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Sperm *Mesoderm Specific Transcript* Gene Methylation Status in Infertile Patients: A Systematic Review and Meta-Analysis

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Purpose: The *mesoderm specific transcription* (*MEST*) gene is a paternally expressed imprinted gene that appears to play a role in embryo survival. The latest meta-analysis on *MEST* methylation pattern in spermatozoa of infertile patients found higher methylation in spermatozoa from infertile patients than fertile controls. To provide an updated and comprehensive systematic review and meta-analysis on the *MEST* gene methylation pattern in patients with abnormal sperm parameters compared to men with normal parameters.

Materials and Methods: This meta-analysis was registered in PROSPERO (CRD42023397056) and performed following the MOOSE guidelines for Meta-analyses and Systematic Reviews of Observational Studies and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P). Only original articles evaluating *MEST* gene methylation in spermatozoa from patients with infertility or abnormalities in one or more sperm parameters compared to fertile or normozoospermic men were included.

Results: Of 354 abstracts evaluated for eligibility, only 6 studies were included in the quantitative synthesis, involving a total of 301 patients and 163 controls. Our analysis showed significantly higher levels of *MEST* gene methylation in patients compared with controls (standard mean difference [SMD] 2.150, 95% confidence interval [CI] 0.377, 3.922; p=0.017), although there was significant heterogeneity between studies (Q-value=239.90, p<0.001; I^2=97.91%). No significant evidence of publication bias was found, although one study was sensitive enough to skew the results, leading to a loss of significance (SMD 1.543, 95% CI –0.300, 3.387; p=0.101). In meta-regression analysis, we found that the results were independent of both ages (p=0.6519) and sperm concentration (p=0.2360).

Conclusions: Sperm DNA methylation may be associated with epigenetic risk in assisted reproductive techniques (ART). The *MEST* gene could be included in the genetic panel of prospective studies aimed at identifying the most representative and cost-effective genes to be analyzed in couples undergoing ART.

Keywords: Epigenomics; Infertility; Oligospermia; Methylation

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INTRODUCTION

Infertility is the inability to conceive after 1–2 years of regular unprotected sexual intercourse. It affects up to ~15% of couples and therefore this high prevalence makes it a problem for industrialized countries [1].

Cumulatively, male infertility occurs in about half of the infertile couples and it is the only cause of couple infertility in 30% of cases [1]. It is usually diagnosed in the presence of abnormal sperm parameters (oligozoospermia, teratozoospermia, asthenozoospermia, or a combination of them, and azoospermia) [2]. However, normal sperm parameters do not always ensure fertilization or fertility [3]. Several factors could be responsible for male infertility, such as primary (due to testicular dysfunction) or central (due to hypothalamic-pituitary disorders) hypogonadism, or seminal duct obstruction [4].

It is concerning that, despite a careful diagnostic workup, the etiology of male infertility remains undiagnosed in a significant number of cases which therefore constitutes idiopathic infertility. In such cases, more complex and incompletely understood factors may come into play, including genetic and epigenetic causes. Notably, only 28% of infertile patients receive a specific causal diagnosis for their condition which, very often, is due to previous gonadotoxic chemo- or radiotherapy, chronic diseases such as diabetes mellitus [5], and testosterone abuse [2]. Meanwhile, the prevalence of idiopathic cases was reported to reach 72% despite careful diagnostic workup, in a single-center retrospective study of more than 25,000 infertile patients [2]. Similarly, in a single-center prospective study of a total of 1,737 patients, the primary cause of infertility was found in 695 patients (40.0%), while approximately 75% of oligozoospermia cases remained unexplained [6]. This evidence strongly justifies the need for further research.

In recent years, epigenetics has been suggested as an important aid in helping to understand the causes of apparently idiopathic male infertility [3]. Epigenetics includes all the molecular changes regulating gene expression without modification of the DNA sequence [7]. DNA methylation, histone modification, and chromatin remodeling are the most common epigenetic modifications occurring in spermatozoa [3,8,9]. Several genes are associated with abnormal sperm parameters or male infertility when undergoing to epigenetic changes, mainly hypermethylation [8]. These genes include *methylenetetrahydrofolate reductase (MTHFR)*, *paired box 8 (PAX8)*, *neurotrophin 3 (NTF3)*, *stratifin (SFN)*, *Harvey Rat sarcoma virus (HRAS)*, *JHM2DA*, *insulin-like growth factor 2 (IGF2)*, *H19*, *Ras protein specific guanine nucleotide releasing factor 1 (RASGRF1)*, *maternally expressed gene 3 (MEG3 or GTL2)*, *pleomorphic adenoma gene 1 (PLAG1)*, *DIRAS family, GTP-binding RAS-like protein 3 (DIRAS3)*, *potassium voltage-gated channel subfamily Q member 1 (KCNQ1)*, *long QT Intronic Transcript 1 (LIT1)*, *small nuclear ribonucleoprotein polypeptide N (SNRPN)*, and *mesoderm specific transcript (MEST)* [8]. The latter is the objective of this meta-analysis.

The *MEST* gene also known as *paternally expressed gene 1* (*PEG1*), is an imprinted gene predominantly expressed from the paternal allele, mapping on chromosome 6 in mice [10], and in chromosome 7q32 in humans [11]. The maternal allele is methylated and therefore not expressed in the embryo, while the paternal allele is unmethylated and fully expressed [10]. *MEST* encodes for a protein similar to the α/ß-hydrolase fold family, suggesting an enzymatic activity [10]. It seems to be involved in obesity because of a positive association between the variation of MEST mRNA and the rate of fat mass deposition [12]. *MEST* is also expressed in neuronal tissue and could be involved in maternal behavior, although this aspect is still controversial [10]. This gene, as well as other imprinted genes, appears to play a role in embryo survival. Indeed, parthenogenesis (a type of reproduction characterized by the development of unfertilized oocytes, therefore containing only the maternal genome [13]) is lethal for the mouse embryo, in part because of the lack of paternally-imprinted genes which contribute to the embryo growth and development [14], such as *MEST* gene. Albeit this gene has been proposed to be involved in different functions, its biochemical role is not yet fully understood.

During preimplantation, genome-wide demethylation and *de novo* methylation occur in the embryo. However, the methylation pattern of imprinted genes is not altered during this process to ensure their parent-specific expression. Thus, *MEST* gene imprinting, even if abnormal, is transmitted to offspring. In recent years, attention has been paid to assisted reproductive techniques (ART) safety, as some studies have shown a greater risk of adverse outcomes in offspring conceived with ART than in spontaneously-conceived offspring.

Epigenetic dysregulation has been proposed as responsible for this. Regarding *MEST* methylation, two studies demonstrated that this gene was hypomethylated in ART offspring compared to spontaneously-conceived offspring [15]. Whether the epigenetic dysregulation is due to ART manipulation or epigenetic dysregulation already present in the gametes is still not clear [15]. Indeed, as mentioned before, abnormal methylation patterns have been reported in spermatozoa of infertile patients. These patients could be referred to ART, thereby transmitting their abnormal epigenetic pattern to their offspring, resulting in poor ART outcomes or adverse effects in the offspring.

Few data are available on sperm *MEST* gene methylation and pregnancy outcome. An abnormal pattern of methylation appears to occur in the spermatozoa of the male partners of women with recurrent pregnancy loss [16]. More data are available on the methylation pattern in spermatozoa of patients with abnormal conventional parameters than in normozoospermic men. In detail, the latest meta-analysis on this issue has been published in 2017 and showed that sperm *MEST* gene methylation is significantly higher in patients with abnormal sperm parameters than in normal ones [17]. However, several weaknesses are present in this meta-analysis [17], such as discrepancies between the data analyzed in the plots and those published in the original articles, absence of data although reported in the original articles, or statistical mistakes like the use of the median as the mean, to name a few (Supplement Table 1).

Emerging evidence suggests a role of age in sperm quality [18] as older men have poorer sperm parameters and a higher DNA fragmentation rate [19]. Furthermore, the latest meta-analysis on sperm DNA methylation in male infertility mainly included patients with reduced sperm counts, thus increasing the possibility that the abnormality may be associated with the sperm count [17]. A comprehensive meta-regression analysis has not yet been performed to understand whether *MEST* gene methylation status changes with advancing age or sperm concentration.

To sum up, considering 1) the role of the *MEST* gene in embryo survival [14], 2) that the pattern of sperm methylation of this gene is inherited by the offspring, and 3) that a hypermethylation at the sperm level would reduce the gene expression in the embryo, thus compromising its growth and development, we choose the methylation of this gene as target of the present meta-analysis, whose aims are: 1) to provide an update on the difference in its methylation status in patients with abnormal or normal conventional sperm parameters, and 2) to evaluate whether age and sperm concentration can influence the methylation rate of this gene at the sperm level.

MATERIALS AND METHODS

1. Search strategy

This meta-analysis was registered on PROSPERO (CRD42023397056) and performed following the MOOSE guidelines for Meta-analyses and Systematic reviews of Observational Studies [20] and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) [21]. The MOOSE and PRISMA checklists were reported in Supplement Table 2 and 3.

The Scopus, Pubmed, Cochrane, and Embase databases were searched from their inception through January 2022. The search strategy used the following combination of Medical Subjects Heading (MeSH) terms and keywords: "MEST," "gene methylation," "fertilization rate," "sperm DNA fragmentation," "assisted reproductive technique," "pregnancy rate," "abortion," and "miscarriage". Additional manual searches were conducted using the relevant studies reference lists. The search was restricted to original articles in humans only, and no language restrictions were applied in any literature searches. After duplicate removal, identified abstracts were screened for eligibility.

2. Selection criteria

Studies were selected based on the Population, Exposure, Comparison/Comparator, Outcome, and Study type (PECOS) model system (Table 1) [22]. Briefly, only articles aimed at evaluating *MEST* gene methylation in spermatozoa of patients with infertility or abnormalities in one or more sperm parameters, and in fertile controls or men with normozoospermia were included. The search was limited to human studies and only English articles were selected. Original articles were considered for inclusion, while reviews, meta-analyses, case reports, commentaries, editorials, and animal or *in vitro* studies were excluded. The selection of eligible studies was carried out by two researchers (F.B. and A.C.) who worked independently and not blindly.

Table 1. Inclusion and exclusion criteria according to the PECOS model [22]

	Inclusion criteria	Exclusion criteria
Population	Male patients	Adolescents, female, and patients with azoospermia
Exposure	Abnormal sperm parameters (oligo and/or astheno and/or teratozoospermia) or infertility	
Comparison	Normal sperm parameters (normozoospermia) or fertility	
Outcomes	*MEST* gene methylation	
Study type	Observational studies, randomized controlled studies, case-control studies	Animal studies, *in vitro* studies, review & meta-analyses, case reports, book chapters, editorials

MEST: mesoderm specific transcript, PECOS: Population, Exposure, Comparison/Comparator, Outcomes, Study type.

This resulted in two reviews for each article. The researchers first screened titles and abstracts for inclusion. In case of uncertainty, each researcher reviewed the full text. Any disagreement between the two reviewers was followed by a discussion between the two; if consensus was not reached, a third researcher (R.C.) was called to evaluate the article and make the final decision. Afterward, the full texts of the selected articles were downloaded and underwent data extraction.

3. Data extraction

The following data were collected: age, sperm concentration (mean and standard deviation [SD]), levels of methylation of *MEST* (mean and SD), number of patients, number of controls, characteristics of patients and controls (oligo-, astheno-, and/or terato-zoospermia and fertility status). For studies reporting data as the median and interquartile range, the formula by Wan and colleagues was used to estimate the mean and SD [23].

In case of missing information in the original articles, we used the data included in the meta-analysis by Santi et al [17] for our first preliminary analysis. We then contacted all authors to confirm the correctness of the data. If there was no reply, a reminder was sent after 10 days and we waited up to two weeks after the second reminder before we considered the data to be missing. Only information sent by email by the authors was included in the final analysis.

4. Quality assessment

The quality of evidence (QoE) of each study was assessed by two other researchers (C.L. and R.C.), using the Cambridge Quality Checklists [24]. This checklist consists of three domains designed to assess the quality of studies correlates, risk factors, and random risk factors. The correlate checklist evaluates the appropriateness of sampling methods and sample size, as well as the quality of outcome and measurement of correlates. It consists of five items, each of which can be assigned a score of 0 or 1, for a total score of 5. The risk factors checklist can be rated 1, 2, or 3, respectively, if the data are cross-sectional, retrospective, or prospective, predicting higher scores for those studies with appropriate time-ordered data. The third checklist is for casual risk factors. It evaluates the type of study design by assigning a score from 1 (cross-sectional study without a control group) to 7 (randomized clinical trials study).

The higher the Cambridge Quality Checklist total score (the sum of the three subscores) is the higher the quality of the study.

5. Statistical analysis

Comprehensive Meta-Analysis Software (Version 3; Biostat Inc.) was used to perform a meta-analysis of quantitative data. Standard mean difference (SMD) was used as the effect size for statistical comparison between patients and controls, and the data were considered statistically significant for $p \leq 0.05$. Cochran's Q test and heterogeneity index (I^2) were used to assess inter-study heterogeneity and $p<0.10$ was considered statistically significant. The I^2 value is between 0 and 100%. Values <25% indicate low heterogeneity, 50% moderate heterogeneity, and 75% high heterogeneity. Pooled effect size was calculated using fixed or random effect models, depending on the level of heterogeneity. The fixed model was used in case of low heterogeneity and the random one for significant heterogeneity. The pooled effect size and corresponding confidence interval (CI) were also calculated after the exclusion of one study at a time (sensitivity analysis). A study resulting in inference change after its exclusion was labeled a "sensitive study". Publication bias was qualitatively analyzed by funnel plot skewness, which suggested some

Table 2. Characteristics of the included studies

Study	Study design	Population (n)		Characteristics		Age (y)		Sperm concentration (million/mL)		*MEST* methylation (%)		Method of evaluation of the outcome
		Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	
El Hajj et al (2011) [25]	Retrospective case-control study	106	28	Infertile	Presumably fertile (female infertility)	38.10±5.60	38.30±5.60	11.40±5.90	56.20±24.10	6.3±2.4	6.2±1.6	Bisulfite Pyrosequencing
Laurentino et al (2015) [26]	Retrospective case-control study	7	5	OAT	N	35.33±1.70	33.68±1.58	2.35±0.73	65.53±10.49	22.66±4.43	0.76±0.12	Bisulfite Pyrosequencing
Richardson et al (2014) [27]	Retrospective case-control study	95	45	Abnormal semen parameters	N	35.3±5.3[a]	36.7±4.6[a]	20.4±18.4[a]	61.8±46.5[a]	16±1.2	10±0.93	Pyrosequencing and Bisulfite sequencing
Xu et al (2016) [28]	Retrospective case-control study	46	49	A	N	31.95±2.21	32.16±3.26	43.93±3.37	63.31±3.27	14.91±1.36	15.8±1.06	Bisulfite conversion and MassARRAY quantitative methylation analysis
Hammoud et al (2010) [31]	Retrospective case-control study	10	5	O	Fertile	-	-	-	-	8.33±3.29[b]	0.38±1.10[b]	Bisulfite Pyrosequencing
Kläver et al (2013) [34]	Prospective case-control study	37	31	Idiopathic infertility	N	-	34.5±7.2[c]	-	45.3±35.9	11.39±9.88[c]	7.9±5.1	Bisulfite Pyrosequencing

Values are presented as mean±standard deviation.

A: asthenozoospermia; N: normozoospermia; *MEST*, *mesoderm specific transcript* gene; OAT: oligoasthenoteratozoospermia; O: oligozoospermia.

[a]Data expressed as median (interquartile range) in the original article and converted in mean±standard deviation using the calculation provided by Wan et al [23]. [b]Data calculated as the mean of methylation of each CpG islet (Table 3, from Hammoud et al. [31]; [c]Data calculated as the mean of values indicated in Supplement Table 2 (Kläver et al [34]).

missing studies on one side of the graph. For quantitative analysis of publication bias, we used Egger's intercept test, which assessed the statistical significance of publication bias. In case of publication bias, unbiased estimates were calculated using the "trim and fill" method. Finally, using a meta-regression approach, we evaluated the correlation between *MEST* gene methylation and age (model 1) or sperm concentration (model 2) overall, in patients and controls.

RESULTS

Using the above search strategy, 354 abstracts were extracted. After removing duplicates (n=221), 133 articles were selected based on title and abstract. A total of 74 abstracts were excluded, while 59 full-text articles were evaluated for eligibility. Of these, 24 were not pertinent to our study, 20 were animal studies, and 5 were review articles. Of the 10 studies initially included in this meta-analysis, 9 were retrospective case-control studies [25-33], and 1 was a prospective case-control study [34] (Table 2). Although a total of 10 studies met our inclusion criteria, data on *MEST* methylation were available, after contacting all authors, for only six studies [25-28,31,34], for a total of 301 patients and 163 controls (Fig. 1).

1. Results of the QoE

All included studies were assessed using the Cambridge Quality Checklist. Although this scale does not establish a precise threshold for differentiating between high and low-quality studies, out of a total score of 15, 1 study scored 11 [34], 2 studies scored 10 [25,28], 6 studies scored 9 [27,31], and 1 study scored 8 [26] (Table 3).

2. Differences in patients versus controls

Due to the presence of significant inter-study heterogeneity, as demonstrated by the Q-test (Q-value=239.90; $p<0.001$) and $I^2=98\%$, the random effect model was used. Overall, patients with infertility/abnormal sperm parameters showed significantly higher levels of *MEST* gene methylation than the control population (SMD 2.02, 95% CI: 0.27, 3.76; $p<0.01$) (Fig. 2). In the subanalysis, based on patient phenotype (oligozoospermia, asthenozoospermia, infertile), *MEST* methylation levels were significantly higher in patients with oligozoospermia than in controls (SMD 1.31, 95% CI: 0.18, 2.45;

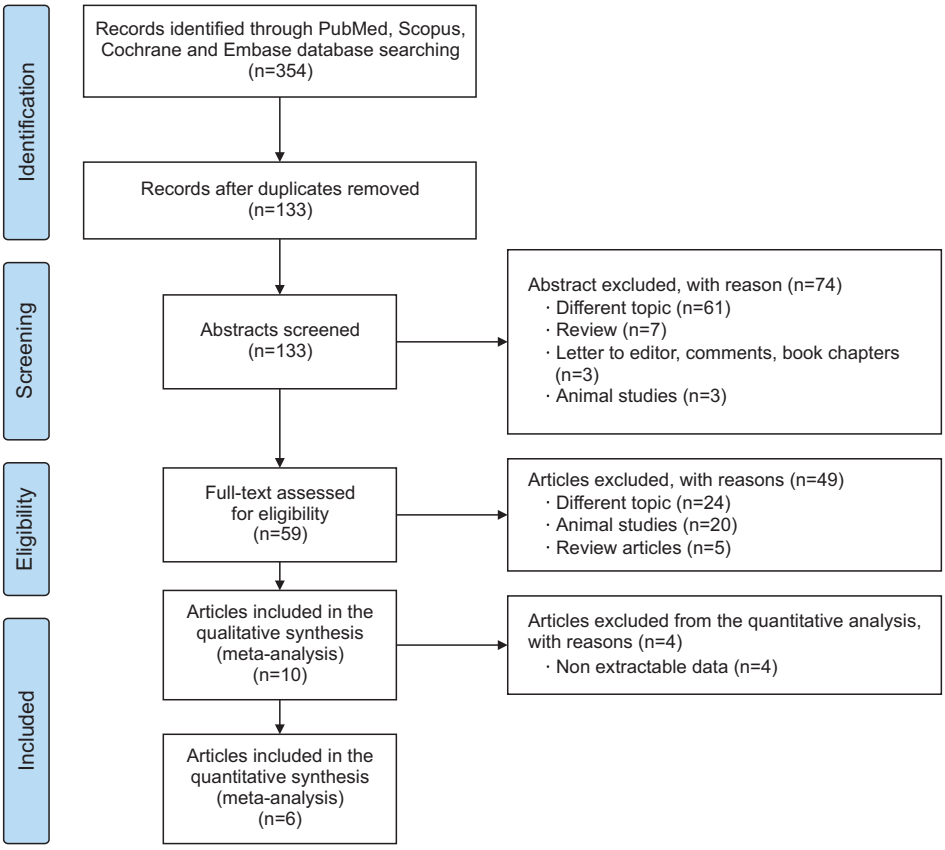

Fig. 1. PRISMA flow-chart of the included studies.

Table 3. Quality of evidence assessment of the included studies (results of the Cambridge Quality Checklist [24])

Study name	Type of study	Cambridge quality checklists		
		Checklist for correlate	Checklist for risk factor	Checklist for causal risk factor
El Hajj et al (2011) [25]	Retrospective case-control study	3	2	5
Laurentino et al (2015) [26]	Retrospective case-control study	1	2	5
Richardson et al (2014) [27]	Retrospective case-control study	2	2	5
Xu et al (2016) [28]	Retrospective case-control study	3	2	5
Hammoud et al (2010) [31]	Retrospective case-control study	2	2	5
Kläver et al (2013) [34]	Prospective case-control study	2	3	6

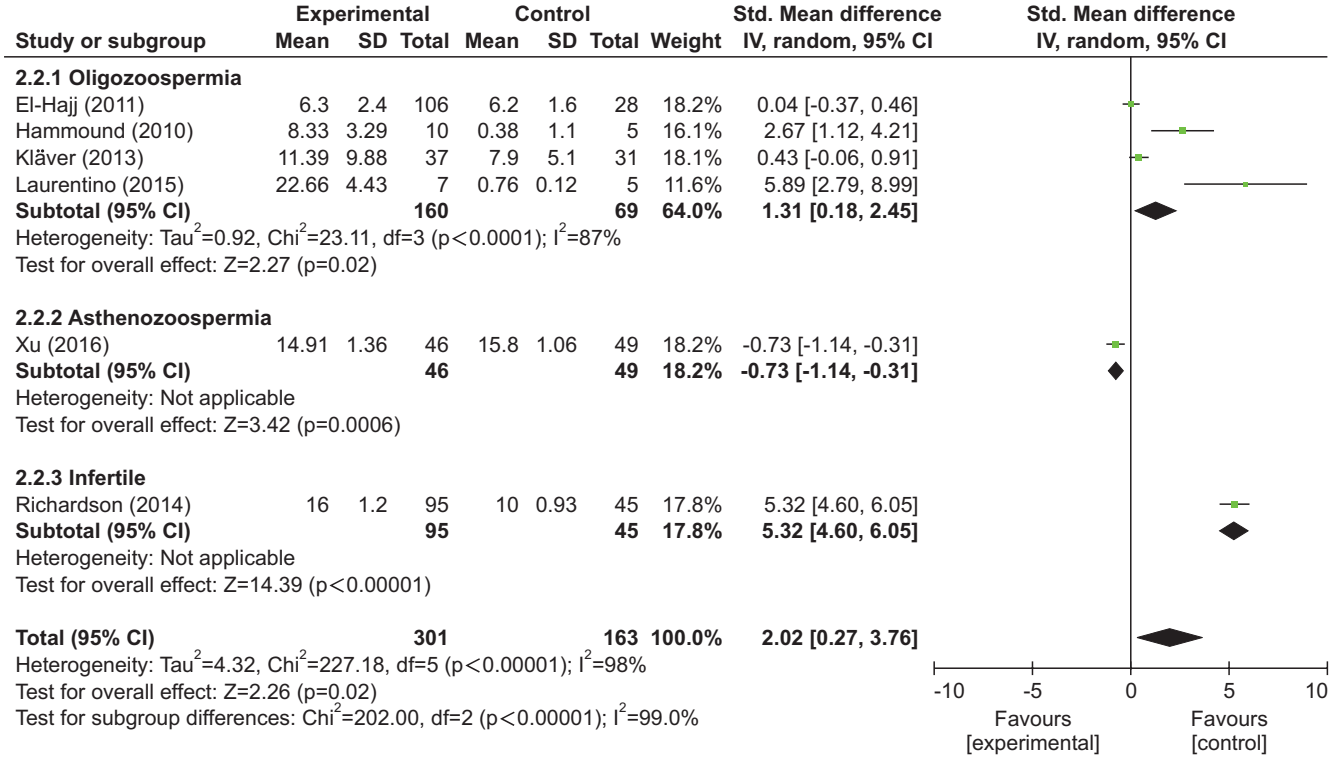

Fig. 2. *MEST* gene methylation in spermatozoa of patients with infertility/abnormal sperm parameters *versus* controls. MEST: mesoderm-specific transcription, SD: standard deviation, CI: confidence interval.

p<0.01).

Egger's test showed no significant evidence of publication bias (intercept 8.60152, 95% CI: -6.07112, 23.27415, p=0.08947), as confirmed by the symmetry of the funnel plots (Fig. 3A). One study was sensitive enough to alter the results [27]. Its removal led to the loss of significance of the analysis (SMD 1.543, 95% CI: –0.300, 3.387, p=0.101) (Fig. 3B).

3. Meta-regression analysis

To investigate whether the differences we found between patients and controls could be influenced by age or sperm concentration, we built a meta-regression model, in which the SMD of *MEST* gene methylation rate in spermatozoa was correlated with the difference in the mean age (Fig. 4A) and with the mean sperm concentration (Fig. 4B) between patients and controls. We found that the difference in methylation was independent of both the age (p=0.6519) and the sperm concentration (p=0.2360).

DISCUSSION

In line with previous evidence, our analysis has shown that patients with infertility and/or abnormal sperm parameters have higher levels of *MEST* gene

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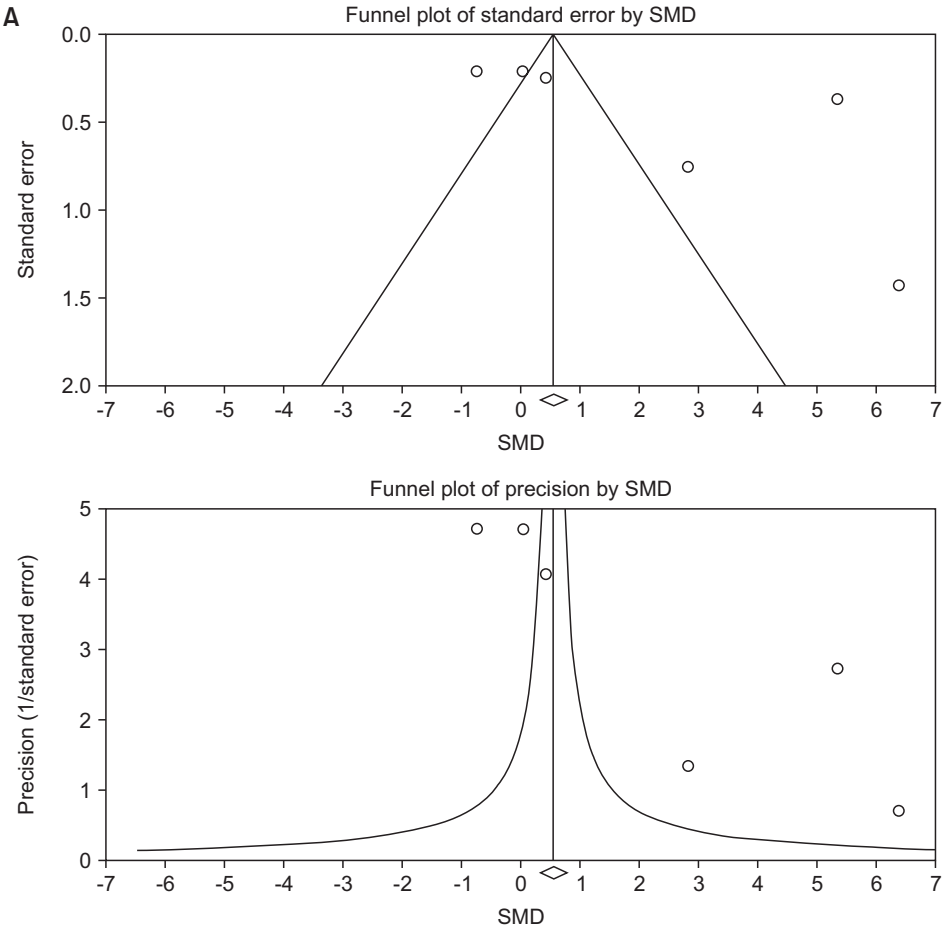
A



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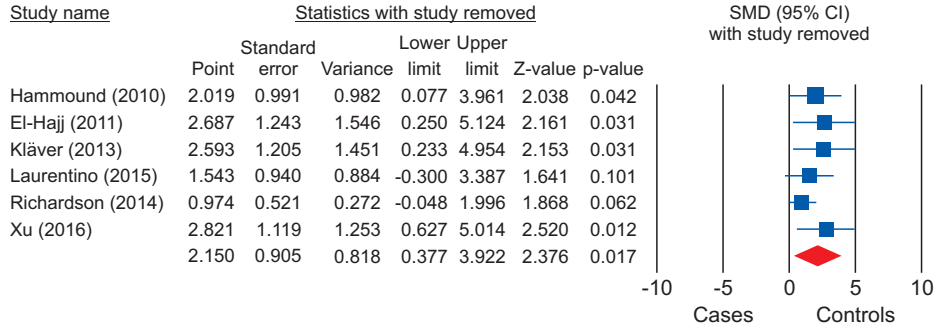

Fig. 3. Funnel plot (A) and sensitivity analysis (B) of *MEST* gene methylation in spermatozoa from patients with infertility/abnormal sperm parameters and controls. SMD: standard mean difference, MEST: mesoderm-specific transcription.

methylation than the control population. Methods of assessing *MEST* methylation are similar among the included studies (bisulfide pyrosequencing in four out of six studies), thus making the finding of the present meta-analysis unlikely biased by this methodological aspect. Furthermore, by performing a meta-regression analysis, we found that this difference in methylation degree was unaffected by neither age nor sperm concentration, confirming a direct and independent role of the *MEST* gene methylation pattern in male infertility. To the best of our knowledge, no comprehensive meta-regression analysis has been performed to date to evaluate whether *MEST* gene methylation status changes with advancing age. This is noteworthy, as age is known to correlate with poorer sperm parameters and other elements that influence male fertility, such as DNA sperm fragmentation [19].

Methylation is one of the epigenetic changes that regulate the differential gene expression. It consists of a dynamic mechanism of gene expression regulation,

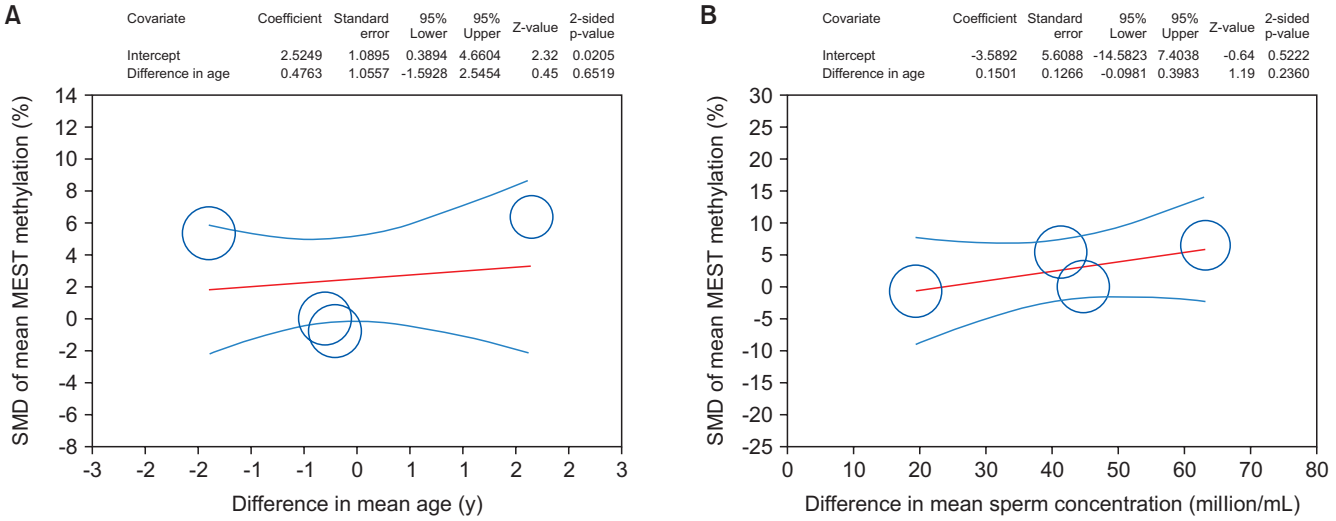

Fig. 4. Meta-regression analysis between the SMD of the mean *MEST* gene methylation and the difference in mean age (A) and sperm concentration (B). SMD: standard mean difference, MEST: mesoderm-specific transcription.

catalyzed by DNA methyltransferases, and involves cytosine-guanine dinucleotides, often located near the gene promoter region or others involved in the regulation of gene expression [35]. Hypermethilation leads to gene silencing, while hypomethylation to gene hypoexpression [36].

DNA methylation includes global DNA methylation, methylation of imprinted and non-imprinted genes, and methylation of repetitive elements [3]. It plays an important role in mammalian spermatogenesis and studies suggest that abnormal methylation in germ cells may lead to impaired spermatogenetic [35]. In particular, hypo- or hypermethylation can impair the cellular differentiation of spermatogonia into spermatozoa, causing infertility or subfertility [35]. Indeed, according to a meta-analysis, infertile patients showed a higher level of aberrant DNA methylation of *H19*, *SNRPN*, and *MEST* genes [17].

Abnormal methylation could impair embryo development and growth or invalidate the outcome of intracytoplasmatic sperm injection [35]. For example, imprinted genes encode proteins and transcription factors involved in embryo development, so aberrant methylation of imprinted genes in germ cells can lead to aberrant methylation in the embryo, pregnancy loss, and even imprinting disorders of the offspring [37]. Indeed, the methylation pattern of imprinted genes is established during human spermatogenesis [38] and it is not altered during preimplantation. Therefore, the *MEST* gene imprinting, even if abnormal, is transmitted to the offspring. Examples of imprinted genes whose aberrant methylation is associated with a high rate of pregnancy loss or sperm DNA fragmentation (SDF) are the *H19*/*IGF2* genes [37].

Another interesting aspect is the role of sperm epigenetic dysregulation in the controversial association between ART and adverse outcome of offspring conceived with these techniques [15]. The literature suggests that ART may affect epigenetics, including DNA methylation, in the fetus and the placenta. This is often due to the manipulation and processes used in these techniques [39]. However, based on our results, the possibility that the association between ART and aberrant DNA methylation at imprinted loci may be due to the already altered methylation pattern of paternal gametes should be considered. *MEST* methylation status could be preliminarily assessed in patients seeking ART to predict the outcomes in terms of birth rate and offspring health. Indeed, several imprinting disorders, such as Prader-Willi, Angelmann, Beckwith-Wiedemann, and Silver-Russell syndromes appears to be related to ART use [40]. In particular, literature evidence suggests that *MEST* is hypomethylated in ART offspring compared to spontaneously-conceived offspring [15], while the epigenetic alteration in infertile male gametes consists of *MEST* hypermestilation. These conflinctin results should be better investigated.

To our knowledge, this represents the second meta-analysis performed so far to investigate *MEST* methylation levels in spermatozoa of infertile patients. Our

study has some limitations. First, by focusing on cross-sectional studies, a direct cause-and-effect relationship between *MEST* methylation levels and infertility cannot be established. Second, the number of studies is low, as well as the studies' sample size, and the data are heterogeneous. On the other hand, the robust methodology used represents one of the strengths of this study. Consequently, the use of several databases during the search strategy phase allowed us to collect the majority of studies on this topic, while minimizing the possibility of missing likely eligible studies. Furthermore, the data extraction and collection phase was accurate. Each author was contacted individually when data were not extractable in the included original articles. Finally, both qualitative (Funnel plot) and quantitative (Egger's test) analysis of publication bias were performed, as well as sensitivity analysis, making the statistical methodology robust. Even though the results of the previous meta-analysis are similar to ours (as both studies suggest higher methylation levels in infertile patients), there are some shortcomings and discrepancies in data collection in the latter study (Supplement Table 1) [25-32,34,41]. Therefore, this represents the most accurate systematic review and meta-analysis performed to date focusing specifically on *MEST* gene methylation levels in spermatozoa of infertile patients.

CONCLUSIONS

Methylation levels of the *MEST* gene are higher in infertile patients and/or with abnormal sperm parameters than in controls and this difference is not affected by age or sperm concentration. This suggests that the *MEST* methylation pattern in germ cells is not influenced by the aging process, unlike other parameters such as SDF.

Despite some limitations, mainly deriving from the paucity and heterogeneity of data publiched in the literature, the results of the present study may open new avenues for the diagnosis and treatment of male infertility and also for ART. Indeed, on the basis of our findings, the *MEST* gene could be included in the genetic panel of prospective studies aimed at identifying the most representative and cost-effective genes to analyze in couples undergoing ART cycles. In fact, aberrant methylation of *MEST* could be transferred to the embryo, compromising its development. The possibility that *MEST* methylation alterations present in spermatozoa could be transmitted to the offspring should be considered, since some evidence in the literature suggests a possible association between ART and some imprinting disorders such as Prader-Willi, Angelmann, Beckwith-Wiedemann, Silver-Russell syndromes [40]. However, further studies are needed to better understand whether *MEST* methylation status is altered in children conceived by fathers with gametes carrying aberrant methylation of this gene, as well as the consequence of this altered methylation status on ART outcome and/or offspring health.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: RC, AEC. Data curation: FB. Formal analysis: RC, CL, AC. Writing – original draft: CL. Writing – review & editing: RC, AC, AEC. Supervision: SLV, RAC. Project administration: RC, AEC. All aurthors approved the final version of the article.

Supplementary Materials

Supplementary materials can be found via https://doi.org/doi.org/10.5534/wjmh.230094.

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