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Impact of DNA methylation of the human mesoderm-specific transcript (*MEST*) on male infertility

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ABSTRACT

Male infertility accounts for nearly 40%–50% of all infertile cases. One of the most prevalent disorders detected in infertile men is errors in the MEST differentially methylated region (DMR), which has been correlated with poor sperm indexes. The aim of our study was to characterize the methylation pattern of the MEST gene, along with assessing seminal factors and chromatin condensation in sperm samples from both infertile patients and fertile cases, all of whom were candidates for intracytoplasmic sperm injection. We collected forty-five semen specimens from men undergoing routine spermiogram analysis at the Infertility Treatment Center. The specimens consisted of 15 samples of normospermia as the control group, 15 individuals of asthenospermia, and 15 individuals of oligoasthenoteratospermia as the cases group. Standard semen analysis and the chromatin quality and sperm maturity tests using aniline blue staining were performed. The DNA from spermatozoa was extracted and treated with a sodium bisulfite–based procedure. The methylation measure was done quantitatively at the DMRs of the *MEST* gene by quantitative methylation-specific polymerase chain reaction (qMSP). The mean percentages of total motility, progression, and morphology in normospermia were significantly higher than oligoasthenoteratospermia and asthenospermia, and they were substantially higher in asthenospermia compared to oligoasthenoteratospermia ($P \leq 0.05$). The mean percentages of histone transition abnormality and *MEST* methylation in oligoasthenoteratospermia were significantly higher than asthenospermia and normospermia ($P \leq 0.05$). A negative correlation existed between the histone transition abnormality and *MEST* methylation with sperm parameters. In conclusion, chromatin integrity, sperm maturity, and *MEST* methylation may be considered important predictors for addressing male factor infertility. Therefore, we suggest that male infertility may be linked to methylation of the imprinted genes.

1. Introduction

Sexual disorders in men cover a wide range of issues, varying from Erectile Dysfunction (ED) to infertility, indicating the diverse and complex nature of male-related problems in this domain in various nations [1–3]. Infertility is a serious health problem globally

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affecting 15 % of reproductive-aged couples [4,5]. It is worth noting that male infertility is accounted for approximately 40%–50 % of infertile persons [6]. The heritable and acquired disturbances of the hypothalamic-pituitary-testicular endocrine, anatomical disorders, chromosomal abnormalities, and mutations are well-introduced as potential causes of male infertility. Nevertheless, the etiology remains unclear for most infertile men [7]. Assisted reproductive technology (ART) has significantly developed new procedures and therapies for male infertility. Since the first child was born via in vitro fertilization, ART has contributed to more than 5 million birth and continued to progress quickly [8]. However, ART and infertility may be related to epigenetic disorders [9]. Epigenetics is assigned to mitosis or meiotic heredity variations in genes that cannot be illustrated by alterations in the sequence of DNA, comprising a wide range of modifications such as histone transition abnormality, DNA methylation, and noncoding RNAs. Any disturbances of epigenetic adjustment caused by the ART approach or abnormal spermatogenesis disrupt sperm function and transfer to future generations and lead to epigenetic diseases [10].

Cancer epigenetics play a crucial role in the continuous quest for discovering biomarkers in different types of cancer [11,12]. In germ cell epigenetics, recent progression also has facilitated the detection of male factor infertility predictive markers. Differential cytosine methylation in CpG islands, especially by imprinted genes e.g., *MEST* gene influences gene regulation [13].

The congenital imprints are eliminated in the fetus and created again based on embryo sexuality in gametogenesis. Uncorrected imprinting leads to aberrant fetal growth and virulent diseases [14]. In addition, imprinting disorders have been found in classical genetic disturbances for instance Silver–Russell. The prevalence of classical genetic diseases is elevated in those who received ART [15]. Furthermore, prior documents have reported aberrant methylation within imprinting and non-imprinting genes related to various sorts of infertility in men [16,17].

By next-generation bisulfite sequencing, another study demonstrated that differentially methylated regions (DMRs) of imprinted genes, including *SNRPN, MEG8, GNAS*, and *H19* revealed distinct methylation patterns in the abnormal semen groups [18].

The PEG1 gene, paternally expressed gene 1, known as the human mesoderm-specific transcript (MEST) gene, is an imprinted gene placed on chromosome 7q32.2 [19]. An enzyme of the α/β-hydrolase fold family is encoded by the *MEST* gene. *MEST* comprises two basic isoforms, which show mono-allelic and bi-allelic expressions. The *MEST* gene promoter of the active paternal alleles is completely hypomethylated in normal status [20]. Although the MEST gene promoter of the silent maternal alleles is transcriptionally hypermethylated and it regulates the monoallelic expression of the MEST gene, the imprinted *MEST* gene is also a candidate gene for Silver-Russell syndrome [21].

Unregulated expression of the MEST gene has been related to breast cancer, colorectal cancer, uterine leiomyoma, and lung cancer cell lines in humans [22,23], and growth retardation and elevated mortality in mice [24].

According to documents, ART procedures affect *MEST* gene methylation [25]. Likewise, aberrant expression of the *MEST* gene is responsible for some forms of male infertility. In previous studies, oligozoospermic men and those with idiopathic infertility have reported hypermethylation at the MEST differentially methylated region (DMR) [26,27]. MEST DMR methylation errors were the prevalent disorders detected in infertile men compared to other imprinted genes [28]. Moreover, a previous study illustrated that *MEST* DMR methylation is correlated with poor sperm indexes [29].

On the other hand, sperm chromatin condensation is another crucial factor that can serve as an index of sperm quality. Consequently, it may have a negative impact on both natural fertility outcomes and ART results, such as pregnancy failure and decreased birth rate [30,31]. In spermatogenesis, DNA histones are predominantly substituted with protamines, leading to a tightly condensed sperm chromatin structure. An alternative indirect method involves using aniline blue staining to identify any surplus histones, thereby indirectly suggesting lower levels of protamines within the sperm nucleus. To put it simply, aniline blue staining detects the lingering histones by binding to the lysine-rich histones present. This technique helps in assessing the chromatin remodeling process during sperm development [32,33].

This study aimed to characterize the methylation status of the MEST gene, seminal parameters, and chromatin integrity in sperm samples from infertile patients and fertile cases who were candidates for intracytoplasmic sperm injection (ICSI). For the first time, the current study evaluated the MEST methylation in infertile groups, especially oligoasthenoteratospermia.

2. Materials and methods

2.1. Sample collection

This research as a case-control study was carried out on infertile cases who were candidates for intra cytoplasmic sperm injection (ICSI) in the Infertility Treatment and Andrology Institute located in Kermanshah-Iran. Participants with a history of previous varicocele surgery, smoking, alcohol consumption, and drug abuse were excluded from the study. Eventually, fifteen men with asthenospermia and fifteen men with oligoasthenoteratospermia were included in the analysis as the case groups. The normozoospermic group ($n = 15$) was considered the control group. All participants were required to sign a written consent form and were provided with a participant information statement that thoroughly explained the consent process. The Research Ethics Committee at the Deputy of Research of Islamic Azad University approved the study protocol (IR.IAU.SRB.REC.1401.184). Additionally, patients who either refused to participate or did not meet the necessary criteria based on sperm characteristics were excluded from the study. The sampling process lasted from August to November 2021.

2.2. Sperm parameters analysis

All samples were collected following the standard method and an abstinence protocol, with intervals ranging from 2 to 7 days [34].

Next, incubation at 37 °C with 5% CO2 was carried out for 20–60 min, depending on the sample, to complete liquefaction [35]. Sperm parameters, including ejaculate volume, count, concentration, motility, and morphology were assessed based on the WHO laboratory guidelines [36]. About 10 μl of the seminal specimen was loaded into a micro-Cell sperm-counting chamber and assessed for concentration and motility. Total sperm count was accounted as volume × concentration [36].

2.3. The chromatin quality and maturity of samples

To determine chromatin quality and sperm maturity, the Aniline Blue (AB) coloring of sperm heads was applied. To make a seminal smear, 10 μl of the specimen was loaded on a slide. Slides were air-dried and then plunged in the fixative (Formalin 4 %) for 5 min, and the slides were placed vertically to drain excess solution on absorbing paper. In step 1, they were immersed in AB 5 % for 5 min, and in step 2, in Eosin 5 % for 1 min. To prepare AB stain 5 %, 5 g powder was solved in 96 ml distilled water and then in 4% of acetic acid (pH = 3.5). Next, the washed slides were air-dried. In × 1000 magnification of bright field microscopy, all slides were assessed [37]. To report the percentage of aberrant spermatozoa, a minimum of 200 sperm were numbered on each slide [38].

2.4. DNA extraction and sodium bisulfite treatment

Extraction of DNA from semen samples was done using the DNA extraction Kit (DNrich sperm kit, Cat. No. AESDX1012, Azma Elixir Pajooh Company, Iran) based on the manufacturer's guidance. The DNA extraction was also confirmed by gel electrophoresis. Then, extracted DNA was stored at −20 °C. The sodium bisulfite treatment was applied on the extracted DNA using the Bisulfite modification genomic DNA (Lot N: MSP0027, AnaCell company, Iran). For all samples, the same amount of genomic DNA (1000 ng) was used for bisulfite treatment. In that process, unmethylated cytosines changed to uracil, whereas methylated cytosines were unaltered [39]. Sensitivity assay for bisulfite modification was carried out. For this assay, untreated DNA was used as a template for PCR on set of MSP primers.

2.5. DNA methylation analysis

The methylation was measured quantitatively at the DMRs of the MEST gene by real-time methylation-specific PCR (MSP) to discern methylated from unmethylated DNA [40]. Primer sequences of the MEST gene are shown in Table 1. The analysis of samples was independently applied by two MSP reactions. The PCR reaction was 25 μl containing 12.5 μl of 2X PCR master mix (Cat No. MM2011, SinaClon company, Iran), 0.5 μM of each M-forward and M-reverse primer that amplify the methylated imprint specifically or 0.5 μM of each U-forward and U-reverse primer that amplify the unmethylated gene and 50 ng of bisulfite-treated DNA. The PCR program conducted under this thermal cycler situation as followed: denaturation at 95 °C (5 min), next 40 cycles at 94 °C (1 min), annealing at 58 °C (1 min), and a final extension at 72 °C (5 min). Then, the amplified fragment was electrophoresed on 2 % agarose gel and was stained with DNA safe stain. PCR amplification presented 300-bp PCR products in Fig. 1.

2.6. Data analysis

The quantitative variables were descripted as the mean ± standard deviation (SD). Homogeneity and normality were assessed using the Kolmogorov-Smirnov test. However, all data sets did not meet the normality assumption (Data not shown). Consequently, the Mann-Whitney *U* test was employed for comparing means between two distinct groups. For comparing means across multiple groups, the Kruskal-Wallis test was utilized, followed by Dunn's multiple comparisons test. Non-parametric Spearman correlation was employed for conducting correlation analyses. A probability value (*P*-value) lower than 0.05 was defined as a significant value. The data analysis was performed by the Stata software (version 14.1) (Stata Corp, College Station, TX, USA).

3. Results

The mean age of the participants did not differ significantly, 38.24 ± 4.18 in normospermia, 36.78 ± 4.81 in asthenospermia, and 37.17 ± 5.46 in oligoasthenoteratospermia (p = 0.166). As shown in Table 2, the percentages of spermatozoa count, concentration, total motility, progressive, and morphology in the infertile were lower than normozoospermic group, but the mean percentages of histone transition abnormality (immature sperm) were higher in the infertile compared to normospermia (P < 0.05).

The mean percentages of sperm count and sperm concentration in oligoasthenoteratospermia were significantly lower than in asthenospermia (P = 0.001) and normospermia (P = 0.001). Likewise, the mean percentages of total motility, progression, and morphology in normospermia were significantly higher than oligoasthenoteratospermia and asthenospermia, and they were significantly higher in asthenospermia compared to oligoasthenoteratospermia (P < 0.05). The mean percentages of histone transition

Table 1
Primer sequences for *MEST* gene [67].

Gene	Methylation status	Forward Primer	Reverse Primer	Annealing temperature for PCR	Product length
MEST	**Un-methylated**	5′-gtggtagttgtgttttgtaagtgtagtgtt-3′	5′-cacacaatcctccactcacctaca-3′	58 °C	300 bp
	Methylated	5′-tagttgcgtttcgtaaggtagtgtc-3′	5′-acacaatcctccgctcgccta-3′		

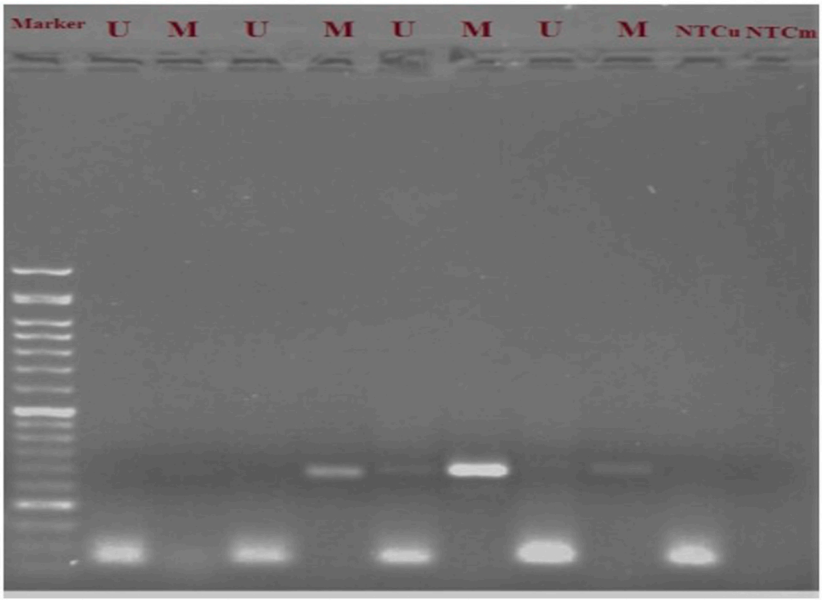

Fig. 1. PCR products from a bisulfite-modified DNA using the methylated and unmethylated primer pairs were electrophoresed on a 2% agarose gel.

abnormality in oligoasthenoteratospermia were significantly higher than asthenospermia and normospermia ($P < 0.05$) **(Table 2)**.

Respectively, normal sperm were known as unstained or pale blue and dark blue cells, and aberrant spermatozoa were recognized as dark blue stained cells. Table 3 illustrates a negative relationship between abnormal spermatozoa chromatin condensation with spermatozoa count ($r = -0.373$; $P = 0.012$), concentration ($r = -0.350$; $P = 0.019$), total motility ($r = -0.406$; $P = 0.006$), progressive ($r = -0.405$; $P = 0.006$), and normal morphology ($r = -0.426$; $P = 0.004$).

Table 4 demonstrates that the mean percentages of *MEST* methylation were higher in infertile compared to normospermia ($P < 0.05$). The mean percentages of *MEST* methylation in oligoasthenoteratospermia were significantly higher than in asthenospermia and normospermia ($P < 0.05$).

Table 5 illustrates a negative correlation between *MEST* methylation with spermatozoa count ($r = -0.374$; $P = 0.011$), concentration ($r = -0.325$; $P = 0.029$), progressive ($r = -0.323$; $P = 0.030$), and normal morphology ($r = -0.384$; $P = 0.009$).

4. Discussion

In our study, the seminal assessment was carried out for normozoospermia, which is categorized as the case group, and asthenospermia and oligoasthenoteratospermia were considered as case group(s). For the first time, this research evaluated the status of the *MEST* gene and sperm maturity simultaneously in abnormal samples, especially in oligoasthenoteratospermia groups, and normal sperm samples. Furthermore, the research considered assessing the role of chromatin maturity in categorizing the sperm as typical and atypical as another aim.

To opt for choosing the best evaluation, there were three factors related to sperm that were considered in this study: sperm parameters, chromatin quality, and status of *MEST* methylation in fertile and infertile groups.

According to sperm parameters, results demonstrated a statistically considerable difference in spermatozoa parameters between the normozoospermic (control) and the infertile group, as the case group, and among oligoasthenoteratospermia, asthenospermia, and normozoospermia (Table 2). These findings are in accordance with a retrospective study done by Pourmasumi et al. indicating a remarkable difference in sperm parameters between normozoospermia and infertile groups [41]. Moreover, a study on Erbil population showed a considerable negative association between infertility and sperm characteristics, e.g., viability, volume, concentration, total count, total motility, progressive motility, and morphology [42].

In spermiogenesis, histones are replaced by protamines in the chromatin of the nucleus, which is crucial for chromatin condensation and stability. Therefore, mature spermatozoa (normal) were characterized by pale blue and dark blue sperm heads, while immature sperm (abnormal) were identified by dark blue or gray coloration.

The findings of our study illustrated a significantly negative relationship between histone transition abnormality via AB^{+} cells with spermatozoa count, concentration, total motility, progressive, and normal morphology (Table 3). Pourmasumi and colleagues reported similar findings with 1386 overall sample size [41]. Moreover, Al-Fahhamet al. observed a considerable negative association between chromatin results of AB and toluidine blue (TB) staining with spermatozoa concentration, morphology, and progressive motility too [43]. Additionally, in a study done by Kim and coworkers, a remarkable correlation between chromatin maturity with normal sperm stained by AB and TB staining was reported [44]. Sellami et al. demonstrated a negative relationship between sperm maturity and histone transition abnormality [45]. Furthermore, a study performed by Hammadeh and coworkers reported a meaningful difference

Table 2
Sperm parameters in normospermia group and infertile subgroups.

Spermparameters	Normozoospermic(n = 15)	95 %CI	Infertile(n = 30)	95 %CI	P.[a]	Normozoospermic(n = 15)	95 %CI	Asthenospermia(n = 15)	95 %CI	Oligoasthenoteratospermia(n = 15)	95 %CI	P.[b]
Sperm Count (10^6 cells per ejaculate)	144.18 ± 53.44	115.02–169.30	105.0 ± 97.56	72.61–139.51	0.030	144.18 ± 53.44	114.58–173.78	180.73 ± 84.52	133.92–227.54	29.26 ± 16.84	19.93–38.59	0.001
Sperm Concentration (10^6 cells/mL)	56.85 ± 15.18	49.91–65.20	28.36 ± 22.92	20.10–36.37	0.001	56.85 ± 15.18	48.44–65.26	47.66 ± 16.35	38.60–56.72	9.06 ± 4.78	6.41–11.71	0.001
Total Motility (%)	54.64 ± 8.86	50.21–59.88	27.50 ± 10.32	23.84–30.89	0.001	54.64 ± 8.86	49.73–59.54	33.40 ± 4.27	31.03–35.76	21.60 ± 11.31	15.33–27.86	0.001
Progressive (%)	32.53 ± 1.24	30.0–33.25	9.53 ± 6.18	7.23–11.48	0.001	32.53 ± 1.24	31.84–33.22	13.93 ± 4.72	11.31–16.55	5.13 ± 3.92	2.95–7.30	0.001
Morphology (%normal)	4.73 ± 0.45	4.50–4.94	1.30 ± 0.87	1.0–1.61	0.001	4.73 ± 0.45	4.47–4.98	2.06 ± 0.25	1.92–2.20	0.53 ± 0.51	0.24–0.81	0.001
Histone transition abnormality (%)	0.07 ± 0.04	0.01–0.07	0.09 ± 0.05	0.07–0.11	0.068	0.07 ± 0.04	0.04–0.09	0.07 ± 0.03	0.04–0.09	0.12 ± 0.05	0.08–0.15	0.002

[a] Relationships between sperm parameters in normospermia and infertile group were determined with the nonparametric Mann-Whitney *U* test.
[b] *P*-value obtained from the difference between means in normospermia, asthenospermia, and oligoasthenoteratospermia was tested for significance, by Kruskal-Wallis one way ANOVA on ranks and post hoc analysis was performed using Dunn's for all pair-wise comparisons. $p < 0.05$ was considered statistically significant.

Table 3
Correlation between sperm parameters with abnormal sperm chromatin condensation.

Sperm parameters	Total		Normozoospermic		Asthenospermia		Oligoasthenoteratospermia	
	r	P	r	P	r	P	r	P
Sperm Count (10^6 cells per ejaculate)	−0.373	0.012	0.200	0.476	−0.162	0.565	0.262	0.345
Sperm Concentration (10^6 cells/mL)	−0.350	0.019	−0.023	0.936	0.185	0.508	0.151	0.591
Total Motility (%)	−0.406	0.006	0.300	0.277	−0.130	0.644	−0.339	0.216
Progressive (%)	−0.405	0.006	−0.027	0.923	−0.179	0.523	−0.039	0.891
Morphology (%normal)	−0.426	0.004	0.126	0.655	0.435	0.105	0.063	0.823

Spearman's nonparametric correlation coefficient was calculated for data.

in spermatozoa parameters between the normozoospermic and infertile groups [46]. Besides, Kazerooni et al. found a meaningfully negative relationship between histone transition abnormality with sperm morphology and motility [47].

In contrast, the study conducted by Dehghanpour et al. [48] found no significant correlation between sperm chromatin maturity and the parameters of men with teratospermia, as determined by the CMA3 test. Moreover, the incidence of spermatozoa with abnormal chromatin condensation showed a positive correlation with sperm concentration but did not exhibit any correlation with other semen parameters, including motility and morphology [49]. Currently, the role of abnormal DNA methylation in the occurrence of human diseases is well documented [50]. The significance of methylation status in biology lies in its capacity to control gene expression [20]. Several studies found that spermatozoa from infertile patients often show higher levels of aberrant DNA methylation at imprinted loci [51,52]. When considering *MEST* methylation, the maternally methylated *MEST* DMR must have a completely unmethylated pattern [20]. Our findings indicated that the mean percentage of methylation related to *MEST* was significantly higher in the infertile group, which comprised asthenospermia and oligoasthenoteratospermia, in comparison to the fertile group (normospermia) (Table 4). Given the novelty of the study's design, it seems that the *MEST* status in oligoasthenoteratospermia cases has not been studied anywhere else in the world. However, a recent study evaluating it in the asthenospermia group was published [53].

In the aforementioned study, authors investigated MEST methylation in 74 men with asthenozoospermia and 92 normozoospermia individuals recruited between November 2017 and May 2019. The result confirmed a significant difference between the mild asthenospermia and fertile samples [53].

Until now, various research regarding other types of infertile groups, especially oligozoospermia, have illustrated that abnormal methylation of imprinted genes might disrupt spermatozoa production and/or lead to male factor infertility [54–57]. *MEST*, as an imprinted gene, severally has been reported as hypermethylated in male infertility [26,52,58–60]. The sample size in these studies ranges from 10 to 175 for the case group and from 5 to 119 for the control group. Santi et al. performed meta-analysis research on the impairment of spermatozoa DNA methylation in male infertility. In this comprehensive meta-analysis, comprising twenty-four studies, it was revealed that male infertility is significantly correlated with increased *MEST* methylation [61]. Klaver & Gromoll reported a greater odds ratio of abnormal DNA methylation on the *MEST* gene in infertile patients compared the healthy men [62]. Kobayashi et al. [16] investigated 97 samples, consisting of 79 normal individuals and 18 patients with oligospermia. Similarly, Poplinski et al. [27] conducted a study using bisulfite sequencing, while Sato et al. [63] analyzed 61 cases with severe oligozoospermia, 67 cases with moderate oligozoospermia, and 209 cases with normozoospermia using the combined bisulfite PCR restriction analysis (COBRA) method. These studies collectively revealed a robust association between abnormal MEST gene methylation and idiopathic male infertility. The literature review could not find any study that revealed a non-significant relation between *MEST* methylation pattern and infertility. Overall, correct levels of sperm DNA methylation are critical for potential fertility.

Since imbalances in the methylation levels have been diagnosed in the case of imprinting genes like *MEST* in the spermatozoa DNA of infertile patients, the importance of DNA methylation in fertility and pregnancy requires considerable consideration [55,64]. Our results suggested a negative relationship between *MEST* methylation with spermatozoa count, sperm concentration, progressive, and normal morphology (Table 5). Our results are in accord with prior research done by Song et al. [53], Laqqan et al. [65], Santana et al. [66], and Tang et al. [28] found that abnormal DNA methylation patterns were related to the weak spermatozoa parameters in infertile patients. However, a study conducted by Louie et al. could not establish a significant relationship between *MEST* methylation across sperm concentrations [20].

The following items can be nominated as limitations of the present study, and researchers can consider these suggestions for future investigations;

1- Recruiting additional samples from multiple treatment centers can increase the overall sample size and, as a result, improve the ability to determine the role of effect size. 2- Chromomycin A3 (CMA3) is another way to detect DNA and chromatin defects in sperm more accurately and sensitively compared to Aniline Blue. Additionally, the combined use of these two staining methods can increase the accuracy of chromatin assessment and aid in identifying sperm issues. 3- Bisulfite sequencing of specific genes offers the advantage of providing accurate quantification of multiple CpG methylation sites in the same reaction. In contrast, the qMSP assay can be designed to target one or more CpG sites within a specific genomic region, but it can only determine the overall methylation status of the entire targeted region as a whole [62]. This makes bisulfite sequencing of specific genes a more detailed and informative method for studying epigenetic modifications in DNA. 4- Follow-up with the participating individuals to determine the outcomes, such as embryo quality and birth rate, is recommended.

Table 4
The methylation status of the *MEST* gene DMRs in normospermia and infertile subgroups.

Variable	Normozoospermic(n = 15)	95 %CI	Infertile(n = 30)	95 %CI	P.[a]	Normozoospermic(n = 15)	95 %CI	Asthenospermia(n = 15)	95 %CI	Oligoasthenoteratospermia(n = 15)	95 %CI	P.[b]
MEST methylation (%)	10.35 ± 15.96	3.15–19.20	21.35 ± 27.99	11.37–31.88	0.045	10.35 ± 15.96	1.51–19.19	10.24 ± 18.0	0.27–20.21	32.47 ± 32.16	14.66–50.29	0.014

[a] Relationships between sperm parameters in normospermia and infertile group were determined with the nonparametric Mann-Whitney *U* test.
[b] P-value obtained from the difference between means in normospermia, asthenospermia, and oligoasthenoteratospermia was tested for significance, by Kruskal-Wallis one way ANOVA on ranks and post hoc analysis was performed using Dunn's for all pair-wise comparisons. $p < 0.05$ was considered statistically significant.

Table 5
Correlation between sperm parameters with *MEST* methylation.

Sperm parameters	Total (N = 45)		Normozoospermic (N = 15		Asthenospermia (N = 15)		Oligoasthenoteratospermia (N = 15)	
	r	P	r	P	r	P	r	P
Sperm Count (10^6 cells per ejaculate)	−0.374	0.011	0.143	0.611	−0.393	0.147	0.089	0.752
Sperm Concentration (10^6 cells/mL)	−0.325	0.029	0.249	0.371	−0.118	0.674	0.224	0.422
Total Motility (%)	−0.234	0.121	−0.068	0.810	0.343	0.211	0.475	0.073
Progressive (%)	−0.323	0.030	0.321	0.244	−0.140	0.619	0.283	0.307
Morphology (%normal)	−0.384	0.009	0.174	0.534	−0.434	0.106	0.303	0.272
Histone transition abnormality (%)	0.139	0.363	0.096	0.734	−0.077	0.784	−0.282	0.309

Spearman's nonparametric correlation coefficient was calculated for data.

5. Conclusion

A negative correlation existed between the histone transition abnormality and *MEST* methylation with sperm parameters. In addition, chromatin integrity, sperm maturity, and *MEST* methylation may be considered important predictors for the assessment of male fertility potential.

Author contribution statement

Tayebeh Amjadian: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Parichehreh Yaghmaei: Analyzed and interpreted the data; Wrote the paper.
Nasim Hayati Roodbari: Contributed reagents, materials, analysis tools or data; Wrote the paper.
Kheirollah Yari: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This study was not founded by anywhere/anyone.

Data availability statement

Data will be made available on request.

Ethics statement

The Research Ethics Committee at the Deputy of Research of Islamic Azad University approved the study protocol (IR.IAU.SRB.REC.1401.184). All participants were required to sign a written consent form and were provided with a participant information statement that thoroughly explained the consent process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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