	Yes; 102	CPR	Lower CPR for NOA compared with other groups	Miscarriage rate and multiparity rates presented but not compared
Ghazzawi et al. 1998 (22)	Prospective	Jordan	19 *vs.* 30	Yes; 28	LBR	Lower LBR when testicular sperm from men with NOA was used (10%) compared with ejaculated (21%) or epididymis (22%)	Increased miscarriage rate when testicular sperm from NOA men were used
Ubaldi et al.; 1999 (23)	Prospective controlled	Italy	33 *vs.* 29	Yes; 62	Ongoing pregnancy and LBR	Similar results among groups	Implantation rate lower in NOA (13.4%) *vs.* ejaculated sperm or OA (~26%)
Palermo et al.; 1999 (24)	Retrospective	USA	255 *vs.* 53	No	LBR	Lower LBR with testicular sperm from NOA *vs.* epididymal sperm from OA	Similar malformation rate between groups
De Croo et al.; 2000 (25)	Retrospective	Belgium	139 *vs.* 54	No	LBR	Similar LBR between OA (16.2%) and NOA (22.6%)	Miscarriage and multiparity described but not compared
Bukulmez et al.; 2001 (26)	Retrospective	Turkey	43 *vs.* 53	Yes; 780	CPR	No difference in outcome	NR
Schwarzer et al. 2003 (27)	Retrospective	Germany	300 *vs.* 414	No	LBR	Lower LBR in NOA (19%) *vs.* OA (28%)	NR
Ghanem et al.; 2005 (28)	Case series and meta-analysis of cohort studies	Egypt	48 *vs.* 42	No	CPR	Similar CPR between OA (25%) and NOA (23.1%)	Lower fertilization rate in NOA
La Sala et al.; 2006 (29)	Retrospective	Italy	NA	NA	CPR	Similar CPR in OA (12.9%) *vs.* NOA (15.4%)	NR
Verza Jr & Esteves; 2008 (15)	Retrospective	Brazil	39 *vs.* 54	Yes; 220	CPR	Lower pregnancy rates (25.9%) in NOA compared with OA (51.3%) and ejaculated sperm (36.6%)	Miscarriage rates did not differ between groups
Semião-Francisco et al.; 2010 (30)	Retrospective	Brazil	274 *vs.* 102	No	CPR	No differences in CPR between groups	Higher miscarriage rate in OA with the use of testicular sperm compared with epididymal sperm
He et al., 2010 (31)	Retrospective	China	112 *vs.* 42	No	CPR	Lower CPR in NOA (21.4%) than OA (40.2%)	Similar miscarriage rates

AO = obstructive azoospermia; NOA = nonobstructive azoospermia; LBR = live birth rate; CPR = clinical pregnancy rate; NR = not reported; NA = not available.

and 3.8% in children born after the use of testicular and epididymal spermatozoa, respectively; these values were not significantly different than the rate of 8.4% with ejaculated sperm (37). Other investigators reached similar conclusions and reported even lower rates of congenital anomalies in those groups (38-40). These publications, however, failed to discriminate between the subgroups of men with OA and NOA.

Nonetheless, few studies to date have addressed outcomes by making a systematic distinction between OA and NOA. This prevents consideration regarding the severity of spermatic defects on ICSI results. Whereas men with OA have normal sperm production, those with NOA have severely defective spermatogenesis and a very limited amount of sperm within the testes, if any, which may result in an increased risk of genetic and epigenetic defects (41).

In this study, we compared the reproductive potential of azoospermic men undergoing sperm injections according to the type of azoospermia. A subgroup of non-azoospermic infertile men treated with ICSI was included for comparison. We noted that sperm injections with testicular sperm of men with NOA resulted in lower fertilization and embryo development compared with either the sperm of OA individuals or ejaculated sperm of non-azoospermic men. Moreover, clinical pregnancy and live birth rates were lowest in the NOA group, whereas no difference was observed between the groups of OA and ejaculated sperm. We also reported the neonatal profile of babies born with ICSI and showed similar outcomes from using NOA, OA or ejaculated sperm. Although we noted that the preterm birth rates were greatest for singletons from OA and twins from both OA and NOA and that the gestational age was lowest for twins from OA, our data involved a limited population. In our series, congenital malformation (1.6%) and perinatal death rates (2.8%) did not differ between groups and are in agreement with those reported in larger cohorts (32-34). Given the relative rarity of specific birth defects, identifying an association between a specific exposure and subsequent risk is difficult. Moreover, not all major malformations are found at birth, and a proportion of children were lost to follow-up

Table 5 - Studies Comparing Neonatal and Developmental Outcomes of Children Born Following Intracytoplasmic Sperm Injection using Surgically-retrieved Spermatozoa from Men with Obstructive and Nonobstructed Azoospermia.

Authors; Year (reference)	Design	Region	OA *vs.* NOA; Number of children	Control group of children born with ejaculated sperm; yes/no	Outcomes assessed	Main findings
Palermo et al. 1999 (24)	Retrospective	USA	158 *vs.* 22	No	Malformation rate	Congenital malformation rate did not differ in OA (1.3%) compared with NOA (4.5%)
Vernaeve et al. 2003 (32)	Retrospective	Belgium	196 *vs.* 61	No	Multiple pregnancy rates, gestational age, birth weight, preterm delivery, low birth weight, malformation rate	Similar multiple birth, overall preterm delivery, low birth weight, perinatal death and malformation rates (3% OA; 4% NOA). Lower gestational age in singletons and increased frequency of premature twins in the NOA group
Fedder et al 2007 (33)	Retrospective	Denmark	282 *vs.* 76	No	Multiple pregnancy rates; congenital anomalies	Malformation rate in OA (4.0%); No malformations reported in NOA group
Belva et al.; 2011 (34)	Prospective	Belgium	474 *vs.* 193	No	Multiple pregnancy rates, gestational age, birth weight, preterm delivery, low birth weight, malformation rate	Similar multiple birth, overall preterm delivery, low birth weight, perinatal death and malformation rates (5.2% OA; 4.2% NOA)

(Table 3). It is therefore possible that the number of malformations is underestimated.

We also presented a systematic review of published data comparing the pregnancy outcomes following ICSI for the treatment of male infertility due to obstructive and nonobstructive azoospermia and the short- and long-term safety of such interventions. We noted that few studies have specifically compared sperm injection outcomes taking into account the type of azoospermia. Moreover, these studies had several shortcomings. The majority were retrospective case studies that only provided data on pregnancy rates (clinical or live birth). We were unable to identify follow-up studies on the physical, neurological, and developmental outcomes of children from fathers with these categories of male infertility. In general, the clinical pregnancy and live birth rates reported in the literature range from 26-57% and 18-55% in NOA and OA, respectively, and the results are similar to those reported with ICSI using ejaculated sperm (15,21-31). Although the assessment of fertilization and implantation rates was not the scope of this study, we noted that such parameters were lower with ICSI using testicular spermatozoa of men with NOA compared with ejaculated sperm or epididymal/testicular sperm of men with OA (15,23). Nonetheless, conflicting reports exist regarding whether clinical pregnancy and live birth rates are affected by the type of azoospermia. Studies have reported either a decrease (15,21,22,24,27,31) or no difference (23,25,26,28-30) in pregnancy outcomes with ICSI in cases of NOA and OA, respectively. Such decreased reproductive potential of men with NOA seen in some studies may be explained by the fact that testicular spermatozoa from men with severely impaired spermatogenesis have a higher tendency to carry deficiencies, such as the those related to the centrioles and genetic material, which ultimately affect the capability of the male gamete to activate the egg and trigger the formation and development of a normal zygote and viable embryo (32).

Palermo et al. (1999), Vernaeve et al. (2003), and Belva et al. (2011) were among the few researchers that differentiated between men with OA and NOA. In the first study, the frequency of congenital malformation did not differ in relation to the sperm source or type of azoospermia. In the study of Vernaeve et al., malformation rates of 4% and 3% were reported after the use of testicular sperm of NOA and OA patients, respectively. Recently, Belva et al. reported on the neonatal outcome of 724 children born after ICSI using non-ejaculated sperm and included a subgroup analysis of the type of azoospermia in which the frequencies of major malformation and karyotype anomalies were not different in OA and NOA.

Despite the limited population analyzed, some differences observed in our study and that of Vernaeve et al. (32) regarding the gestational age and birth weight of babies born call for continuing monitoring. Because intrauterine growth is strongly dependent on placental function, these observations may suggest increased abnormalities of implantation/placentation in such pregnancies. The extent to which this is a function of treatment, maternal/embryonic factors, or both is yet to be determined.

This report has several limitations, including the restriction of studies to English, Portuguese and Spanish languages, the potential for missing relevant studies, and the lack of studies with large patient samples and meta-analysis. As noted, the relative lack of data on fetal, neonatal and long-term outcomes in the studied male infertility categories should be identified as a major research priority. As such, future research considerations should include the use of multi-center trials with adequate sample sizes, the development of standard data sets to differentiate between the groups of men with OA and NOA, and control groups of children conceived with ICSI using ejaculated sperm to facilitate meta-analyses and reach a consensus on significant clinical differences to aid sample size estimates, especially for less common outcomes.

Key issues

- In our series of 1,092 ICSI cycles performed in 835 male infertility patients, live birth rates were lowest in the

NOA group. Miscarriage, ectopic pregnancy and multiple pregnancy rates were not different between clinical pregnancies obtained using ejaculated or non-ejaculated sperm from men with OA or NOA.

- Of the 427 babies born following ICSI using sperm from non-azoospermic infertile fathers and azoospermic fathers with OA and NOA, the short-term neonatal outcomes were similar among groups, despite a tendency towards higher preterm births in both azoospermia categories and lower gestational age for twins from OA. The overall perinatal death and malformation rates were 2.8% and 1.6%, respectively, and our results did not differ regarding whether deliveries following ICSI used ejaculated or non-ejaculated sperm from men with OA or NOA.
- Most published studies that addressed pregnancy and neonatal outcome of children born after the use of non-ejaculated sperm suffer from methodological shortcomings. The population included is small, and in general, no discrimination is made between OA and NOA.
- To date, few studies have directly compared pregnancy outcomes between OA and NOA, and the data are limited. Most of the studies were not designed to detect differences in pregnancy and live birth rates and had low power to detect differences in less-frequent outcomes, such as multiple births and complications.
- In general, clinical pregnancy and live birth rates reported in the literature range from 26-57% for NOA and 18-55% for OA, and the results are similar to those reported with ICSI using ejaculated sperm. Published studies have shown either a decrease or no difference in pregnancy outcomes with ICSI in cases of NOA and OA. No major difference was noted in short-term neonatal outcomes and congenital malformation rates between children from fathers with NOA and OA. However, these results are based on a very limited population, and tendencies towards lower gestational age and birth weight of babies born from azoospermic fathers call for continued monitoring.
- No follow-up study has yet compared the long-term physical, neurological and developmental outcomes of children born with ICSI using sperm from azoospermic men with OA and NOA.
- Due to the relative lack of data on fetal, neonatal and long-term outcomes of children born from azoospermic fathers, future studies should include the use of multicenter trials with adequate sample size and development of standard datasets to differentiate between the groups of men with OA and NOA. Efforts should also be made to reach a consensus on significant clinical differences regarding sample size estimates, especially for less common outcomes, thus facilitating meta-analyses.
- Currently, the limited evidence regarding pregnancy and postnatal outcomes of ICSI using surgically-derived sperm from azoospermic men is reassuring, but a call for continuous monitoring is of utmost importance to support the recommendation of sperm retrieval and ICSI in such male infertility categories.

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AUTHOR CONTRIBUTIONS

Esteves SC was involved in the acquisition and analysis of the data; both authors were involved in the drafting and revision of the manuscript.

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