Original Article
Male reproductive health and infertility

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health Published online Sep 2, 2022
https://doi.org/10.5534/wjmh.220069

The World Journal of MEN's HEALTH

Is There a Relevant Clinical Impact in Differentiating Idiopathic *versus* Unexplained Male Infertility?

Christian Corsini[1,3,*], Luca Boeri[2,*], Luigi Candela[1,3], Edoardo Pozzi[1,3], Federico Belladelli[1,3], Paolo Capogrosso[4], Giuseppe Fallara[1,3], Nicolò Schifano[1,3], Daniele Cignoli[1,3], Eugenio Ventimiglia[1,3], Alessia D'Arma[1], Massimo Alfano[1], Francesco Montorsi[1,3], Andrea Salonia[1,3]

[1]*Division of Experimental Oncology/Unit of Urology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele,* [2]*Department of Urology, Foundation IRCCS Ca' Granda – Ospedale Maggiore Policlinico, University of Milan,* [3]*University Vita-Salute San Raffaele, Milan,* [4]*Department of Urology and Andrology, Ospedale di Circolo and Macchi Foundation, Varese, Italy*

Purpose: Overall, male factor infertility (MFI) accounts for up to 50% of etiologies of couple's infertility, with almost 30% of MFI cases being idiopathic in nature. Idiopathic MFI does not support a tailored treatment work-up in clinical practice. To investigate rates of and characteristics of men presenting for idiopathic *versus* unexplained primary infertility as compared with same-ethnicity, age-comparable fertile men.

Materials and Methods: Demographic, clinical and laboratory data from 3,098 primary infertile men consecutively evaluated were analyzed and compared with those of 103 fertile controls. Idiopathic male infertility (IMI) was defined for abnormality at semen analysis with no previous history of diseases affecting fertility and normal findings on physical examination and genetic and laboratory testing. Unexplained male infertility (UMI) was defined as infertility of unknown origin with completely normal findings at semen analysis. Descriptive statistics and logistic regression models tested the association between clinical variables and idiopathic infertility status.

Results: Overall, 570 (18.5%) and 154 (5.0%) patients depicted criteria suggestive for either IMI or UMI, respectively. Groups were similar in terms of age, BMI, CCI, recreational habits, hormonal milieu, and sperm DNA fragmentation indexes. Conversely, testicular volume was lower in IMI ($p<0.001$). Vitamin D3 levels were lower in IMI *vs*. UMI *vs*. fertile controls ($p=0.01$). At multivariable logistic regression analysis only vitamin D3 deficiency (OR, 9.67; $p=0.03$) was associated with IMI. Characteristics suggestive for IMI versus UMI were observed in almost 20% and 5% of men, respectively. Overall, clinical differences between groups were slightly significant and certainly not supportive of a tailored management work-up.

Conclusions: Current findings further support the urgent need of a more detailed and comprehensive assessment of infertile men to better tailoring their management work-up in the everyday clinical setting.

Keywords: Andrology; Infertility; Infertility etiology; Testis abnormalities; Vitamin D

Received: May 9, 2022 **Accepted:** Aug 10, 2022 **Published online** Sep 2, 2022
Correspondence to: Andrea Salonia https://orcid.org/0000-0002-0595-7165
Division of Experimental Oncology/Unit of Urology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, University Vita-Salute San Raffaele, Via Olgettina 60, 20132 Milan, Italy.
Tel: +39-02-26436763, **Fax:** +39-02-26436352, **E-mail:** salonia.andrea@hsr.it
*These authors contributed equally to this work as co-first authors.

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INTRODUCTION

Compelling evidence over the last decades suggests that infertility affects about 15% of couples, and in one of two cases a male factor can be identified [1]. Several causes have been ascribed in the context of male factor infertility (MFI), ranging from clinical, hormonal and genetic conditions; however, almost 30% of men show impaired sperm parameters without an identifiable cause even in the most comprehensive currently available real-life diagnostic work-up, thus defining the condition of idiopathic male infertility (IMI) [1,2]. Recent evidence suggests that IMI may be linked with numerous unidentified pathological conditions that may perturb the testicular micro-environment and spermatozoa characteristics (*e.g.*, pollution exposure, reactive oxygen species), causing DNA damages and genetic/epigenetic abnormalities, thus decreasing overall sperm quality and fertility potential [3]. In light of this, current European Association of Urology (EAU) guidelines strongly suggest that every infertile man should be evaluated with a detailed medical history, an accurate physical examination and at least a semen analysis with adherence to World Health Organization (WHO) reference values [1,4,5], in order to screen for potential causes of male infertility. Routine semen analysis is amongst the cardinal points in MFI investigation, being significantly related to conception chance [1,6,7]. However, the individual parameter at semen analysis provides only a partial indication of actual fertility potential. In this context, data would suggest that normal sperm parameters themselves do not reliably account for fertility in the real-life setting [8]. Indeed, approximately 15% to 40% of men are infertile despite having normal sperm parameters, normal medical history and normal physical examination; overall, this condition is currently defined as unexplained male infertility (UMI) [1,9,10].

Despite the underlying causes and the eventual biopathology of both idiopathic and unexplained infertility remain unclear, to date, there has been no sufficiently detailed investigation of specific clinical and laboratory characteristics of infertile men belonging to the two groups, which could be easily performed on a routine basis and eventually relevant in the everyday clinical practice. A more detailed characterization of both conditions could be of actual support in better tailoring infertile men throughout the management work-up of such a delicate condition.

Hence, we aimed to (1) define the prevalence of both IMI and UMI in a relatively large, homogeneous cohort of non-Finnish white-European men seeking first medical attention for couple's primary infertility; (2) investigate potential useful differences in clinical and laboratory parameters between the two categories; and (3) compare patients' characteristics with those of a cohort of same-ethnicity, age-comparable fertile controls.

MATERIALS AND METHODS

In this case-control retrospective study, demographic, clinical and laboratory data from 3,098 primary infertile men (according to WHO definition) consecutively assessed at a single academic center between 2012 and 2020 were analyzed. Patients were enrolled if they were ≥18 and ≤55 years old and had pure MFI, defined after a comprehensive diagnostic evaluation of all the female partners. Complete data from 103 same-ethnicity, age-comparable fertile controls (*i.e.*, men who had fathered at least one child, spontaneously conceived, with a time-to-pregnancy within 12 months, as for WHO criteria) were also collected [11]. According to our research protocol, fertile men were recruited *via* their partners who had been expectant and new mothers at Department of Obstetrics and Gynecology, IRCCS San Raffaele Hospital.

All participants were assessed with a thorough medical history. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI). Likewise, waist circumference, weight, and height were measured, calculating body mass index (BMI). Testicular volume (TV) was assessed with a Prader's orchidometer by a single expert uro-andrologist in all cases, calculating the mean value between the two sides [12]. Length of infertility was calculated in months. Varicocele was also clinically assessed in every patient [13].

Venous blood samples were drawn from each patient between 7 a.m. and 11 a.m. after an overnight fast. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), sex hormone binding globulin (SHBG), prolactin (PRL), inhibin B, and thyroid-stimulating hormone (TSH) levels were measured for every individual. Likewise, 25-OH vitamin D (vitamin D3) levels were evaluated for those infertile men consecutively evaluated in our center from 2015 to 2020 [14]. Vitamin D3 deficiency was considered for levels <20 ng/mL [15]. Chromosomal analysis and genetic test-

ing were performed in every infertile man (karyotype analysis and tests for Y-chromosome microdeletions and cystic fibrosis mutations) [16]. According to our research protocol, fertile men were evaluated with TT, FSH, and LH only.

Participants underwent at least two consecutive semen analyses [4]. As for clinical practice, we considered semen volume, sperm concentration, rates of progressive sperm motility and normal morphology. Likewise, total motile sperm count (TMSC) was calculated according to Hamilton et al [17]. Semen analyses were based on 2010 WHO reference criteria [4]. Accordingly, oligozoospermia was defined as <15 million spermatozoa per mL; asthenozoospermia as <32% progressive motility; and teratozoospermia <4% of typical forms. Oligoasthenoteratozoospermia was defined when all three abnormalities occurred simultaneously. Azoospermia was considered as the complete absence of spermatozoa in semen after centrifugation [4]. Sperm DNA fragmentation (SDF) index was measured by sperm chromatin structure assay in infertile men only.

For the specific purpose of this study, we selected only infertile men with either IMI or UMI [1]. We excluded azoospermic men, patients with known genetic diseases (any type), hypogonadal men [18], cases when other known causes were potential responsible factors (*e.g.*, hormonal causes, other than hypogonadism; cancer and cancer therapies; infectious conditions; immunological disorders; drugs, recreational drugs, and illicit substances; and erectile or ejaculatory dysfunction) and men with varicocele.

Data collection followed 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 protocol was approved by the Institutional Review Board of IRCCS San Raffaele Hospital Ethical Committee (IRB Prot. 2014—Pazienti Ambulatoriali).

Table 1. Clinical characteristics of participants according to fertility status (n=827)

Variable	Idiopathic MFI	Unexplained MFI	Fertile	p-value[a]
No. of individuals (%)	570 (68.9)	154 (18.6)	103 (12.5)	
Age (y)				0.3
Median (IQR)	37 (34–41)	36 (33–41)	37 (33–40)	
Range	21–54	25–54	25–48	
BMI (kg/m^2)				0.6
Median (IQR)	24.7 (23.1–26.3)	24.5 (23.1–26.2)	24.5 (23.1–27.9)	
Range	18.5–30.0	17.8–29.9	18.7–28.8	
CCI (score)				0.6
Median (IQR)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	
Mean±SD	0.10±0.1	0.10±0.2	0.06±0.1	
Range	0–7	0–4	0–2	
Mean testes volume (Prader's estimation)				<0.001
Median (IQR)	20 (15–23)	20 (15–25)[b]	23 (20–25)[b,c]	
Range	7–25	8–25	10–25	
Mean testes volume <15 mL, n (%)	135 (23.6)	21 (13.6)	5 (4.9)	<0.001
Waist circumference (cm)				0.7
Median (IQR)	92 (86–97)	91 (85–97)	89 (81–95)	
Range	54–119	75–114	75–120	
Length of infertility (mo)				0.6
Median (IQR)	18 (12–32)	24 (12–26)		
Range	12–192	12–135		
Partner's age (y)				0.1
Median (IQR)	35 (32–38)	34 (31–37)	35 (31–37)	
Range	19–47	23–45	20–44	

MFI: male factor infertility, IQR: interquartile range, BMI: body mass index, CCI: Charlson Comorbidity Index, SD: standard deviation.
[a]p-value according to the Kruskal–Wallis test and Fisher exact test, as indicated. [b]p<0.01 *vs.* idiopathic group. [c]p<0.01 *vs.* unexplained group.

Statistical methods

Distribution was tested with the Shapiro–Wilk test. Data are presented as medians (interquartile range, IQR) or frequencies (proportions). Demographic characteristics, hormonal values and sperm parameters were compared among the three groups (*i.e.*, IMI, UMI, and fertile) with the Kruskal–Wallis test and Fisher exact test, when appropriate. Lastly, univariable (UVA) and multivariable (MVA) logistic regression models were used to identify potential predictors (*e.g.*, age, BMI, CCI, TV, FSH, TT, and vitamin D levels) of IMI within the infertile cohort. For completeness, same analyses were performed also for UMI in the same cohort.

Statistical analyses were performed using SPSS v.26 (IBM Corp., Armonk, NY, USA) and Stata 14.0 (StataCorp, College Station, TX, USA). All tests were two-sided, and the statistical significance level was determined at $p<0.05$.

RESULTS

Of all, 570 (18.4%) and 154 (5.0%) men depicted criteria suggestive for IMI and UMI, respectively.

Table 1 details the clinical characteristics of the entire cohort of participants, as segregated according to fertility status. Groups were similar in terms of age, BMI, CCI, waist circumference, and partner's age. TV was lower in IMI compared to UMI patients ($p<0.001$); conversely, TV was greater in fertile controls than in both IMI and UMI cases (all $p<0.01$). A higher percentage of IMI men depicted TV <15 mL compared to both UMI patients and fertile controls ($p<0.001$) (Table 1).

Table 2. Serum hormones of participants according to fertility status (n=827)

Variable	Idiopathic MFI	Unexplained MFI	Fertile	p-value[a]
No. of individuals (%)	570 (68.9)	154 (18.6)	103 (12.5)	
TT (ng/mL)				0.2
Median (IQR)	5.2 (4.2–6.2)	5.4 (4.0–6.3)	4.9 (4.1–5.9)	
Range	3.1–14.3	3.1–9.9	2.4–9.2	
FSH (mUI/mL)				0.1
Median (IQR)	4.5 (3.0–7.7)	4.6 (2.3–6.2)	4.1 (3.0–5.6)	
Range	1.0–19.6	1.0–13.1	1.4–12.6	
LH (mUI/mL)				0.02
Median (IQR)	3.9 (2.8–5.4)	3.5 (2.8–5.1)	4.3 (3.6–5.5)[b,c]	
Range	0.9–16.0	1.6–8.7	1.5–10.4	
Prolactin (ng/mL)				0.5
Median (IQR)	8.8 (6.5–12.5)	8.5 (6.6–11.1)		
Range	1.9–59.4	2.6–75.3		
SHBG (nmol/L)				0.6
Median (IQR)	34.1 (27–42)	36.0 (27–45)		
Range	11.1–154.0	15.0–75.0		
TSH (mUI/L)				0.1
Median (IQR)	1.7 (1.2–2.3)	1.6 (1.1–2.2)		
Range	0.3–9.0	0.9–5.4		
Inhibin B (pg/mL)				0.2
Median (IQR)	141 (87–199)	150 (123–190)		
Range	0.7–538.0	18.0–305.0		
Vitamin D (ng/mL)				0.01
No. of individuals (%)	106 (18.6)	87 (56.5)		
Median (IQR)	22.3 (17.8–28.05)	31.6 (21.4–45.2)		
Range	8.8–128.0	18.7–52.2		
Vitamin D <20 ng/mL, n (%)	215 (37.7)	13 (8.4)		<0.01

MFI: male factor infertility, TT: total testosterone, IQR: interquartile range, FSH: follicle-stimulating hormone, LH: luteinizing hormone, SHBG: sex hormone binding globulin, TSH: thyroid-stimulating hormone.
[a]p value according to the Kruskal–Wallis test and Fisher exact test, as indicated. [b]$p<0.01$ *vs.* idiopathic group. [c]$p<0.01$ *vs.* unexplained group.

Table 2 reports serum hormones in all participants as segregated according to fertility status. Circulating LH values were higher in fertile than infertile men (p=0.02), but TT was comparable among groups. Conversely FSH levels did not significantly vary among groups. Likewise, IMI and UMI patients did not differ in terms of other hormonal levels. When evaluated, serum vitamin D was significantly lower in IMI *vs*. UMI (p=0.01), with a higher rate of vitamin D <20 ng/mL in IMI *vs*. UMI (p<0.01) (Table 2).

As expected, sperm parameters were different among the three groups (Table 3), with lower values for sperm concentration, TMSC, rates of progressive sperm motility, and normal sperm morphology observed in IMI compared to both UMI patients and fertile controls (all p<0.001).

Table 4 depicts logistic regression models testing the association between clinical and biochemical predictors with IMI status. At UVA model, pathologic vitamin D levels (p<0.001) and TV <15 mL (p=0.02) were found to be associated with IMI. Conversely, age, BMI, CCI, FSH, and TT were not. At MVA model, only vitamin D deficiency (p=0.03) was found to be independently associated with IMI, after accounting for age, BMI, TT, and TV.

Supplement Table illustrates the results of logistic regression analysis used to test the association between the same latter predictors with UMI status in the infertile cohort.

DISCUSSION

In an attempt to be effective in the management of males with primary infertility for whom a certain cause of the problem is not identified, and a consequent adequate therapy is eventually unavailable, any effort for a more comprehensive characterization is obviously of enormous importance in the everyday clinical practice. In this sense, great effort is made to try and peculiarize the most difficult and insidious cases, such as men with idiopathic or unexplained infertility. Current study showed that in a relatively large same-ethnicity cohort of primary infertile men, approximately 20% and 5% of patients depicted clinical criteria suggestive for either idiopathic or unexplained infertility, respectively. Surprisingly, but given the problematic nature

Table 3. Sperm parameters of the whole cohort according to fertility status (n=827)

Variable	Idiopathic MFI	Unexplained MFI	Fertile	p-value[a]
No. of individuals (%)	570 (68.9)	154 (18.6)	103 (12.5)	
Semen volume (mL)				0.1
Median (IQR)	3.0 (2.0–4.0)	3.0 (2.0–5.0)	2.0 (1.0–2.5)	
Range	0.1–13.0	1.0–8.0	1.0–7.0	
Sperm concentration ($\times 10^6$/mL)				<0.001
Median (IQR)	17.0 (6.0–46.0)	50.0 (29.4–80.0)	26.1 (18.1–50.3)	
Range	0.5–305.9	15.2–220.8	2.1–155.4	
Progressive sperm motility (%)				<0.001
Median (IQR)	25.0 (11–38)	49.0 (40–58)	36.2 (26–43)	
Range	0.0–96.0	32.0–84.0	0.0–78.2	
TMSC ($\times 10^6$)				<0.001
Median (IQR)	14.9 (3.5–45.0)	81.4 (48.8–127.1)	38.4 (25.1–84.6)	
Range	0.0–287.4	7.2–351.0	2.1–356.9	
Normal sperm morphology (%)				<0.001
Median (IQR)	2.0 (1–6)	8.0 (5–22)	3.0 (1–8)	
Range	0.0–92.0	4.0–94.0	0.0–91.0	
SDF (%)				0.8
Median (IQR)	36.5 (13–45)	31.9 (20–48)		
Range	5.5–72.2	0.3–97.7		
SDF >30%, n (%)	311 (54.6)	84 (54.5)		0.7

MFI: male factor infertility, IQR: interquartile range, TMSC: total motile sperm count, SDF: sperm DNA fragmentation.
[a]p-value according to the Kruskal–Wallis test and Fisher exact test, as indicated.

Table 4. Logistic regression models predicting idiopathic infertility in the infertile cohort

Variable	Univariable model		Multivariable model	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.01 (0.98–1.05)	0.21	1.01 (0.97–1.15)	0.16
BMI	1.02 (0.95–1.11)	0.51	1.37 (0.94–2.01)	0.11
CCI	0.86 (0.61–1.25)	0.45		
FSH	1.08 (0.99–1.19)	0.09		
Total testosterone	0.98 (0.83–1.16)	0.87	1.05 (0.70–1.59)	0.79
Testicular volume <15 mL	1.92 (1.01–3.36)	0.02	1.02 (0.89–1.14)	0.56
Vitamin D <20 ng/mL	7.92 (4.31–14.56)	<0.001	9.67 (1.21–16.37)	0.03

OR: odds ratio, CI: confidence interval, BMI: body mass index, CCI: Charlson Comorbidity Index, FSH: follicle-stimulating hormone.

of the two clinical pictures, not so much, only few minimal differences could be observed in those two settings compared to the fertile counterpart. Indeed, idiopathic infertile men showed lower TV and lower serum vitamin D levels compared to men with unexplained infertility. No further clinical characteristics emerged to be relevant throughout the real-life diagnostic work-up of patients presenting for primary infertility to support on the one hand the identification of a rationale biopathology in both conditions as compared with fertile controls, and on the other a consequent effective therapeutic approach.

Therefore, beyond a semantic distinction, the first real dilemma is to understand whether it can be imagined that what is currently available in the diagnostic work-up of men belonging to infertile couples is objectively the best to be done [1], without consequently and obligatorily resorting to assisted reproductive technologies. Second relevant query deals with the concept that it is probably the right time to overcome the pure differences in definition and classification of infertility types to invest in technologically more refined (and consequently more useful) diagnostics tools [19]. Our findings would seem to strongly support this second way.

Likewise, current results also advocate a more comprehensive discussion about the importance of testing some analytes apparently unrelated to the difficult process of fathering. Indeed, the potential role of vitamin D in male infertility was analized in a cross-sectional study by Ciccone et al, [20] showing that serum vitamin D levels were significantly lower in men with impaired sperm parameters as compared with normozoospermic men. They found a positive correlation between vitamin D levels and sperm concentration, motility, morphology, and TT. Similarly, Alzoubi et al [21] analyzed a cohort of 117 Jordanian males and, after stratification of participants in IMI, controls, and secondary infertility, they found that serum vitamin D levels were significantly lower in IMI *vs.* fertile controls *vs.* secondary infertile men. Hence, after two months of vitamin D treatment, total, and progressive sperm motility significantly improved compared to baseline values. A subsequent study with UMI men, teratozoospermic infertile men, and fertile controls showed that serum vitamin D levels were significantly lower in men with unexplained infertility and those with teratozoospermia, compared to fertile participants [22]. In contrast, Banks et al [23] demonstrated that vitamin D deficiency in the male partner did not significantly impact sperm parameters. This inconsistency may be due to the poor predictive value of semen analysis per se in terms of diagnostic investigation for male fertility [8].

A possible biopathological explanation of the relationship between vitamin D and male fertility was given by Hussein et al [24]; the author found that methylation of vitamin D receptor gene was significantly higher in patients with IMI compared to control group. Classically, gene expression is associated with hypomethylation, whereas hypermethylation results in gene silencing [25]. In the latter study, a negative correlation was found between methylation of vitamin D receptor gene and both sperm concentration and progressive motility in the overall group, suggesting that vitamin D deficiency and vitamin D receptor gene methylation may be involved in the biopathology of IMI.

Referring back to the doubts previously expressed, although throughout the last decade increasing data suggested that values such as circulating vitamin D

may be clinically relevant, the same recent evidence clearly explains that those parameters *per se* cannot explain the entire biopathology behind both idiopathic or unexplained infertility cases, thus strongly highlighting the importance of a more comprehensive genetic investigation in the management and counselling of infertile males [19]. Our findings suggest that roughly one out of four men presenting for primary couples' infertility depicted criteria suggestive for either idiopathic or unexplained cases; thereof, considering a possible unknown or misdiagnosed genetic cause of infertility, and the obvious rebound in terms of their reproductive health, having more robust information related to the genetic profile emerges of paramount importance to better characterize a causal diagnosis and to more realistically provide every man with an adequate management/therapeutic work-up. According to the EAU guidelines [1] it is recommended to offer standard karyotype analysis and genetic counselling to all men with azoospermia and oligozoospermia, and in those case of a family history suggestive for recurrent spontaneous abortions, malformations, or mental retardation. Similarly, specific recommendations are provided for Y-chromosome microdeletion and cystic fibrosis transmembrane conductance regulator abnormalities testing. However, EAU Guidelines thresholds might present a low sensitivity and specificity and will still theoretically miss one out five infertile men with genetic abnormalities [16]. For this specific reason, also according to our current observations, a more refined and extended genetic assessment should be considered in patients with either idiopathic or unexplained infertility since undiscovered alterations may subtend a possible explanation of this conditions.

The second observation outlines the relevance of TV. Indeed, TV has been previously associated with semen quality [26]. A recent study showed that infertile men had smaller TV compared to fertile controls and TV was positively correlated with TT, sperm concentration, and progressive sperm motility in infertile men [12]. Here we further corroborate those results. Indeed, we confirmed that fertile men had larger TV compared to infertile patients. Likewise, men with UMI had larger TV than those with IMI. However, TV was not found to be independently associated with IMI status, after accounting for other possible confounders. Speculatively, a reason for this finding could be that serum TT and FSH levels were similar among groups. In fact, TV is closely related both to the testicular exocrine and the endocrine function and previous data showed that TV is lower in hypogonadal men [27] and TV was strongly associated with TT values in infertile men [28].

Our study has a main clinical implication. We carried out the first retrospective case-control study with a comprehensive hormonal and clinical investigation performed on same-ethnicity, age-comparable cohorts of fertile, UMI or IMI infertile males. From the laboratory standpoint, the only difference was found in vitamin D levels. As such, it is worth noting the large and overlapping range of vitamin D levels in both UMI and IMI groups. Moreover, the findings that IMI had lower semen parameters and TV than UMI are not surprising. Taken together, these findings would probably suggest that the definition of UMI should be reconsidered. UMI indicates that the male is the primary driver, however it is likely that hidden female factors may also cause couple infertility. In a previous study, it has been demonstrated that isolated sperm abnormalities were more frequently found in fertile patients [8], these results corroborate the idea that it is not correct to manage infertile men only because of their sperm parameters. However, the finding that a significant proportion of patients with UMI had elevated SDF rates is of clinical interest. Hence, SDF evaluation in fertile controls would be needed as well.

Our study is not devoid of limitations. First, this was a single-center study, and all data was retrospectively collected, raising the possibility of selection biases. Second, we enrolled participants over a year-long period, spanning the four seasons. Unfortunately, we were unable to avoid the well-documented seasonal variations of vitamin D levels [29]; a problem we would consider in our subsequent long-term cohort. Third, the lack of a different and more complete genetic profile probably represents a significant limitation of this and of all the studies dedicated to infertile men for whom, not recognizing a cause, it is not possible to be effective in setting up a therapy. Fourth, TV was evaluated with an orchidometer, which has been recognized as a largely acceptable method of assessment and a valid alternative for US-measured TV in the everyday clinical practice [1]; however, the use of an orchidometer may under or overestimate the true TV.

CONCLUSIONS

Data from this relatively large, homogeneous, case-control study suggest that almost one fourth of men presenting for primary couple's infertility associated with pure male factors have clinical criteria suggestive for either idiopathic or unexplained infertility, therefore greatly limiting the actual therapeutic possibilities in the real-life scenario. Men with idiopathic infertility showed lower TV than those with unexplained infertility and fertile controls. Moreover, idiopathic infertile men displayed lower levels of serum vitamin D and a higher rate of vitamin D deficiency, when compared to those with unexplained infertility conditions. The real clinical importance of these differential findings is obviously questionable, but it is certainly true that this extensive analysis did not highlight other differences among groups. Thereof, it certainly cannot be said that unexplained and idiopathic cases should be differently addressed according to those variables. Thus, current observations support the urgent need for more comprehensive investigations which may help to reduce the dramatic gap that prevents rational therapeutic protocols in male infertility based on causal factors.

Conflict of Interest

The authors have nothing to disclose.

Funding

None.

Author Contribution

Conceptualization: AS, LB, CC. Data curation: CC, LB. Formal analysis: AS, LB. Investigation: All authors. Methodology: AS, LB. Supervision: AS, LB. Validation: All authors. Writing–original draft: CC, LB. Writing–review & editing: AS.

Supplementary Material

Supplementary material can be found *via* https://doi.org/10.5534/wjmh.220069.

Data Sharing Statement

The data analyzed for this study have been deposited in HARVARD Dataverse and are available at https://doi.org/10.7910/DVN/X5X5IT.

REFERENCES

1. Minhas S, Bettocchi C, Boeri L, Capogrosso P, Carvalho J, Cilesiz NC, et al.; EAU Working Group on Male Sexual and Reproductive Health. European Association of Urology guidelines on male sexual and reproductive health: 2021 update on male infertility. Eur Urol 2021;80:603-20.
2. Garolla A, Pizzol D, Carosso AR, Borini A, Ubaldi FM, Calogero AE, et al. Practical clinical and diagnostic pathway for the investigation of the infertile couple. Front Endocrinol (Lausanne) 2021;11:591837.
3. Agarwal A, Parekh N, Panner Selvam MK, Henkel R, Shah R, Homa ST, et al. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. World J Mens Health 2019;37:296-312.
4. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16:231-45.
5. World Health Organization (WHO). WHO laboratory manual for the examination and processing of human semen. 6th ed. Geneva: WHO; 2021.
6. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. J Urol 2021;205:36-43.
7. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. Lancet 2021;397:319-33.
8. Boeri L, Belladelli F, Capogrosso P, Cazzaniga W, Candela L, Pozzi E, et al. Normal sperm parameters per se do not reliably account for fertility: a case-control study in the real-life setting. Andrologia 2021;53:e13861.
9. Hamada A, Esteves SC, Agarwal A. Unexplained male infertility: potential causes and management. Hum Androl 2011;1:2-16.
10. Hamada A, Esteves SC, Nizza M, Agarwal A. Unexplained male infertility: diagnosis and management. Int Braz J Urol 2012;38:576-94.
11. Boeri L, Capogrosso P, Cazzaniga W, Ventimiglia E, Pozzi E, Belladelli F, et al. Infertile men have higher prostate-specific antigen values than fertile individuals of comparable age. Eur Urol 2021;79:234-40.
12. Boeri L, Capogrosso P, Ventimiglia E, Cazzaniga W, Pozzi E, Belladelli F, et al. Testicular volume in infertile versus fertile white-European men: a case-control investigation in the real-

life setting. Asian J Androl 2021;23:501-9.
13. Boeri L, Capogrosso P, Ortensi I, Miacola C, Cai T, Verze P, et al. Diagnostic and therapeutic workup of male infertility: results from a Delphi consensus panel. Int J Impot Res 2021. doi: 10.1038/s41443-021-00511-x [Epub].
14. Pagliardini L, Vigano' P, Molgora M, Persico P, Salonia A, Vailati SH, et al. High prevalence of vitamin D deficiency in infertile women referring for assisted reproduction. Nutrients 2015;7:9972-84.
15. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30. Erratum in: J Clin Endocrinol Metab 2011;96:3908.
16. Ventimiglia E, Capogrosso P, Boeri L, Pederzoli F, Cazzaniga W, Scano R, et al. When to perform karyotype analysis in infertile men? Validation of the European Association of Urology guidelines with the proposal of a new predictive model. Eur Urol 2016;70:920-3.
17. Hamilton JA, Cissen M, Brandes M, Smeenk JM, de Bruin JP, Kremer JA, et al. Total motile sperm count: a better indicator for the severity of male factor infertility than the WHO sperm classification system. Hum Reprod 2015;30:1110-21.
18. Ventimiglia E, Ippolito S, Capogrosso P, Pederzoli F, Cazzaniga W, Boeri L, et al. Primary, secondary and compensated hypogonadism: a novel risk stratification for infertile men. Andrology 2017;5:505-10.
19. Nagirnaja L, Mørup N, Nielsen JE, Stakaitis R, Golubickaite I, Oud MS, et al. Variant PNLDC1, defective piRNA processing, and azoospermia. N Engl J Med 2021;385:707-19.
20. Ciccone IM, Costa EM, Pariz JR, Teixeira TA, Drevet JR, Gharagozloo P, et al. Serum vitamin D content is associated with semen parameters and serum testosterone levels in men. Asian J Androl 2021;23:52-8.
21. Alzoubi A, Mahdi H, Al Bashir S, Halalsheh O, Al Ebbini M, Alzarir M, et al. Normalization of serum vitamin D improves semen motility parameters in patients with idiopathic male infertility. Acta Endocrinol (Buchar) 2017;13:180-7.
22. Albaldawy MT, Alsalami AS. Study of association among vitamin D, testosterone and semen quality in fertile and Iraqi infertile men. J Pharm Sci Res 2017;9:1067-71.
23. Banks N, Sun F, Krawetz SA, Coward RM, Masson P, Smith JF, et al. Male vitamin D status and male factor infertility. Fertil Steril 2021;116:973-9.
24. Hussein TM, Eldabah N, Zayed HA, Genedy RM. Assessment of serum vitamin D level and seminal vitamin D receptor gene methylation in a sample of Egyptian men with idiopathic infertility. Andrologia 2021;53:e14172.
25. Saccone D, Asani F, Bornman L. Regulation of the vitamin D receptor gene by environment, genetics and epigenetics. Gene 2015;561:171-80.
26. Condorelli R, Calogero AE, La Vignera S. Relationship between testicular volume and conventional or nonconventional sperm parameters. Int J Endocrinol 2013;2013:145792.
27. Salonia A, Rastrelli G, Hackett G, Seminara SB, Huhtaniemi IT, Rey RA, et al. Paediatric and adult-onset male hypogonadism. Nat Rev Dis Primers 2019;5:38.
28. Ruiz-Olvera SF, Rajmil O, Sanchez-Curbelo JR, Vinay J, Rodriguez-Espinosa J, Ruiz-Castañé E. Association of serum testosterone levels and testicular volume in adult patients. Andrologia 2018;50:e12933.
29. Lombardi G, Vitale JA, Logoluso S, Logoluso G, Cocco N, Cocco G, et al. Circannual rhythm of plasmatic vitamin D levels and the association with markers of psychophysical stress in a cohort of Italian professional soccer players. Chronobiol Int 2017;34:471-9.