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ORIGINAL ARTICLE

Male Reproduction

PGT or ICSI? The impression of NGS-based PGT outcomes in nonmosaic Klinefelter syndrome

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This retrospective study demonstrates the clinical outcomes of patients with nonmosaic Klinefelter's syndrome (KS) who underwent preimplantation genetic testing (PGT) with frozen-thawed testicular spermatozoa. Microdissection testicular sperm extraction (micro-TESE) was performed for sperm retrieval. Next-generation sequencing (NGS) was conducted for embryo analysis. A total of 18 couples aged ≤35 years were included, and 22 oocyte retrieval cycles were completed. Euploidy was detected in 29 of 45 (64.4%) embryos. Additionally, the numbers of aneuploid and mosaic embryos detected were 8 (17.8%) and 8 (17.8%), respectively, regardless of a lack of sex chromosome abnormalities. Finally, 13 couples with euploid embryos completed 14 frozen embryo transfer (FET) cycles. Ten couples had clinical pregnancies, and 6 of them had already delivered 5 healthy babies and 1 monozygotic twin. There were also 4 ongoing pregnancies and 2 biochemical pregnancies, but no early pregnancy loss was reported. Based on our results, we speculate that for KS patients, when sperm can be obtained by micro-TESE, the cryopreservation strategy makes the ovarian stimulation procedure more favorable for female partners. The paternal genetic risk of sex chromosome abnormalities in their offspring is extremely low in men with KS. In addition to PGT, the intracytoplasmic sperm injection (ICSI) procedure is comparably effective but more economical for young nonmosaic KS couples. ICSI should be offered as an option for such couples, but monitoring by prenatal genetic diagnosis is recommended.

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INTRODUCTION

Klinefelter's syndrome (KS) is the most common chromosomal aneuploidy in males, with an incidence of 1 in 500–1000 males, and it frequently causes azoospermia. KS is characterized by the presence of at least one supernumerary X chromosome. A 47,XXY karyotype is detected in 80%–90% of all KS cases, whereas the remaining cases show a mosaic karyotype (46,XY/47,XXY) or the presence of additional X chromosomes (such as 48,XXXY or 48,XXYY).[1] Notably, 75% of men with KS are undiagnosed, and most of them are not diagnosed until adulthood because of infertility.[2]

KS is characterized generally by primary testicular failure with reduced testicular volume, hypergonadotropic hypogonadism, and tall stature, regardless of its variable phenotype. The testicular function of KS shows progressive degeneration with age.[3] Reduced numbers of germ cells are already found in the biopsies of 47,XXY fetal testes from 18 weeks and 22 weeks of gestation.[4] Moreover, transcriptomic analysis has revealed the impaired development of spermatogonia to mature spermatozoa, defects in the testicular architecture, pathophysiology of the testicular environment, and apoptosis of the germinal and somatic cells responsible for hypospermatogenesis in men with KS.[5]

Sperm can be retrieved by means of surgical testicular sperm extraction (TESE) or advanced microdissection TESE (micro-TESE) using a high-power surgical microscope (magnification of 20–25 times).[6,7] For this reason, men with KS require assisted reproductive technology (ART) to father their own children. Intracytoplasmic sperm injection (ICSI), a successful fertility treatment, has been applied to men with KS worldwide, and hundreds of healthy babies have been born.[8–11] Even so, regarding the risk of transmission of this genetic pathology to the new-born, the use of preimplantation genetic testing (PGT) technology for such couples is worthy of attention. However, PGT is a more expensive procedure,[12] and recommendation of ICSI or PGT for such couples is still a dilemma.

Next-generation sequencing (NGS) is the latest and most popular method utilized for identifying and screening embryos with reduced viability, such as mosaic embryos and those with partial aneuploidy in PGT laboratories at present, and it has been routinely adopted for testing embryos by massively parallel genome sequencing.[13] This article presents the clinical outcomes of NGS-based PGT in nonmosaic KS males with infertility and aims to shed more light on the reproductive treatment plan for such cases.

PATIENTS AND METHODS

Study population

Eighteen couples where the male partners were diagnosed with nonobstructive azoospermia (NOA) and nonmosaic KS and who received PGT treatment in Center for Reproductive Medicine, Ren

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Ji Hospital, School of Medicine, Shanghai Jiao Tong University (Shanghai, China) from January 2018 to December 2020 were recruited into this study. All males underwent semen analysis according to the 5th edition of the World Health Organization (WHO) criteria[14] to confirm azoospermia. The nonmosaic 47,XXY karyotype was proven by G-binding chromosome analysis based on peripheral blood. The testicular volume and serum follicle-stimulation hormone (FSH), luteinizing hormone (LH), and testosterone levels in each man were measured. None of these men had previously received human chorionic gonadotropin (hCG) or androgen replacement therapy. All female partners had a 46,XX normal karyotype, as verified by G-binding chromosome analysis using peripheral blood. They underwent routine analysis before the initiation of ovarian stimulation.

Data collection in this retrospective study was approved by the Ethics Committee of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University School of Medicine ([2020] IRB approval No. 43). Informed consent from patients was exempted due to the retrospective nature and anonymous data analysis.

Micro-TESE, sperm processing, and freezing

Micro-TESE was performed under general anaesthesia as previously described by Dabaja and Schlegel[15] with a few modifications. The procedure began on the testicle with obviously larger volume or on the right testicle if the volumes of the two testicles were not different. A longitudinal incision was made along the tunica vaginalis to allow for a thorough inspection of the entire testis and epididymis. Testicular parenchyma was observed directly at 20×–25× magnification (Nikon ECLIPSE Ti, Nikon, Tokyo, Japan) to locate and collect the wider, full-appearing, and opaque seminiferous tubule with a higher chance of harboring spermatozoa.

Testicular fragments were washed in Earl's balanced salt solution (EBSS; Sigma, St. Louis, MO, USA) medium, placed in 0.5 ml of Sperm Washing Medium™ (Vitrolife, Gothenburg, Sweden) and finely minced by sterile hypodermic injection needles in sterile tissue culture dishes (Falcon, Franklin Lakes, NJ, USA). Then, an inverted microscope (Nikon ECLIPSE Ti-U, Nikon) at 200× magnification was used to examine the presence of spermatozoa. If sperm was seen, the routine cryopreservation process was then performed. Specifically, testicular tissues were mixed 1:1 with sperm freezing medium (Origio, Malov, Denmark) in 0.5 ml straw (Croy Bio System, L'Aigler, France) after labeling for 30 min in liquid nitrogen vapor before being stored in liquid nitrogen. If sperm were not found on the date of surgery, the search for the remaining cell suspensions continued the next morning. Spermatozoa were retrieved successfully from all male cases and frozen for future ICSI.

Ovarian stimulation and PGT procedure

Basically, the stimulation protocol using gonadotrophin-releasing hormone (GnRH) analogues and gonadotrophins was decided depending on female age, female ovarian response, and clinician experience.

ICSI was applied to all mature (meiosis II stage) oocytes with frozen-thawed micro-TESE spermatozoa, and only spermatozoa with normal morphology could be used for ICSI; 17–20 h later, fertilization was confirmed by the presence of pronuclei, and the presence of two visible distinct pronuclei was considered normal. Next, embryo cleavage of the two pronuclear oocytes was evaluated at 41–44 h (day 2) and 65–68 h (day 3) after ICSI. All embryos were then cultured to the blastocyst stage under assisted hatching, and trophectoderm biopsies were carried out on day 5 or day 6. Thereafter, DNA was extracted from the trophectoderm-biopsied specimens and quantified. After biopsy, blastocysts were vitrified using a Kitazato vitrification kit (Kitazato Biopharma Co., Ltd., Shizuoka, Japan) in combination with Vitrification Cryotop (Kitazato Biopharma Co., Ltd.), according to the protocol recommended. Each blastocyst was stored in an individual straw.

In this study, NGS technology was adopted for the direct readings of sequenced DNA fragments together with their quantification based on the sequence read numbers. Briefly, the NGS protocol was completed in five steps: (i) sample processing; (ii) initial quality analysis; (iii) library preparation; (iv) sequencing; and (v) data analysis, in accordance with the Illumina NGS platform protocol (https://www.illumina.com). For the sake of cost-effectiveness, couple 7 agreed to an analysis of 6 of the 7 embryos.

Embryo transfer and outcome assessment

Embryo transfer was performed in line with the specific policy, and one euploid embryo was transferred for one patient per hormone replacement cycle. All patients received progesterone supplementation after embryo transfer, and luteal support was continued for the pregnant cases until 12 weeks of gestation.

Pregnancy was first evaluated by the serum hCG concentration at 14 days after embryo transfer. Biochemical pregnancy was defined as a pregnancy that failed to progress to the point of ultrasound confirmation of the gestational sac despite a positive pregnancy test. Clinical pregnancy was defined as the presence of a gestational sac and heartbeat detected by ultrasonography at 4–5 weeks after embryo transfer. Additionally, ongoing pregnancy was defined as the presence of a fetus with heartbeat at 11–12 weeks of gestation. Early pregnancy loss was defined as pregnancy loss before 20 weeks of gestation after confirmation of a gestational sac by ultrasound.

RESULTS

In total, 18 couples were included in this analysis, and they completed 22 oocyte retrieval cycles with frozen-thawed micro-TESE spermatozoa. Among them, 13 couples obtained euploid embryos after PGT and completed 14 frozen embryo transfer cycles (couple 18 had two cycles of embryo transfer). In the end, 10 couples had clinical pregnancies, and 6 of them had already delivered healthy babies. At present, there are also 2 biochemical pregnancies and 4 ongoing pregnancies without early pregnancy loss.

The baseline clinical characteristics of the patients are listed in **Supplementary Table 1**. As obtained from the table, the median age of the males was 29 (range: 23–35) years, and the mean testis volume was 2.0 (range: 1.0–3.8) ml. The median serum levels of FSH, LH and testosterone were 27.0 (range: 2.2–59.1) IU l^{-1}, 18.6 (range: 6.6–41.8) IU l^{-1} and 5.2 (range: 0.3–13.6) pg ml^{-1}, respectively. All male cases were identified as having nonmosaic KS according to the peripheral blood karyotypes and exhibited the clinical features of a small testicular size and hypergonadotropic hypogonadism. The spermatozoa status before freezing and after recovery is also shown in **Table 1**. Fortunately, enough spermatozoa were preserved in straw tubes for future ICSI in every male patient.

The median age of the females was 29 (range: 24–35) years. The median serum levels of anti-Müllerian hormone (AMH), basal FSH, basal LH, and basal estrogen and the median body mass index (BMI) and thyroid-stimulating hormone (TSH) level were 3.2 (range: 0.6–9.2) ng ml^{-1}, 6.4 (range: 3.6–8.6) IU l^{-1}, 5.4 (range: 1.6–12.0) IU l^{-1}, 36.5 (range: 20–120) pg ml^{-1}, 22.7 (range: 16.8–29.3) kg m^{-2}, and 1.8 (range: 0.3–3.6) mIU ml^{-1}, respectively. All female cases had normal karyotypes. Except for case 18, which had diminished ovarian function, other females had a normal ovarian response.

The ovarian stimulation outcomes of 22 cycles are shown in **Table 1**. On average, 1387.5 (range: 675–3000) IU FSH and 8 (range: 6–11) days were consumed during the ovarian stimulation procedure. The median numbers of oocytes retrieved, MII oocytes, normal fertilized oocytes (two pro-nucleate, 2PN), fertilized oocytes per cycle that cleaved, and blastocysts were 12 (range: 3–37), 11 (range: 3–21), 6 (range: 2–13), 6 (range: 2–13), and 1.5 (range: 0–7), respectively. Unfortunately, 3 couples (case 8, case 10, and case 15) failed to obtain blastocysts after 3 oocyte retrieval cycles, which might be ascribed to a low fertilization rate and poor embryo quality.

The PGT screening data are summarized in **Table 2**. Embryo biopsies and chromosomal structural rearrangement tests were performed on 45 embryos, among which 29 (64.4%) were euploid. In addition, the numbers of aneuploid and mosaic embryos detected were 8 (17.8%) and 8 (17.8%), respectively. Moreover, none of the aneuploid and mosaic embryos had abnormal sex chromosomes. Two couples (case 5 and case 11) failed to obtain one euploid embryo and cancelled the subsequent embryo transfer.

All clinical outcomes are displayed in **Table 3**. At the time of the writing of this manuscript, 10 couples had clinical pregnancies, and 6 of them had

Table 1: Ovarian stimulation outcomes undergoing preimplantation genetic testing for nonmosaic Klinefelter's syndrome

Couple	*Ovarian stimulation cycle (n)*	*Duration of stimulation (day)*	*Dose of FSH used (IU)*	*Oocytes retrieved (n)*	*MII (n)*	*2PN (n)*	*Fertilized oocytes that cleaved (n)*	*Blastocysts (n)*
1	1	9	1950	13	13	9	8	1
2	1	8	1400	18	15	10	10	4
3	1	8	1200	28	21	12	11	6
4	1	8	1575	7	7	4	4	2
5	1	11	3000	12	11	9	8	2
6	1	10	1700	15	15	10	10	6
7	1st	10	1500	5	3	2	2	1
	2nd	7	1275	12	12	10	10	7
8	1st	8	1325	10	9	6	5	0
	2nd	7	1050	13	12	3	3	0
9	1st	8	2388	14	14	10	9	2
	2nd	7	1463	14	13	13	13	1
10	1	10	3000	37	18	4	4	0
11	1	8	1650	9	9	6	6	1
12	1	8	1375	12	10	6	6	2
13	1	9	1800	11	9	5	5	3
14	1	8	1200	17	11	6	6	1
15	1	6	825	10	9	6	6	0
16	1	8	1275	12	11	11	5	1
17	1	8	1050	14	11	3	4	2
18	1st	6	675	5	5	4	4	2
	2nd	8	862.5	3	3	2	2	1

FSH: follicle-stimulation hormone; 2PN: two pro-nucleate; MII: meiosis II

Table 2: Preimplantation genetic testing screening data

Couple	*Ovarian stimulation cycle (n)*	*Embryos biopsied and diagnosed (n)*	*Euploid (n)*	*Aneuploid (n)*	*Mosaicism (n)*
1	1	1	1	0	0
2	1	4	3	0	1
3	1	6	4	1	1
4	1	2	1	0	1
5	1	2	0	0	2
6	1	6	5	0	1
7	1st	1	0	1	0
	2nd	6	4	2	0
9	1st	2	0	1	1
	2nd	1	1	0	0
11	1	1	0	1	0
12	1	2	1	0	1
13	1	3	3	0	0
14	1	1	1	0	0
16	1	1	1	0	0
17	1	3	1	2	0
18	1st	2	2	0	0
	2nd	1	1	0	0
Total	18	45	29	8	8

Table 3: Clinical outcomes for nonmosaic Klinefelter's syndrome undergoing preimplantation genetic testing

Couple	*FET cycle (n)*	*Biochemical pregnancy*	*Clinical pregnancy*	*Ongoing pregnancy*	*Baby delivery details*	*Mode of delivery and gestational week*
1	1	NA	NA	NA	NA	NA
2	1	NA	Yes	NA	1 female (3200 g)	CS (39+5)
3	1	NA	Yes	NA	2 female (2200 g, 1850 g)	CS (33+2)
4	1	NA	Yes	Yes	NA	NA
6	1	NA	Yes	NA	1 male (3900 g)	VD (40+3)
7	1	NA	Yes	NA	1 male (3050 g)	CS (38+2)
9	1	NA	Yes	NA	1 male (4100 g)	CS (40+6)
12	1	NA	Yes	Yes	NA	NA
13	1	NA	Yes	NA	1 female (2900 g)	CS (39+3)
14	1	NA	Yes	Yes	NA	NA
16	1	Yes	NA	NA	NA	NA
17	1	NA	Yes	Yes	NA	NA
18	1st	Yes	NA	NA	NA	NA
	2nd	NA	NA	NA	NA	NA
Total (*n*)	14	2	10	4	7	NA

FET: frozen embryo transfer; CS: cesarean section; VD: vaginal delivery; NA: not available

already delivered healthy babies. There are also 4 ongoing pregnancies and 2 biochemical pregnancies, with no early pregnancy loss reported.

DISCUSSION

Our study confirms that men with nonmosaic KS men can father healthy children. Moreover, the cryopreservation strategy of micro-TESE spermatozoa ensures more favorable ovarian stimulation for female partners. Notably, the paternal genetic risk of sex chromosome abnormalities in the offspring seems to be extremely low in men with KS. Although NGS-based PGT can guarantee the safety of offspring, ICSI is comparably effective but more economical for young-aged couples.

Due presumably to the presence of a redundant X chromosome, most nonmosaic KS cases suffer from progressive testicular damage, leading to azoospermia and infertility. However, because of the development of TESE or micro-TESE combined with ICSI within the past decade, males with KS can sire their own children. Spermatozoa can be retrieved in approximately 50% of KS males because of the presence of residual foci with preserved spermatogenesis.[16,17] Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University has performed micro-TESE for nonobstructive azoospermia (NOA) patients since 2003, and the sperm retrieval rate (SRR) in the KS group is 51.5%.[18] In our study, enough spermatozoa were obtained from all males and frozen via micro-TESE. Typically, such a cryopreservation strategy of micro-TESE spermatozoa ensures the timing and flexibility of ovarian stimulation for the female partners rather than based on the possibility or the number of available spermatozoa. Due to the coronavirus (COVID-19) pandemic in China as of January 2020, our center had postponed the micro-TESE procedures until June 2020, which resulted in the small sample available for this study.

It is believed that maternal errors contribute to more than 95% of autosomal trisomies in embryos. Aneuploidy rates increase steadily with maternal age, reaching >80% in women >42 years old,[19] but the effect of paternal age on the prevalence of embryo aneuploidy is not specific.[20] In 50% of KS cases, the supernumerary X originates from paternal nondisjunction, which is hypothesized to be caused by environmental factors interfering with paternal meiosis I.[21,22] 47,XXY germ cells may undergo and complete meiosis, which increases the aneuploidy rate in spermatozoa such as XX- and XY-disomies.[23,24] Theoretically, half of the sperm should be sex chromosomal disomic, and hence, half of the embryos should be aneuploid. However, the observed frequency of sex chromosomal abnormalities in sperm is much lower. Analysis of sperm karyotypes from KS patients showed that the frequency of sex chromosomal disomy in sperm is only 3% to 4% in XXY males, in contrast to 1% in infertile XY males.[25–28]

Numerous studies have already reported that routine clinical ICSI approaches are sufficient for infertility treatment among men with KS.[8–10,29–31] Obviously, genetic screening of sperm cells does not always definitively determine the genetic situation of embryos. As Stassen *et al*.[32] reported in 2003, 34 healthy children were born through ICSI without PGT, and only one of them was a 47,XXY fetus. The number of healthy children climbed up to 101, as summarized by Fullerton *et al*.[11] in 2010. Later, in 2013, one study[8] reported that 16 healthy babies with a normal karyotype were born after ICSI treatment with fresh or frozen-thawed TESE or micro-TESE spermatozoa to couples where the male partners were KS. Two studies[29,31] in the last year reported 34 babies born to KS couples via ICSI cycles without PGT, and 6 babies were diagnosed as normal in prenatal screening.

In our center, micro-TESE was applied in combination with ICSI as a principal strategy for couples suffering from KS between January 2003 and December 2017 and achieved a satisfactory effect. Specifically, the sperm qualified for ICSI rate was 88.2%; moreover, the fertilization rate, cleavage rate, high qualified embryo rate and clinical pregnancy rate did not decrease when using our frozen-thawed technology.[18] However, the possibility of men with KS transmitting sex chromosomal abnormalities to offspring makes the safety of ICSI treatment doubtful. To address the crucial concerns of genetic health in offspring, we modified our protocol to provide PGT combined with micro-TESE for couples with KS in the last two years. However, we observed no sex chromosome abnormality in the embryos of couples with KS in this study, suggesting that the paternal genetic risk of sex chromosome abnormality is not increased in men with KS. Moreover, the aneuploidy rate and the mosaic rate in embryos seem to be equivalent to those of other age-matched patients seeking ART.[33] Coincidentally with our results, another study in 2017 found no gametes with sex chromosomal abnormalities in a fluorescence *in situ* hybridization (FISH) analysis and concluded that the risk of ART for patients with KS was not as high

as previously expected.[34] A study in 2003[32] demonstrated embryonic detection outcomes with FISH and indicated that the proportion of normal embryos in KS cases (54.0%) was significantly lower than that in controls (77.2%). Among the abnormal embryos, the rate of sex chromosomal abnormalities was 13.2%, which was much higher than that of the controls (3.1%). We reanalyzed their data and found that the rates of sex chromosomal trisomy, including XYY, XXX, XXY, mosaic XY/XXY, and mosaic XY/XYY, were not significantly different from those of the controls. Overall, these embryonic detection outcomes, combined with the fact that hundreds of healthy babies are born via ICSI, indicate that the paternal genetic risk of sex chromosome abnormalities in their offspring seems to be extremely low in KS cases. From the cost-effectiveness perspective, ICSI is also an appropriate option.

Of note, 8 (17.8%) mosaic embryos were detected according to our results. Mosaic embryos characterized by the copresence of cells with two (or more) different chromosomal constitutions are not usually transferred due to the unknown developmental potential and the effect on implantation.[35] Since Greco *et al.*[36] first reported healthy live births from mosaic monosomy embryos at different mosaic levels and affecting different chromosomes, emerging evidence has shown that the transfer of mosaic embryos could result in viable and genetically normal live births.[37–39] However, the transfer of mosaic embryos, now a major shift in current PGT practice, should be considered with extreme caution due to the major concern of the lowered implantation potential and the antenatal and postnatal health of babies derived from mosaic embryos. Although it would increase the likelihood of a live birth for couples who failed to obtain a euploid embryo for transfer, most experts insist that this option should be provided only under the condition of comprehensive genetic counseling and recommendation for follow-up with a prenatal diagnosis.[40,41]

CONCLUSIONS

Although this is a small retrospective clinical observation study, it can still suggest that if spermatozoa are available, the cryopreservation strategy makes the ovarian stimulation procedure more favorable for female partners. More importantly, in addition to PGT, the ICSI procedure is comparably effective but more economical for young nonmosaic KS couples. ICSI should be offered as an option for such couples, but monitoring via prenatal genetic diagnosis is recommended.

AUTHOR CONTRIBUTIONS

JT and TZ carried out the study design and drafted the manuscript. ARW participated in data collection. XMZ revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Clinical characteristics of couples recruited

Couple	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Male									
Male age (year)	29	33	32	29	28	34	32	25	28
Left testis volume (ml)	3.3	1	2	1	1	2	2	2	1
Right testis volume (ml)	4.2	1	2	1	1	2	2	2	1
Serum FSH concentration (IU l^{-1})	20	27	23	59.1	26	25.6	36.9	12.3	27.7
Serum LH concentration (IU l^{-1})	19	22	21	41.8	20	13.6	24.3	8	22.2
Serum testosterone (pg mL^{-1})	13	3	4	4.7	5.1	9.8	7.7	0.3	3.9
Spermatozoa status									
Amount (before frozen)	0–8/dish	0–1/10–15 LPF	0–2/dish	0–1/20–30 LPF	0–2/dish	0–1/5 LPF	0–1/5 LPF	0–1/dish	0–4/dish
Motility (before frozen)	M	IM	M	M	IM	M	M	M	M
Amount (after recovery)	0–1/2 slides	0–1/30–35 LPF	0–1/3 slides	0–1/2 slides	0–1/3 slides	0–1/10–15 LPF	0–1/10–15 LPF	0–1/2 slides	0–1/2 slides
Motility (after recovery)	IM	IM	IM	IM	IM	IM	IM	IM	IM
The number of straw tubes finally obtained	4	6	2	4	2	6	6	4	4
Female									
Female age (year)	29	35	31	32	28	29	29	24	29
AMH (ng ml^{-1})	3.0	5.2	6.9	2.1	4.2	3.4	5.6	1.1	2.8
Basal FSH concentration (IU l^{-1})	3.6	6.1	5.7	5.1	5.1	7.2	5.3	8.3	6.6
Basal LH concentration (IU l^{-1})	1.6	8.4	12	3.8	5.1	3.5	2.4	7.6	5.3
Basal estradiol concentration (pg ml^{-1})	36	52.9	30	34	33	24	120	29	37
BMI (kg m^{-2})	29.3	24.8	19.7	22.9	27.1	21.1	19.6	18.9	22.9
TSH (mIU ml^{-1})	2.0	1.5	1.8	0.8	2.2	2.5	2.6	2.4	1.3
Couple	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Male									
Male age (year)	28	32	27	32	30	35	27	28	23
Left testis volume (ml)	2	2	1	2	2	2.6	1	1	2
Right testis volume (ml)	2	2	1	2	2	2.3	1	1	2
Serum FSH concentration (IU l^{-1})	31	32.6	29	15.9	50.7	2.2	8.1	47.1	26.9
Serum LH concentration (IU l^{-1})	17.8	18.1	23	12.8	15.8	6.6	8	27.9	12.6
Serum testosterone (pg mL^{-1})	7	5.5	8	6.6	1.6	13.6	2.3	5.2	2.2
Spermatozoa status									
Amount (before frozen)	0–4/40–50 LPF	0–1/10–15 LPF	2–8/dish	0–1/5 LPF	0–1/10 LPF	0–1/40–50 LPF	2–4/dish	0–1/10–15LPF	0-1/15-20 LPF
Motility (before frozen)	M	M	M	M	M	M	IM	IM	IM
Amount (after recovery)	0–1/1 slide	0–1/20–25 LPF	0–1/2 slides	0–1/10–15 LPF	0–1/15–20 LPF	0–1/2 slides	0–1/5 slides	0–1/40 LPF	0-1/40 LPF
Motility (after recovery)	IM	IM	IM	IM	IM	IM	IM	IM	IM
The number of straw tubes finally obtained	3	7	2	6	4	4	2	5	5
Female									
Female age (year)	32	34	25	28	24	31	26	26	25
AMH (ng ml^{-1})	9.2	2.2	1.8	2.2	3.0	4	5.2	5.6	0.6
Basal FSH concentration (IU l^{-1})	7	6.4	6.9	8.6	6.4	6.2	5.9	6.7	8.2
Basal LH concentration (IU l^{-1})	6.5	3.3	5.4	6.1	7.0	3.4	6.3	6.7	4.5
Basal estradiol concentration (pg ml^{-1})	20	34	45	30	61	53	48	52	41
BMI (kg m^{-2})	23.5	25.6	19.4	22.5	17.5	16.8	23.2	25.6	18
TSH (mIU ml^{-1})	0.3	1.7	1.3	1.3	3.6	0.8	1.1	2.8	2.4

Amount description was defined as following: 100–150 μl centrifugation deposit from micro-TESE sample was loaded on circular droplet (diameter 40 mm) with about 1000 LPFs with ×200 magnification. M: mobility; IM: immobility; AMH: anti-Müllerian hormone; BMI: body mass index; TSH: thyroid-stimulating hormone; FSH: follicle-stimulation hormone; LH: luteinizing hormone; Micro-TESE: microdissection testicular sperm extraction; LPFs: low power fields