



## Original article

## Two novel mutations in exon 2 of bone morphogenetic protein (BMP) 15 gene in Pakistani infertile females

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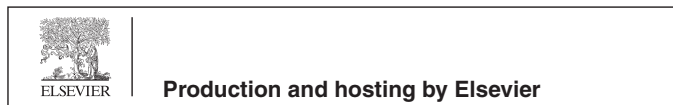
## ABSTRACT

**Objective:** To determine the proportion of fertility in Pakistani infertile females and discover if there are considerable connection among *BMP15* gene polymorphism, follicle maturation and hormonal regulation in Pakistani infertile females.**Methods:** All selected participants were initially examined through follicle-stimulating hormones (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), Prolactin, and Trans-vaginal scan (TVS). *BMP15* gene polymorphism among infertile and fertile females was done by extracted Genomic DNA from whole blood. Sanger sequencing was performed for the identification of mutation in exons-intron boundaries of the *BMP15* gene. Bioinformatics tools were used to assess the protein structure.**Results:** The total five mutations including two novel missense variants of *BMP15* in exon 2, whereas three previously reported i.e. two cosmic mutations (c.615delC), (c.584InsG) and one frame shift mutations (p.346Gln < Gly) in which the insertion of GG at DNA position 1038 of exon 2 resulting in a substitution of glutamine into glycine at 346th amino acid of BMP15 protein. The second novel variant (c.1049delT) (p. Ser334Pro) was also observed in exon 2 of the *BMP15* gene, which substituted serine into proline at 334th amino acid of the BMP15 protein.**Conclusion:** It is concluded that there are various missense mutations present in exon 2 of the *BMP15* gene of Pakistani infertile females, consequently expected function of protein changes due to change in codons of amino acids. Provean and SIFT suggest the two novel variants as potentially deleterious. Although three other variants were also found in Pakistani infertile females which were previously reported. These mutations may result in early blockage of folliculogenesis and ovaries become streaky. Further research is required to resolve the actual allusion of these variations in the *BMP15* gene.© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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## 1. Introduction

Infertility is a noticeable and most important fitness problem of the world due to countless reasons such as an abnormal function of testes and ovaries, maternal age, excess weight, an abnormal number of chromosomes, genetic and epigenetic mutations, polycystic ovarian syndrome, cystic fibrosis, and premature ovarian failure. Several genetic features from both males and females are also involved in the reproduction process (Zorrilla and Yatsenko 2013). Gene mutation is also a reason for infertility because it always creates a problem in the pubertal development of a female, hence she has to face the deficiency of pituitary hormones or disturbing the ovarian functions. Consequently, the patient suffers from hypogonadotropic hypogonadism, a situation of missing or

lacking puberty due, their blood serum also show less amount of reproductive hormones such as follicle-stimulating hormone (FSH) or luteinizing hormone (LH) (Badar et al. 2018). These are two main hormones for a female reproductive function that assist the ovaries in regulating their function by the mutual action of the most important ovarian partners such as activins, inhibins, growth differentiation factor 9 (GDF9), and bone morphogenetic protein 15 (BMP15) (Dixit et al. 2010). *BMP15* gene helps in follicle growth and continues to grow up the primary gonadotrophin-independent phases of folliculogenesis, this regulates the production of follicular granulosa cell (GC) and also protect the GC from the apoptosis process, and support oocyte maturity proficiency (Persani et al. 2014).

For the normal reproduction process, it is crucial that *BMP15* and *GDF9* genes function properly; an abnormal function of these two important genes can exhibit premature ovarian failure (POF), an ovarian deficiency in which ovaries become weakened to produce mature follicles in young females (Kumar et al. 2017). *BMP15* gene has been revealed to play its role to excite the growth of granulosa cells and regulate the sequence of folliculogenesis in combination with the FSH dependent stage (Galloway et al. 2000; Chang et al. 2002; Moore and Shimasaki 2005; Crawford et al. 2011; Kumar et al. 2017). For many years scientists are exploring the possible participation of the *BMP15* gene in POI (Di Pasquale et al. 2004; Tiotiu et al. 2010). Bone morphogenetic protein (BMP) 15 gene (locus Xp11.2) determined in 1998 (Dube et al. 1998) is considered as an important part of the transforming growth factor (TGF)- $\beta$  super family which helps in growth and differentiation of granulosa cells (GCs), which gives essential support to the oocyte for upcoming healthy embryo/ fetal growth (Gilchrist et al. 2008; Sugimura et al. 2014). *BMP15* protein is composed of an N-terminal prodomain and a C-terminal mature domain. In the event of *BMP15* molecule composition, the prodomain caused to folds and make dimmers of the mature growth factor (Shi et al. 2011). Later *BMP15* protein is split into several parts by the furin enzyme-like proteases; this enzyme was released from the oocyte which is non-covalently attached with its prodomain. Normally *BMP15* lacks the cysteine particles which helps to make an intermolecular disulfide bond, and that's why function as a non-covalent dimer (Mottershead et al. 2013). Due to prodomain dislocation, human BMP (hBMP)15 connects to a group of gene-like type I (ALK6) and type II (BMPRII) receptors on the outside of GCs.

The cause of mutation in the *BMP15* gene and the relation of the gene with female infertility has still obscure, might be because of changes among contests and likely investigation inaccuracy. Until now, no report was published in Pakistani women on SNPs within the *BMP15* gene. Therefore, this investigation was attempted to take a step ahead to resolve this problem and was intended to examine variations in the exons of bone morphogenetic protein (BMP) 15 gene in Pakistani infertile females.

## 2. Material and methods

### 2.1. Subjects

A total of 160 (110 cases and 50 control) individuals were analyzed. All obese infertile females who were aged between 18 and 40 years, suffering from disturbed hormones having amenorrhea were included whereas the females above the age of 40 years who were pregnant, had nephritis, cardiac and hypertensive conditions were excluded in this study. After identification of the patient, written consent was taken from all participants and their family partners and parents/guardians. An authenticated questionnaire had been prepared to save the information related to the causes of infertility, a period of infertility, diet, age, *trans*-vaginal

scan (TVS) and lifestyles, besides anthropometric measurements. Clinical investigations for hormones; FSH, LH, TSH, and Prolactin, and genetic polymorphism of the *BMP15* gene of blood samples was carried out. The ethics research committee and board of research and graduate studies of University of Sindh, Jamshoro, approved this study.

### 2.2. Blood sample collection

For hormonal investigation and DNA extraction, 6.0 ml venous blood was drawn from selected infertile and fertile females and stored as 3.0 ml in 50 ml falcon tubes containing 0.2 ml Ethylene diamine tetra acetic acid (EDTA) as anticoagulant for DNA extraction whereas 3.0 ml of blood was collected in plain tubes and serum was separated for hormonal assays.

### 2.3. Hormones analysis

The hormones (FSH, LH, TSH, and Prolactin) concentrations in serum samples were determined in duplicate using the Cobas e411 immunoassay analyzer. The immunoassay sandwich principle technique was used for about 18 min. Results were obtained by a standard curve, which is instrument-specifically generated by 2-point calibration and a master curve provided through the reagent barcode on Elecsys 2010 MODULAR ANALYTICS E170.

### 2.4. Amplification of *BMP15* gene and sequencing

Genomic DNA was extracted from whole blood with the genomic DNA preparation kit (Jena Bioscience, Germany). The polymerase chain reaction (PCR) of the *BMP 15* gene was performed using the extracted genomic DNA with designed primers and PCR master mix in Eppendorf gradient thermo cycler as described by Mullis et al. (Mullis et al. 1986). PCR was performed in a 25  $\mu$ l reaction volume containing 18.2  $\mu$ l deionized distilled water, 2.5  $\mu$ l of 10X Buffer, 1.5  $\mu$ l of 25 mM MgCl<sub>2</sub>, 0.5  $\mu$ l of 2.5 mM dNTPs mixture, 0.5  $\mu$ l of each forward and reverse primers, 0.3  $\mu$ l Taq polymerase (5U/ $\mu$ L), and 1  $\mu$ l sample DNA. The designed sequences of primers of *BMP15* gene exon 2 were F; 5'GGTCTGGAATAACAAGGGAC3', R; 5'CCACCACTGATTGATAAGG 3' with amplified product size 468 bp (Al-ajoury et al. 2015).

The cycling program was set as follows; initial denaturation at 94 °C for 4 min followed by 35cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 secs, and extension at 72 °C for 1 min and the final extension was done at 72 °C for 8 min. The PCR products were resolved by the electrophoresis with 2.5% agarose. PCR products were observed under ultraviolet *trans*-illumination in Gel Documentation equipment (Bio-Rad).

For direct mutation screening of PCR products of designated *BMP15* gene exon 2, automated DNA Sequencer (ABI Genetic Analyzer) following Sanger's dideoxy chain termination method was used. Sequence alignment report was generated by chromas software and online BLAST sequence analyzer.

### 2.5. Statistical analysis

The statistical analysis was carried out by SPSS 20.0 (SPSS Inc., Chicago, IL., USA). On the whole distributed data were articulated as mean  $\pm$  SD, categorical variables are expressed as percentage, relative frequency. Provean (<http://provean.icvi.org/index.php>) and SIFT (software implement fault tolerance) software were used to find out the possible harmful consequences of the amino acid alterations. Provean software calculates whether an amino acid substitution or deletion has any contact with the natural function of a protein (Choi 2012). The SIFT software check the effects of

altered nucleotide bases in amino acid codons on protein function (Ng and Henikoff 2003).

### 3. Results

In the present study, *BMP15* gene was analyzed to check the variants concerning infertility in 110 infertile females and 50 control fertile females. The PCR and DNA sequencing was performed for all exon–intron boundaries of the *BMP15* gene of infertile females. Results showed that 13.6% of infertile females were carrying different variants in the exon 2 of the *BMP15* gene, resulting in nucleotide and amino acid changes in cDNA and protein respectively (Table 1).

The direct sequencing of exon 2 and exon–intron boundaries of *BMP15* gene in 110 infertile females revealed five mutations including two novel missense variants. The first novel mutation was found at (c.1038InsGG)(p.346Gln < Gly) in which the insertion of GG at DNA position 1038 of exon 2 resulting in a substitution of glutamine into glycine at 346th amino acid of BMP15 protein (Table 1, Fig. 1D). The score certified by the Provean software was deleterious (PROVEAN score is  $-4.172$  and the cutoff value is  $-2.5$ ). The second novel variant (c.1049delT)(p. Ser334Pro) was also observed in exon 2 of the *BMP15* gene, which substituted serine into proline at 334th amino acid of the BMP15 protein (Table 1, Fig. 1E). This *BMP15* gene variants acquire the potentially deleterious effect on female fertility, was confirmed via bioinformatics tool SIFT and PROVEAN (Provean score is  $-4.681$ ), mentioned in Table 1. Three previously reported mutations in *BMP15* gene exon 2 were also found in studied cases of infertile females including two were cosmic mutations and one was frame shift mutation. The previously studied frame shift variant (c.635delA)(p.212Gln < Lys)(Q212K), resulting in substitution of glutamine into lysine was observed in six affected (infertile) females which was confirmed deleterious by provean and SIFT with provean score  $-1.015$  (Table 1, Fig. 1C). On the other hand, the cosmic mutation (c.584InsG)(p.W195W) was found in two infertile females (Table 1, Fig. 1B) in which insertion of G was observed and there was no change of amino acid occurred with the non-pathogenic effect of this variant confirmed through the bioinformatics tool Provean, however, the pathogenic effect of this variant is low with a neutral value as calculated via bioinformatics tool Provean. Another cosmic mutation (c.615delC)(p.L205L) deletion of C occurred in the *BMP15* gene at nucleotide position 615, hence no amino acid change at this position but provean score is  $-8.350$  showing the deleterious effect of this previously reported mutation (Table 1, Fig. 1A).

According to Table 2, the total of 15 infertile, out of 110 females was carrying different gene mutations. All the females were near to 30 or above 30 years of their age, the menstrual cycle was also found disturbed or need to induce by medicines, hormonal secretion and follicle size were measured significantly lower, all three hormones, FSH, LH and Prolactin were found abnormal in range the comparison of infertile hormones and follicle with control females were shown in Table 3, according to present study all

control females were mostly have normal hormonal secretion and their TVS were also shown their mature follicle at 14 day of cycle (Table 3). All the infertile females were frequently eating junk food, broiler chicken, meat, the follicles of these females were also measured either multiple small size (polycystic) or immature in growth through TVS ultrasound (Fig. 2, B & C, Table 2). These findings indicate that an improper diet might be one of the reason for disturbed hormonal secretion and an immature follicle in the infertile females. Changes in amino acid codon can cause hormonal disturbance due to which follicles do not reach their desired size and also affect the menstrual cycle. TVS Fig. 2D is showing fully grown mature fertile follicle, ready to fertilize with sperm. The follicle average size is (4 cm).

### 4. Discussion

Infertility may occur due to various health issues. In this study we have demonstrated that most of the female were having irregular menstrual cycle (41.8%) and the trans vaginal scan (TVS) showed that majority of patients (86.4%) have immature small size follicles ranged from 0.5 cm to 1.2 cm which fail to achieve either ovulation or fertilization as compared to control group. It was also confirmed by two other studies that the follicles those are either too small or too large are not considered as mature oocytes and also not suitable for fertilization (Revelli et al. 2014; Karavani et al. 2019). Shimasaki et al. (2004) and Hashimoto et al. (2005) also mentioned the role of *BMP15* gene in follicle development as it play an important role in folliculogenesis and GC (granulosa cell) activities, the gene important function can be highlighted as under; a) this gene helps to promote maturity of follicles from the primary stage of follicles development to final folliculogenesis step; b) instruct follicular GC to react on FSH action; c) help to stop GC apoptosis; d) enhance oocyte growth ability; and e) regulates the process of ovulation.

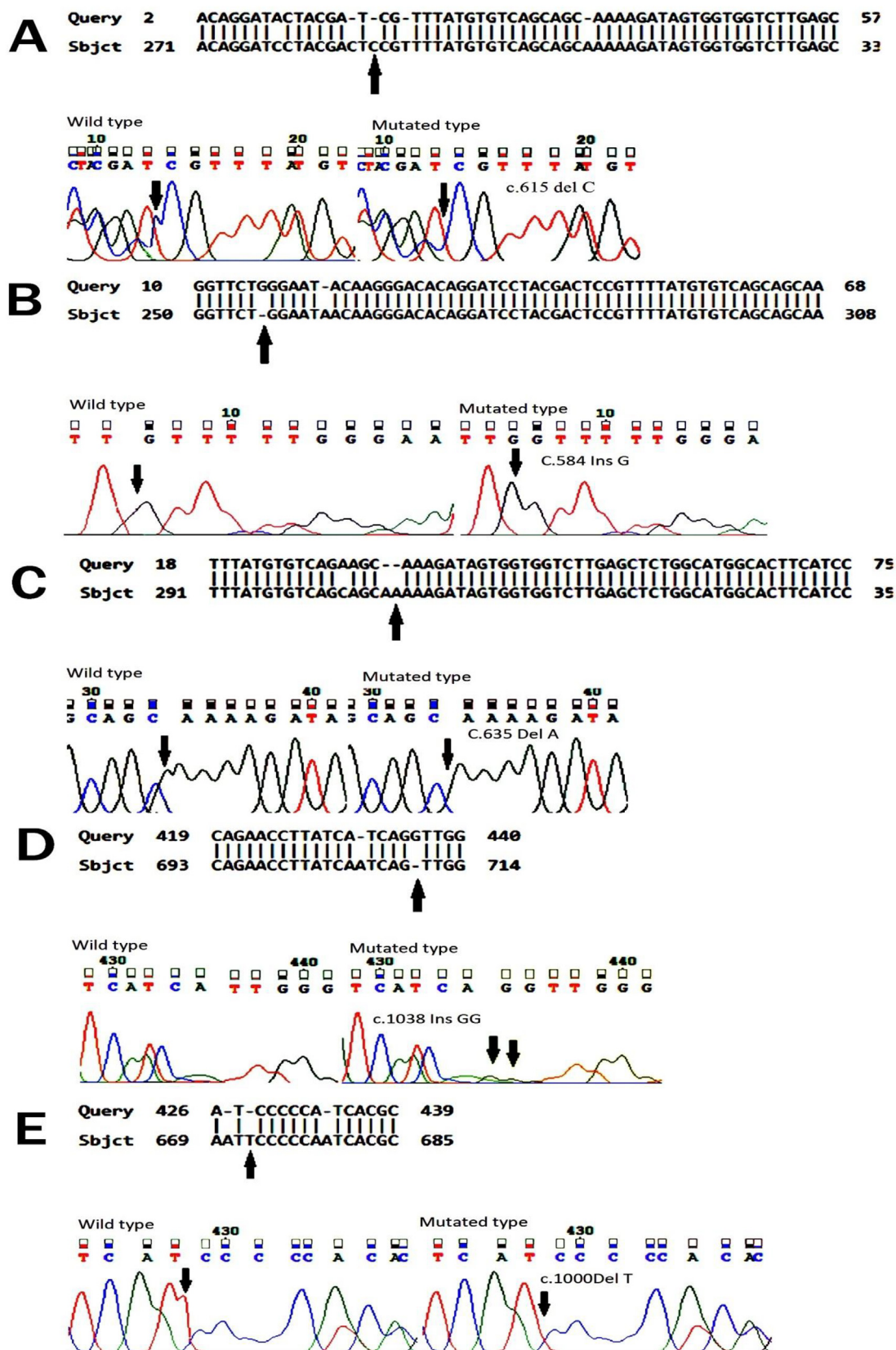
Liu's research specified that FSH concentration increase with increasing age, which confirms the ovarian dysfunction and female does not able to conceive (Liu et al. 2015). It is reported that normal level of FSH is generally 10–13 mIU/ml if FSH level drops down, it decreases pregnancy rates (Anwar and Anwar 2016). The results of Table 2 demonstrated that many of the infertile females were showing normal range of reproductive hormones according to the standard reference value, even they were unable to conceive due to follicles immaturity because of *BMP15* gene mutation, (Sudha and Reddy 2013) have also the same opinion. Since last 20 years it has been accepted that older age women face more problem in conception as compare to younger age women, as ability of reproduction decreases, the efficiency of ovary decreases, sexual craving reduces and chromosomal abnormalities and miscarriage chances increases with increasing age (Collins et al. 2005; Abic and Yilmaz 2019).

Many studies have been conducted in order to check the connection between *BMP15* gene variants and infertility, not in human but also in animals as well. As it has been proved by many

**Table 1**  
BMP 15 gene exon 2 variants showing mutations identified in the present study.

| S. no | Gene  | Exon | Nucleotide Variant | Protein Variant | Sift        | Provean     |
|-------|-------|------|--------------------|-----------------|-------------|-------------|
| 1     | BMP15 | 2    | c.615delC          | p.L205L         | Deleterious | Deleterious |
| 2     | BMP15 | 2    | c.584InsG          | p.W195W         | Neutral     | Neutral     |
| 3     | BMP15 | 2    | c.635delA          | p.Q212K         | Deleterious | Deleterious |
| 4     | BMP15 | 2    | c.1038InsGG        | p.Q346G         | Deleterious | Deleterious |
| 5     | BMP15 | 2    | c.1000delT         | p.S334P         | Deleterious | Deleterious |

The intron–exon boundaries of *BMP15* gene was screened through sanger sequencing of all the participants of the study. Total five mutations were observed in exon 2 of *BMP15* gene, two were newly identified missense variants c.1038InsGG(p.Q246G) and c.1000delT (p.S334P) and three were reported variants. Silico analysis was performed on novel variants which declared to be deleterious effect on phenotype of individual.



**Fig. 1.** A Sequence chromatogram of affected individual in which deletion of C is taking place but no change into amino acid leucine remain same L205L. Protein structure in Fig. 1A shows mutation with deleterious effect by SIFT and provean. Fig. 1B. Sequence chromatogram of affected individual in which insertion of G is taking place but no change into amino acid tryptophan remain same W195W protein fig shows mutation with neutral effect by SIFT and provean. Fig. 1C. Sequence chromatogram of affected individual into which deletion of Adinine. Protein Model via SWISS-MODEL bioinformatics tool showing the glutamine change into lysine at the position of 212. Fig. 1D. Sequence chromatogram of the affected individual in which insertion of GG, change the amino acid glutamine to glycine at 346 positions. Protein model via SWISS-MODEL, showing the change of amino acids of glutamine to glycine at the position of 346. Fig. 1E. Sequence chromatogram of individual in which deletion of T is mentioned which alters the amino acid Serine into Proline at the position of 334. Protein models via SWISS-MODEL in that amino acids serine change into proline at the position of 334.

**Table 2**  
The changes in hormonal levels and follicle size in the infertile females showing *BMP15* gene exon 2 variants.

| Sr. no | P. ID | <i>BMP15</i> variants | Age | Menstrual cycle | Hormonal level (In follicular phase) |           |                 | Diet           | Follicle size (cm) |
|--------|-------|-----------------------|-----|-----------------|--------------------------------------|-----------|-----------------|----------------|--------------------|
|        |       |                       |     |                 | FSH mIU/ml                           | LH mIU/ml | Prolactin ng/ml |                |                    |
| 1.     | 2 s   |                       | 35  | Regular         | 5.21                                 | 15.67     | 0.858           | Veg + meat     | 0.8                |
| 2.     | 40 s  | 615del C              | 29  | Irregular       | 4.51                                 | 12.43     | 20.46           | Chi + meat     | 1.2                |
| 3.     | 16 s  |                       | 33  | Irregular       | 6.83                                 | 10.45     | 19.12           | veg + chi      | 1.0                |
| 4.     | 17 s  |                       | 29  | Regular         | 4.21                                 | 8.63      | 40.16           | Chi + meat     | Multi 0.5          |
| 5.     | 11 s  | 584InsG               | 30  | Regular         | 8.43                                 | 2.46      | 21.22           | Veg + chi      | Multi 0.5          |
| 6.     | 20 s  |                       | 35  | Irregular       | 5.65                                 | 18.84     | 24.16           | Veg + chi      | 1.0                |
| 7.     | 33 s  |                       | 30  | Regular         | 10.67                                | 7.84      | 10.14           | Chi + meat     | 0.9                |
| 8.     | 41 s  |                       | 29  | Regular         | 8.78                                 | 2.86      | 20.34           | Veg + meat     | 1.4                |
| 9.     | 10 s  | 635delA               | 31  | Regular         | 9.34                                 | 4.67      | 23.45           | Chi + meat     | Less than 0.5      |
| 10.    | 12 s  |                       | 29  | Induce          | 7.45                                 | 3.21      | 0.986           | Chi mostly     | PCO                |
| 11.    | 6 s   |                       | 30  | Regular         | 6.83                                 | 15.48     | 19.18           | Veg + chi      | 1.3                |
| 12.    | 7 s   | 1038Ins GG            | 33  | Induce          | 7.21                                 | 9.73      | 20.76           | Chi + meat     | Less than 0.5      |
| 13.    | 19 s  |                       | 34  | Irregular       | 4.21                                 | 14.67     | 23.32           | Chi + meat     | PCO                |
| 14.    | 27 s  |                       | 30  | Irregular       | 6.21                                 | 5.38      | 23.35           | veg + meat     | 1.2                |
| 15.    | 21 s  | 1000delT              | 35  | Irregular       | 6.56                                 | 12.43     | 18.67           | J. food + meat | PCO                |

Results described the information about those infertile females who got the genetic polymorphism in exon 2 of *BMP15* gene. Total 15 individual's *bmp15* gene was found mutated out of 110 infertile females. All 15 infertile females were about to or cross 30 years, they were mostly facing irregular menstrual cycle or need to induce after a long interval, their reproductive hormones were not in normal. These females were frequently eating junk food, chicken and meat and they were not producing mature follicle as their trans vaginal scan showed.

**Table 3**  
Comparison of menstrual regularity, hormonal secretion and follicle size between control and infertile female.

| Sr. no | Attributes (Hormones) | Groups     | Control Freq. (50) | Control % | P-value | Chi -sq | Infertile Freq. (110) | Infertile % | P-value | Chi -sq |
|--------|-----------------------|------------|--------------------|-----------|---------|---------|-----------------------|-------------|---------|---------|
| 1.     | Periods               | Regular    | 50                 | 100       | 0.000   | 100     | 64                    | 58.2        | 0.086   | 2.94545 |
|        |                       | Irregular  | 0                  | 0         |         |         | 46                    | 41.8        |         |         |
| 2.     | FSH                   | Normal     | 45                 | 90        | 0.001   | 11.428  | 33                    | 30          | 0.021   | 7.76364 |
|        |                       | Borderline | 00                 | 00        |         |         | 50                    | 45.5        |         |         |
|        |                       | Abnormal   | 05                 | 10        |         |         | 27                    | 24.5        |         |         |
| 3.     | LH                    | Normal     | 50                 | 100       | 0.000   | 16.667  | 36                    | 32.7        | 0.371   | 1.98182 |
|        |                       | Borderline | 00                 | 00        |         |         | 43                    | 39.1        |         |         |
|        |                       | Abnormal   | 00                 | 00        |         |         | 31                    | 28.2        |         |         |
| 4.     | Prolactin             | Normal     | 41                 | 82        | 0.005   | 7.757   | 63                    | 57.3        | 0.000   | 29.0364 |
|        |                       | Borderline | 3                  | 6         |         |         | 27                    | 24.5        |         |         |
|        |                       | Abnormal   | 6                  | 12        |         |         | 20                    | 18.2        |         |         |
| 5.     | Follicle size         | Normal     | 50                 | 100       | 0.000   | 100     | 15                    | 13.6        | 0.000   | 58.1818 |
|        |                       | Abnormal   | 00                 | 00        |         |         | 95                    | 86.4        |         |         |

Showing the comparison between control and infertile females in accordance of periods regularity, normal hormonal secretion and follicle size. Results shows the significant differences in all parameters in control whereas P-value shows the non significant differences in periods regularity, FSH and LH secretion in infertile females, although prolactin and follicle size shows significant results.

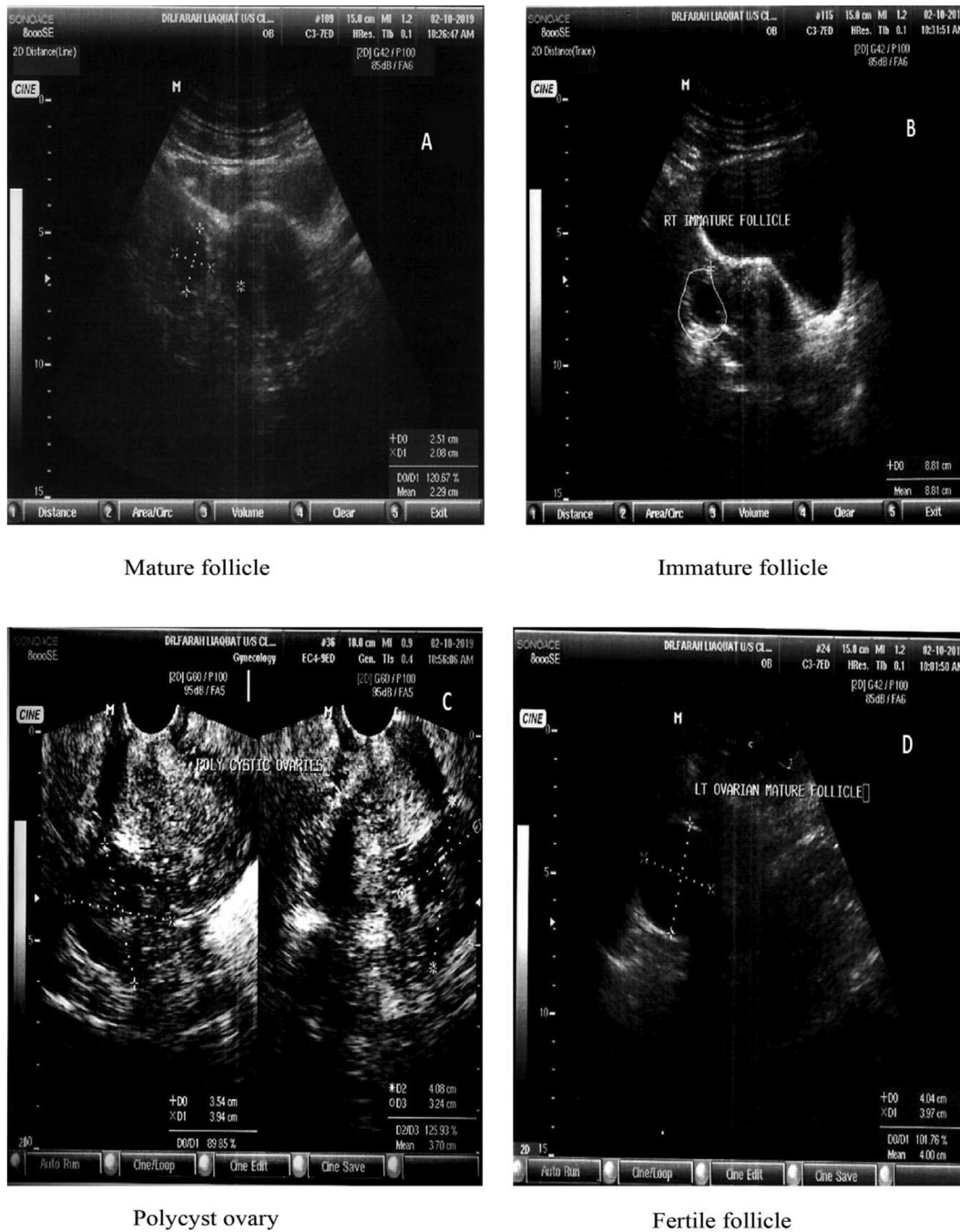
scientists that *BMP15* gene actively takes part in the maturation of the oocytes and normal growth of follicles, all the way through granulosa cells activation (Database resources of the national center for biotechnology information, 2018). This gene is located on the X chromosome (Xp11.22), consisting of 2 exons; 17 amino acids are encoded by exon 1 in the first portion of the polypeptide region, whereas the remaining peptide region contains 125 amino acids encoded by exon 2 (Persani et al. 2014). Ovarian dysgenesis and premature ovarian failure can be caused due to imperfection in this gene. Laissue et al. (2006) also proved by his study that change in amino acid codon (L148P) in exon 2 of *BMP-15* gene can cause POF, in French women, but this mutation cannot be considered as a main cause of ovarian insufficiency. Another study revealed that sufficient fluid secretion from *BMP15* gene in the follicular phase is important for female fertility. However Heterozygous mutations in *BMP15* gene may cause defect in the production of bioactive protein which may cause primary ovarian insufficiency in humans (Rossetti et al. 2009).

Any change in the codon of *BMP15* gene can become a reason for primary ovarian insufficiency (POI) in result very small amount of mature protein produces, which are less active or make a weak connection to work with GDF9. As *BMP15* protein and GDF-9

works together for oocyte-specific growth, hence confirm the regulation of follicle production and the process of ovulation (Patino et al., 2017).

According to Di Pasquale et al. (2004), the first heterozygous *BMP15* mutation (p.Cys235Tyr) linked with POF was found in the protein's pro-domain, region and causing the reduction of granulosa cell multiplication by governing deleterious effect. Many variants showed the polymorphism in the *BMP15* gene with poor functional consequences in women diagnosed with physiological menopause at an early young age (Moron et al. 2006; Rossetti et al. 2009). In this study, we also observed some patients with menopause in early young age and need medication to induce menstrual cycle, among them two females also showed the deletion of A at 635th nucleotide position with QK Gln < Lys at 212th amino acid of the protein. Another mutation was found by the addition of GG at 1038, 1039 nucleotide positions of *BMP15* gene which was not reported before with Q < G 346th amino acid in protein and substituted Gln < Gly.

Variations in *BMP15* gene nucleotides can affect ovarian function and can cause female infertility. There are many interesting scientific interrelation characters of *BMP15* gene which are supposed to be a valuable marker in IVF techniques (Persani et al.



Mature follicle

Immature follicle

Polycyst ovary

Fertile follicle

**Fig. 2.** 2A was taken from affected individual which shows the mature follicle, another affected individual TVS showed the immature follicle in Fig 2B whereas some individual's TVS were showing the polycystic ovary Fig 2C but Fig. 2D is showing the fully grown mature follicle which were ready to fertilize, this scan was taken from fertile female at 14 days of her menstrual cycle.

2014). According to Karavani et al. (2019), both the quality and quantity of oocytes must be perfectly measured before going to *in vitro* fertilization. Present results demonstrated that many of the infertile females were showing a normal range of reproductive hormones, even their follicles were not found mature enough for fertilization or females got polycystic ovaries. Sudha and Reddy (2013) have also reported same finding.

Some changes in the *BMP15* gene are favorable such as AG in contrast to AA codon may produce defensive results that help to avoid anovulation and infertility (Gonzalez et al., 2008), whereas on the other side, a frequent increase of TC and TT nucleotide in the *BMP15* genotype can cause risk factors for premature ovarian

insufficiency (POI) and menopause (Tiotiu et al. 2010). Other researchers also suggested that single nucleotide deletion (c.-8 del C) does not hamper the reading frame, but may involve in early steps of the translation (Rossetti et al. 2009). Based on these pieces of evidence, several mechanisms were checked in light of previous findings in animal species. It was observed that *BMP15* works as a powerful stimulator of granulosa cell growth and supports the sequence of follicles formation and growth from the primary stage to the FSH-dependent stage reported by Otsuka (Otsuka et al. 2000).

In the present study, five different variants were found in fifteen patients, out of which two were novel variants, whereas other

three different variants were previously reported. In earlier research scientists reported another variant c. 538G > A (p. Aln180Thr) in the same exon 2 of BMP 15 which has no harmful effect on the biological function of the protein (Laissue et al. 2006; Tiotiu et al. 2010). Another study showed one of the other variant 788 in TCT was most commonly found in POF patients and considered as polymorphism, this insertion was also found in many control, as previously reported (Kumar et al. 2017). An early study identified BMP15 gene mutations in basic regions of the prodomain (Coulam et al. 1986) which play a role in suppression of the expression of BMP15, either reduced the activity of BMP15 (Nelson 2009) or inhibited BMP15 synergy with GDF9 (Di Pasquale et al. 2004).

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#### Authors' contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be submitted for publication and agree to be accountable for all aspects of work.

#### Declaration of Competing Interest

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