Evaluation of the association between polymorphisms of PRM1 and PRM2 and the risk of male infertility: a systematic review, meta-analysis, and meta-regression

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Studies have reported the genetic gives rise to male infertility. The aim of the present meta-analysis was to evaluate the association between PRM1 (rs737008 and rs2301365) and PRM2 (rs1646022 and rs2070923) polymorphisms and susceptibility to male infertility. The association between PRM1 and PRM2 polymorphisms and the risk of male infertility was evaluated using specific search terms in the Web of Science, Cochrane Library, PubMed, and Scopus databases without language restriction until January 28, 2020. The association was determined by odds ratio (OR) and 95% confidence interval (CI) on five genetic models using Review Manager 5.3 software. The funnel plot analysis and sensitivity analysis were done by the Comprehensive Meta-analysis 2.0 software. Out of 261 records retrieved from the databases, 17 studies were analyzed in the meta-analysis, including the four PRM polymorphisms. The pooled results as OR (P-value) showed 0.96 (0.44), 1.04 (0.70), 0.94 (0.51), 0.94 (0.48), and 1.03 (0.72) for PRM1 rs737008 polymorphism and 1.67 (0.0007), 1.73 (0.06), 1.50 (0.007), 1.56 (0.004), and 1.62 (0.33) for PRM1 rs2301365 polymorphism in allele, homozygous, heterozygous, recessive, and dominant models, respectively. Moreover, the pooled results as OR (P-value) showed 1.19 (0.004), 1.15 (0.26), 1.08 (0.70), 1.05 (0.76), and 0.98 (0.82) for PRM2 rs1646022 and 0.88 (0.04), 0.84 (0.10), 1.05 (0.81), 0.90 (0.24), and 0.80 (0.02) for PRM2 rs2070923 in allele, homozygous, heterozygous, recessive, and dominant models, respectively. The results showed PRM1 rs2301365 and PRM2 rs1646022 polymorphisms were associated with an elevated risk of male infertility and PRM2 rs2070923 polymorphism had a protective role in infertile men.

Infertility is defined as couples' inability to have a baby after one year of regular unprotected intercourse1. Male factor infertility affects up to 50% of couples' infertility and accounts for only 20% of total infertility2. Recently, however, the male factor infertility incidence has increased3,4. Male infertility is currently assessed through routine analysis according to sperm concentration/number, motility, and sperm morphology. However, there is a significant integration of semen characteristics between fertile and infertile males. In fact, around 15% of patients with male factor infertility according to WHO guidelines5 have normal semen parameters6. Thus, there are several limitations to routine conventional semen analysis in assessing male infertility, indicating that conventional semen parameters are poor predictors of reproductive outcome and that definitive diagnosis of male infertility cannot be made by routine analysis alone7. These limitations have led to the development of advanced methods for the study of sperm function, oxidative stress, fragmentation and DNA packing8. Non-obstructive azoospermia and severe oligozoospermia are two of the dominant phenotypes associated with severe spermatogenesis9. However, many factors relate to male infertility, like to reproductive tract disorders, chemical exposure, and infection8.

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Genetic factors account for 50% or more of all male infertility etiology, and approximately 7% of men worldwide suffer from infertility. In order to indicate the underlying causes, extensive research has been done on the genetic reasons of male infertility in recent years.

There are two types of protamines (PRMs), PRM1 and PRM2, which are encoded by two genes, PRM1 and PRM2, located on chromosome 16. In human sperm cells, 85% of histones are replaced by PRM and from DNA in Protect against harmful agents. Altered ratio of histones to proteins has been shown to increase chromatin deficiency in sperm, increasing the risk of DNA damage and male infertility. In addition, an adequate ratio of PRM1 and PRM2 (normal 0.8–1.2) is needed for normal sperm function. The expression of these two proteins in the sperm nucleus is approximately equal. The complete translation of PRM1 and PRM2 mRNA happens throughout the elongated spermatids development, occurring in the production of positively charged PRMNs as a result of the high arginine content and this allows for strong binding to negatively charged DNA.

It was noticed a significantly diminished level of PRM1 mRNA in spermatozoa isolated from crossbred Frieswal bulls with poor semen parameters, mostly featured by low progressive motility, in comparison to a group with good semen features and decreased PRM2 levels have been reported in various studies in infertile patients. PRMs are believed to play a significant role in chromatin aggregation, transcriptional repression, haploid male genome conservation, sperm formation, and offspring production. There were two previous meta-analyses reporting an association between PRM polymorphisms and the risk of male infertility including 8 studies and checking one PRM polymorphism and another included 13 studies with six PRM polymorphisms. Therefore, in the present meta-analysis including a meta-regression analysis of 17 studies, we investigated 13 PRM polymorphisms and then focused on the association between four functional PRM1 (rs737008 and rs2301365) and PRM2 (rs1646022 and PRM2 rs2070923) polymorphisms and male infertility susceptibility in case–control studies.

Materials and methods
The meta-analysis was done based on PRISMA statement, and the study question was formulated based on the PICO framework. Participants (P): Men with infertility
Interventions (I): Prevalence of PRM1 and PRM2 polymorphisms
Comparisons (C): Male healthy controls
Outcomes (O): Risk of PRM1 and PRM2 polymorphisms
Study design (S): Case–control studies

Literature search. To search the association of PRM1 and PRM2 polymorphisms with the risk of male infertility, one author used the search terms ("male infertility") and ("PRM1" or "PRM2" or "Protamine 1" or "Protamine 2") and ("gene" or "variant" or "polymorphism" or "single-nucleotide polymorphism") in the Web of Science, Cochrane Library, PubMed, and Scopus databases without language restriction until January 28, 2020. Another author checked the titles and abstracts to exclude the duplicates and irrelevant records and checked the full-texts of eligible studies. The databases were searched manually by crosschecking the references of original papers, review papers, and previous meta-analyses related to our topic in this meta-analysis to find the possibly missed studies. In addition, among studies retrieved, two previous meta-analyses had reported an association between PRM polymorphisms and the risk of male infertility. One of them included 8 studies checking PRM1 rs2301365 polymorphism and showed an association between this polymorphism and the risk of male infertility just in Caucasians. Another included 13 studies (11 studies on PRM1 and 7 studies on PRM2 polymorphisms) with six PRM polymorphisms and showed an association between PRM1 rs737008, PRM1 rs2301365, and PRM2 rs1646022 polymorphisms and the risk of male infertility.

Inclusion and exclusion criteria. The inclusion criteria included (1) study focus on PRM1 polymorphisms rs35576928, rs737008, rs35262993, rs2301365, rs140477029, and rs193922261 and also PRM2 polymorphisms of rs1646022, rs779337774, rs545828790, rs201933708, rs115686767, rs200072135, and rs2070923 with male infertility susceptibility; (2) case–control studies on human beings that the cases were infertile patients with idiopathic infertility and including all subtypes (mainly azaospermia, cryptozoospermia, and oligozoospermia) and the controls were fertile; (3) including the details of genotype or allele frequency of cases and controls; (4) studies with complete full-text, and (5) studies with every language, (6) studies with or without deviation from the Hardy–Weinberg equilibrium (HWE) in controls. The exclusion criteria included (1) studies not concerning the association between PRM polymorphisms mentioned above and male infertility susceptibility; (2) animal articles, review studies, meta-analyses, and conference papers or editorial articles; (3) duplicate studies; and (4) studies with irrelevant data.

Data extraction and verification. The information retrieved from each study is mentioned in Tables 1, 2, and 3, including: (I) the first author’s name, (II) publication year, (III) region of origin and ethnicity, (IV) genotyping methods, (V) number of both cases and controls, (VI) HWE in the controls, (VII) control sources, and (VIII) prevalence of genotypes and alleles. Two authors independently extracted all the data of the studies included in the meta-analysis. In the case of disagreement between the two authors, another author resolved the disagreement by review and discussion.

Statistical analysis. The evaluation of the strength of association between PRM1 and PRM2 polymorphisms and male infertility risk was performed by odds ratio (OR) and 95% confidence interval (CI). Review
Table 1. Main characteristics of all studies entered to the meta-analysis. PCR Polymerase chain reaction, RFLP restriction fragment length polymorphism, SSCP single-strand conformation polymorphism, HB hospital-based, PB population-based.

| First author, publication year | Country | Ethnicity | No. of patients to controls | Method | Control source |
|-------------------------------|---------|-----------|----------------------------|--------|----------------|
| Tanaka, 2003^24               | Japan   | Asian     | 226/270                    | PCR sequence | PB              |
| Aoki, 2006^12                 | USA     | Mixed     | 192/96                     | PCR sequence | HB              |
| Ravel, 2007^26                | France  | Caucasian | 281/111                    | PCR-RFLP and sequence | PB              |
| Gazquez, 2008^27              | Spain   | Caucasian | 220/101                    | PCR-RFLP and sequence | PB              |
| Imklen, 2009^28               | Morocco | Caucasian | 135/160                    | PCR sequence | PB              |
| Tuttelmann, 2010^29           | Germany | Caucasian | 171/77                     | PCR sequence | PB              |
| Jodar, 2011^25                | Spain and Sweden | Caucasian | 156/102 and 53/50 | PCR sequence | HB              |
| Venkatesh, 2011^18            | India   | Caucasian | 100/100                    | PCR sequence | PB              |
| Grasetti, 2012^21             | Italy   | Caucasian | 110/53                     | PCR sequence | HB              |
| He, 2012^22                   | China   | Asian     | 304/369                    | Mass ARRAY  | HB              |
| Stasi, 2012^23                | Iran    | Caucasian | 96/100                     | PCR-RFLP, PCR-SSCP and PCR sequencing | HB              |
| Yu, 2012^24                   | China   | Asian     | 157/37                     | Mass ARRAY  | HB              |
| Jamali, 2016^25               | Iran    | Caucasian | 130/130                    | PCR-RFLP    | PB              |
| Jiang, 2017^26                | China   | Asian     | 636/442                    | Mass ARRAY  | HB              |
| Aydos, 2018^27                | Turkey  | Caucasian | 100/100                    | PCR        | HB              |
| Nabi, 2018^28                 | Iran    | Caucasian | 100/100                    | PCR sequence | HB              |
| Dehghanpour, 2019^39          | Iran    | Caucasian | 65/65                      | PCR sequence | HB              |

Manager 5.3 software was applied to calculate the summary ORs based on five genetic models (allele, heterozygous, homozygous, recessive, and dominant). In this state, the statistical significance of pooled results was illustrated with the Z-test. P-value < 0.05 was considered statistically significant. In addition, heterogeneity across the studies was estimated by the Chi-square-based Q test^26. If the P or $P_{heterogeneity}$ was > 0.10 and heterogeneity or I^2 < 50%, showing lack of heterogeneity between studies, we should use the fixed-effects model, but conversely, we used the random-effects model^21.

The thirteen polymorphisms were assessed for the association with susceptibility to male infertility based on five genetic models. Among them, four polymorphisms were included in the meta-analysis: PRM1 (rs737008 and rs2301365) and PRM2 (rs1646022 and rs2070923). The prevalence rates of GG (wild-type homozygote), CA (heterozygote), and AA genotype (rare homozygote) were calculated for PRM1 rs737008, PRM1 rs2301365, and PRM2 rs2070923 polymorphisms. Further, the GG (wild-type homozygote), GC (heterozygote), and CC (rare homozygote) were calculated for PRM2 rs1646022 polymorphism. Subgroup analyses were further performed based on ethnicity, method, and control source. A sensitivity analysis was conducted in which the studies with deviation from HWE in the controls were deleted. A meta-regression analysis was performed to detect the confounding factors affecting the pooled results by IBM SPSS 22.0 software. In addition, sensitivity analyses, including “one remove study” and “cumulative analysis”, were conducted each time on previous analyses to determine the stability of the pooled results. Funnel plots and Egger’s liner regression test were used to examine the publication bias. The funnel plot analysis and sensitivity analysis were done by Comprehensive Meta-analysis 2.0 software.

Results

Out of 261 records retrieved in the databases, 25 articles including full-texts were evaluated for eligibility after excluding the duplicates and irrelevant records (Fig. 1). Among these full-texts, 7 of them were excluded with reasons (2 meta-analyses, 2 reviews, 1 animal study, and 2 studies with no control groups). Therefore, 18 studies were included in the systematic review, from which one study^22 was excluded because it did not include four eligible polymorphisms. Finally, 17 studies including four polymorphisms of PRM1 rs737008, PRM1 rs2301365, PRM2 rs1646022, and PRM2 rs2070923 were analyzed in the meta-analysis. One study^23 checked the rs737008 and rs2301365 polymorphisms in two different populations (13 for polymorphism of PRM1 rs737008, 10 for PRM1 rs2301365, 9 for PRM2 rs1646022, and 8 for PRM2 rs2070923).

Table 1 presentations the features of studies entered to the meta-analysis. The studies^3–9 were published from 2003 to 2019. Twelve studies^25–31,33,35,37–39 were reported in Caucasian, four studies^24,32,34,36 in Asian, and one^26 in mixed ethnicities. The genotyping method was PCR-based in fourteen studies^23–31,33,35,37–39 and Mass ARRAY in three studies^32,34,36. The source of controls was hospital-based in ten studies^25,31–33,34,36–39 and population-based in seven studies^24,26–30,35.

Tables 2 and 3 show the prevalence of the genotypes and alleles of PRM1 and PRM2 polymorphisms. We included four polymorphisms (PRM1 rs737008, PRM1 rs2301365, PRM2 rs1646022, and PRM2 rs2070923) in the meta-analysis mentioned in Table 2. The other polymorphisms mentioned (PRM1 rs35262993, rs140477029, rs5576928, and rs193922261 polymorphisms) and PRM2 rs779337774, rs545828790, rs201933708, rs115686767, PRM1 rs140477029, rs5576928, and rs193922261 polymorphisms and PRM2 rs779337774, rs545828790, rs201933708, rs115686767, PRM1 rs140477029, rs5576928, and rs193922261 polymorphisms and PRM2 rs779337774, rs545828790, rs201933708, rs115686767,
and rs200072135 polymorphisms) in Table 3 were excluded from the meta-analysis because a lot of studies had no mutation or the percentage of mutation was very low. The P-values of HWE were less than 0.05 for the controls of PRM1 rs737008 polymorphism in two studies30,33, PRM2 rs1646022 in six studies25,29,30,36,38,39, and PRM2 rs2070923 in four studies25,30,32,38.

The pooled results of PRM1 rs737008 polymorphism based on five genetic models are illustrated in Fig. 2. The pooled results as OR (95%CI; P-value) showed 0.96 (0.87, 1.06; 0.44) with I² = 44% (Pheterogeneity or Ph = 0.04), 1.04 (0.84, 1.30; 0.70) with I² = 19% (Ph = 0.25), 0.94 (0.79, 1.12; 0.51) with I² = 35% (Ph = 0.10), 0.94 (0.80, 1.11; 0.48) with I² = 39% (Ph = 0.07), and 1.03 (0.87, 1.21; 0.72) with I² = 7% (Ph = 0.37) in the allele, homozygous, heterozygous, recessive, and dominant models, respectively. Based on the results, this polymorphism was not associated with the male infertility susceptibility.

Table 2. Prevalence of genotypes and alleles of PRM1 and PRM2 polymorphisms. HWE Hardy–Weinberg equilibrium. *P-values of HWE for control group. The study of Jodar et al.17 included two studies.
The pooled results of \( PRM1 \) rs2301365 polymorphism based on five genetic models are indicated in Fig. 3. The pooled results as OR (95% CI; \( P \)-value) showed the 1.67 (1.24, 2.25; 0.0007) with \( I^2 = 82\% \) (\( Ph < 0.00001 \)), 1.73 (0.98, 3.04; 0.06) with \( I^2 = 50\% \) (\( Ph = 0.03 \)), 1.50 (1.12, 2.00; 0.007) with \( I^2 = 70\% \) (\( Ph = 0.004 \)), 1.56 (1.15, 2.10; 0.004) with \( I^2 = 74\% \) (\( Ph < 0.0001 \)), and 1.62 (0.61, 4.29; 0.33) with \( I^2 = 83\% \) (\( Ph < 0.00001 \)) in the allele, homozygous, heterozygous, recessive, and dominant models, respectively. Based on the results, C allele and CA genotype of \( PRM1 \) rs2301365 polymorphism were associated with the elevated risk of male infertility.

The pooled results of \( PRM2 \) rs1646022 polymorphism based on five genetic models are shown in Fig. 4. The pooled results as OR (95% CI; \( P \)-value) showed the 1.19 (1.06, 1.34; 0.004) with \( I^2 = 44\% \) (\( Ph = 0.08 \)), 1.15 (0.91, 1.48; 0.26) with \( I^2 = 31\% \) (\( Ph = 0.17 \)), 1.08 (0.74, 1.56; 0.70) with \( I^2 = 68\% \) (\( Ph = 0.002 \)), 1.05 (0.77, 1.43; 0.76) with \( I^2 = 60\% \) (\( Ph = 0.010 \)), and 0.98 (0.82, 1.17; 0.82) with \( I^2 = 0\% \) (\( Ph = 0.54 \)) in the allele, homozygous, heterozygous, recessive, and dominant models, respectively.

### Table 3. Prevalence of genotypes and alleles of other \( PRM1 \) and \( PRM2 \) polymorphisms. The study of Jodar et al.\(^{17} \) included two studies.

| First author, publication year | PRM1 polymorphism | Case | Control | Case | Control |
|------------------------------|-------------------|------|---------|------|---------|
| Aoki, 2006\(^{25} \) | rs35262993 | 189 | 3 | 0 | 94 | 2 | 0 | 381 | 3 | 190 | 2 |
| Ravel, 2007\(^{26} \) | rs35262993 | 111 | 0 | 0 | 281 | 0 | 0 | 222 | 0 | 562 | 0 |
| Imken, 2009\(^{28} \) | rs35262993 | 133 | 2 | 0 | 155 | 5 | 0 | 315 | 5 | 271 | 2 |
| Tuttelmann, 2010\(^{29} \) | rs35262993 | 167 | 4 | 0 | 75 | 2 | 0 | 338 | 4 | 152 | 2 |
| Grassetti, 2012\(^{31} \) | rs35262993 | 109 | 1 | 0 | 53 | 0 | 0 | 106 | 1 | 119 | 0 |
| He, 2012\(^{32} \) | rs35262993 | 292 | 1 | 0 | 373 | 1 | 0 | 385 | 1 | 747 | 1 |
| First author, publication year | PRM2 polymorphism | Case | Control | Case | Control |
|------------------------------|-------------------|------|---------|------|---------|
| First author, publication year | PRM1 polymorphism | GG | GA | AA | GG | GA | AA | C | A | G | A |
| First author, publication year | PRM2 polymorphism | CC | CT | TT | CC | CT | TT | C | T | G | T |
| First author, publication year | PRM1 polymorphism | GG | GT | TT | GG | GT | TT | G | T | G | T |
| First author, publication year | PRM1 polymorphism | GG | GC | CC | GG | GC | CC | G | C | G | C |
| First author, publication year | PRM2 polymorphism | CC | CT | TT | CC | CT | TT | C | T | C | T |
| First author, publication year | PRM2 polymorphism | GG | GA | AA | GG | GA | AA | G | A | G | A |
| First author, publication year | PRM2 polymorphism | GG | GC | CC | GG | GC | CC | G | C | G | C |
| First author, publication year | PRM2 polymorphism | CC | CT | TT | CC | CT | TT | C | T | C | T |
| First author, publication year | PRM2 polymorphism | GG | GA | AA | GG | GA | AA | G | A | G | A |
| First author, publication year | PRM2 polymorphism | GG | GC | CC | GG | GC | CC | G | C | G | C |
| First author, publication year | PRM2 polymorphism | CC | CT | TT | CC | CT | TT | C | T | C | T |
| First author, publication year | PRM2 polymorphism | GG | GA | AA | GG | GA | AA | G | A | G | A |
recessive, and dominant models, respectively. Based on the results, the G allele of PRM2 rs1646022 polymorphism was associated with the elevated risk of male infertility.

The pooled results of PRM2 rs2070923 polymorphism based on five genetic models are demonstrated in Fig. 5. The pooled results as OR (95% CI; P-value) showed the 0.88 (0.78, 0.99; 0.04) with $I^2 = 1\%$ ($P_h = 0.43$), 0.84 (0.68, 1.04; 0.10) with $I^2 = 0\%$ ($P_h = 0.59$), 1.05 (0.71, 1.56; 0.81) with $I^2 = 63\%$ ($P_h = 0.009$), 0.90 (0.76, 1.07; 0.24) with $I^2 = 35\%$ ($P_h = 0.15$), and 0.80 (0.67, 0.97; 0.02) with $I^2 = 23\%$ ($P_h = 0.25$) in the allele, homozygous, heterozygous, recessive, and dominant models, respectively. Based on the results, the C allele and CC genotype of PRM2 rs2070923 polymorphism were associated with the reduced risk of male infertility.
Figure 2. Forest plot of analysis of PRM1 rs737008 polymorphism based on five genetic models.
Subgroup analysis. The results of subgroup analysis for PRM1 rs737008, PRM1 rs2301365, PRM2 rs2070923, and PRM2 rs1646022 polymorphisms are shown in Table 4. The AA + CA genotype in the studies with population-based controls was associated with the reduced risk of male infertility (OR 0.77; 95% CI 0.60,
**Figure 4.** Forest plot of analysis of PRM2 rs1646022 polymorphism based on five genetic models.
Figure 5. Forest plot of analysis of PRM2 rs2070923 polymorphism based on five genetic models.
0.98; \( P = 0.04 \)) without heterogeneity. With regard to \( PRM1 \) rs2301365 polymorphism, the C allele and CA genotype in the Caucasian ethnicity were associated with the elevated risk of male infertility (OR 1.96; 95% CI 1.29, 2.97; \( P = 0.002 \) and OR 1.79; 95% CI 1.13, 2.83; \( P = 0.01 \), respectively). Also, the C allele (OR 1.59; 95% CI 1.15, 2.20; \( P = 0.005 \)) and CC (OR 1.44; 95% CI 1.02, 2.03; \( P = 0.04 \)) and CA (OR 1.39; 95% CI 1.01, 1.92; \( P = 0.04 \)) genotypes in the studies with hospital-based controls were associated with the elevated risk of male infertility. For \( PRM1 \) rs2301365 polymorphism, the C allele and CA genotype in the studies with PCR-based method were associated with the elevated risk of male infertility (OR 1.96; 95% CI 1.29, 2.97; \( P = 0.002 \) and OR 1.79; 95% CI 1.13, 2.83; \( P = 0.01 \), respectively). About \( PRM2 \) rs2070923 polymorphism, the G allele had an elevated risk in male infertility compared to male fertility (OR 1.38; 95% CI 1.18, 1.63; \( P < 0.0001 \), which was similar to the G allele (OR 1.26; 95% CI 1.09, 1.46; \( P = 0.001 \)) and GG genotype (OR 1.43; 95% CI 1.06, 1.94; \( P = 0.02 \)) in the studies with hospital-based controls. With regard to mass ARRAY, the G allele (OR 1.49; 95% CI 1.23, 1.82; \( P < 0.0001 \)) and GG (OR 1.93; 95% CI 1.21, 3.08; \( P = 0.006 \)) and GC (OR 2.20; 95% CI 1.37, 3.56; \( P = 0.001 \)) genotypes had an elevated risk in male infertility compared to male fertility. As for \( PRM2 \) rs1646022 polymorphism, the CC genotype was associated with a reduced risk of male infertility (OR 0.69; 95% CI 0.51, 0.94; \( P = 0.02 \)) in the Caucasian ethnicity and C allele (OR 0.65; 95% CI 0.46, 0.93; \( P = 0.02 \)) in the mixed ethnicity. Further, the C allele (OR 0.86; 95% CI 0.74, 0.99; \( P = 0.04 \)) and CC genotype (OR 0.72; 95% CI 0.57, 0.92; \( P = 0.009 \)) in the PCR-based method had a reduced risk of male infertility.

**Meta-regression analysis.** The results of meta-regression analysis for four polymorphisms based on publication year are shown in Table 5. The publication year could be a confounding factor for \( PRM1 \) rs737008, \( PRM1 \) rs2301365, and \( PRM2 \) rs1646022 polymorphisms.

**Sensitivity analysis.** We excluded the studies with a deviation of HWE in the controls, including two studies25,36 for polymorphism of \( PRM1 \) rs737008, six25,29,30,36,39 for \( PRM2 \) rs1646022, and four25,29,30,38 for \( PRM2 \) rs2070923. The results after excluding are presented in Table 6. Moreover, the sensitivity analysis based on “one study removed” and “cumulative analysis” on the previous analyses did not change the results and therefore confirmed the stability of the pooled data.

**Publication bias.** The funnel plots of \( PRM1 \) and \( PRM2 \) polymorphisms based on five genetic models are shown in Figs. 6 and 7, respectively. As the results showed, Egger’s test revealed the publication bias for AA + CA vs. CC (\( P < 0.001 \)) and AA vs. CA + CC models (\( P = 0.04 \)) in \( PRM1 \) rs737008 polymorphism and C vs. G model (\( P = 0.016 \)) in \( PRM2 \) rs1646022 polymorphism. In addition, Begg’s test revealed the publication bias for AA + CA vs. CC (\( P = 0.001 \)) model in \( PRM1 \) rs737008 polymorphism, CA vs. CC (\( P = 0.025 \)) and AA + CA vs. CC models (\( P = 0.039 \)) in \( PRM1 \) rs2301365 polymorphism.

**Discussion**

There is considerable empirical evidence to suggest that PRMs are necessary for male infertility and that \( PRM1 \) and \( PRM2 \) have a fundamental role in sperm chromatin density and spermatogenesis40,41. Any single nucleotide polymorphism in the coding and non-coding areas of \( PRM1 \) and \( PRM2 \) genes may cause significant abnormalities in their expression9. The changes in one set of genes and expression patterns impact the spermatogenesis process and its products, resulting in spermatogenesis dysfunction and leading to male infertility42. Nowadays, the findings on the association of \( PRM \) genes with male infertility are not fully convincing, and there are not sufficient studies on this topic32. A research confirmed that the expression of PRMs is uniquely related to the transcription/translation factors43. The present meta-analysis showed that \( PRM1 \) rs737008 polymorphism was not associated with the risk of male infertility. \( PRM1 \) rs2301365 and \( PRM2 \) rs1646022 polymorphisms were associated with an elevated risk of male infertility and \( PRM2 \) rs2070923 polymorphism had a protective role in infertile men. In addition, the subgroup analysis showed the effect of ethnicity, control source, and genotyping method on the association of \( PRM \) polymorphisms with the risk of male infertility. The results of meta-regression showed that publication year was a confounding factor involved in the association between \( PRM1 \) rs737008, \( PRM1 \) rs2301365, and \( PRM2 \) rs1646022 polymorphisms and susceptibility to male infertility. Although single nucleotide polymorphism of G197T that lead to arginine to serine conversion was required in highly protected clusters of arginine for normal DNA binding has been found in 10% of unrelated infertile cases whose sperms were phenotypically same as those from mice with PRMN deficiency44. It has been shown that \( PRM1 \) and \( PRM2 \) variants are related to male infertility in both humans and animals25,26. In the animal model, reduction of \( PRM \) causes sperm morphology defects due to decreased motility and infertility as a result of haploid germ deficiency45–47. Using gene–gene interaction analysis, Jiang et al.36 examined twelve combined genotypes of \( PRM \) polymorphisms. Their results showed a significant association between the combined genotypes and male infertility. One study reported that sperm concentration, motility, and morphology significantly decreased in patients with an aberrant \( PRM \) ratio38. \( PRM \) protection is very important in mammals and minor alternations in the coding and non-coding regions of \( PRM \) genes may cause important abnormalities in the expression or maintenance of gene expression stability9. PRMs may act as a checkpoint for spermatogenesis, where abnormal \( PRM \) expression causes the induction of an apoptotic process that may explain the decrease in sperm production32. In addition, studies have shown that abnormal \( PRM \) expression is related to defective spermatogenesis32. There is some evidence that \( PRM \) mutations or polymorphisms may induce alternations at the protein level and their composition in sperm chromatin, resulting in sperm deficiency40,47. Semen quality decreases with age and characteristic molecular changes occur during aging (increased damage of sperm DNA, sperm infection changes, and plasma miRNA profile changes). In addition, the logistic regression models have illustrated an association between age and semen parameters49.
| Ethnicity  | Control source | Mass ARRAY (1) | PCR-based (7) | PRM2 rs2301365 | PB (5) | Associated Controls | PRM2 rs1646022 | Heterogeneity statistics |
|-----------|----------------|----------------|---------------|----------------|--------|---------------------|----------------|------------------------|
| Asian (2) | 0.96 (0.65, 1.43), 0.86, 78, 0.03 | 1.04 (0.43, 2.55), 0.93, 75, 0.04 | 0.90 (0.71, 1.15), 0.40, 30, 0.23 | 0.92 (0.61, 1.37), 0.67, 66, 0.09 | 1.10 (0.51, 2.38), 0.80, 68, 0.08 | 0.84 (0.68, 1.04), 0.10, 0.59 | 0.90 (0.76, 1.07), 0.24, 35, 0.09 | P = 0.02, I² = 36, Ph = 0.80 |
| Caucasian (10) | 0.96 (0.84, 1.09), 0.50, 47, 0.05 | 1.08 (0.82, 1.24), 0.60, 10, 0.35 | 1.04 (0.80, 1.34), 0.79, 47, 0.05 | 0.98 (0.78, 1.25), 0.89, 46, 0.06 | 1.01 (0.84, 1.23), 0.90, 5, 0.40 | 0.84 (0.68, 1.04), 0.10, 0.59 | 0.90 (0.76, 1.07), 0.24, 35, 0.09 | P = 0.02, I² = 36, Ph = 0.80 |
| Mixed (1) | 0.92 (0.68, 1.23), 0.57 | 0.74 (0.35, 1.59), 0.44 | 0.69 (0.32, 1.47), 0.34 | 0.71 (0.35, 1.46), 0.36 | 0.98 (0.60, 1.61), 0.93 | 0.84 (0.68, 1.04), 0.10, 0.59 | 0.90 (0.76, 1.07), 0.24, 35, 0.09 | P = 0.02, I² = 36, Ph = 0.80 |

Control source:

- HB (8)
- PB (5)
- Associated Controls

Method:

- PCR-based (12)
- Mass ARRAY (1)

Control source:

- HB (8)
- PB (2)
- Associated Controls

Method:

- PCR-based (7)
- Mass ARRAY (3)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)

Control source:

- HB (5)
- PB (4)
- Associated Controls

Method:

- PCR-based (9)
- Mass ARRAY (1)
As the present meta-analysis demonstrated, ethnicity, control source, and genotyping method of PRM polymorphisms are important and may contribute to the difference in susceptibility to male infertility. A meta-analysis reported an association between PRM1 rs2301365 polymorphism and the risk of male infertility, and PRM2 rs1646022 polymorphism only in Asians. In addition, there was significantly a decreased risk of PRM1 rs737008 in population-based controls, elevated risk of PRM2 rs1646022 in hospital-based controls. Also, with regards to method, an elevated risk of PRM2 rs1646022 in Mass ARRAY method and an increased risk of PRM2 rs1646022 in PCR-based method and an elevated risk of PRM2 rs1646022 in hospital-based controls. Thus, we can conclude that the expression of genes, environmental factors, and spermatogenesis disorder can play an important role in male sterility. Another possible reason for these findings could be the involvement of genetic factors in determining susceptibility to male infertility.
inconsistent findings can be a particular selection of the clinical subtypes of male infertility and PRM1 and PRM2 variations in different populations examined. Therefore, existence of heterogeneity among studies may be due to the differences genotyping method, clinical subtypes of male infertility, ethnicity, publication year, control source, and even number of recruited patients.

This meta-analysis had two significant limitations. First, the clinical data such as age, abstinence time, serum hormone index, and semen quality and parameters were not analyzed due to lack of information. Second, the meta-analysis did not evaluate the gene–gene and gene-environment interactions due to lack of information in the published studies.

Conclusions
The present meta-analysis evaluated four PRM polymorphisms (PRM1 rs737008, PRM1 rs2301365, PRM2 rs1646022, and PRM2 rs2070923). The results showed PRM1 rs2301365 and PRM2 rs1646022 polymorphisms were associated with an elevated risk of male infertility and PRM2 rs2070923 polymorphism had a protective role in infertile men. In addition, ethnicity, control source, and genotyping method impacted the PRM polymorphisms and susceptibility to male infertility. Based on the results, the future studies need to evaluate these
polymorphisms in a large number of participants in various areas, with an emphasis on environmental factors, interactions, age, method, and selection of controls (deviation of HWE and source).

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H.N. designed the study. M.S. analyzed the data and wrote the manuscript. M.N. and M.M. critically revised the work. All authors have approved the final content of the manuscript.

Competing interests
The authors declare no competing interests.

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