



Testicular Sperm Extraction and Intracytoplasmic Sperm Injection in Management of Obstructive Azoospermia: A Two-Year Multicenter Review in Ghana

Promise E. Sefogah¹, Alim Swarray-Deen^{1*}, Edem K. Hiadzi², Rudolph K. Adageba³, Nana Essuman Oduro⁴, Hanson G. Nuamah⁵, Mercy A. Nuamah¹

1- Department of Obstetrics and Gynaecology, College of Health Sciences, University of Ghana Medical School, Accra, Ghana

2- Department of Obstetrics and Gynaecology, Lister Hospital and Fertility Centre, Accra, Ghana

3- Department of Obstetrics and Gynaecology, Ruma Fertility and Specialist Hospital, Kumasi, Ghana

4- Department of Obstetrics and Gynaecology, Korle Bu Teaching Hospital, Accra, Ghana

5- Department of Epidemiology and Disease Control, School of Public Health, University of Ghana, Accra, Ghana

Abstract

Background: The objective of this study was to evaluate treatment outcomes and assess predictors of clinical pregnancy in obstructive azoospermia cases treated with testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) in Ghana.

Methods: This study was a retrospective study conducted on 67 men seeking treatment for obstructive azoospermia at two study sites in Ghana from January 2018 to December 2019. First, archived data were reviewed and treatment outcomes of cases of obstructive azoospermia from the hospital records were evaluated. Infertile men who met the inclusion criteria were recruited. Descriptive data were expressed in the form of frequencies and percentages. The dependent and independent variables were analyzed using multiple logistic regression and reported as odds ratios (ORs). The confidence interval (CI) was set at 95% and a p-value <0.05 was considered significant.

Results: The mean age of male participants was 42.43±9.11 years (mean±SD) while the mean age of their partners was 32.89±5.73 years (mean±SD). The average duration of infertility before intervention was 5.01±3.60 years (mean±SD). Successful pregnancy was observed in 52.2% (35/67) of the participants. After adjusting for confounders, the rate of a successful clinical pregnancy was 0.07 lower for every additional year increase in the male's age [AOR=0.93 (95%CI=0.87-0.99), p=0.02].

Conclusion: Overall the rate of clinical pregnancy following TESE/ICSI from our study was 52.2%. A man's age was a strong predictor of successful clinical pregnancy among couples treated with TESE-ICSI for obstructive azoospermia in Ghana.

Keywords: Azoospermia, Intracytoplasmic sperm injection, Male infertility, Testicular sperm retrieval.

To cite this article: Sefogah PE, Swarray-Deen A, Hiadzi EK, Adageba RK, Oduro NE, Nuamah HG, et al. Testicular Sperm Extraction and Intracytoplasmic Sperm Injection in Management of Obstructive Azoospermia: A Two-Year Multicenter Review in Ghana. *J Reprod Infertil.* 2022;23(4):264-270. <https://doi.org/10.18502/jri.v23i4.10811>.

Introduction

Infertility, the inability of a couple to achieve pregnancy following one year of unprotected intercourse, is a common condition experi-

enced by many men and women. Global trends in infertility report that approximately 48.5 million couples are unable to have a child, consisting of

* Corresponding Author:
Alim Swarray-Deen,
Department of Obstetrics
and Gynaecology, College
of Health Sciences,
University of Ghana Medical
School, Accra, Ghana
E-mail:
asdeen8@gmail.com

Received: Feb. 20, 2022

Accepted: Jul. 17, 2022

19.2 million couples suffering from primary infertility while the remaining 29.3 million have secondary infertility (1). Approximately 10.0 million infertile couples live in sub-Saharan Africa (1). Studies suggest that male factor accounts for up to half of the infertility cases (2, 3) and approximately 10% of infertile men present with azoospermia (4). A prospective study of 110 male patients seeking treatment for infertility at the urology outpatient clinic of the Komfo Anokye Teaching Hospital, Kumasi, Ghana, reported azoospermia in 25.5% of patients (5).

Azoospermia, defined as the complete absence of spermatozoa in ejaculation, is the most severe manifestation of male infertility (6). Although there are several causes of azoospermia, Jungwirth et al. described how the aetiologies of this disorder might be categorized as pre-testicular, testicular, or post-testicular (7). Scrotal and transrectal ultrasound scans can identify structural abnormalities of the epididymis, seminal vesicles, and prostatic cysts, which can confirm the aetiology of azoospermia (8). Azoospermia may also be clinically classified as obstructive or non-obstructive. Obstructive azoospermia, the absence of spermatozoa in the ejaculate despite normal spermatogenesis, is less common than non-obstructive azoospermia and constitutes approximately 15-20% of azoospermic men (7). Significant advancements in treatment strategies for male infertility have been achieved. These advancements are attributed mainly to surgical and microsurgical techniques, including varicocele repair and testicular sperm retrieval (9).

Several factors have been reported to influence successful testicular sperm extraction treatment. Different studies have established testicular histopathology (10-12) and the man's age (14, 15) as the strongest predictors of successful testicular sperm extraction. Other factors such as follicle-stimulating hormone levels (16, 17) and body mass index (BMI) (14, 18, 19) have also been observed to influence the success rate of testicular sperm extraction. It can be concluded from these studies that different factors and patient characteristics play essential roles in influencing the success rate of testicular sperm extraction for fertilization.

Since the desire to have children is both a basic need for many individuals and an instinctive desire for procreation, infertility may cause significant distress to those affected. Unfortunately, despite the increased number of centers providing

assisted conception treatment in Ghana in recent years, there is still a paucity of information on treatment outcomes of obstructive azoospermia cases treated with testicular sperm extraction in Ghana. Therefore, the study sought to evaluate treatment outcomes and assess predictors of pregnancy in cases of obstructive azoospermia treated with testicular sperm extraction and intracytoplasmic sperm injection in Ghana.

Methods

This study was a retrospective cohort study that involved a review of archived data and evaluated treatment outcomes of cases of obstructive azoospermia at the Lister Hospital and Fertility Centre in Accra and Ruma Fertility and Specialist Hospital in Kumasi, Ghana.

A structured data collection form was used to collect and record essential data such as their sociodemographic, treatment details, and outcomes. Infertile men who met the inclusion criteria were added to the study. This study included 67 patients with azoospermia who underwent TESE/ICSI from January 2018 to December 2019 at the Lister Hospital and Ruma Hospital in Ghana. Diagnosis of obstructive azoospermia had been confirmed earlier, based on histological analysis of testicular biopsy specimen taken previously during the work-up stage. Down-regulation, controlled ovarian hyperstimulation, and oocyte retrieval were performed using a routine long protocol of GnRH agonist suppression, followed by follicle stimulating hormone (FSH) for ovarian stimulation. Oocytes were retrieved 36 hours after administration of 5,000–10,000 IU of human chorionic gonadotropin (hCG). Oocyte retrieval was performed through transvaginal ultrasound-guided follicle aspiration. Under aseptic conditions, local anaesthesia was achieved with 10 ml to 20 ml of 1% lidocaine injection around the spermatic cord. A scrotal incision was made to expose the testis, the tunica vaginalis was incised to expose the seminiferous tubes, and a piece of protruding testicular tissue was excised. The tunica was sutured with a 3-0 delayed absorbable suture, and the scrotal incision was closed. The excised testicular tissue was then placed in a dish with a buffered medium and shredded with needles, microscope slides, or scissors to release spermatozoa into the medium. The resulting suspension was examined with an inverted microscope at 200x or 400x magnification. Once spermatozoa were found, the suspension was transferred into the test tube. Af-

ter sedimentation of the remaining tissue pieces during 30–60 s, the supernatant containing free spermatozoa was centrifuged at 750 $\times$ g for 5 min. The resulting supernatant was then removed, the pellet re-suspended, and 10 μ l droplets were smeared on the bottom of a Petri dish and overlaid with oil. The ideal sperm in morphology and motility were then aspirated for ICSI.

Following the oocyte's denudation of the surrounding cumulus and corona cells, nuclear maturation was assessed using an inverted microscope, and then injection of only metaphase II oocytes was performed. Eighteen hours following sperm injection, fertilization was assessed and confirmed with the finding of two distinct pronuclei. After confirmation of fertilization, embryonic cleavage and morphological quality were assessed approximately 24 hr later, and then embryo transfer was performed either at cleavage or blastocyst stage. Luteal phase support involved intramuscular progesterone, 100 mg daily, and vaginal or rectal micro-ionized progesterone, 400 mg twice daily, for 14 days post embryo transfer. Pregnancy test was performed using serum beta hCG measurement 14 days post embryo transfer. This was followed by a transvaginal ultrasound scan done 28 days (4 weeks) after embryo transfer to confirm the presence of a gestational sac. Clinical pregnancy was the dependent variable while characteristics such as the participant's age, duration of infertility, partner's age, number of retrieved oocytes, number of fertilized oocytes, viable day 2, viable day 3, viable day 5 embryos, stage of embryo transfer, and number of transferred embryos were the independent variables.

The data were entered into Microsoft Excel 2016 (Microsoft Corporation, USA) and exported into Stata statistical software v 15.1 for analysis. Descriptive data were expressed in the form of frequencies and percentages. The dependent and independent variables were analyzed using multiple logistic regression and reported as odds ratios

(ORs). The confidence interval (CI) was set at 95%, and $p < 0.05$ was considered significant.

Approval to conduct this study was obtained from the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (Protocol Identification number: CHS-Et/M3-5.15, 2019-2020). Informed consent from patients was not possible, but permission to review the data was obtained from the institutions involved.

Results

A total of 67 men seeking treatment for obstructive azoospermia participated in the study. The mean age of the male participants was 42.43 ± 9.11 years (mean $\pm$ SD), while the mean age of their partners was 32.89 ± 5.73 years (mean $\pm$ SD). The average duration of infertility before the intervention was 5.01 ± 3.60 years (mean $\pm$ SD) and three of the couples used donor eggs for TESE/ICSI. The overall clinical pregnancy rate following TESE/ICSI in our study was 52.2% (35/67). The rate of clinical pregnancy in participants aged below 35 years was 75% (6/8) as compared to other age groups (Table 1).

Participants with less than 2 years of infertility history had the most proportion of successful clinical pregnancies, 11/15 (73.3%), and this value was not statistically significant if the duration of infertility was more than 2 years. The success rate in achieving clinical pregnancy among couples with 10-15 oocytes retrieved was 77.8% (14/18), whereas the rate was 78.5% (11/14) in those with 16-19 oocytes.

There were nine single embryo transfers done in this study (4 were done at the cleavage stage whilst 5 during the blastocyst stage) and there was one clinical pregnancy among clients who had a single embryo transfer. Twenty one of the 35 (60.0%) cases who had two embryo transfers achieved clinical pregnancy, while 13 of 22 (59.1%) of those who had three embryo transfers

Table 1. Comparison between numbers of cleavage and blastocyst stage embryo transfers

Number of embryos transferred	Stage at transfer		Total	Positive clinical pregnancy (%)
	Cleavage stage	Blastocyst stage		
1	4	5	9	1 (11.1)
2	8	27	35	21 (60.0)
3	13	9	22	13 (59.1)
4	1	0	1	0 (0)
Total	26	41	67	35 (52.2%)

achieved clinical pregnancy. The majority (27/62) of participants had two blastocysts transferred. Transferring two embryos resulted in the highest number of clinical pregnancies, 21/34 (61.8%), but this was not statistically significant ($p=0.80$) (Table 2). Following fertilization from TESE/ IC-SI, couples having 5 or more viable embryos on days 2 to 5 did not show any significant improvement in the clinical pregnancy rates. Blastocyst stage embryo transfer resulted in a higher number of clinical pregnancies, 26/41 (63.4%), than cleavage stage embryo transfer, 9/26 (34.6%), (Table 2).

The logistic regression analysis revealed a significant association between male age and clinical pregnancy ($p<0.05$). After adjusting for confounders, the rate of clinical pregnancy was 0.07 lower for every additional year increase in the male's age [AOR=0.93 (95%CI=0.87–0.99), $p=0.02$] (Table 3).

Discussion

In this study, the archived data were reviewed to assess treatment outcomes of obstructive azoospermia cases at the Lister Hospital in Accra and Ruma Hospital in Kumasi. The results revealed that males' age is a significant predictor of clinical pregnancy ($p<0.05$). The findings of our study were in agreement with previous studies on the same subject. Sartorius and Nieschlag reported associations between advancing age and alterations of human sperm apoptosis (13). These alterations adversely impact naturally occurring control mechanisms that select healthy sperm at fertilization. Also, delayed fatherhood has been asso-

Table 2. Relationship between study participant's characteristics and clinical pregnancy

Characteristics	Clinical pregnancy		p-value
	Positive n (%)	Negative n (%)	
Male's age			0.03
≤35	10 (83.3)	2 (16.7)	
>35	25 (47.2)	28 (52.8)	
Duration of infertility			0.14
≤2	11 (73.3)	4 (26.7)	
>2	24 (48.0)	26 (52.0)	
Partner's age			1.00
≤35	25 (53.2)	22 (46.8)	
>35	10 (52.6)	9 (47.4)	
Number of oocytes retrieved			0.81
≤15	19 (54.3)	16 (45.7)	
>15	16 (50.0)	16 (50.0)	
Number of oocytes fertilized			0.34
≤7	16 (47.1)	18 (52.9)	
>7	19 (59.4)	13 (40.6)	
Viable day 2 embryos			0.33
≤5	13 (44.8)	16 (55.2)	
>5	22 (57.9)	16 (42.1)	
Viable day 3 embryos			0.77
≤5	14 (58.3)	10 (41.7)	
>5	12 (52.2)	11 (47.8)	
Viable day 5 embryos			0.27
≤5	22 (66.7)	11 (33.3)	
>5	4 (44.4)	5 (55.6)	
Stage of embryo transfer			0.03
Cleavage	9 (34.6)	17 (65.4)	
Blastocyst	26 (63.4)	15 (36.6)	
Number of embryos transferred			0.80
≤2	22 (50.0)	22 (50.0)	
>2	13 (56.5)	10 (43.5)	

n = number

Table 3. Characteristics associated with clinical pregnancy

Characteristics	Clinical pregnancy			
	COR (95%CI)	p-value	AOR (95%CI)	p-value
Male's age	0.92 (0.86-0.98)	0.01	0.93 (0.87-0.99)	0.02
Duration of infertility	0.89 (0.76-1.04)	0.13	-	-
Partner's age	0.96 (0.88-1.04)	0.32	-	-
Number of oocytes retrieved	0.98 (0.93-1.04)	0.50	-	-
Number of oocytes fertilized	1.07 (0.97-1.18)	0.20	-	-
Viable day 2 embryos	1.08 (0.95-1.22)	0.24	-	-
Viable day 3 embryos	0.98 (0.85-1.13)	0.77	-	-
Viable day 5 embryos	1.02 (0.82-1.26)	0.88	-	-
Stage at transfer	3.27 (1.17-9.15)	0.02	2.85 (0.95-8.52)	0.06
Number of embryo transfers	1.72 (0.83-3.56)	0.14	-	-

CI = Confidence interval, COR = Crude odds ratio, AOR = Adjusted odds ratio

ciated with a significant increase in the proportion of sperm carrying abnormal rates of DNA fragmentations (14), which may account for reduced clinical pregnancy rates with increasing male age. Age was also a significant predictor of successful sperm retrieval in men with non-obstructive azoospermia (15). Similar findings were reported from a prospective study that clinical pregnancy rates declined with increasing paternal age. Specifically, every additional year in the male partner was associated with 11% increased odds of not getting pregnant and 12% odds of failure to achieve a live birth following IVF cycles (20).

However, Mehta et al. found no statistically significant difference between paternal ages of pregnant couples and those who did not get pregnant following TESE/ICSI (21). In Taiwan, a study of 184 TESE/ICSI outcomes indicated that there was inadequate evidence to confirm the effect of advanced paternal age on treatment outcome (22). However, this study used cryopreserved testicular spermatozoa, unlike our study that used only freshly-obtained testicular spermatozoa.

Kavoussi et al., also found that increasing male partner's age was associated with increased odds of reaching embryo transfer stage but it did not affect clinical pregnancy or live birth rates (23). Our study revealed that the association between female partner's age and clinical pregnancy was not significant. While this disagrees with the previous studies, it may result from the relatively young age of the women in this study (below 40 years).

Currently, the practice of blastocyst-stage embryo transfer is being favored. At this stage, the embryos have developed and formed 64 cells. Therefore, the embryos are considered to have achieved maximum developmental and implantation potential. Our study found the odds of clinical pregnancy to be 1.85 times higher in blastocyst-stage embryo transfer compared to cleavage stage transfer [$p < 0.06$]. This agrees with reports by Cameron et al., who found that clinical pregnancy and cumulative live birth rates increased in those who underwent blastocyst transfer (56.5%) compared to cleavage-stage embryo transfer (34.8%). Furthermore, another systematic review of 27 randomized control trials reported higher clinical pregnancy rates of 39%-46%, following fresh blastocyst transfer, compared to 36% following cleavage stage transfer (OR: 1.30, 95%CI 1.14-1.47) (25).

One possible explanation may be that embryos which survive for five days are more likely to be viable and of better quality than those at the cleavage stage. This increases the likelihood of implantation. Additionally, it is likely that blastocyst transfer results in some improved synchronization between endometrial lining and embryo that favors the chances of implantation. Further, embryo culture for 5 days allows for the natural selection of viable embryos, thus resulting in better clinical pregnancy and live birth rates. Consistent with findings from previous studies, the rate of cleavage-stage embryo transfers in our study following TESE/ICSI treatment was low. Physiologically, cleavage-stage embryo transfer implies *in vivo* exposure of premature embryos to the endometrial environment; these embryos should transit through the fallopian tube. According to Blake et al., embryos traverse the fallopian tubes to reach the uterine cavity prior to the morula stage (26). The *in vitro* environment could likely be inferior to *in vivo*, and the *in vitro* culture beyond the embryonic genome activation stage may damage the embryo.

Our study found a statistically significant association between number of embryos transferred and clinical pregnancy rates and, in particular, two embryos conferred the most chance of clinical pregnancy. A similar retrospective analysis of 118 TESE/ICSI treatments identified that the number of embryos transferred is positively associated with clinical pregnancy success (27). A recent Cochrane review found that there was a higher (46%) chance of live birth following a single cycle of transferring two embryos than one embryo (27-35%) (28).

Pandian et al. also reported that a single embryo transfer was associated with lower live birth rates than double embryo transfers. However, they found no evidence of a significant difference in the cumulative live birth rate when a single cycle of double embryo transfer is compared with repeated single embryo transfer cycles (29).

Mehta et al., however, found no significant association between the number of embryos transferred and clinical pregnancy rate among couples who underwent TESE/ICSI (21). The low number of patients due to insufficient availability of the TESE in Ghana may limit the overall power of this study. However, our results agree with previous studies to show the significance of two embryo transfers in achieving optimal results for ICSI in Ghana.

Conclusion

The overall pregnancy rate in our study following TESE/ICSI was 52.2%. A man's age was a strong predictors of clinical pregnancy among couples treated with TESE/ICSI for obstructive azoospermia in Ghana.

Acknowledgement

We are sincerely grateful to the following staff of Lister and Ruma Fertility Centre for their contributions and assistance during data collection, analysis, and editorial support: Ms. Lisa Hughes-Thompsons, Cecilia Anani Akakpo, Joshua Adoboe, Mary Otoo Akuffo, Michael Yakass, and Kingsley Kuma Anti.

Conflict of Interest

There is no conflict of interest.

References

- Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med*. 2012;9(12):e1001356.
- Dupree JM. Insurance coverage for male infertility care in the United States. *Asian J Androl*. 2016;18(3):339-41.
- Mehta A, Nangia AK, Dupree JM, Smith JF. Limitations and barriers in access to care for male factor infertility. *Fertil Steril*. 2016;105(5):1128-37.
- Tüttelmann F, Werny F, Cooper TG, Kliesch S, Simoni M, Nieschlag E. Clinical experience with azoospermia: aetiology and chances for spermatozoa detection upon biopsy. *Int J Androl*. 2011;34(4):291-8.
- Gyasi-Sarpong CK, Manu Maison PO, Koranteng AK. The pattern of male infertility in Kumasi, Ghana. *Afr J Infertil Assist Concept*. 2017;2:3-5.
- Esteves SC, Lee W, Benjamin DJ, Seol B, Verza S, Agarwal A. Reproductive potential of men with obstructive azoospermia undergoing percutaneous sperm retrieval and intracytoplasmic sperm injection according to the cause of obstruction. *J Urol*. 2013;189(1):232-7.
- Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European association of urology guidelines on male infertility: The 2012 update. *Eur Urol*. 2012;62(2):324-32.
- Abdulwahed SR, Mohamed EEM, Taha EA, Saleh MA, Abdelsalam YM, Elganainy EO. Sensitivity and specificity of ultrasonography in predicting etiology of azoospermia. *Urology*. 2013;81(5):967-71.
- Lee HS, Seo JT. Advances in surgical treatment of male infertility. *World J Mens Health*. 2012;30(2):108-13.
- Abdel Raheem A, Garaffa G, Rushwan N, De Luca F, Zacharakis E, Abdel Raheem T, et al. Testicular histopathology as a predictor of a positive sperm retrieval in men with non-obstructive azoospermia. *BJU Int*. 2013;111(3):492-9.
- Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. *Basic Clin Androl*. 2013;23:5.
- Caroppo E, Colpi EM, Gazzano G, Vaccalluzzo L, Scropo FI, D'Amato G, et al. Testicular histology may predict the successful sperm retrieval in patients with non-obstructive azoospermia undergoing conventional TESE: a diagnostic accuracy study. *J Assist Reprod Genet*. 2017;34(1):149-54.
- Sartorius GA, Nieschlag E. Paternal age and reproduction. *Hum Reprod Update*. 2010;16(1):65-79.
- Hammeche F, Laven JSE, Boxmeer JC, Dohle GR, Steegers EAP, Steegers-Theunissen RPM. Sperm quality decline among men below 60 years of age undergoing IVF or ICSI treatment. *J Androl*. 2011;32(1):70-6.
- Enatsu N, Miyake H, Chiba K, Fujisawa M. Predictive factors of successful sperm retrieval on microdissection testicular sperm extraction in Japanese men. *Reprod Med Biol*. 2016;15(1):29-33.
- Gnessi L, Scarselli F, Minasi MG, Mariani S, Lubrano C, Basciani S, et al. Testicular histopathology, semen analysis and FSH, predictive value of sperm retrieval: supportive counseling in case of reoperation after testicular sperm extraction (TES-E). *BMC Urol*. 2018;18(1):63.
- Busch AS, Tüttelmann F, Cremers JF, Schubert M, Nordhoff V, Schüring AN, et al. FSHB -211 G>T polymorphism as predictor for TESE success in patients with unexplained azoospermia. *J Clin Endocrinol Metab*. 2019;104(6):2315-24.
- Belloc S, Cohen-Bacrie M, Amar E, Izard V, Benkhalifa M, Dalléac A, et al. High body mass index has a deleterious effect on semen parameters except morphology: results from a large cohort study. *Fertil Steril*. 2014;102(5):1268-73.
- Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Buck Louis GM. The relationship between male BMI and waist circumference on semen quality: Data from the LIFE study. *Hum Reprod*. 2015;30(2):493-4.
- Klonoff-Cohen HS, Natarajan L. The effect of advancing paternal age on pregnancy and live birth rates in couples undergoing in vitro fertilization or

- gamete intrafallopian transfer. *Am J Obstet Gynecol.* 2004;191(2):507-14.
21. Mehta A, Bolyakov A, Schlegel PN, Paduch DA. Higher pregnancy rates using testicular sperm in men with severe oligospermia. *Fertil Steril.* 2015; 104(6):1382-7.
22. Tsai YR, Lan KC, Kung FT, Lin PY, Chiang HJ, Lin YJ, et al. The effect of advanced paternal age on the outcomes of assisted reproductive techniques among patients with azoospermia using cryopreserved testicular spermatozoa. *Taiwan J Obstet Gynecol.* 2013;52(3):351-5.
23. Kavoussi PK, West BT, Chen SH, Hunn C, Gilkey MS, MacHen GL, et al. A comprehensive assessment of predictors of fertility outcomes in men with non-obstructive azoospermia undergoing microdissection testicular sperm extraction. *Reprod Biol Endocrinol.* 2020;18(1):90.
24. Cameron NJ, Bhattacharya S, McLernon DJ. Cumulative live birth rates following blastocyst-versus cleavage-stage embryo transfer in the first complete cycle of IVF: a population-based retrospective cohort study. *Hum Reprod.* 2020;35(10): 2365-74.
25. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev.* 2016;(6):CD002118.
26. Blake DA, Proctor M, Johnson NP. The merits of blastocyst versus cleavage stage embryo transfer: A Cochrane review. *Hum Reprod.* 2004;19(4):795-807.
27. Rehnitz J, Rösner S, Harsch J, Dietrich J, Bruckner T, Capp E, et al. Factors influencing success rate of intracytoplasmic sperm injection with azoospermic male patients. *Geburtshilfe Frauenheilkd.* 2020;80 (7):713-22.
28. Kamath MS, Mascarenhas M, Kirubakaran R, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev.* 2020;8(8):CD003416.
29. Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev.* 2013(7):CD003416.