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REVIEW ARTICLE

The Role of Protamine in Male Fertility

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Abstract: **Introduction:** Approximately 40-50% of infertilities is related to males. Abnormal sperm chromatin structure is suggested as a significant cause of infertility. Protamines constitute a significant component of the sperm chromatin, and they play a vital role in the proper packaging of chromatin. Numerous studies have shown that protamine deficiency in sperm is associated with low sperm quality and infertility. Given the importance of protamine infertility, the purpose of this article is to investigate the content of protamine and its biological importance in male fertility. **Material and Methods:** In this study, data and information collected on English-language articles from PubMed and MEDLINE databases. For Persian articles, Persian-language databases, including SID Scientific Database, IranMedex Medical Articles Database, IranDoc (Iran Scientific Information and Documents Research Institute), Magiran Publication Information and MedLib investigated. **Results:** Based on previous studies about protamine and its role in spermatogenesis, any disruption in protamine genes, including PRM1 and PMR2, can disrupt histone/protamine ratio, leading to abnormal spermatozoa. **Conclusion:** Most the previous studies approved any mutation in protamine genes correlates with infertility in men. These results can be a potential subject for future researches into infertility treatment in men.

Keywords: Chromatin; Fertility; Protamine; Reproductive system

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1. Introduction

In addition to the nucleosome structure and function of histones, sperm nucleus chromatin health is dependent on another nuclear protein called protamine. Protamine works as a protection for chromatin against denaturation (1). Protamine is a small nuclear protein with a positive electrical charge, which is explicitly expressed in testis tissue. Sperm nucleus density is one of the main events during the process of spermatogenesis. This event is essential in making the sperm head hydrodynamic, facilitating sperm movement, as well as protecting the physical and chemical damage that sperm faces during passage through the male and female reproductive system. Any defect in chromatin packaging can cause a lack of protamine in the chromatin structure of sperm.

Therefore, instead of condensing the chromatin nucleus, it takes on a relaxed state and therefore is not safe from free radical attack (2, 3). Protamine plays a vital role in male fertility and is available in two forms, protamine 1 (P1) and protamine 2 (P2). P1 is present in all mammalian species, while P2 has only been reported in some organisms, including mice, hamsters, horses, and humans (4). In the process of spermatogenesis, this protein binds to the DNA of the sperm nucleus by replacing it with histone proteins, causing the sperm nucleus genome to become denser. The first step in this process occurs in round spermatids and involves replacing somatic histones with transfer proteins (TP1 and TP2). After that, in elongated spermatids, P1 and P2 replace TP1 and TP2, and the resulting chromatin becomes very dense and transcription does not occur in this state (5). (Figure 1)

This phenomenon plays an essential role in the ability of sperm in the process of fertilization and fertilization of eggs. One of the most critical protamine functions is the protection and support of the paternal genome (6, 7). Protamine is rich in the amino acid cysteine, which helps form disulfide bonds between adjacent molecules and leads to more excellent of the DNA-protamine complex stability. Sperm nucleus density requires for protection against external stresses such as oxidative stress (8). P1 and P2 are phosphorylated after synthesis, but after binding to DNA, most of the phosphate

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groups are removed, and the cysteine residues are oxidized to form disulfide bridges that bind protamine. Both P1 and P2 are required for normal sperm function in mammals and many rodents (9). Expression of protamine genes (mRNA) occurs at the stage of spherical spermatids (10). It should be noted that transcription and translation of protamine genes do not occur continuously and continuously during sperm maturation. These mRNAs do not translate into sperm in the form of ribonucleic-protein RNPs in the spherical spermatid stage until sperm elongation in where they are activated during spermatogenesis and go through the stages of translation (11-14) Recent research suggests that any change in the natural expression or function of this protein is likely to lead to infertility in men. Incomplete protamination can make the paternal genome vulnerable to endogenous and exogenous factors such as nucleases, free radicals, and mutagens. Damage or breakdown in sperm DNA depends on the amount of protamine attached to the DNA (15). The transcription of the P1 gene only leads to the production of a single P1 protein that is produced after gene processing. The result of P2 gene processing is a P2 precursor, which is converted to various types of P2, including P2, P3, and P4 during a proteolytic process (16). Both P1 and P2 are involved in the condensation of the sperm nuclear genome. So that the ratio P1 to P2 is approximately equal to one. Recent studies show that a deviation of this ratio from 1 indicates a deficiency of one of these two protamines, which leads to infertility and increased chromosomal defects (17). Also, any abnormalities in the chromosomal and gene positions of P1 and P2 that cause defects in their expression or function can impair male fertility. Therefore, due to the importance of Protamine in fertility, in this study, its role in fertility is discussed.

2. Material and Methods

For this study, as a narrative review, information on English-language articles from PubMed and MEDLINE databases were collected, and for Persian articles, Persian-language databases, including SID Scientific Database, IranMedex Medical Articles Database, IranDoc (Iran Scientific Information and Documents Research Institute), Magiran Publication Information and MedLib were investigated. More than 50 articles on protamine and infertility published during 2000-2020 were studied. The keywords searched in this study were: protamine, DNA, sperm, infertility, and chromatin. To select the documents used, the titles found were reviewed thematically using the search engine. Then, articles' titles were evaluated in terms of relevance to the subject and purpose and were studied after selecting the appropriate items. Findings and results related to the correlation between infertility in men and protamine obtained from 50 articles studied were analyzed as the primary data of this study. The common results obtained from previous studies and their inferences are mentioned as part of the conclusion in this review article. In general, the steps are included: searching for resources, selecting content, determining keywords, categorizing content, summarizing, and concluding.

3. Results

3.1. Infertility and Protamine

Numerous studies have shown that protamine abnormalities are associated with abnormalities in spermatogenesis and physical defects in the sperm (1). The deficiency of protamine in the sperm is the result of increased oxidative stress, which in turn causes DNA damage. Recent studies have found that fetal quality is negatively associated with protamine deficiency (2). Mutations or polymorphisms of the protamine gene can lead to changes in the spatial structure of this protein and affect its function in twisting around the nuclear genome of sperm. A small number of P1 and P2 haplotypes have a protective effect against male infertility, while most haplotypes significantly increase the risk of infertility.
According to previous studies, polymorphism -C190 <A is associated with changes in PRM1 gene expression, abnormal sperm morphology, P1/P2 ratio disturbance, and infertility in men (1). Low levels of PRM2 may be associated with morphological abnormalities and decreased sperm motility. PRM2, as an essential marker, can also help us understand the regulatory mechanisms of spermatogenesis and its essential role in fertilization (18). Four infertile individuals in whom the P1/P2 ratio was altered were analyzed by Yebra and colleagues, but no mutation in the protamine gene was observed (4). Also, in the study of Schelicker et al. 1994, the genomes of 36 infertile individuals were examined , and no mutation was observed (19). Besides the importance of the gene sequence, the region encoding the protamine gene, namely the UTR3 and UTR5 regions, is also essential. Because specific sequences from these regions can affect the synthesis and processing of mRNA from a gene and its translation into a protein. In 1994, a study of the UTR region sequence identified a mutation in the repetitive GA sequence in the upstream transcription starting point in the P2 gene (20).
In 2020, Hammound, and colleagues identified fourteen SNPs in the UTR region of the P1 and P2 genes (21, 22), the most important of which was the GC nucleotide change at the 62-position position in the UTR3 region. This SNP was observed only in infertile men and none of the men with typical protamine ratio or standard sperm parameters carried this SNP. Also, a variation in the repetitive GA sequence was identified in the UTR5 region of the P2 gene, so that the frequency of this variation was not significantly different in infertile men compared to fertile men. One study identified a

mutation in the P1 gene as a transversion mutation (mutations that convert purine to pyrimidine, and vice versa). This mutation was detected in two of the 281 infertile individuals studied (19).

In the study by FaizHamad (2019), the impact of histone and protamine expression pattern on sperm's quality and chromatin integrity were investigated. In this study spermatozoa, from 53 normal and 75 patients were surveyed. He found out that the expression of histones H2A and H2B in patients was significantly higher than normal males, while the level of Protamine PRM2 mRNA in patients was lower in comparison to normal sperms. As mentioned before, the normal ratio of PRM1/PRM2 and H2A/H2B mRNAs is one, but based on FaizHamad results, these ratios were higher than one. According to these data (H2A+H2B)/(PRM1+PRM2) mRNAs ratios can be a criterion for assessing sperm quality because histone and protamine ratio showed an negative correlation to sperm's count, total count, motility, progressive motility, normal morphology, membrane integrity, and positively with chromatin decondensation (23).

3.2. Teratospermia and Protamine

The expression of the protamine gene is practical on all sperm parameters. It has been observed in that patients with morphological defects of the sperm, such as having a round head, they contain less protamine, and more histone than normal sperm. Impaired protamine expression, is also associated with decreased sperm quality, functional ability, and DNA cohesion. Protamine is considered as a controlling point in the process of spermatogenesis and reducing its expression induces apoptosis and reduces sperm quality (18).

In 2006, a heterozygous single nucleotide polymorphism in the P1 gene was reported in the Chinese population (24) This polymorphism, which was observed in 3 out of 30 infertile people studied, led to the conversion of the amino acid arginine to serine. With this transfer, a conserved and arginine-rich sequence is transformed into a serine-arginine sequence, and this change makes this sequence susceptible to phosphorylation by the SRPK1enzyme. This phosphorylation leads to a change in the tendency of this altered protamine to bind to sperm DNA, which is only seen in oligospermic individuals. Down-Regulation has been demonstrated in the expression levels of P1 and P2 in the oligospermia group compared with the fertile group(22).

In 2019, Al Zeyadi et al. performed a study on Iraq population to find valuable evidence of significant relation between polymorphisms of PRM1 and PRM2 with teratozoospermia. They showed three types of polymorphism repeated in all infertile men in this study, significantly. These polymorphisms are including (G197, G197T and G/T197) in PRM1, and (C248, C248TandC/T248) in PRM2 (25).

3.3. Azoospermia and protamine

In 2003, Tanka and colleagues reported five gene polymorphisms in the P1 gene and three polymorphisms in the P2 gene (26). Of the five P1 gene polymorphisms, four were in the exon region without amino acid displacement and one was in the UTR3 region. out of three polymorphisms in the P2 gene, two were in the exon region without amino acid displacement, and one of them produced an early end codon in the P2 gene. This polymorphism was identified among 266 infertile individuals in one case that resulted in azoospermia in that individual. In another study, of 6 polymorphisms in 77 individuals, azoospermia was not observed in any of the subjects(26).

In a case-control study by Talebi et al. (2020), relationships between sperm parameters in infertility with protamine were investigated among 100 oligospermia, 100 asthenospermia, and 100 teratospermia infertile men. Although in this study, correlation between sperm motility and deficiency of protamine is not approved, PRM1 deficiency was related to abnormal morphology of spermatozoa (27).

3.4. Varicocele and protamine

Varicocele-induced infertility in men may be associated with decreased protamine and damage to sperm DNA. P1/P2 mRNA ratio and DNA fragmentation index (DFI) affect fertility in men undergoing varicocelectomy. Varicocelectomy in some patients improves chromatin compression and DNA cohesion (7).

Increased oxidative stress of testicular temperature in people with varicocele, in addition to reducing the quality of sperm parameters, also has a negative effect on sperm chromatin health and, as a result, affects the process of spermatogenesis and fertility. In people with varicocele, the percentage of DNA damage and protamine deficiency is significantly higher (28). mRNAP1/P2 has been suggested as a fertility predictor. Several studies have shown that patients with varicocele have an increased risk of damage to sperm DNA. Sperm DNA damage is multifactorial. One of the most important causes of sperm protamine deficiency is that it affects the density of the sperm nucleus and leads to fragmentation of sperm DNA, impaired sperm concentration during fertilization, and impaired transmission of paternal genetic information to the developing fetus (7).

Two meta-analyses conclude that the pathogenicity of a protamine gene mutation for male infertility has not been proven, and only -190 <C polymorphism can be considered as a risk factor for male infertility. Patients were classified into 13 groups (A to M) according to the type and number of nucleotide substitutions in RPM1 and RPM2 genes. Each patient with varicocele had at least two cases (C and G) and a maximum of six changes (group J) in protamine

genes. According to CMA3 staining, significant differences were observed for changes in PRM1 and PRM2 genes in the varicocele group compared to the control group. Semen parameters in varicocele patients were significantly altered by changes in PRM1 and PRM2 compared to fertile men. Nine different types, including six types in PRM1 and three types in the PRM2 gene, were observed in patients with varicocele. Homozygous g.805C <T was the most common change in PRM2 and was observed in all patients with varicocele. Heterozygous had the lowest frequency (3%) in both g. IVS1+T <C and g. G151>A in PRM1.

In the mentioned study, the prevalence of protamine gene polymorphisms in infertile men with varicocele was evaluated. Alternation of different nucleotides in PRM1 and PRM2 genes was not observed in varicocele patients. While in the control group, no changes in PRM1 and PRM2 genes were observed. This was the first report of protamine gene polymorphism in varicocele patients (29).

In another study by Abdullah et al. (2019), c.-190 C>A transversion in PRM1 gene were related to idiopathic infertility in men. In this study, blood of sixty male patients complaining of idiopathic infertility and forty healthy fertile males were searched for percent of this mutation. Low sperm concentration and decreased sperm motility were associated with AA and CA genotype in the PRM1 gene (30).

Protamine is present in mammals in both PRM1 and PRM2 forms and is involved in DNA compression and spermatid differentiation. Disruption of the structure of this protein is the cause of idiopathic infertility in men (18). Abnormal P1/P2 ratio in sperm disrupts chromatin condensation, DNA breakage, and infertility (7). Moreover, 13-75 infertile individuals did not have protamine 2, compared with 50 fertile individuals (22). All studies about the relation of protamine and infertility in men is summarized in table 1.

4. Discussion

Spermatogenesis is a complex process in which mitotic and meiotic division's occurrence leads to the formation of a male gamete or sperm (31). Male infertility is a multifactorial syndrome that involves a wide range of specific disorders. In this study we explored more than 50 previous studies about the relation between protamine deficiency and infertility in men. Based on these studies, P1/P2 mRNA ratio was significantly higher in infertile men in comparison to normal people. In the spermatogenesis process, sperm chromatin is replaced by testis-specific nuclear proteins at first, then by transitional proteins, and at last by protamine. Imperfect predomination increases DNA damage and causes male infertility (32). On the other hand, some studies showed that protamine deficiency leads to deformed DNA packaging. Which increases susceptibility to damage by external stress resulting. In this mini-review, all these data were collected, and we found that in men with protamine deficiency, the percent of DNA sperm damage is higher than in normal men. Deformed low mobility sperms are the results of DNA sperm damage (7). A meta-analyses found that after using Assisted Reproductive Technology, a significant relationship between increasing sperm DNA damage and decreasing infants' live birth is possible. There are conflicting views on the effect of sperm with damaged DNA on the superiority of ICSI clinical results on IVF; Some views believe that ICSI improves pregnancy rates in people with sperm damage (33, 34),while some other researchers believe that the selection of sperm based on normal morphology for ICSI cannot guarantee DNA health. There are sperms with a normal morphology that have DNA damage (35, 36). Researchers were able to measure sperm protamine deficiency using aniline blue or chromomycin-A3 (CMA3) staining, both methods are unable to discriminate between protamine-1 and protamine-2. For this purpose, real-time RT-qPCR or semi-quantitative western blotting should be performed. The results show that protamine deficiency is associated with the severity of DNA damage, while in the study of P1: P2 ratio with DNA damage, this relation was not observed. Therefore, it is possible to evaluate sperm quality and protamine depletion associated with DNA damage (by examining protamine deficiency (37). Recent studies show that fetal quality is negatively related to sperm protamine deficiency. The sperm have a decrease in sperm fertilization in IVF but not in ICSI. So, any defect in chromatin packaging can cause a lack of protamine in the Chromatin structure. Therefore, instead of condensing the nucleus, chromatin gets into a relaxation state that is not immune to free radical attacks and leads to oxidative stress; so, the DNA structure of sperm is prone to breakage, and these sperms are not able to fertilize and ultimately reduce person's fertility potential. The process of spermatogenesis can be improved by Using antioxidant therapy, so the produced sperm has the proper density of chromatin and better quality in terms of shape, motility and, since changes in the P1:P2 ratio of the early stages of fertilization affect the penetration of sperm into the egg (37). Therefore, according to the available results, if the level of protamine in people decreases, it is recommended to use the ICSI method and antioxidant therapy.

Although, many studies have been performed for demonstration of the relationship between protamines and infertility, there are too many ambiguous points in protamine function. Besides many questions remain to clarify mechanisms of nucleohistone–nucleoprotamine transition, epigenetic impacts and integrity of DNA, and their role in fertility. On the other hand, the demonstration of mutations and polymorphisms in the protamine gene and their relation in infertility require more accurate and comprehensive studies. According

to some studies, environmental factors such as pollution and stress can affect sperm protamination. Therefore, it seems that future studies can focus on environmental factors and their relationship with protamine-related genetic factors to clarify the role of protamine and epigenetic effects on infertility in men as much as possible.

5. Appendix

5.1. Acknowledgements

None.

5.2. Author contribution

All the authors have the same contribution.

5.3. Funding/Support

None.

5.4. Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Table 1: Studies about the relation of protamine and infertility in men.

Reference	Main findings
Schelicker et al. 1994	No mutation was observed
Jonckheere et al. 1994	Mutation in the repetitive GA sequence in the upstream transcription starting point in the P2 gene
Hammound et al. 2020	Identification of 14 SNPs in the UTR region of the P1 and P2 genes
Tanaka et al. 2003	Finding a mutation in the P1 gene as a transversion mutation in 281 infertile men
FaizHamad 2019	A higher ratio of (H2A+H2B)/(PRM1+PRM2) mRNA level in infertile men
Iguchi et al. 2006	SNP which leads to arginine-rich sequence transformation into a serine-arginine sequence in oligospermia individuals
Al Zeyadi et al. 2019	Repeated polymorphisms in all infertile men (G197, G197T and G/T197) in PRM1, and (C248, C248TandC/T248) in PRM2
Talebi et al. 2020	Relation of PRM1 deficiency with abnormal morphology of spermatozoa
Nayeri et al. 2020	Polymorphisms of protamine genes in CMA3 staining and infertile men.
Abdullah et al. 2019	Relation of c.-190 C>A transversions in PRM1 gene with idiopathic infertility in men

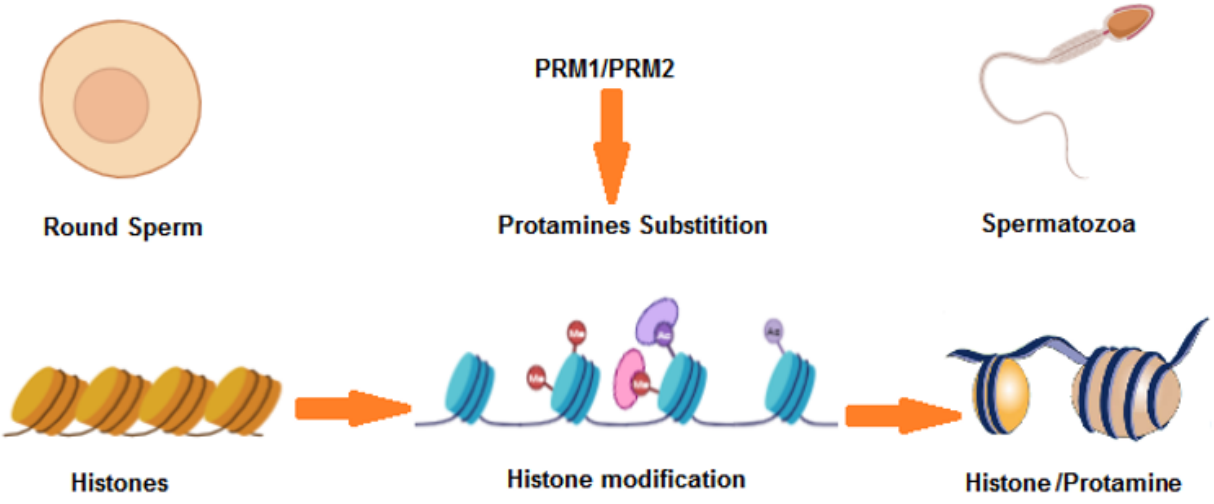

Figure 1: The role of Protamine in spermatozoa maturation. In round sperms, histones are the main components in chromatin condensation. During spermatogenesis, histone modification and protamine, substitution change sperm morphology and constitute spermatozoa.