SPERM RETRIEVAL AND PREGNANCY OUTCOME FOR AZOOSPERMIC INFERTILE MEN

AVEEN MUNIB MAHMOUD, MBCHB, MSC*
SHAKIR SALEEM JABALI, MBCHB, FICMS, FACS**
ABDULGHAFOOR S. ABDULKAREEM, MBCHB, FICMS***
OMAR HASSAN KHANAGA, B SC, PH D****

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ABSTRACT

Background: surgical sperm retrieval and in vitro fertilization using intracytoplasmic sperm injection is the only hope for the infertile men with permanent azoospermia to father a biologic child since its introduction by the Palmero group in 1992.

Aim of the study: To study the testicular sperm retrieval rate of azoospermic infertile men and the pregnancy outcome by intracytoplasmic sperm injection at Azadi infertility center.

Patients and methods: A retrospective study of 220 azoospermic infertile patients were evaluated and dealt with at Azadi infertility center at Duhok, Kurdistan, Iraq, during the period from 2011–2020. They were diagnosed with either obstructive or nonobstructive azoospermia. One hundred forty-three of them were subjected to conventional testicular sperm extraction for sperm retrieval, and the sperms, when detected, were cryopreserved if the surgery was performed in advance. Sixty-one couples accepted the option of intracytoplasmic sperm injection at the Azadi infertility center. Ovarian stimulation performed by the gonadotrophin-releasing hormone agonist and antagonist protocol, and intracytoplasmic sperm injection was performed by the embryologist. The pregnancy result was regarded successful if serum βhCG level is more than 25 mIU/ml two weeks after embryo transfer or miscarriage if serum βhCG level is below 25 mIU/ml or lack of fetal heart beating in trans vaginal ultrasound examination. The pregnancy is then followed regularly through the antenatal care unit. All the completed pregnancies were delivered by the Caesarian section.

Results: Out of 1301 infertile couples, 220 (16.9%) were azoospermic. Testicular sperm extraction was performed for 143 men. One hundred and one men (70.6%) were positive and 42 men (29.3%) were negative for sperm retrieval and 61 of them accepted to proceed for intracytoplasmic sperm injection, 47 (77%) with obstructive azoospermia and 14 (23%) with nonobstructive azoospermia. Freshly extracted sperms were used in 40 (65.6%) ICSI cycles while cryopreserved sperms in 21(34.4%) cycles. The number of ova retrieved ranged from 2-17 with a mean of 8.6. Successful fertilization confirmed in 57 cycles and positive biochemical pregnancy confirmed in 27 (47.3%), miscarriage in 4 out of 27 pregnancies (14.8%) and 16/57 (28%) gave live birth of 11 singletons, 4 twins and one triplet and seven still with ongoing pregnancy. Two birth defects were reported among the delivered babies.

Conclusions: The sperm retrieval rates in obstructive azoospermia were lower than the usual rates using the conventional testicular sperm extraction. Microsurgical testicular sperm extraction is advised for nonobstructive azoospermia. The fertilization rates, biochemical pregnancy, miscarriage and baby take home were within the usual rates no increase in the birth defects rates in ICSI.

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Fertility and childbirth have been one of the main basic needs throughout the human life. World health organization (WHO) defines infertility as a disease characterized by the failure to establish a clinical pregnancy after one year of

* Assistant Lecturer, College of Pharmacy, Duhok University, University of Duhok, Kurdistan Region, Iraq
** Assistant Prof, Department of Surgery, College of Medicine, University of Duhok, Kurdistan Region, Iraq
*** Assistant Prof, Department of Surgery, College of Medicine, University of Duhok, Kurdistan Region, Iraq
**** Assistant Prof., Biology. Azadi infertility center for reproductive medicine
Correspondence author: *Aveen M. Mahmoud, aveen.mahmoud@uod.ac, Mobil +96407504586974*
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regular, unprotected sexual intercourse or due to an impairment of a person's ability to reproduce either as an individual or with his/her partner[1, 2].

Many factors are behind infertility, such as; ovulatory factor (about 20%), utero-tubal peritoneal factors (30%), semen migration factor (10%) and male factor (30%). Around 40% of all infertile couples exhibit a combination of factors and about 15% of couples may not display any objective alterations leading to a definite diagnosis (unexplained infertility) [3].

Globally, assisted reproductive technologies (ARTs) are increasingly used to overcome fertility problems which include in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), oocyte donation, embryo cryopreservation, treatment with artificial gametes and surrogacy. Many adverse outcomes may associate ART such as miscarriage, low birth weight, multiple pregnancy, preterm membrane rupture, preterm delivery and congenital malformations[4,5].

Azoospermia, defined as the absence of sperm in the ejaculate, is found in around 1% of all men and in 10-16% of infertile males. Cryptozoospermia means sperm concentration always less than (0.001×106/mL) with occasional azoospermia[6,7]. Azoospermia is either obstructive one (OA) as a consequence of epididymal or vas obstruction or nonobstructive one (NOA) due to deficits in the spermatogenesis whereby there is no enough sperm production to be detected in the ejaculate, which could be either idiopathic or caused by genetic causes with absence or near absence of mature spermatids[8].

Once infertile men with azoospermia had no other options than adopting a child or using donor sperm to father a child. However, nowadays, they are provided with other alternatives, which are given to them by introducing sperm retrieval from their testes and then entering an Intracytoplasmic sperm injection (ICSI) cycle. Sperm donation and surrogacy are forbidden by Islamic Shareea'a according to the 3rd international Islamic Fiqh Academy Conference outputs in Amman, 1986[9].

In obstructive azoospermia, sperm is usually obtained by percutaneous or microsurgical epididymal sperm aspiration (PESA) or (MESA). In nonobstructive azoospermia, sperm is obtained directly from the testis. Micro testicular sperm extraction (m-TESE) yields the greatest number of sperms than conventional TESE[10].

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technology (ART) procedure where a single sperm is injected directly into a mature egg, is indicated for the treatment of male-factor infertility when sperm parameters are inadequate for intrauterine insemination (IUI) or conventional in vitro fertilization (IVF). ICSI is the most used ARTs (66%) worldwide for male infertility since its introduction by Palmero et al. group in 1992 and resulted in the birth of millions of babies[11-13].

Aim of the study:

To analyze the success rate of testicular sperm retrieval through TESE of obstructive and nonobstructive azoospermic men and pregnancy outcome by intracytoplasmic sperm injection (ICSI).

PATIENTS AND METHODS

A total of 220 azoospermic patients out of 1301 infertile couples were retrospectively evaluated and dealt with at Azadi infertility center at Duhok, Kurdistan Region, Iraq, during the period from 2011–2020. All infertile couples were evaluated clinically, biochemically, and radiologically and accordingly were diagnosed with either obstructive or nonobstructive azoospermia (testicular size and consistency, FSH level, and past testicular biopsy results reviewed). One hundred forty-three patients with azoospermia accepted the option of conventional TESE to find out the presence of sperm inside the testicle. The procedure of ordinary TESE was performed uni or bilaterally according to the results under general anesthesia. The tunica albuginea was incised for 0.5 cm at the avascular plane. The part of the protruded seminiferous tissue was excised by the scissor and placed in Petri dishes containing Eagle's solution and delivered to the embryologist for evaluation of the minced seminiferous tissue under a phase-contrast microscope. If the spermatozoa were found in the material submitted, the procedure is terminated; if not, the procedure is then repeated in other sites of the same testis or the other testis. If the wife was not prepared initially, the retrieved sperms were cryopreserved by consent.

The option of ICSI was given to those azoospermic infertile patients with positive testicular sperm retrieval to achieve pregnancy, and only those who accepted the procedure were included in the study.

Preparation of female partners is usually done at the start of the ICSI program, which includes ovarian stimulation or induction of ovulation performed using the short and long gonadotropin-releasing hormone GnRH agonist and GnRH antagonist protocol. The ova were retrieval by transvaginal ultrasound guide and under general anesthesia.

ICSI is performed by the embryologist in the ICSI lab in which a single sperm (fresh or cryopreserved) is injected into a single oocyte under an inverted ICSI microscope and incubated in a special incubator at 37O C. The number of embryo transfers (1-3) usually performed either on day 3 (morula) or day 5 (blastocyst) according to the patient's condition, quality of fertilization, and the patient's wish.

The pregnancy result is usually evaluated two weeks after embryo transfer and regarded successful biochemically (clinical pregnancy) if serum βhCG level is more than 25 mIU/ml or miscarriage if serum βhCG level is below 25 mIU/ml or lack of fetal heart beating in trans vaginal US examination. Then the pregnancy is followed up by ultrasound evaluation 6 weeks later for gestational sac, and fetal heart beating then followed regularly through the antenatal care unit. All the completed pregnancies were delivered by the Caesarian section at a pre-scheduled time.

RESULTS

Out of 1301 infertile couples, 220 (16.9%) were diagnosed with azoospermia, 146 (66.3%) with clinical OA, and 74(33.6%) with NOA. Testicular sperm extraction (TESE) was performed for 143 men (129 with primary and 14 with secondary infertility), their ages ranged from 22-55 years with a mean of 33.96 ± 6.8 SD,

while the age range of their wives were 18-43 years with a mean of 30.43 ± 6.25. One hundred and one men (70.6%) were positive and 42 men (29.3%) were negative for sperm retrieval and 61 of them accepted to proceed for ICSI, 47 (77%) with OA and 14 (23%) with NOA (table 1). Freshly extracted sperms were used in 40 (65.6%) ICSI cycles while cryopreserved sperms in 21(34.4%) cycles. The number of ova retrieved ranged from 2-17 with a mean of 8.6. Successful fertilization confirmed in 42.7% of total ova retrieved, and the embryo was transferred to 57 wives.

Biochemical pregnancy was confirmed in 27 (47.3%) of the transferred embryos with s BhCG>25mIU/ml. Abortion or miscarriage documented in 4/27 (14.8%) and fetal loss of 2 embryos during the follow-up periods and 16/27 (59.2%) completed uneventful pregnancy and delivery of 20 babies (11 singletons, 4 twins and one triplet), 7/61 still with an ongoing pregnancy at the time of writing this article and the overall pregnancy and delivery rates were 37.7% and 26.2% respectively (Table 2).

Table 1: Azoospermic patients' demographic, sperm retrieval, and TESE outcomes.

Male partner age (n; 143)	22-55	33.96 ± 6.8 SD
Female partner age (n;143)	18-43	30.43 ± 6.25 SD
Sperm retrieval by TESE (n;143)	+ve 101 (70.6%)	-ve 42 (29.3%)
+ve sperm retrieval (n;101)	OA 44/49 (89.7%)	NOA 57/94 (60.6%)

OA: obstructive azoospermia; NOA: nonobstructive azoospermia

Table 2: fertilization rate and pregnancy outcomes of azoospermic patients by ICSI

Sperm type used	Fresh TESE 40	Cryopreserved TESE 21
Successful fertilization rate (525 oocytes)	42.7%	
Biochemical pregnancy (n;57) s BhCG>25mIU/ml	+ve 27 (47.3%)	-ve 30 (52.6%)
Miscarriage	4/27 (14.8%)	
Fetal loss	2/23	
Live birth	16/61 (26.2%)	singleton 11, twin 4 and triplet 1
Ongoing pregnancy	7/61 (11.4%)	
Birth defects	2/16	

DISCUSSION

Recent advances in the assisted reproductive techniques have made it quite possible to identify and overcome most of the previously untreatable causes of male infertility, specially azoospermia. To properly utilize the available techniques and improve clinical results of azoospermic infertile men, it is of the utmost importance that patients are adequately diagnosed and evaluated.

The treatment of infertility due to azoospermia has undergone a radical change over the past decades, firstly by the introduction of microsurgery, which has increased the success rates of reproductive tract reconstruction and sperm retrieval either from the testicle or the epididymis and gamete micromanipulation and secondly by the result of ICSI by which many obstacles will be eliminated in the way of fatherhood for men with severe male factor infertility. Today, more than 95% of infertile men can father their own genetic child without the need for donor sperm[14,15].

In our study, the sperm retrieval rates were lower than the expected rates as it should be (89.7% vs. 100 %) in OA and was higher in NOA (60.6% vs. 52.4%).16 In a study performed by Erdem et al., and Vloeberghs et al., found sperm retrieval rates related to TESE procedure was 40.5% in NOA, and two reasons were attributed for this variation results first, the use the conventional TESE rather than m TESE and second, some of the OA were diagnosed as NOA.16,17 In a meta-analysis performed by Bernie et al., the superiority of m-TESE over conventional TESE for sperm retrieval rates (52 vs. 35%) was demonstrated, and (50.8 vs. 33.7%) by Turunc 2010. The testis volume and FSH level were important predictive factors for positive sperm retrieval results in NOA[18,19].

Although the m TESE has been recognized to be better for sperm retrieval and less invasive with less detrimental consequences and complications, none of these early complications were recorded in our patients and with similar positive sperm retrieval results.[16]

It has been stated that ICSI results are generally not affected by the immaturity of the male gamete retrieved from the testis. However, fertilization rates are shown to vary according to the type of azoospermia (OA or NOA) and whether the sperm used is fresh or frozen-thawed. Also, it depends on the maturity stage of the retrieved oocyte. Habermann et al. 2000 reported fertilization rates of 56% with fresh vs. 61% with frozen-thawed testicular sperm. Ohlander et al. 2014 study showed no statistical difference between the use of fresh or cryopreserved-thawed testicular sperm when assessing fertilization rates (52 vs. 54%) and clinical pregnancy (28.7 vs. 28.1%) in couples undergoing ICSI due to higher sperm DNA fragmentation in the ejaculated or fresh sperms[20,21].

The pregnancy and giving live birth rates are variable by ICSI among studies of surgically retrieved sperms in azoospermic infertile men. The published literature reveals that overall clinical pregnancy and live-birth rates are 2.4-fold and 2.6-fold higher, respectively when Testi-ICSI is performed as compared with ICSI using ejaculated sperm (ICSI is also not affected by the immaturity of the male gamete and SDF rate is lower in testi-sperm than ejaculated sperm) Basukarno et al. 2016 reported success rate of ICSI 72.5% and most of the gave live births[22].

Turunc et al. reported 39.1% and 37.1 live birth rates when conventional TESE compared with m TESE, respectively. Erdem et al. reported clinical pregnancy and live birth rates were 29.9% and 25.6% in OA and 27.1% and 23.7% in NOA, respectively. Gunby et al. reported 27.1% live birth rate in Canadian ICSI centers[16,23].

Generally, the incidence of miscarriage is slightly higher after IVF than spontaneous pregnancy. The reasons behind higher miscarriage rates in IVF are the maternal age (3-5 years older in IVF), earlier pregnancy detection, intrinsic abnormalities within the embryo (higher sperm DNA fragmentation index, non-disjunction and aneuploidy), improper culture conditions, singleton versus twin pregnancy (miscarriage is about 21% for singleton and 11% for twin pregnancy at 6th wk of gestation and the incidence gradually fall to reach about 2% at 13th wk for both) [24].

Esteves et al. 2017 reported a statistically significant and clinically important 72% reduction of miscarriage likelihood in the Testi-ICSI cohort compared with ejaculated sperms ICSI[18,25].

Evidence-based strategies to improve sperm DNA quality and decrease miscarriage rates include varicocelectomy, antioxidant therapy, and treatment of genitourinary infections prior to ICSI may be beneficial and has been supported by multiple studies[26].

It's hard to evaluate the birth defects in our small sample study as many studies have shown that infants born after ART have poorer birth outcomes than spontaneously conceived babies (4% vs. 2.9%), especially in a singleton pregnancy and large sample size researches are actually needed to clarify effects from fresh- or frozen-embryo cycles after ICSI[27,28].

In conclusion: in our center, TESE is a relatively successful surgical procedure for infertile males with obstructive and nonobstructive azoospermia to retrieve sperms for ICSI procedures with variable but within the general fertilization clinical pregnancy and live birth rates.

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پوختە

دەرئینانا تۆڤی زەلامی (سپێرم) ئو ئەنجاما دووگیانی ژبو مێرین نەزوک ئو بێ تۆڤ

بنەکوک وپاشکیتی :دەرئینانا تۆڤی(سپێرم) بریکا نشتەرگەریی ئوبکارێینانا کوتان کرن لناف سایتوپلازمێ دبیتە هیڤیا بتنی بو میرین نەزوک ین بێ تۆڤ دا ببیتە باب. ئەڤ ریکە هاتە بکار ئینان بو جارا ئیکی ژ لای گروپا پالمیرو لسالا ۱۹۹۲

ئارمانجا لیکولینی :بو خیندنا دەرئینانا تۆڤی(سپیرم) ل گونی ل میرین نەزوک ئو بێ تۆڤ. هەروەسا ئەنجامین دووگیانی ب رێکا تۆڤ کوتان لناڤ سایتوپلاسمێ ل سەنتەرێ ئازادی یی نەزوکی

نەخوش و رێک:

دناڤبەینا سالێن ۲۰۱۱-۲۰۲۰ دا لیکولینەکا پاشپیرتوی ۲۲۰ نەخوشین نەزوک ین بی تۆڤ ل ناڤەندا سەنتەرێ ئازادی یی نەزوکی ل دهۆکی (کوردستانا عیراقی).

نەخوش هاتنە دەست نیشان کرن . دوو جورین بی تۆڤ هەبوون ریکا سپیرما گرتی ئو ریکا سپیرما نە گرتی. نشتەرگەریا دەرئینانا تۆڤی ل گونی هاتە ئەنجامدان بو ۱٤۳ نەخوشا. ئو سپێرمیت دەرئینایین هاتنە فریز کرن ئەگەر نشتەرگەری هاتنە ئەنجامدان بەروەخت. شێست و ئێک ژن و مێرا پەسەند کر کو بو بیتە ئەنجامدان ل سنتەرێ ئازادی یێ نەزوکی. هێلک هاتنە هاندان ب دوو رێکا :

GnRH agonist and antagonist protocols

ئو کوتانا تۆڤی دناڤ سایتوپلاسمێ هاتە ئەنجامدان ژلایێ پزیشکی کورپەلناس. ئەنجامێن دووگیانی هاتە تومارکرن سەرکەفتی ئەگەرپشکنینا دووگیانی پتر بو ژ۲۵یەکە دوو حەفتیا پشتی کورپەلە تێتە ڤەگوهاستن بو ژنکێ یان ژی بچیک ژبەر چوون ئەگەرپشکنینا دووگیانی کیمتر بو ژ۲۵ یەکە یان ژی دلی بچیکی نە قوتیت وەختی سۆنەر بو ژنکێ تێتە کرن. دوو گیانی ب رێک و پێک ژ لایێ ئێکەتیا لێنیرینا ناتانی ڤە تیتە شوپاندن. ژنێن ماوی دووگیانی کوتایی کریندی هێنە هنارتن بو هێلا زارۆک بوونی بریکا نشتەر گەریی قەیسەری

ئەنجام :ژ ۱۳۰۱ مێرێن بی زاروک ۲۲۰ ژوان بێ تۆڤ بوون دەرئینانا سپێرما ل گونی بریکا نشتەرگەریی هاتە ئەنجامدان بو ۱٤۳ مێرا. سەدوو ئیک ژ وان (۷۰،٦٪) تۆڤ هەبوون بریکا نشتەرگەریی ئو ٤۲(۲۹،۳%) ژ وان تۆڤ نەبوون. شێست و ئێک ژوان ئەوین تۆڤ هەین پەسەندکرن کو بو وان بێتە ئەنجامدان ژ وان ٦۱ پتنێ ٤۷(۷۷%) ریکا تۆڤی گرتی بوئو ۱٤(۲۳%) رێک نە گرتی بو. ئەو تۆڤی تازە ئینایە دەری هاتنە بکار ئینان بو ٤۰(٦٥،٦%) خولین دەما کو تۆڤی فریز کری ها تیە بکار ئینان بو ۲۱(۳٤،٤%) خولەکین .

ژماریت هیلکا بیت هاتینە دەرئینان د ناڤبەرا (۲-۱۷) بو ئو تیکرا د بیتە (۸،٦). کوتان سەرکەفتی بو ل ۵۷ خولەکان ئو دووگیانی بریکا پشکنینا دووگیاني سەرکەفتی بو ل ۲۷(٤۷،۳%).بچیک ژ بەر چوون ل ٤(۱٤٪) ژوان ۲۷ یت دووگیان. شازدە ل وان ۲۷ بیت دووگیان زارۆک بوون. ٤ جێمک بوو ئو ئێکی سێ بچیک بوون. حەفت ژوان دووگیانی یابەردەوامە. دوو بچیکیت پەیدا بووین کیموکوری لی بوون

دەرئەنجام :دەرئینانا توڤی ژ میرین ریکا توڤی نە گرتی کیمتر بو ژ ریژا یاسای یا دەرئینانا توڤی بریکا نشتەرگەری. ژبەر هندی ئەم پیشنیار تکەین کو نشتەرگەری بریکا میکروسکوپی بو وان بیتە ئەنجامدان. ریژا کوتانی, دووگیانیا زیندویا کیمیاوی, بچیک ژبەر چوون ئو بچیک برنە مالی وەکی ریژیت یاسای بوون.. ریژا کیماسیین ژدایک بوونی زیدە نەبوون

الخلاصة

سترجاع النطف المنوية ونتائج الحمل عند العقيمين اللانطفيين

الخلفية: إسترجاع النطف المنوية جراحيا والإخصاب الخارجي بالحقن المجهري هي السبيل الوحيد للعقيمين اللانطفيين الدائميين لإنجاب أطفال من أصلابهم منذ استحداثها عام 1992 من قبل مجموعة بالميرو.

الهدف: دراسة معدل إسترجاع النطف المنوية جراحيا من العقيمين اللانطفيين ونتائج الحمل بطريقة الإخصاب الخارجي بواسطة الحقن المجهري للحيمن في مركز آزادي للعقم.

المرضى وطرق البحث: دراسة مستعادة ل 220 عقيم لانطفي تم تقييمهم وعلاجهم في مركز آزادي للعقم مستشفى آزادي/ محافظة دهوك / كردستان العراق خلال الفترة من 2011-2020. تم تشخيصهم الى لانطفيين انسدادي أو غير إنسدادي.تم إخضاع 143 مريض لإستخراج الحيامن من الخصية جراحيا وتجميد الحيامن في حالة وجودها. تم إجراء الحقن المجهري ل 61 عقيم لانطفي وإعتبار فحص الحمل موجبا اذا كانت نتيجة مستوى المنشط المنسلي المشيمي في الدم أكثر من 25 وحدة عالمية في الملتر. تمت الولادات بعملية قيصرية.

النتائج: أظهرت الدراسة 220(16%) عقيم لانطفي من أصل 1301عقيم تم علاجهم في المركز. تم العثور على الحيامن في الخصى جراحيا في 70% من أصل 143 الذين تم إخضاعهم جراحيا , 77% لانطفي إنسدادي و23% لانطفي غير إنسدادي. تم استخدام النطف المنوية الطازجة في 65,6% بينما المجمدة سابقا في 34.4. %
عدد البويضات المسحبة كانت بين 2-17 وبمعدل 8.6 . تم تأكيد الإخصاب في42,7% من مجموع البويضات المسحوبة بينما كان فحص الحمل ايجابيا في 47.3% من العقيمين. اسقاط جنيني في 14,8% كما تمت ولادة 28% من أصل 57 على شكل ولادات أحادية في 11 حالة وتوائم ثنائية وثلاثية في 5 حالات . تم تسجيل تشوهات خلقية في حالتين.

الإستنتاجات: معدل إسترجاع النطف من الخصية جراحيا في اللانطفيين الإنسدادي كان اقل من المعدلات الإعتيادية وننصح باستخدام المجهر الجراحي بينما كانت نسبة الإخصاب والحمل والإسقاطات والولادات الحية ضمن المعدلات ولاتوجد زيادة في معدل اتشوهات الخلقية عند الولادات