Infertile couples still undergo assisted reproductive treatments without initial andrological evaluation in the real-life setting: a failure to adhere to guidelines?

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ABSTRACT

Background: The EAU Guidelines on Male Sexual and Reproductive Health state that both partners of the infertile couple should undergo simultaneous investigation.

Objectives: To assess the prevalence and the characteristics of infertile men who were referred for an andrological evaluation after failed attempts of Assisted Reproductive Technology (ART) with those who were evaluated at the beginning of their infertility pathway at a single academic centre over a 17-year period.

Materials and methods: Data of 3213 primary infertile couples assessed between 2003 and 2020 were analysed. Descriptive statistics compared the overall characteristics of male partners of couples with (+ART) or without (–ART) previous ART prior to andrological consultation. Logistic regression models analysed variables associated with +ART. Local polynomial regression models explored the probability of +ART over the analysed time frame.

Results: Of all, 493 (15.3%) participants were +ART. Patients and female partners' age were higher in +ART couples (all $p \leq 0.04$). Sperm concentration, progressive sperm motility and normal sperm morphology were lower in +ART than in –ART patients (all $p<0.001$), along with a greater percentage of non-obstructive azoospermia in +ART compared to –ART men ($p<0.0001$). At univariable analysis, patient age and partner age >35 years and a less recent assessment were associated with +ART status (all $p \leq 0.04$). Male age and less recent years of assessment were also independent predictors of +ART, after accounting for partner's age >35 years (all $p<0.01$). A not significant decrease of this pattern was observed throughout the last 7 years at local polynomial regression models.

Discussion: Overall awareness towards the importance of a comprehensive evaluation for the male partner of every infertile couple should therefore be further strengthened.

Conclusions: Approximately 15% of couples still undergo ART without any initial andrological evaluation in the real-life setting. A not significant decrease in this trend was observed over most recent years.

INTRODUCTION

Infertility is defined as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy within one year [1]. Globally, it has been estimated that approximately 48 million couples and 186 million individuals currently suffer of fertility issues [2–4]. Infertility is caused by a number of different conditions, and a male factor infertility (MFI) can be identified in about half of all cases [1,4–6]. As such, it is plausible to assume that the inability to conceive is a consequence of both partners having reduced fertility potential [4]. Moreover, over the last decades, couples have been delaying having a child, thus increasing the time at which paternity is achieved [7,8]; in this context, while women have been warned of the consequences for their fertility, the fact that men will experience a similar fall in fertility has been given considerably less attention [9–12]. Accordingly, numerous scientific societies recommend that both male and female partners should undergo concurrent assessment in the initial fertility evaluation [5,13]. For instance, the European Association of Urology (EAU) guidelines on Male Sexual and Reproductive Health strongly advise that in infertile couples, all male partners should undergo an andrological assessment to categorise the cause of infertility and develop a strategy for patient management [5]. Furthermore, infertility is now recognised as a disease [14], and emerging evidence has demonstrated a link between MFI and health status in men, suggesting that andrological examination goes beyond purely a reproductive investigation but should also be considered as a unique opportunity to assess the male partners health [13,15,16]. In spite of this, studies suggest that many couples will use Assisted Reproductive Technology (ART) treatments before any andrological evaluation [17,18]. As such, a number of ART could be avoided if the male partners had been assessed prior to undergoing ART. In fact, a comprehensive medical history, a semen analysis and, when necessary, a sperm DNA fragmentation test together with male genetic profiling, is integral to planning successful ART [5], which will also be more timely and cost effective for the couple [19]. Therefore, considering the established importance of an initial diagnostic work-up of both partners of every infertile couple and the growing epidemiologic evidence of an association between MFI and overall men's health, we aimed to investigate the prevalence of and the characteristics of infertile men who were referred for an andrological evaluation after failed attempts of ART with those who were evaluated at the beginning of their infertility pathway at a single academic centre over a 17-year period.

MATERIALS AND METHODS

A comprehensive database of 3213 men consecutively assessed for primary infertility at a single academic tertiary-referral andrology centre between January 2003 and October 2020 was analysed. Infertility was defined as not conceiving a pregnancy after at least 12 months of unprotected intercourses regardless of whether a pregnancy ultimately occurred [1], according to the World Health Organization (WHO) criteria. Primary and secondary infertility were defined according to the inability to conceive after a previous pregnancy [1]. MFI diagnosis was achieved after a thorough and comprehensive andrological assessment of the male partner by the same expert academic urologist (A.S.) and after an extensive diagnostic evaluation of the female partner performed by trained reproductive gynaecologists. During the diagnostic evaluation, all patients were assessed with a thorough and focused medical and fertility history. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI) [20] and body mass index (BMI) was calculated for each patient. A comprehensive physical exam was performed in all cases, and testes volume was assessed using a Prader orchidometer, calculating the mean value between the two sides. Endocrinological evaluation was undertaken in all patients [5]. Hypogonadism was defined as total testosterone (tT) ≤3.0 ng/mL [21]. Participants underwent at least two consecutive semen analyses, analysed according to the 2010 WHO reference criteria [22]. For the specific purposes of this study, we considered semen volume, sperm concentration, progressive sperm motility, normal morphology and total motile sperm count (TMSC) [23]. The same laboratory was used for analyses of all parameters. Chromosomal analysis and genetic testing (i.e., karyotype analysis and tests for Y-chromosome microdeletions and cystic fibrosis mutations) were performed according to guideline recommendations [5,24]. Data collection adhered to the principles outlined in the Declaration of Helsinki. All men signed an informed consent agreeing to share their own anonymous information for future studies. The study was approved by the IRCCS San Raffaele Hospital Ethical Committee (Prot. 2014—Pazienti Ambulatoriali).

Statistical analysis

Distribution of data was tested with the Shapiro–Wilk test. Data are presented as medians (interquartile range [IQR]) or frequencies (proportions). For the specific purpose of the study, the entire cohort was segregated into: i) patients belonging to infertile couples referring to the andrology clinic for the first time after one or more previous failed attempts of any type of ART (+ART); and, ii) patients belonging to infertile couples referring to the andrology clinic for the first time without any previous attempts of ART (–ART). First, demographics and clinical characteristics, hormonal values, and semen parameters were compared between +ART and –ART couples using the Mann-Whitney and Chi-square tests. Second, logistic regression models were applied to identify variables associated with the probability of being referred to andrology evaluation after previous failed ART (one or more cycles). Third, local polynomial regression models explored and graphically displayed the probability of couples to report ART before any andrological consultation over the analysed time frame (2003 – 2020).

Statistical analyses were conducted using R studio Inc. (2016) integrated development environment for R software version 3.5.3, Boston, MA (USA) and GraphPad Prism version 8.0.0, GraphPad

Software, San Diego, CA (USA). All tests were two sided, and statistical significance level was determined at $p < 0.05$.

RESULTS

Table 1 lists the characteristics of the entire cohort of patients. Overall, 493 (15.3%) couples reported at least one previous ART cycle before any andrological consultation, throughout the study period. Of them, 62 (12.6%) underwent previous in vitro fertilization and embryo transfer (IVFET), 296 (60.0%) with intracytoplasmic sperm injection (ICSI), 65 (13.2%) with artificial insemination (AIH), 45 (9.1%) intrauterine insemination (IUI), and 25 (5.1%) reported previous multiple miscarriages.

Table 2 depicts the descriptive statistics according to previous ART status in the whole cohort. Both +ART partners were older than –ART partners (all $p \leq 0.04$). Overall, men belonging to +ART cohort had a lower sperm concentration ($p<0.0001$), a greater rate of non-obstructive azoospermia (NOA) ($p<0.001$) and a lower TMSC ($p<0.0001$). Furthermore, a pure MFI component was more likely to be reported in the +ART group than in the –ART one. Conversely, groups did not differ in terms of all other variables (Table 2).

Table 3 details the logistic regression models testing the association between clinical variables and +ART. At univariable analysis, male age, partner's age >35 years, patient's CCI at presentation, and the year of first andrological assessment (all $p \leq 0.04$) were associated with +ART. At multivariable analysis, male age ($p=0.04$) and less recent years of andrological assessment (both 2003-2009 and 2010-2015 vs. 2016-2020, all $p<0.01$) were predictors of +ART, after accounting for partner's age >35 years.

At local polynomial regression models, the likelihood of assessing the male partner belonging to +ART infertile couples remained almost constant throughout the entire analysed time frame, with a slight but not significant decrease over the last 7 years (Figure 1).

DISCUSSION

As a socio-cultural taboo, for decades the burden of couples' infertility has been often and disproportionately assumed as the women responsibility. As such, for biological and social reasons, a couple's infertility has been unequally shared, with the tendency to investigate the female partner over the male, with the potential to miss important treatable pathologies in the infertile couple [12,25]. In this regard, a large WHO multinational study showed that, among infertile couples, a major factor responsible for infertility status in the female, with no demonstrable cause in the male, was diagnosed in only 12.8% of cases, and a major factor in the male, with no demonstrable cause in

the female, was diagnosed in only 7.5% of cases [4]. In this context, epidemiological studies have shown that MFI has been estimated to account for up to 50% of the cases [6], affecting approximately 7% of all men [26]. As such, the true underlying cause of a couple's infertility is fairly distributed between both male and female partners [6]. For this reason, the most recent EAU guidelines strongly recommend to investigate all male patients belonging to the infertile couple in order to streamline care and increase the chances of conceiving [5]. Likewise, the newly released AUA/ASMR guidelines suggest that for initial infertility evaluation, both male and female partners should undergo concurrent assessment. In this context, men with one or more abnormal semen parameters or presumed male infertility should be evaluated by a male reproductive expert to collect a complete medical and fertility history and perform a physical examination, as well as other directed tests when indicated [27]. Moreover, in the ESHRE Special Interest Group of Embryology report, it is mentioned a general concern that semen analysis reference values have little or no value for ART procedures [28,29]. In this regard, the WHO reference values for sperm concentration, motility and vitality were derived from populations of men who had achieved in vivo conceptions [30], and therefore these cut-off values have no a priori relevance in regard to ART patients, and hence the need or suitability for any form of ART treatment should not be decided based on these reference values [31]. This further strengthens the importance of expert andrological evaluations of every infertile man before any ART attempt. So far, ARTs' success rates have been fairly constant, with an approximate 23% live birth rate [32]. In addition, a 10-year cohort study of 3394 women undergoing 8048 ART cycles demonstrated that live birth rates were 52% after 3 cycles, 72% after 6 cycles and 85% after 12 cycles, with a cumulative rate of 50% for patients under 40 years [33]; still, an overall failure rate of around 50% from ART. Hence, the tendency of only concentrating on female reproductive health has a major impact on the success rates of ART when it comes to evaluate both partners [34]. Taking together these observations, we have investigated the trends of infertile men presenting for their first andrological evaluation after previous ART (one or even more cycles), assessing potential differences with a cohort of patients belonging to infertile couples that, conversely, have been not submitted to (any) ART throughout the same time frame. Of major clinical importance, we found that approximately 15% of couples presented for a first andrological evaluation after previous failed attempts of ART (one or even more) during the entire 17-year time frame. Overall, the likelihood of assessing male patients belonging to +ART infertile couples remained almost constant throughout the years, with a slight decrease in the last 7 years (Figure 1).

Indeed, it was observed that even during the last 5 years of analysis, even 147 (14.5%) couples attended for their first andrological assessment after at least one previous ART cycle. In this context, infertile men belonging to +ART couples were older than those in –ART, and this was also clearly observed for their female counterpart at presentation. Accordingly, male age was found to be independently associated with +ART, after accounting for partner's age >35 years; this finding further corroborates the hypothesis that there could have been a tendency to prioritise the investigation of the female partner over the male, despite a clear recommendation from guidelines [5,27]. Whist, compared to –ART couples, men belonging to +ART couples had worse semen parameters. This in itself does not justify or support primary referral for ART without andrological assessment, as these patients may have significant abnormalities in their reproductive health (i.e., hypogonadism, varicocele).

Furthermore, even men with normal semen parameters may not be able to achieve a successful physiological pregnancy due to underlying semen abnormalities (i.e., oxidative stress) [34]. This

further highlights the importance of specialistic andrological evaluation of infertile couples. Of relevance the less recent years of assessment (i.e., 2003-2009 and 2010-2015 vs. 2016-2020) were found to be independently associated with +ART, even after accounting for the female partner's age >35 years; we hope that the slight observed decreased trend in assessing +ART couples over the last 5 years could reflect a change in clinical practice by virtue contemporary guidelines stressing the importance of assessing both partners of an infertile couple prior to treatment.

The evaluation of the male partner of every infertile couple is important for a number of reasons. Firstly, even though a semen analysis has been considered historically the cornerstone to establishing the reproductive potential of the male, several studies have shown than a significant proportion of men are infertile despite having a normal sperm analyses [34–36]. Of clinical relevance, a recent study comparing sperm parameters from 1,957 infertile men with those from 103 age-comparable fertile controls showed that 12% of infertile men and only 41% of fertile men had normal sperm parameters [34]. Moreover, among fertile men, 36.9% had isolated sperm abnormalities and 22.3% men had two or more concomitant sperm abnormalities. As such, authors concluded that normal sperm parameters per-se are not an adequate surrogate marker for male fertility [34]. This may also explain why semen analysis alone is insufficient in predicting success when the couple is planning to undergo ART. Secondly, it is also known that the spectrum of potential causes of MFI has changed overtime [37], with an ever more growing number of men with more severe cases and many more unexplained or idiopathic cases for which at least a basic andrological consultation should be considered. Thirdly, the incorrect timing of assessment - i.e., after ART - can not only delay the couple's fertility management but may also not be cost-effective. In this regard, it has been recently demonstrated that the duration of male infertility was negatively associated with semen parameters [38]. Indeed, the length of infertility was correlated with higher risks of oligozoospermia, lower TMSC, and a higher prevalence of NOA making "time" either a precious ally or a dangerous enemy to plan a successful procreation path [9,38]. Lastly, and possibly the most important, an in-depth assessment of the male partner may also play a major role in disease prevention and detection [39]. Indeed, several studies have confirmed the association between male infertility and overall general health, therefore considering male infertility as an "opportunity" for a comprehensive medical assessment to screen for comorbid conditions and to improve male health status, with either lifestyle changes or therapies that could also improve reproductive function [15,40,41]. Therefore, a comprehensive andrological evaluation goes beyond the "simple" assessment of reproductive parameters, but it gains even more importance in terms of evaluation of long-term health.

Our study is certainly not devoid of limitations. Of relevance, it is a cross-sectional retrospective analysis at a single, tertiary-referral academic centre, thus raising the possibility of selection biases; in this context, we fear that in unselected centres these percentages may be considerably higher. Therefore, larger multi-centre cohort studies are needed to validate our findings. In spite of this, all patients have been consistently analysed over time with a rigorous comprehensive work-up according to scientific guidelines, thus limiting potential heterogeneity associated with differences in diagnostic work-up methodology and treatment decision making as a confounding variable.

CONCLUSIONS

Despite contemporary guidelines strongly recommending the importance of a simultaneous evaluation of both partners of the infertile couple, approximately 15% of couples are still referred directly to ART without an initial andrological evaluation in the real-life setting. This is despite the fact that infertile partners of couples reporting failed ART previous prior to andrological evaluation were older and their male partners had worse sperm parameters than those seeking expert andrological consult before an attempt at ART. In recent years we found a small decrease in this referral pattern, which may reflect changes in public and speciality awareness towards the importance of a comprehensive evaluation for the male partner of every infertile couple.

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Table 1: Descriptive characteristics of the whole cohort of patients (n. 3213)

Age (years)	
Median (IQR)	37 (33 – 41)
Range	27 – 55
Partner's age (years)	
Median (IQR)	34 (31-37)
Range	18 – 53
Pure MFI [n. (%)]	2609 (81.20)
Mixed infertility [n. (%)]	958 (29.82)
BMI (kg/m^2)	
Median (IQR)	25.03 (23.18 – 27.17)
Range	17.00 – 35.00
CCI [n. (%)]	
0	2930 (91.19)
1	144 (4.48)
≥ 2	140 (4.36)
Arterial hypertension [n. (%)]	
Yes	219 (6.82)
No	2995 (93.22)
History of cryptorchidism [n. (%)]	344 (10.71)
Cigarette smoking [n. (%)]	
Yes	908 (28.26)
No	1928 (60.00)
Ex-smokers	375 (11.67)
Testis volume, right (Prader)	
Median (IQR)	15 (12 – 20)
Range	0 – 25
Testis volume, left (Prader)	
Median (IQR)	15 (12 – 20)
Range	0 – 25
FSH (mIU/mL)	
Median (IQR)	5.9 (3.4 – 12.5)
Range	0.00 – 198.00
LH (mIU/mL)	
Median (IQR)	4.30 (3.00 – 6.30)
Range	0.00 – 434.0
InhB (pg/mL)	
Median (IQR)	104 (41.65 – 163.45)
Range	0.01 – 670.96
AMH (ng/mL)	
Median (IQR)	4.79 (2.71 – 7.90)
Range	0.01– 113.00)
tT (ng/mL)	
Median (IQR)	4.55 (3.46 – 5.80)
Range	0.00 – 26.47
tT <3 ng/mL [n. (%)]	328 (10.21)
SHBG (nmol/L)	
Median (IQR)	31 (23 – 41)
Range	2.44 – 790.00
Prolactin (ng/mL)	
Median (IQR)	8.70 (6.40 – 12.40)
Range	0.01 – 751.00
TSH (mIU/mL)	
Median (IQR)	1.65 (1.18 – 2.32)
Range	0.01 – 77.88
PSA (ng/mL) [n. 978 (30.44%)]	
Median (IQR)	0.71 (0.48 – 1.08)

Range	0.01 – 90.70
Semen volume (mL)	
Median (IQR)	3 (2 – 4)
Range	0 – 16
Semen volume <1.5 mL [n. (%)]	327 (10.18)
Sperm concentration	
Median (IQR)	17 (4.79 – 45.00)
Range	0.00 – 455.30
Sperm concentration <15 x 10^6/mL [n. (%)]	779 (24.25)
Total motile sperm count	
Median (IQR)	25.65 (5 – 81.82)
Range	0 - 400
Progressive motility <32% [n. (%)]	1272 (39.59)
Normal morphology	
Median (IQR)	3 (1 – 10)
Range	0 – 100.00
Normal morphology <4% [n. (%)]	1124 (34.98)
Nonobstructive azoospermia [n. (%)]	509 (15.84)
Obstructive azoospermia [n. (%)]	96 (2.99)
Normal karyotype [n. (%)]	3109 (96.76)
Klinefelter (any karyotype) [n. (%)]	40 (1.24)
Chromosomal abnormalities (any type) [n. (%)]	61 (1.90)
Y-chromosome microdeletions (any type) [n. (%)]	29 (0.90)
CFTR mutations (any type) [n. (%)]	205 (6.38)
Couples +ART [n. (%)]	
Total	493 (15.34)
No previous ART	2720 (64.66)
IVFET	62 (2.09)
ICSI	296 (9.21)
AIH	65 (2.02)
IUI	45 (1.40)
Multiple abortions (≥1)	25 (0.77)

Keys: AMH = anti-mullerian hormone; BMI = body mass index; CCI = Charlson comorbidity index; CFTR = cystic fibrosis transmembrane conductance regulator; FSH = follicle-stimulating hormone; InhB = inhibin B; LH = luteinizing hormone; PRL = prolactin; tT = total testosterone; SHBG = sex hormone–binding globulin; TSH = thyroid-stimulating hormone; PSA = prostate specific antigen; MFI = male factor infertility; +ART = assisted reproductive technology prior than any andrological evaluation; IVFET = Fertilization in Vitro and Embryo Transfer; ICSI = Intracytoplasmic sperm injection; AIH = artificial insemination, homologous; IUI= IntraUterine Insemination.

Table 2: Characteristics of the whole cohort of patients according to previous ART (n. = 3213)

	+ART	-ART	p value*
No. of participants [no. (%)]	493 (15.3)	2720 (84.7)	
Age (years)			
Median (IQR)	38 (34 - 41)	37 (33 – 40)	0.01
Range	19 – 55	18 – 55	
Partner's age (years)			
Median (IQR)	35 (32 – 37)	34 (31 – 37)	0.04
Range	20 – 48	18 - 53	
Pure MFI [n. (%)]	427 (86.61)	2182 (80.22)	<0.0001
Mixed infertility [n. (%)]	119 (24.14)	656 (24.12	0.36
BMI (kg/m^2)			
Median (IQR)	25.06 (23.15 – 27.08)	25.03 (23.20 – 27.17)	0.96
Range	17.51 – 44.98	17.0 – 35.0	
CCI [n. (%)]			
0	440 (89.25)	2398 (87.83)	
1	27 (5.48)	118 (4.33)	0.32
≥ 2	26 (5.27)	114 (4.19)	0.96
Arterial hypertension [n. (%)]			
Yes	36 (7.17)	203 (7.35)	0.88
No	467 (43.03)	2561 (28.48)	
History of cryptorchidism [n. (%)]	67 (13.59)	277 (6.88)	0.08
Cigarette smoking [n. (%)]			
Yes	146 (29.68)	763 (27.99)	
No	295 (59.84)	1633 (60.03)	0.60
Ex-smokers	52 (10.54)	323 (11.88)	
Testis volume, right (Prader)			
Median (IQR)	15 (12 – 20)	15 (12 – 20)	0.52
Range	0 – 25	0 - 25	
Testis volume, left (Prader)			
Median (IQR)	15 (12 – 20)	15 (12 – 20)	0.53
Range	0 – 25	0 – 25	
FSH (mIU/mL)			
Median (IQR)	5.6 (3.40 – 11.35)	6.00 (3.40 – 12.80)	0.41
Range	0.01 – 198	0.00 – 99.10	
LH (mIU/mL)			
Median (IQR)	4.40 (2.90 - 6.30)	4.30 (3.00 - 6.30)	0.79
Range	0.10 – 37.4	00.00 – 434	

InhB (pg/mL)			
Median (IQR)		102.55 (41.80 – 163.65)	112.39 (104.90)
Range		0.70 – 400.49	0.01 – 670.96
	0.68		
AMH (ng/mL)			
Median (IQR)		4.82 (2.75 – 7.63)	4.79 (2.71 – 7.91)
	0.86		
Range		0.10 – 112.50	0.01 – 113.00
tT (ng/mL)			
Median (IQR)		4.37 (3.36 – 5.70)	4.6 (3.50 – 5.83)
	0.08		
Range		0.01 – 13.10	0.00 – 26.47
tT <3 ng/mL [n. (%)]		50 (10.14)	278 (10.22)
	0.71		
SHBG (nmol/L)			
Median (IQR)		30.55 (23.00 – 41.00)	31.55 (23.00 – 41.00)
	0.75		
Range		10.00 – 790.00	2.44 – 154.00
PRL (ng/mL)			
Median (IQR)		8.2 (6.23 – 12.15)	8.80 (6.42 – 12.50)
	0.15		
Range		0.91 – 319.00	0.01 – 751.00
TSH (mIU/mL)			
Median (IQR)		1.69 (1.19 – 2.21)	1.65 (1.18 – 2.34)
	0.79		
Range		0.08 – 11.54	0.01 – 77.88
PSA (ng/mL) [n. 978 (30.44%)]			
Median (IQR)		0.74 (0.45 – 1.13)	0.70 (0.48 – 1.08)
	0.66		
Range		0.01 – 90-70	0.02 – 15.30
Semen volume (mL)			
Median (IQR)		3 (2 – 4)	3 (2 - 4)
	0.74		
Range		0 – 13	0-16
Semen volume <1.5 mL [n. (%)]		42 (8.52)	285 (10.48)
	0.21		
Sperm concentration			
Median (IQR)		10 (0.25 – 34.45)	10 (0.7 – 35)
	<0.0001		
Range		0.00 – 240.80	0.00 – 455.30
Sperm concentration <15 x 10^6/mL [n. (%)]		114 (23.12)	887 (32.61)
	0.26		
Low total motile sperm count [n. (%)]		149 (30.22)	128 (4.70)
	<0.0001		
Progressive motility <32% [n. (%)]		204 (41.38)	1068 (39.26)
	0.09		
Normal morphology			
Median (IQR)		2 (1.00 – 9.50)	3 (1 – 10)
	<0.0001		
Range		0 – 94	0 - 100
Normal morphology <4% [n. (%)]		176 (35.70)	948 (34.85)
	0.58		
Nonobstructive azoospermia [n. (%)]		50 (10.14)	79 (2.90)
	<0.0001		

Obstructive azoospermia [n. (%)]	7 (3.44)	79	(2.90)
0.65			
Normal karyotype [n. (%)]	459 (93.10)	2633	(96.80)
0.43			
Klinefelter (any karyotype) [n. (%)]	10 (2.03)	30	(1.10)
0.04			
Y-chromosome microdeletions (any type) [n. (%)]	2 (2.02)	27	(0.99)
0.22			
CFTR mutations (any type) [n. (%)]	31 (6.30)	174	(6.39)
0.92			

Keys: +ART = assisted reproductive technology prior than any andrological evaluation ; AMH = anti-müllerian hormone; BMI = body mass index; CCI = Charlson comorbidity index; CFTR = cystic fibrosis transmembrane conductance regulator; FSH = follicle-stimulating hormone; InhB = inhibin B; LH = luteinizing hormone; PRL = prolactin; tT = total testosterone; SHBG = sex hormone–binding globulin; TSH = thyroid-stimulating hormone; PSA = prostate specific antigen; MFI = male factor infertility.
*P value according to Mann-Whitney test for continuous variables. Chi-square test for categorical variables, as indicated

Table 3: Univariable and multivariable logistic regression models predicting previous ART

	UVA			**MVA**
	OR	95% CI	p value	OR
95% CI	p value			
Age	1.02	1.05; 1.12	0.02	1.02
1.10; 1.90	0.04			
Partner ‘s age (>35 yr)	1.24	1.01; 1.53	0.04	1.16
0.90; 1.48	0.24			
CCI	1.15	1.01; 1.31	0.03	1.14
0.98; 1.39	0.07			

N. sperm parameters alterations

Azoospermia	Ref.						
Any alterations	0.96	0.75; 1.24	0.77				
Normal sperm parameters	0.91	0.62; 1.31	0.61				
Year of first presentation for couple's infertility							
2016-2020	Ref.						Ref.
2010-2015	1.69	1.28; 2.16	<0.001				1.98
1.50; 2.68	<0.001						
2003-2009	1.36	1.05; 1.77	0.02				1.66
1.26; 2.23	0.01						

Keys: ART = Assisted reproductive technology; UVA = Univariate analysis; MVA = Multivariate analysis; tT = total testosterone.

Figure 1. Probability of infertile couples to report different assisted reproduction techniques (ART)/multiple abortions over time (2003 – 2020) before any andrological assessment. Local polynomial regression models were applied to explore and graphically display patients' likelihood of reporting different ART/multiple abortions over the analysed time frame.

Keys: ART = Assisted reproductive technology; IVFET = Fertilization in Vitro and Embryo Transfer; ICSI = Intracytoplasmic sperm injection; AIH = artificial insemination, homologous; IUI= Intrauterine Insemination.