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RESEARCH ARTICLE

GENETIC ANALYSIS OF FEMALE INFERTILITY WITH THE EFFECT OF HUMAN BMP15 AND GDF9 GENES: A REVIEW

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ABSTRACT

Infertility is a major health problem now a days and it was described as the condition of healthy individual who is unable to conceive or produce or sustain a successful pregnancy during a one year period. A large number of genes are found on X chromosome and autosomes. Two closely related members of the transforming growth factor-β (TGF- β) super family: growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) originates from oocytes are played a very important role in determining follicle growth and these two genes drastically altered fertility and ovulatory rate and early folliculogenesis in humans. In this review, we focused on genetic analysis of female infertility that indicated mutations in these two genes can decrease the inhibin production and increase follicle stimulating hormone levels and lead to ovarian failure causes infertility. Blockage of folliculogenesis in human with a low ovulation rate and the development of monoclonal antibodies may block the activities of these two genes might be useful in the development of novel contraceptive tools for human purposes.

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INTRODUCTION

The world's population is increasing at an alarming rate despite this, 15% of couples world-wide remain childless because of infertility (Matzuk and Lamb, 2002). Infertility as in many countries it affects about 15% of couples trying for a child. It can be observed that 10% of female infertile subject's genetic abnormalities could be present, including chromosome aberrations and single gene mutations. Genetic tests are now available to explore the cause of the infertility and assess the risk of a given couple to transmit its genetic characteristics. The genetics of infertility is dependent on different factors. Genetic factors can affect the production of the germ cells, the ability of the gametes to meet or embryonic development. Infertility can be due to the woman, the man, or both; primary or secondary. In human ovary, the number of follicles is finite. Thus, there is a subsequent loss of follicles during female development and puberty, and eventual follicle loss leads to infertility .Few genes have been identified that can give a substantial proportion in the cases of infertility. Among these genes a large number are found on X chromosome and autosomes. Two closely related members of the transforming growth factor-β (TGF- β) super family: growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are interestingly play a important role in causing infertility in female and ovulation rate in humans (Simpson *et al*., 2014). Infertility is an important issue for most women with POF. Treatments with gonadotropins, GnRH agonists, and immunosuppressant have not been effective in restoring fertility (Karlberg, 2012) and the reliable treatment is the use of donor eggs and IVF. Embryo cryopreservation is already an established method for fertility-preservation.

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Causes of infertility

Infertility can be caused by defects in the development of the urogenital system and in its function, by genetic defects of the endocrine system, including the hypothalamic-pituitary-gonadal axis, and by defects in gametogenesis, gamete function, fertilization or early embryonic development. Infertility may be caused by medical condition that may damage the fallopian tubes, interferes with ovulation, or causes hormonal complications. These medical conditions include pelvic inflammatory disease, endometriosis, polycystic ovarian syndrome, premature ovarian failure, uterine fibroids and environmental factors. Other causes of infertility in females include ovulation problems, tubal blockage, age-related factors,

uterine problems, previous tubal ligation and hormone imbalance.

BMP15 gene and infertility

Bone morphogenetic protein 15 (OMIM 300247) gene is located at Xp11.22 chromosome. It is a member of the TGFβ super family and is specifically expressed in the oocyte during early folliculogenesis and involved in the determination of the ovarian defect in Turner syndrome and significantly contributes to the determination of ovarian reserve. The BMP15 gene has two exons and the major site of expression is in oocytes within the ovary (Dube *et al*., 1998). In women undergoing infertility controlled ovarian stimulation indicates that BMP15 may represent a marker of ovarian response or oocyte quality. In other review phylogenetic analysis indicates that the BMP15 gene diverged very early and exhibited a rapid evolution compared with other BMP members. However, only BMP15 was submitted to a positive selection pressure in the human and closely related to hominidae clade (Auclair *et al*., 2013).

Spontaneous mutations in the ovine and murine of BMP-15 (*FecX*) genes and in the ovine GDF-9 (*FeGC*) gene may drastically alter fertility and ovulatory rate in mammalian species (Vireque *et al*., 2008). Mutations in *BMP15* gene can be associated with hypergonadotropic ovarian failure in humans and that BMP15 is one of the factors whose function is relevant for Turner syndrome. Infertility is due to delayed follicular development and histological examination reveals large oocytes, absent or abnormal granulosa proliferation. The first heterozygous mutation in BMP15 gene (p.Y235C) was carried out with two Italian sisters with hypergonadotropic ovarian failure characterized by ovarian dysgenesis, who inherited the genetic alteration from the unaffected father. Some review have investigated in human ovaries the correlation between mutations in the gdf9 and bmp15 genes and diseases such as premature ovarian failure (POF) and polycystic ovary syndrome (POS) had occurred (Laissure *et al*., 2006; Otsuka *et al*., 2011) (Dixit *et al*., 2011). The detection of BMP-15 mRNA in normal human oocytes indicates that these factors may play a fundamental role in folliculogenesis and fertility in women (Vireque *et al*., 2008). (de Resende *et al*., 2012) examined that, expression of bone morphogenetic protein15 (BMP15) and growth differentiation factor 9 (GDF9) in oocytes, and their receptor type 2 receptor for BMPs (BMPR2) in cumulus cells in women with polycystic ovary syndrome (PCOS) undergoing in vitro fertilization (IVF), and determine if BMP15, and GDF9 expression correlate with hyperandrogenism in PCOS patients. (Hafezian, 2011) proposed that, different mutations in the bone morphogenetic protein 15 (BMP15) gene cause increased ovulation rate and infertility. Large numbers of mutations in the GDF9 and BMP15 genes have been identified in women with premature ovarian failure and in mothers of dizygotic twins. It was observed that, among 24 mammalian species, it was detected positive selection in the *hominidae clade* related to residues in the human BMP15 pro-region (Auclair *et al*., 2013). A proteomic analysis determines that BMP15 regions are very much affected by post-translational modifications such as phosphorylation on Ser residue and N or O glycosylation on Asn or Thr residues. (Saito *et al*., 2008)

Role of GDF9 gene in female infertility

The GDF9 gene is located on human chromosome 5 (5q31.1). It has two exons encoding a 454 amino acid peptide (Lledo *et al*., 2014). GDF9 is a member of the transforming growth factor b (TGF) superfamily and is exclusively expressed in oocytes. GDF9 is involved in oocyte control of cumulus expansion, regulation of several key enzymes of granulosa cells, and granulosa cell proliferation and differentiation (Vireque *et al*., 2008). (Laissue *et al*., 2006) reported that the first mutational screening of the GDF9 gene was reported in Japanese women with ovarian failure. Here they observed the mutational screening results of the GDF9 gene, which show two rare missense mutations in propeptide region associated with ovarian failure. Haplotype analysis and statistical observations showed that the GDF9 gene is an important candidate gene for ovarian failure. In female, growth and differentiation factor plays a critical role during early folliculogenesis in mammals. GDF9 differentially affects granulosa cell function in different species. These review proposed that GDF9 is implicated all along the folliculogenesis process. It controls the early steps of follicular growth closely linked to GC proliferation. In later stages of follicular development, GDF9 gene might modulate the differentiative effect of FSH and control follicular maturation.

In GDF9, the Inhibins are produced by granulosa cells surrounding oocytes in the ovary. They regulate FSH production by gonadotrophs in the anterior pituitary through a negative feedback mechanism. Inhibin production is positively regulated by GDF9. This review indicate that mutations in this factor can decrease the inhibin production and increase FSH levels and lead to ovarian failure causes infertility (Shi *et al*., 2003; Prakash *et al*., 2010). Growth differentiation factor 9 (GDF9) plays an important role in normal growth, differentiation, and proliferation of granulosa cells surrounding the oocyte in the human ovary. In humans, BMP15 and GDF9 are specifically expressed in the oocytes in a similar pattern throughout folliculogenesis (Al-Ajoury and Rana 2015).

Genetic disorders of the female reproductive system (includes infertility)

Premature ovarian failure: Premature ovarian failure, including abnormal apoptosis, an abnormal number of primordial follicles and/or abnormal follicular maturation. POF has been linked with several X chromosome-linked defects and it has been suggested that GDF9 and BMP15 mutations are involved in POI. In humans, a natural heterozygous mutation in the propeptide region of the BMP15 gene has been associated with hypergonadotrophic ovarian failure due to ovarian dysgenesis. The mutation appeared to be associated with reduced granulosa cell growth (Martin, 2001). Women with POF probably have follicles of lower quality.

Endometriosis: Endometriosis is an oestrogen-dependent condition that is characterized by the presence of endometrium-like tissue at ectopic sites such as the pelvic peritoneum and ovaries. Pelvic pain and infertility are the most common features of endometriosis. An abnormal immune response and a genetic predisposition to developing endometriotic lesions.

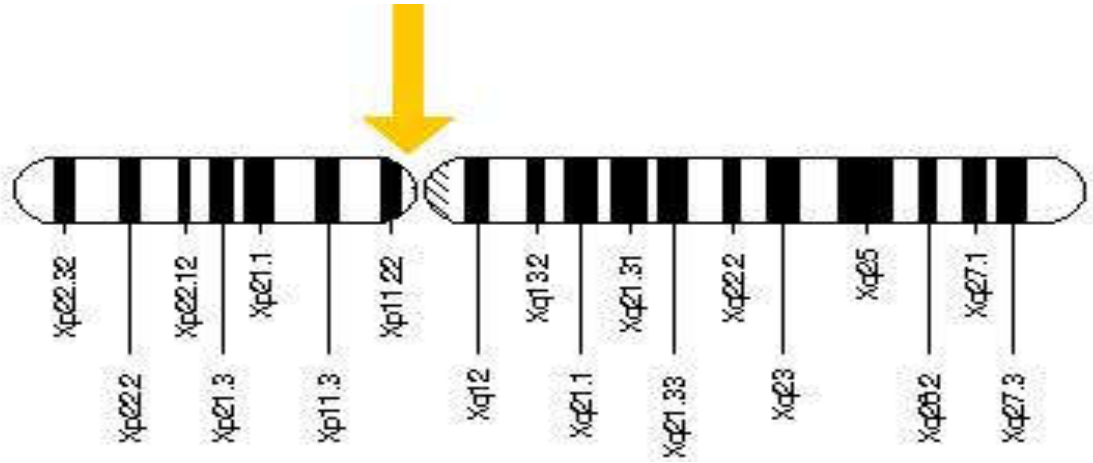

Figure 1. Location of bone morphogenetic protein 15 in short arm of X chromosome at position 11.2. (Ref: www.ghr.nlm.nih.gov)

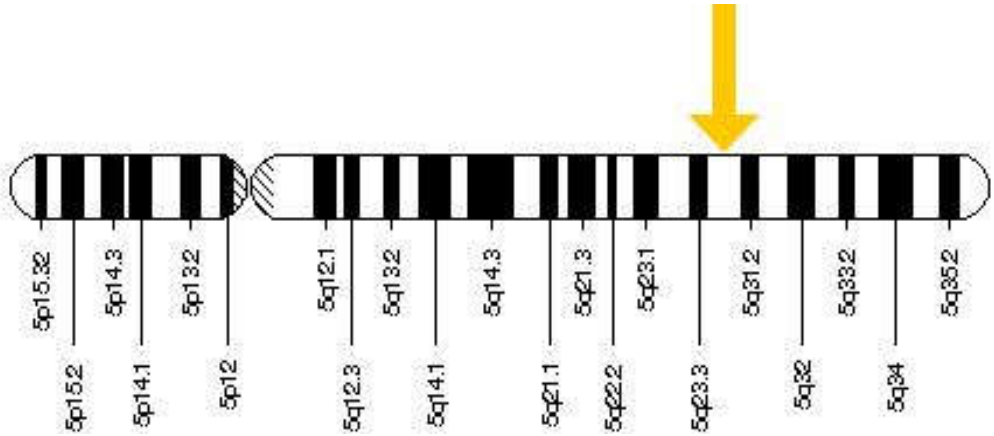

Figure 2. Location of growth differentiation factor 9 in long arm of chromosome 5 at position 31.1. (Ref: www.ghr.nlm.nih.gov)

Table 1. Various genes and their genetic syndrome associated with female infertility

Syndrome	Genes	Causes and features	Reference
Non-classical adrenal hyperplasia	i) Chromo domain helicase DNA-binding protein 7 (CHD7). ii) Diaphanous homolog 2 (DIAPH2).	Women have an increased risk of having children with genital abnormalities if the father is a carrier. It includes charge syndrome, premature ovarian failure (POF2A).	Hickey et al., 2002
Kallmann syndrome	iii) Bone morphogenetic protein receptor 1B (BMPR1B). iv) Fibroblast growth factor 8 (FGF8).	Also known as hypogonadotropic hypogonadism, can be inherited in an X-linked, autosomal dominant and Ovarian dysfunction.	Nudell and Turek, 2000
Immotile cilia syndrome	v) Fibroblast growth factor receptor 1 (FGFR1). vi) Premature ovarian failure 1B (POF1B).	A recessive disorder resulting in absent ciliary or flagellar motility. Characterized by primary amenorrhea (POF2B).	Nudell and Turek, 2000
Noonan syndrome	vii) Bone morphogenetic protein 15 (BMP15). viii) Growth differentiation factor 9 (GDF9).	An autosomal dominant disorder, similar to Turner syndrome. ovarian failure(POF4), Endometriosis, POS, folliculogenesis	Tartaglia et al., 2001
Denys–Drash syndrome (DDS) and Frasier syndrome	ix) Chromo box homolog 2, Drosophila poly comb class (CBX2; M33). x) Prokineticin (PROK2).	DDS is characterized by severe genito–urinary malformations characterized by mutations in the WT1 gene, XY males develop as females and donor splice mutations Autosomal 46,XY, male-to-female sex reversal have been found.	Swain and Lovell-Badge, 1999
Androgen insensitivity syndrome	xi) Sex-determining region Y (SRY). xii) Prokineticin receptor 2 (PROKR2).	It includes non-sense mutations, point mutations. Features include defective spermatogenesis oligozoospermia, azoospermia. Mutations lead to 46,XY females.	Patrizio and Broomfield, 1999b

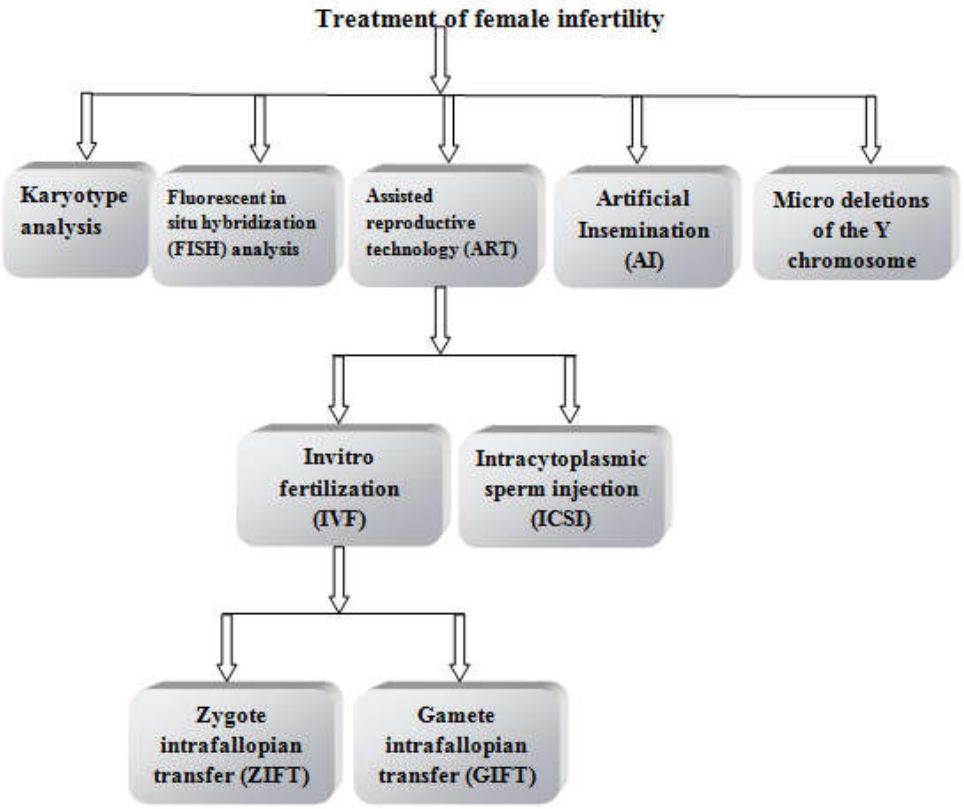

Figure 3. Flow chart of diagnosis of infertility (female)

The initial mutation may be either somatic or heritable. endometriosis has focused mainly on genes involved in inflammation, steroid hormone regulation, metabolism, biosynthesis (Martin, 2001).

Polycystic ovary syndrome: Polycystic Ovary Syndrome, more commonly referred to as PCOS, is an endocrine, reproductive and metabolic disorder that is characterized by diverse clinical symptoms including irregular menstrual cycles (Persani, L *et al*., 2014). It is very commonly seen in PCOS that LH hormone is higher than average and FSH is lower than average. This alone does not allow for proper hormone feedback between the ovaries and pituitary. High LH causes the ovarian follicles to attempt to develop but without the FSH balance.

Human gene mutations causing infertility: Mutation in genes expressed in the hypothalamic-pituitary-gonadal cause pubertal and reproductive deficiencies in humans. Mutation are categorized into three categories which are useful in the endocrinological diagnosis of infertility (Layman, 2002)

In pituitary: Mutations in pituitary genes causing infertility result either in deficiency of all or some of the pituitary hormones, thyroid stimulating hormone (TSH), prolactin, growth hormone, FSH, and LH. In general, therapy for pituitary causes of infertility is very successful.

In gonad: Gonadal causes of infertility constitute the Mutations affecting gonadal function include gonadotrophin receptors, steroid hormone receptors, steroid synthesis defects, as well as miscellaneous causes. The best therapy usually involves the use of donor gametes (sperm or oocytes).

In hypothalamus: Mutations of genes expressed in the hypothalamus generally result in hypogonadotrophic hypogonadism, a condition of absent or deficient of puberty owing to low serum gonadotrophin, follicle stimulating hormone (FSH), and luteinising hormone. Decrease in gonadotrophin secretion results in reduced production of sex steroids (estrogen in women, testosterone in men), which ultimately results in aberrant pubertal development causes infertility.

Diagnosis and genetic tests of Infertility: Treatment of infertility is unique in medical condition as it offers individuals radical invasive treatment. Clearly the hope is that a greater understanding of the genetic control of infertility will bring low-risk treatments that are effective and easy for diagnosis (Karlbag, 2012). Now a days there are several genetic tests for prevention of female infertility. One of the tests is karyotype analysis which is strongly recommended during the diagnostic workup of patients with azoospermia and severe oligozoospermia. In this case the cytogenetic screening is mandatory. Some karyotype abnomalies (for example 47, XYY, some translocations and other structural aberrations) may cause female infertility (Carlo Foresta *et al*., 2002). Another diagnosis is micro deletions of the long arm of the Y chromosome. In this, screening is strongly recommended during the diagnostic workup of infertile patients with non obstructive azoospermia and severe oligozoospermia. Fluorescent in situ hybridization (FISH) for the analysis of

chromosome content in individual spermatozoa, FISH analysis could be indicated after chemo-radiotherapy. IVF is the advanced clinical treatment for infertility. It involves the fertilization of eggs and sperm outside the body in a laboratory and once, an embryo or embryos form; they are then placed in the uterus (Rani and Paliwal, 2014). Assisted reproductive technologies include any fertilization involving manipulation of gametes/ embryos outside the human body and transfer of gametes/embryos into the body. Artificial Insemination (AI) involves manipulation of fertilization by injecting of a sperm artificially through a needle into the vagina/cervix/uterus/ fallopian tubes of the patients directly without sexual intercourse (Chaudhary, 2012). Zygote Intrafallopian Transfer (ZIFT) and Gamete Intrafallopian Tube Transfer (GIFT) are variations of IVF. With ZIFT, eggs are fertilized in a Petri dish and the resulting zygote(s) (a one cell embryo) is placed directly into the woman's fallopian tube through laparoscopic surgery (as opposed to IVF, in which an embryo is placed in the woman's uterus). ZIFT is used when a woman has problems ovulating (Olooto, Wasiu Eniola *et al*., 2012).

Conclusion

The results obtained in the studies mentioned above that support BMP15 and GDF9 as a marker of regulating female infertility. Finally, if mutation of a gene in humans results in infertility, the protein product of that gene may be a future target for novel contraceptives. Therefore, two genes BMP15 and GDF9 plays a important role in ovarian function, and actions in follicle growth and development at all stages of folliculogenesis.

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REFERENCES

Al-Ajoury, R., Kassem, E., Al-Halabi, B., Moassess, F., & Al-Achkar, W 2015. Mutations Analysis of the Growth Differentiation Factor 9 Gene in Syrian Women with Ovarian Failure. *Int J Hum Genet*, 15(3): 139-144.

Auclair s, Rossetti R, Meslin C, Monestier O, Dipasquale E, Pascal G, Persani L, Fabre S 2013. Positive selection in bone morphogenetic protein 15 targets a natural mutation associated with primary ovarian insufficiency in human. PLoS One; 8:e78199

Chaudhary, B. L 2012. Assisted Reproductive Techniques Ethical and Legal Issues. *J Indian Acad Forensic Med,* 34(4): 350-354

de Resende, L. O. T., Vireque, A. A., Santana, L. F., Moreno, D. A., de Sá Rosa, A. C. J., Ferriani, R. A, Reis, R. M 2012. Single-cell expression analysis of BMP15 and GDF9 in mature oocytes and BMPR2 in cumulus cells of women with polycystic ovary syndrome undergoing controlled ovarian hyperstimulation. *Journal of assisted reproduction and genetics,* 29(10): 1057-1065.

Dixit, H., Rao, L. K., Padmalatha, V., Kanakavalli, M., Deenadayal, M., Gupta, N., Singh, L. 2005. Mutational screening of the coding region of growth differentiation factor 9 gene in Indian women with ovarian failure. *Menopause, 12*(6): 749-754.

Dube., J. L,Wang., P, Elvin., J, Lyons., K. M. ,Celeste., A. J, Matzuk., M. M 1998. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. *Mol Endocrinol*; 12:1809–1817.

Foresta, C., Ferlin, A., Gianaroli, L., & Dallapiccola, B. 2002. Guidelines for the appropriate use of genetic tests in infertile couples. *European journal of human genetics: EJHG, 10*(5): 303-312.

Hafezian, S. H. 2011. Genetic polymorphism BMP15 and GDF9 genes in Sangsari sheep of Iran. *International Journal of Genetics and Molecular Biology, 3*(1): 31-34

Hickey T, Chandy A and Norman R. J 2002. The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. *Endocrinology and Journal of Clinical Metabolism* 87:161–165.

Karlberg, S. 2012. Mulibrey nanism: Characterization of hypogonadism, infertility and tumors.

Lledo., B, Ortiz., J. A, Llacer J, Bernabeu., R 2014. Pharmacogenetics of ovarian response. *Pharmacogenomics*, 15(6): 885-893.

Laissue, P., Christin-Maitre, S., Touraine, P., Kuttenn, F., Ritvos, O., Aittomaki, K., Frydman, R. 2006. Mutations and sequence variants in GDF9 and BMP15 in patients with premature ovarian failure. *European Journal of Endocrinology,* 154(5):739-744.

Layman, L. 2002. Human gene mutations causing infertility. *Journal of medical genetics,* 39(3): 153-161.

Martin, E. 2001. The woman in the body: A cultural analysis of reproduction: Beacon Press.

Matzuk, M. M., & Lamb, D. J. 2002. Genetic dissection of mammalian fertility pathways. *Translocations, 45*, 46XY. *Nat Cell Biol,* 4 Suppl: 41-49

Nudell DM and Turek PJ 2000. Genetic causes of male infertility: current concepts Current Urology Reports 1: 273–281

Olooto, W. E., Amballi, A. A., & Banjo, T. A. 2012. A review of Female Infertility; important etiological factors and management. *J Microbiol Biotech Res,* 2(3): 379-385.

Otsuka, F., McTavish, K. J., & Shimasaki, S. 2011. Integral role of GDF□9 and BMP□15 in ovarian function. *Molecular reproduction and development, 78*(1): 9-21.

Patrizio P and Broomfield D 1999. Male Fertility and Infertility. *Cambridge University Press*, *Cambridge*: 164–173.

Persani, L., Rossetti, R., Di Pasquale, E., Cacciatore, C., & Fabre, S 2014. The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. *Human reproduction update* 20: 869–883.

Prakash GJ, Ravi Kanth VV, Shelling AN, Rozati R, Sujatha M 2010. Mutational analysis of inhibin alpha gene revealed three novel variations in Indian women with premature ovarian failure. *Fertil Steril*, 94(1): 90-98.

Rani, K. and Paliwal, S. 2014. A brief review on in-vitro fertilization (ivf): an advanced and miraculous gateway for infertility treatments. *World journal of pharmacy and pharmaceutical sciences*, 3(4): 647-658.

Saito S, Yano K, Sharma S, Mcmahon HE, Shimasaki S 2008. Characterization of the post-translational modification of recombinant human BMP-15 mature protein. *Protein Sci*;17:362–370.

Shi Y, Massagué J 2003. Mechanisms of TGF-beta sig- naling from cell membrane to the nucleus, *Cell*, 113: 685–700.

Simpson., C.M, Robertson., D. M, Al-Musawi., S. L, Heath D. A., McNatty., K. P., Ritter., L. J., Mottershead., D. G., Gilchrist R. B., Harrison., C. A, Stanton, P. G. 2014.Aberrant GDF9 expression and activation is associated with common human ovarian disorders. *J Clin Endocrinol Metab*; 99: E615–624

Swain A and Lovell-Badge R 1999. Mammalian sex determination: a molecular drama Genes and Development 13: 755–767

Tartaglia., M, Mehler., E. L., Goldberg., R *et al*. 2001. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome .*Nature Genetics* 29:465–468.

Vireque, A., Reis, R., e Silva, A. R., Resende, L., Ferreira, E., e Silva, A. R., & Ferriani, R. 2008. Involvement of Bone Morphogenetic Proteins (BMPs) in Ovarian Function and Infertility. *Open Reproductive Science Journal,* 1: 11-15.

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