Open Access Full Text Article

Association of *P73*, *BMP15*, and *GDF9* gene polymorphisms with diminished ovarian reserve

Sina Ghasemifar[1], Seyed Mehdi Kalantar[1], Emad Babakhanzadeh[1,2], Seyed Hamidreza Mirabutalebi[1], Ali Khodadadian[1], Majid Nazari[1], Nasrin Ghasemi[1,3,*]

ABSTRACT

Introduction: Diminished ovarian reserve (DOR) is a condition in which the quantity or quality of oocytes results in impaired fertility. The prevalence of DOR in infertile women is estimated to be around 10%. Our aim was to investigate the polymorphisms rs3810682, rs10491279, and rs4648551 in association with the genes *BMP15*, *GDF9*, and *p73* in patients with DOR and control samples. **Methods**: Genomic DNA was isolated from 5 cc of peripheral blood from 300 participants (150 patients with DOR and 150 women without a history of DOR). After ARMS-PCR assay and sequencing of a series of samples to confirm results, data were analyzed using GraphPad Prism software. **Results**: A significant difference was found between the frequencies of the different polymorphisms associated with the *BMP15* gene (P value = 0.002). However, for the polymorphisms of the *GDF9* and *p73* genes, no significant difference was found between the frequencies of these polymorphisms in the control and patient groups. **Conclusion**: Determining the frequency of gene variants in case and control groups helps in understanding the potential risk factors for the development of DOR. Understanding these factors is very important in different populations that naturally have different lifestyles, especially in a country like Iran, where a large number of pregnancies occur at older ages due to the use of contraceptives. Determining risk factors can help women decide when to become pregnant. Finally, further validation is needed to obtain more information on the use of the polymorphisms investigated in this study as an indicator of DOR in Iranian populations.
Key words: Diminished ovarian reserve, BMP15, GDF9, and p73, polymorphism

[1]*Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

[2]*Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[3]*Abortion Research Center, Yazd Reproductive Sicences Institue, Shahid sadoughi University of Medical Sciences, Yazd, Iran*

Correspondence

Nasrin Ghasemi, Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abortion Research Center, Yazd Reproductive Sicences Institue, Shahid sadoughi University of Medical Sciences, Yazd, Iran

Email: nghasemi479@gmail.com

Check for updates

BioMedPress
The Open Access Publisher

INTRODUCTION

Diminished ovarian reserve (DOR) is a condition where the quantity or quality of oocytes leads to abnormal fertility [1]. DOR is clinically characterized by signs such as low levels of anti-Müllerian hormone (AMH) (< 0.5 – 1.1 ng/ml), low antral follicle count (AFC) (5–7 follicles), and elevated levels of follicle-stimulating hormone (FSH) ($\geq$ 10 mIU/ml) [2–5]. Aging is one of the main causes of DOR; smoking, and invasive treatments such as chemotherapy and radiotherapy are other causes. Genetic conditions such as fragile X syndrome, ovarian surgery, endometriosis, and autoimmune diseases are additional examples of causes of DOR [6,7]. Unfortunately, most women do not exhibit any symptoms of DOR. Only as the condition progresses over time do women notice a shortening of their menstrual cycles.

In general, there is no cure to delay or prevent DOR. Reportedly, 33% of patients with DOR are able to become pregnant with their own eggs after treatment [8,9]. However, reports emphasize that early diagnosis is important as it increases the chances of conception. In the meantime, the main treatment for DOR is to preserve the ability to conceive by stimulating the ovaries and using assisted reproductive techniques such as IVF [10,11]. Another treatment option for women who wish to delay pregnancy is the freezing of eggs and embryos. To treat DOR, medication is administered to stimulate the ovaries to grow eggs, which can then be frozen or used for IVF. Considering the difficulty and complexity of treating diminished ovarian reserve, it seems that studying genetic and epigenetic factors could shed more light on improving our current knowledge in this field.

GDF9 encodes a secreted ligand of the TGF-beta superfamily [12,13]. GDF9 is essential for the development of surrounding somatic cells, particularly granulosa, cumulus, and theca cells [14]. Paracrine interactions between the developing oocyte and the surrounding follicle cells are crucial for proper follicle and oocyte development. GDF9 is essential for the entire process of follicle maturation, ovulation, and egg maturation and thus plays a central role in female fertility [15]. GDF9 binds to the receptors for bone morphogenetic protein (BMPR) and ALK5 via two receptors on oocytes. It promotes the secretion of inhibin A, thus enhancing the ability of the follicle to develop from an early growth stage. GDF9 promotes the

growth of prenatal follicles by preventing apoptosis of granulosa cells[16–18]. In preovulatory follicles, GDF9 induces progesterone production by stimulating the prostaglandin EP2 receptor signaling pathway. Bone morphogenetic protein 15 (BMP-15), also part of the TGF-β family, is involved in folliculogenesis and is expressed only after the early stages of oocyte development[19]. The promotion of ovarian follicle growth and maturation in the early stages of gonadotropin-independent folliculogenesis, the regulation of granulosa cell sensitivity to FSH, and the prevention of granulosa cell apoptosis are important functions of BMP15[20]. In 2004, Hanrahan and colleagues discovered a mutation in the GDF9 gene that increases ovulation and infertility rates in a manner similar to inactivating mutations in BMP15[21]. In 2006, Morón *et al.* analyzed various SNPs in the BMP15 gene in 307 Spanish women treated with rFSH. The results showed a direct correlation between the increased number of follicles and various SNPs in the BMP15 gene in response to rFSH[22]. In 2016, Mehdizadeh *et al.* investigated the association of rs3810682 of the BMP15 gene with polycystic ovary syndrome (PCOS) in the Iranian population. The results showed that the genotype was heterozygous (CG) in 28% of women with PCOS and homozygous (GG) in 2.8%. In a 2017 paper by Peluso *et al.*, no association was found between the rs3810682 SNP of BMP15 and ovarian stimulation in Brazilian infertile women[23].

In 2019, Santos *et al.* investigated variants of the GDF9 and BMP15 genes in women with primary ovarian insufficiency (POI) and those with high FSH levels compared to the control group. The results showed that the frequency of CT and TT genotypes in BMP15:c.852C> T (rs17003221) was higher in the POI group than in the control group, whereas the analysis of GDF9 variants showed no significant difference in genotype distribution[24]. In 2021, Meireles *et al.* conducted a study to investigate the correlation between factors associated with follicular growth and reduced ovarian response in ovaries overstimulated for IVF, such as LHR, GDF9, FSHR, AMHR2, and BMP15 polymorphisms. The results suggest that the polymorphism in the GDF9 gene may be a double-edged sword that can have both a positive (C447T) and a negative effect (398-39 C> G) on ovarian reserve[25].

P73 is involved in cell cycle control and promotes apoptosis. Mouse models have shown that the *p73* gene is involved in female infertility[26]. In 2008, Richard Tomasini *et al.* reported that the suppression of p73 expression in mice can lead to infertility[27]. Their study showed that female mice carrying deletions of exons 2 and 3 of the p73 gene had normal fertility but ovaries with reduced growth capacity and follicle size. In a 2011 study by Feng *et al.* on women undergoing IVF, an association was found between a SNP (rs4648551, A> G) in the p73 gene and infertility. The results showed that infertile women over the age of 35 had the "G" allele[28]. In 2015, a study by Vagnini *et al.* in 605 Brazilian women showed that a polymorphism (rs4648551, A > G) in the p73 gene could be used as a potential marker for DOR[29]. In the present study, our aim was to investigate the polymorphisms rs3810682, rs10491279, and rs4648551 in relation to the genes *BMP15, GDF9,* and *p73* in patients with DOR.

METHODS

After explaining the general steps of the experiment to the participants (with ethics approval code: IR.SSU.MEDICINE.REC.1398.186), 5 cc of peripheral blood was collected from 300 participants (150 patients with DOR and 150 women without a history of DOR) referred to the Yazd Reproductive Sciences Research Institute between September 15, 2020, and April 11, 2021, and placed in falcons containing 1.8 mg/ml K_2 EDTA. Inclusion criteria included age under 35 years, normal karyotype, AMH < 2 μg/L, and no history of chemotherapy. Exclusion criteria were age over 35 years, abnormal karyotype (*e.g.*, fragile X syndrome), and AMH > 2 μg/L. The samples were stored at -20 degrees for DNA extraction.

Genomic DNA extraction from whole peripheral blood

DNA isolation from peripheral blood was performed using the SimEX kit (Cat.: SBL15-2136) according to the manufacturer's procedure. The concentration and purification of the DNA were tested with a spectrophotometer (Thermo Scientific, Wilmington, USA) and validated by 1% agarose gel electrophoresis.

ARMS-PCR

The ARMS-PCR assay was used to specifically amplify the sequences of interest in two tubes. A 100 ng DNA template and Amplicon Master Mix Kit as well as specific primers (**Table 1**) were used for amplification. The primers contained mismatches to maximize discrimination between wild-type and mutant alleles. The PCR settings were: primary denaturation at 95 °C for 1 minute, 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 45 seconds, and extension at 72 °C for 40 seconds. The last extension

was carried out at 72 °C for 10 minutes. The products were loaded onto a 2% agarose gel for 50 minutes at 110 V with a 100 bp ladder and imaged under ultraviolet light after staining with SYBR Safe DNA Gel Stain (Cat. 881603PR).

Statistical analysis

Following the ARMS-PCR assay and sequencing of a number of samples to confirm the results, statistical analysis was completed using the one-way ANOVA test followed by Dunnett's multiple comparison test. P values less than 0.05 were considered statistically significant. Statistical analysis was performed using GraphPad Prism 6 software.

RESULTS

After sampling, DNA extraction, and preparation of the PCR reaction, genotyping of samples for the *BMP15, GDF9*, and *p73* genes was performed by ARMS-PCR in two tubes, and several samples were sequenced to confirm the results.

BMP15 Gene Genotyping

The ARMS-PCR reaction was performed for all samples, and the results were observed and analyzed on agarose gel (2%). With respect to the rs3810682 polymorphism of *BMP15*, the primers were designed so that the length of the PCR product of the external control primer was 661 bp and the length of the internal primer (indicating the presence or absence of the polymorphism) was 396 bp. The results of the rs3810682 polymorphism gel electrophoresis are shown in **Figure 1 A**. This shows that in the first lane of each sample, the external control primer (product length = 661 bp) was used with the normal primer (acting with the wild-type nucleotide "C" in the samples), and in the second lane, the external control primer was used with the mutation-specific primer (G nucleotide). Therefore, 6 wells (3 samples) belonged to samples that were normally homozygous (C nucleotide), as only the lanes with the normal primer (product length = 396 bp) were used. The next four lanes (2 samples) belonged to heterozygous samples, as both normal and mutant-specific primers resulted in a PCR product. The next four wells also belonged to mutant homozygous samples. The last two wells were negative control samples for normal and mutant-specific primers. In the next step (to confirm the gel electrophoresis results), a series of samples were sequenced, and the sequencing result of this polymorphism is also shown in **Figure 2A** and **B**.

GDF9 Gene Genotyping

For the rs10491279 polymorphism of GDF9, the primers were designed so that the PCR product length of the external control primer was 368 bp, and the length of the internal primer (indicating the presence or absence of the polymorphism) was 236 bp. The gel electrophoresis results of the rs10491279 polymorphism are shown in **Figure 1 B**, where the external control primer (product length = 368 bp) with the normal primer (acting with the wild-type nucleotide "C" in the samples) was used in the first lane of each sample, and the external control primer with the mutation-specific primer (nucleotide G) was used in the second lane. Consequently, 6 wells (3 samples) belonged to samples that were normally homozygous (C nucleotide), as only the lanes were linked to the normal primer (product length = 236 bp). The next four lanes (2 samples) belonged to heterozygous samples, as both normal and mutant-specific primers resulted in a PCR product. The next four wells also belonged to mutant homozygous samples. The last two wells were negative control samples for normal and mutant-specific primers. In the next step (to confirm the gel electrophoresis results), a series of samples were sequenced, and the sequencing result of this polymorphism is also shown in **Figure 2C** and **D**.
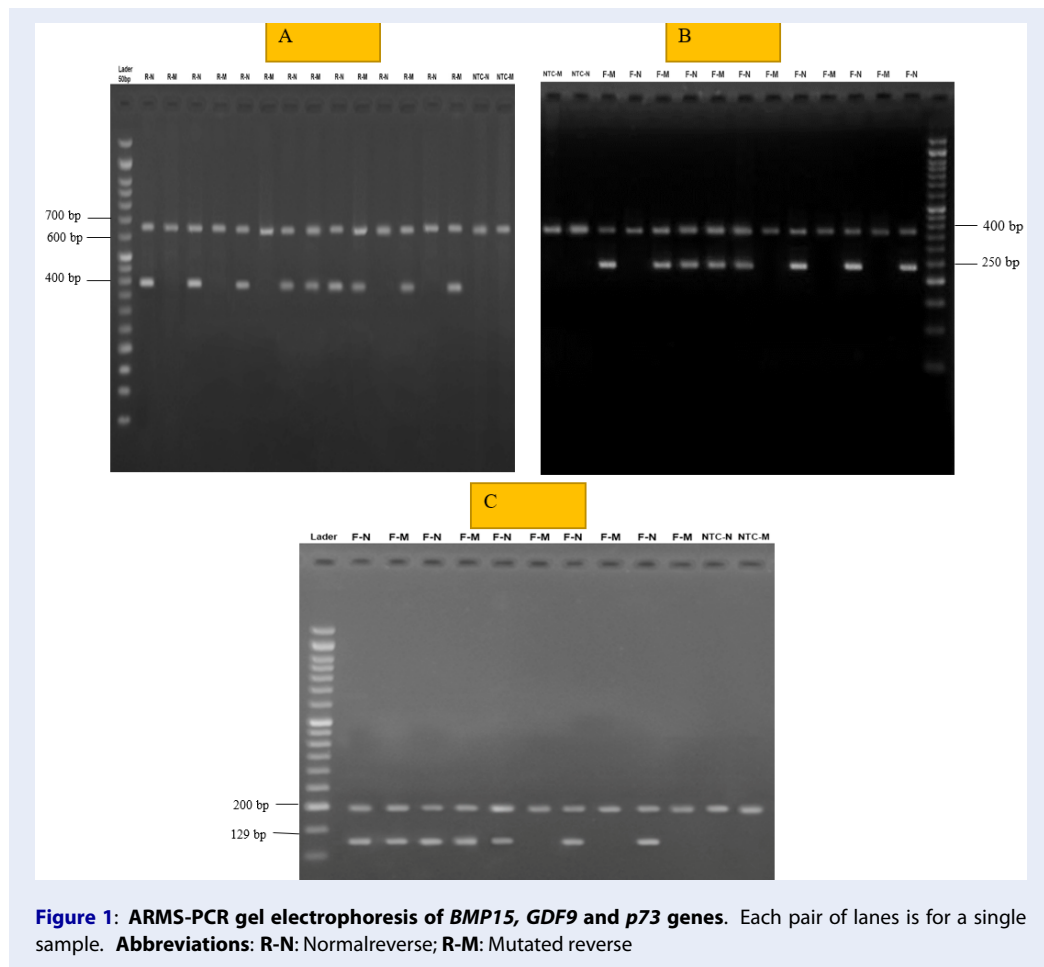
p73 Gene Genotyping

For the rs4648551 polymorphism associated with the *p73* gene, the primers were designed so that the PCR product length of the external control primer was 200 bp, and the length of the internal primer (indicating the presence or absence of the polymorphism) was 129 bp. The gel electrophoresis results of the rs4648551 polymorphism are shown in **Figure 1C**. In the first lane of each sample, the external control primer (product length: 200 bp) was used with the normal primer (acting with the wild-type nucleotide "G" in the samples), and in the second lane, the external control primer was used with the mutation-specific primer (A nucleotide). Thus, 4 wells (2 samples) belonged to samples that were normally heterozygous, as both normal and mutation-specific primers resulted in a PCR product. Similarly, the next 6 lanes (3 samples) belonged to normal homozygotes (G nucleotide), as only the lanes with the normal primer (product length: 129 bp) were used. The next four wells also belonged to the mutant homozygous specimens. The last two wells were negative control samples for normal and mutant-specific primers. In the next step (to confirm the gel electrophoresis results), a series of samples were sequenced, and the sequencing result of this polymorphism is also shown in **Figure 2E** and **F**.

Table 1: Sequence of primers used in the study

Gene	Primers Sequence (5'-3')	Product (bp)
BMP15	F (Ex): AGCCATACTCAGTAGTTTCC	Ex: 661
	F (In): TAGGGGCAAGTAGGATCAGG	In: 396
	R (N): AGGAGGACCATCTTGAAAGG	
	R (M): AGGAGGACCATCTTGAAAGC	
GDF9	F (M): AGTCCTGCTAGAAGACTTTGGT	Ex: 368
	F (N): AGTCCTGCTAGAAGACTTTGGC	In: 236
	R (In): TTGACTTGACTGCCTGTTGTG	
	R (Ex): CAGTAAGGTTGCTGGGAATT	
P73	F (N): TCCGTCAGGGCTGAGGATCG	Ex: 200
	F (M): TCCGTCAGGGCTGAGGATCA	In: 129
	R (In): GGAGACAGATGTGTCTGCTGGC	
	R (Ex): GCCAGTGCCAGGCCCAGCGC	

N: Normal; **M**; Mutant; **Ex**: External; **In**: Internal

Figure 1: **ARMS-PCR gel electrophoresis of *BMP15, GDF9* and *p73* genes**. Each pair of lanes is for a single sample. **Abbreviations**: **R-N**: Normalreverse; **R-M**: Mutated reverse
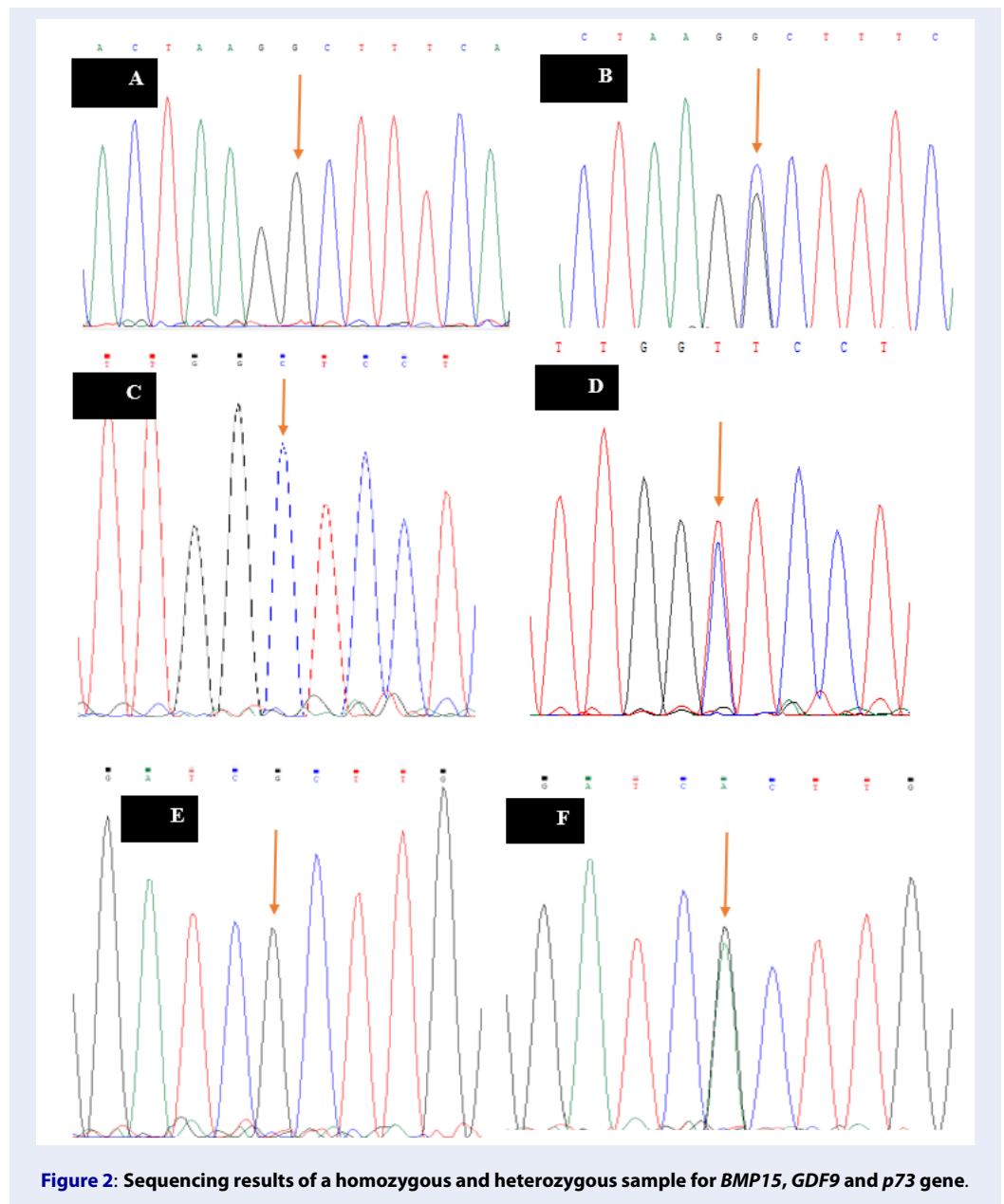
Figure 2: **Sequencing results of a homozygous and heterozygous sample for *BMP15, GDF9* and *p73* gene**.

DISCUSSION

The reason for DOR could be attributed to many genetic changes and is still under investigation. According to Olsen *et al.*, various causes of DOR could lead to a reduced follicle number or a defect in the follicle-stimulating mechanisms. Regarding the rs3810682 polymorphism in BMP15, the results of the present experiment were not consistent with the results reported in some other studies, including the study by Gonzalez *et al.* and the study by Sproul *et al.*; however, it should be noted that both studies were performed in patients with polycystic ovary syndrome[30,31]. In contrast, the study by Moron *et al.* found a statistically significant association between the high response to ovarian stimulation and BMP15 polymorphism (rs3810682), confirming the results of the present study. Decreased BMP15 levels or activity in the ovary may lead to increased FSHR expression in the granulosa cells, which could result in ovarian hyperstimulation syndrome (OHSS)[32]. In a study, Fonseca *et al.* showed that the rs3810682 in BMP15 increases the incidence of premature ovarian failure, which is due to the large number of follicles that are utilized during a woman's lifetime. A study

using transgenic mice overexpressing the *Bmp15* gene showed that wild-type and transgenic mice produced the same number of primary follicles, but immature transgenic mice had more atretic follicles than wild-type mice[33]. In general, in mice with transgenic *Bmp15* expression, follicle growth was greater, atresia increased, and FSHR mRNA levels decreased[34]. This indicates that BMP15 increases follicle growth but not follicle maturation, which ultimately has a negative effect on ovarian reserve[35].

The study by Hanevik *et al.* found a positive correlation between rs3810682 and OHSS[36]. In the present study, an inverse relationship was observed between AMH levels and the rs3810682 polymorphism; AMH levels were lower in homozygous individuals than in normal individuals. This finding contradicted the results of the study by Peluso *et al.* In the results of the study by Peluso *et al.*, it was reported that AMH levels were higher in patients with the rs3810682 polymorphism than in normal individuals[23]. In general, polymorphisms of the *BMP15* gene, especially those occurring in the promoter region, can have a significant impact on gene expression and gene function. In our study, only one of the best-known BMP15-related polymorphisms was investigated, and since BMP15 also contains other important polymorphisms, we cannot assess the effects of the entire BMP15 on infertility based on the information obtained in this study. In this work, we investigated the rs3810682 polymorphism of *BMP15*, the rs10491279 polymorphism of *GDF9*, and the rs4648551 polymorphism of *P73* in women with DOR and normal women. Individuals with elevated FSH levels and decreased AMH levels are potential candidates for DOR. The results of the DOR group were compared with the results of the control group to represent potential risk factors for DOR in the study population and to identify women at higher risk. Galloway *et al.* conducted a study in sheep with mutations in *BMP15*. In this study, a significant increase in ovulation and the birth rate of twins and triplets was observed in heterozygous models. In addition, there was ovarian failure in homozygous sheep, which could be due to incomplete follicular growth. In data from previous studies, Dixit *et al.* have shown that the three variants c.-9C> G, c.308A> G, and c.852C> T of BMP15 are associated with DOR[37]. In the case of the c.-9C> G polymorphism, Leidig *et al.* found no correlation between this polymorphism and DOR[38]. Ma *et al.* also found that the frequency of the C allele varied in the case group, and Dixit *et al.* also confirmed the association between this polymorphism and ovarian failure[39]. In the current study, analysis of BMP15 genotypes and

alleles showed that CT and TT genotypes for BMP15: c.852 C> T were higher in the patient groups than in the control groups.

In this study, no significant difference in the frequency of the rs10491279 polymorphism was found between patients and the control group with respect to GDF9. Furthermore, no association between the polymorphism studied and DOR was found in previous studies. In 2011, Chand *et al.* conducted a study on 3616 individuals with normal menopause to investigate the association between 23 single nucleotide changes in five genes— *AMH, AMHR2, BMP15, FOXL2,* and *GDF9*—and menopause and evaluated rs3897937 in *BMP15*, the rs3810682 polymorphism being similar to our study[40]. In addition, they calculated six different polymorphisms related to GDF9, including rs10491279, rs30177, rs803224, rs11748063, and rs4705974, with the rs10491279 polymorphism being similar to the present study. This research team's analysis also showed no significant association between GDF9 changes and age at menopause, but rs6521896 in *BMP15* was associated with late menopause.

Members of the p53 family are involved in many vital processes that contribute to the regulation of the cell cycle and apoptosis in response to DNA damage[41]. In addition, the p53 family has been defined as a regulatory factor for vital processes related to human reproduction[42]. Interpretations have shown that p73 plays an important role in maintaining follicle pool size and ovulation rate, as well as at checkpoints, and influences egg quality. Recent experiments have shown that numerous polymorphisms in this gene may play an essential role in human reproduction. In the current study, the association between the rs4648551 polymorphism and ovarian reserve in Iranian women with DOR was investigated. Since the *p73* gene is related to fertility, small changes in its function could lead to individual changes in ovarian reserve and could be potential targets for reproductive diseases. However, little is known about how this polymorphism acts in female fertility. Our results showed no significant association between DOR and different genotypes of the rs4648551 polymorphism related to the *p73* gene. A study conducted by Feng *et al.* investigated the association between this polymorphism and infertile patients aged 35 years, and the results indicated a significant association between infertility and polymorphism. Tomasini *et al.* had previously demonstrated a link between this gene and the number of follicles in mice. In contrast to the two studies mentioned, the present study shows that the

rs4648551 polymorphism is not significantly associated with the DOR pattern.

Comprehensive genomic sequencing and GWAS studies can reveal several variations associated with DOR. Understanding these factors is essential in different populations that naturally have different lifestyles, and especially in a country like Iran where a large number of pregnancies occur at older ages due to contraceptive use. Determining risk factors can help women decide whether to become pregnant. Finally, further validation is needed to obtain more information on the use of the polymorphisms investigated in this study as an indicator of DOR in Iranian populations.

CONCLUSION

One of the limitations of this work is the small number of samples, which led to difficulties in the selection of cases and controls. Another limitation was the reason and method for selecting the polymorphisms of each gene, which must be such that both an innovative aspect is present and sufficient information about it was available in previous studies. The difference between the results of the current survey and the results of the different populations indicates the different ethnic composition of the Iranian population, especially the population in the Yazd urban area. There are large inter-ethnic and indigenous differences in Iran. Therefore, caution should be exercised when using international models to assign risk factors. One of the limitations of the present experiment is the number of participants, which may be increased regardless of the larger population in other demographic studies, and more informative studies may confirm the results reported in the present study with greater certainty. It is possible that other changes in the genes examined are associated with DOR but were not examined in the present study.

ABBREVIATIONS

AFC: Antral Follicle Count, **AMH**: Anti-Müllerian Hormone, **AMHR2**: Anti-Müllerian Hormone Receptor Type 2, **ARMS**: Amplification Refractory Mutation System, **BMP15**: Bone Morphogenetic Protein 15, **DOR**: Diminished Ovarian Reserve, **FSH**: Follicle-Stimulating Hormone, **FSHR**: Follicle-Stimulating Hormone Receptor, **GDF9**: Growth Differentiation Factor 9, **GWAS**: Genome-Wide Association Studies, **IVF**: In Vitro Fertilization, **LHR**: Luteinizing Hormone Receptor, **OHSS**: Ovarian Hyperstimulation Syndrome, **PCR**: Polymerase Chain Reaction, **POI**: Primary Ovarian Insufficiency, **P73**: p73 gene, **RS**: RefSeq (e.g., rs3810682), **SNP**: Single Nucleotide Polymorphism, **TGF-β**: Transforming Growth Factor Beta

AUTHOR'S CONTRIBUTIONS

S.G, A.K and E.B performing the main steps of essay, writing the manuscript and statistical tests. S.K, S.M and M.N Collecting the samples and helping to perform DNA extraction and ARMS technique. N.G. Head of team and monitoring and fixing technical errors during all steps of the study. The authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval code: IR.SSU.MEDICINE.REC.1398.186.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Busnelli A, Somigliana E, Cirillo F, Levi-Setti PE. Is diminished ovarian reserve a risk factor for miscarriage? Results of a systematic review and meta-analysis. Human Reproduction Update. 2021;27(6):973–88. PMID: 34254138. Available from: https://doi.org/10.1093/humupd/dmab018.

2. Fàbregues F, Ferreri J, Méndez M, Calafell JM, Otero J, Farré R. In vitro follicular activation and stem cell therapy as a novel treatment strategies in diminished ovarian reserve and primary ovarian insufficiency. Frontiers in Endocrinology (Lausanne). 2021;11:617704. PMID: 33716954. Available from: https://doi.org/10.3389/fendo.2020.617704.

3. Huang P, Zhou Y, Tang W, Ren C, Jiang A, Wang X, et al. Long-term treatment of Nicotinamide mononucleotide improved age-related diminished ovary reserve through enhancing the mitophagy level of granulosa cells in mice. The Journal of Nutritional Biochemistry. 2022;101:108911. PMID: 34801690. Available from: https://doi.org/10.1016/j.jnutbio.2021.108911.

4. Olsen KW, Castillo-Fernandez J, Chan AC, la Cour Freiesleben N, Zedeler A, Bungum M. Identification of a unique epigenetic profile in women with diminished ovarian reserve. Fertility and Sterility. 2021;115(3):732–41. PMID: 33272626. Available from: https://doi.org/10.1016/j.fertnstert.2020.09.009.

5. Jahangir M, Nazari M, Babakhanzadeh E, Manshadi SD. Where do obesity and male infertility collide? BMC Medical Genomics. 2024;17(1):128. PMID: 38730451. Available from: https://doi.org/10.1186/s12920-024-01897-5.

6. Hoseini SH, Enayati P, Nazari M, Babakhanzadeh E, Rastgoo M, Sohrabi NB. Biomarker Profile of Colorectal Cancer: Current Findings and Future Perspective. Journal of Gastrointestinal Cancer. 2024;55(2):497–510. PMID: 38168859. Available from: https://doi.org/10.1007/s12029-023-00990-9.

7. Alipourfard I, Khorshidian A, Babakhanzadeh E, Nazari M. Susceptibility to azoospermia by haplotype analysis of protamine 1 and protamine 2 variants. Human Gene. 2023;37:201200. Available from: https://doi.org/10.1016/j.humgen.2023.201200.

8. Fan Y, Chang Y, Wei L, Chen J, Li J, Goldsmith S. Apoptosis of mural granulosa cells is increased in women with diminished ovarian reserve. Journal of Assisted Reproduction and Genetics. 2019;36(6):1225–35. PMID: 30980221. Available from: https://doi.org/10.1007/s10815-019-01446-5.

9. Nazari M, Khorshidian A, Alizadeh S, Falahati AM, Haghparast A, Ghasemifar S, et al. Association between peroxisome proliferator activated receptor gamma coactivator 1 gene with overweight and obesity risk: casestudy and meta-analysis. Human Gene. 2022;34:201123. Available from: https://doi.org/10.1016/j.humgen.2022.201123.

10. Qin JC, Fan L, Qin AP. The effect of dehydroepiandrosterone (DHEA) supplementation on women with diminished ovarian reserve (DOR) in IVF cycle: evidence from a meta-analysis. Journal of Gynecology Obstetrics and Human Reproduction. 2017;46(1):1–7. PMID: 28403950. Available from: https://doi.org/10.1016/j.jgyn.2016.01.002.

11. Babakhanzadeh E, Danaei H, Abedinzadeh M, Ashrafzadeh HR, Ghasemi N. Association of miR-146a and miR196a2 genotype with susceptibility to idiopathic recurrent pregnancy loss in Iranian women: A case-control study. International Journal of Reproductive Biomedicine. 2021;19(8):725–32. PMID: 34568733. Available from: https://doi.org/10.18502/ijrm.v19i8.9620.

12. Belli M, Shimasaki S. Molecular aspects and clinical relevance of GDF9 and BMP15 in ovarian function. Vitamins and Hormones. 2018;107:317–48. PMID: 29544636. Available from: https://doi.org/10.1016/bs.vh.2017.12.003.

13. Khodadadian A, Varghaiyan Y, Babakhanzadeh E, Alipourfard I, Haghi-Daredeh S, Ghobadi A. Fertility preservation in women with ovarian cancer: Finding new pathways: A case-control study. International Journal of Reproductive Biomedicine. 2021;19(2):157–66. PMID: 33718760. Available from: https://doi.org/10.18502/ijrm.v19i2.8474.

14. Liu MN, Zhang K, Xu TM. The role of BMP15 and GDF9 in the pathogenesis of primary ovarian insufficiency. Human Fertility (Cambridge, England). 2021;24(5):325–32. PMID: 31607184. Available from: https://doi.org/10.1080/14647273.2019.1672107.

15. Alam MH, Lee J, Miyano T. GDF9 and BMP15 induce development of antrum-like structures by bovine granulosa cells without oocytes. The Journal of Reproduction and Development. 2018;64(5):423–31. PMID: 30033985. Available from: https://doi.org/10.1262/jrd.2018-078.

16. Mottershead DG, Sugimura S, Al-Musawi SL, Li JJ, Richani D, White MA. Cumulin, an oocyte-secreted heterodimer of the transforming growth factor-β family, is a potent activator of granulosa cells and improves oocyte quality. The Journal of Biological Chemistry. 2015;290(39):24007–20. PMID: 26254468. Available from: https://doi.org/10.1074/jbc.M115.671487.

17. Heath DA, Pitman JL, McNatty KP. Molecular forms of ruminant BMP15 and GDF9 and putative interactions with receptors. Reproduction (Cambridge, England). 2017;154(4):521–

34. PMID: 28733348. Available from: https://doi.org/10.1530/REP-17-0188.

18. Nazari M, Babakhanzadeh E, Zarch SMA, Talebi M, Narimani N, Dargahi M. Upregulation of the RNF8 gene can predict the presence of sperm in azoospermic individuals. Clinical and Experimental Reproductive Medicine. 2020;47(1):61–7. PMID: 32146775. Available from: https://doi.org/10.5653/cerm.2019.03111.

19. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. Molecular Reproduction and Development. 2011;78(1):9–21. PMID: 21226076. Available from: https://doi.org/10.1002/mrd.21265.

20. Moore RK, Shimasaki S. Molecular biology and physiological role of the oocyte factor, BMP-15. Molecular and Cellular Endocrinology. 2005;234(1-2):67–73. PMID: 15836954. Available from: https://doi.org/10.1016/j.mce.2004.10.012.

21. Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). Biology of Reproduction. 2004;70(4):900–9. PMID: 14627550. Available from: https://doi.org/10.1095/biolreprod.103.023093.

22. Morón FJ, de Castro F, Royo JL, Montoro L, Mira E, Sáez ME. Bone morphogenetic protein 15 (BMP15) alleles predict over-response to recombinant follicle stimulation hormone and iatrogenic ovarian hyperstimulation syndrome (OHSS). Pharmacogenetics and Genomics. 2006;16(7):485–95. PMID: 16788381. Available from: https://doi.org/10.1097/01.fpc.0000215073.44589.96.

23. Peluso C, Goldman C, Cavalcanti V, Gastaldo G, Trevisan CM, Christofolini DM. Use of bone morphogenetic protein 15 polymorphisms to predict ovarian stimulation outcomes in infertile Brazilian women. Genetic Testing and Molecular Biomarkers. 2017;21(5):328–33. PMID: 28410456. Available from: https://doi.org/10.1089/gtmb.2016.0074.

24. Santos M, Cordts EB, Peluso C, Dornas M, Neto FH, Bianco B. Association of BMP15 and GDF9 variants to premature ovarian insufficiency. Journal of Assisted Reproduction and Genetics. 2019;36(10):2163–9. PMID: 31392662. Available from: https://doi.org/10.1007/s10815-019-01548-0.

25. Meireles AJ, Bilibio JP, Lorenzzoni PL, Conto E, Nascimento FC, Cunha-Filho JS. Association of FSHR, LH, LHR, BMP15, GDF9, AMH, and AMHR polymorphisms with poor ovarian response in patients undergoing in vitro fertilization. JBRA Assisted Reproduction. 2021;25(3):439–46. PMID: 33739800. Available from: https://doi.org/10.5935/1518-0557.20210004.

26. Rozenberg JM, Zvereva S, Dalina A, Blatov I, Zubarev I, Luppov D. The p53 family member p73 in the regulation of cell stress response. Biology Direct. 2021;16(1):23. PMID: 34749806. Available from: https://doi.org/10.1186/s13062-021-00307-5.

27. Rosenbluth JM, Pietenpol JA. mTOR regulates autophagy-associated genes downstream of p73. Autophagy. 2009;5(1):114–6. PMID: 19001857. Available from: https://doi.org/10.4161/auto.5.1.7294.

28. Abed F, Novin MG, Omrani MD, Bandehpour M, Norozian M, Hosseini A. Evaluation the Effect of Female Age Factor on Human Oocyte Aneuploidy and Its Relation to P73 Gene Expression. Journal of Applied Environmental and Biological Sciences. 2015;5(4):216–21.

29. Vagnini LD, Renzi A, Oliveira-Pelegrin GR, Canas MC, Petersen CG, Mauri AL, et al. The TP73 gene polymorphism (rs4648551, A>G) is associated with diminished ovarian reserve. PLoS One. 2015;10(3):e0120048. PMID: 25794170. Available from: https://doi.org/10.1371/journal.pone.0120048.

30. Gónzalez A, Ramírez-Lorca R, Calatayud C, Mendoza N, Ruiz A, Sáez ME. Association of genetic markers within the BMP15 gene with anovulation and infertility in women with polycystic ovary syndrome. Fertility and Sterility. 2008;90(2):447–9. PMID: 17905236. Available from: https://doi.org/10.1016/j.fertnstert.2007.06.083.

31. Sproul K, Jones MR, Mathur R, Azziz R, Goodarzi MO. Association study of four key folliculogenesis genes in polycystic ovary syndrome. BJOG. 2010;117(6):756–60. PMID: 20236105. Available from: https://doi.org/10.1111/j.1471-0528.2010.02527.x.

32. Santiquet NW, Greene AF, Becker J, Barfield JP, Schoolcraft WB, Krisher RL. A pre-in vitro maturation medium containing cumulus oocyte complex ligand-receptor signaling molecules maintains meiotic arrest, supports the cumulus oocyte complex and improves oocyte developmental competence. MHR: Basic science of reproductive medicine. 2017;23(9):594–606. Available from: https://doi.org/10.1093/molehr/gax032.

33. Fonseca-Mendoza DJ, Ortega-Recalde O, Esteban-Perez C, Moreno-Ortiz H, Patiño LC, Bermúdez OM, et al. BMP15 c.-9C> G promoter sequence variant may contribute to the cause of non-syndromic premature ovarian failure. Reproductive BioMedicine Online. 2014;29(5):627–33. Available from: https://doi.org/10.1016/j.rbmo.2014.07.01.

34. Otsuka F, Inagaki K. Unique bioactivities of bone morphogenetic proteins in regulation of reproductive endocrine functions. Reproductive Medicine and Biology. 2011;10(3):131–42. PMID: 29662354. Available from: https://doi.org/10.1007/s12522-011-0082-9.

35. Kirkpatrick BW, Morris CA. A major gene for bovine ovulation rate. PLoS One. 2015;10(6):e0129025. PMID: 26046917. Available from: https://doi.org/10.1371/journal.pone.0129025.

36. Hanevik HI, Hilmarsen HT, Skjelbred CF, Tanbo T, Kahn JA. A single nucleotide polymorphism in BMP15 is associated with high response to ovarian stimulation. Reproductive Biomedicine Online. 2011;23(1):97–104. PMID: 21565556. Available from: https://doi.org/10.1016/j.rbmo.2011.02.015.

37. Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, Jokiranta TS. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nature Genetics. 2000;25(3):279–83. PMID: 10888873. Available from: https://doi.org/10.1038/77033.

38. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Human Reproduction Update. 2015;21(6):787–808. PMID: 26243799. Available from: https://doi.org/10.1093/humupd/dmv036.

39. Tiotiu D, Mercadal BA, Imbert R, Verbist J, Demeestere I, Leener AD. Variants of the BMP15 gene in a cohort of patients with premature ovarian failure. Human Reproduction (Oxford, England). 2010;25(6):1581–7. PMID: 20364024. Available from: https://doi.org/10.1093/humrep/deq073.

40. Zhang P, Shi YH, Wang LC, Chen ZJ. Sequence variants in exons of the BMP-15 gene in Chinese patients with premature ovarian failure. Acta Obstetricia et Gynecologica Scandinavica. 2007;86(5):585–9. PMID: 17464588. Available from: https://doi.org/10.1080/00016340701269492.

41. Tabibzadeh S. Signaling pathways and effectors of aging. Frontiers in Bioscience (Landmark Edition). 2021;26(1):50–96. PMID: 33049665. Available from: https://doi.org/10.2741/4889.

42. Kang HJ, Rosenwaks Z. p53 and reproduction. Fertility and Sterility. 2018;109(1):39–43. PMID: 29307398. Available from: https://doi.org/10.1016/j.fertnstert.2017.11.026.