An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

GENETIC EVALUATION OF FEMALE PARTNERS OF INFERTILE COUPLES

Manjuvani .S, Dr.N.Chandra, Dr.E.P.Sabina, K. Shoba*

Department of Biotechnology, D.K.M. College for woman, Vellore – 632001,
Department of Genetics, Madras University, Taramani, Chennai – 600113,
School of Bioscience, VIT, Vellore

*Corresponding author E-mail: **danishoba@gmail.com**

ARTICLE INFO

Key Words
Assisted reproductive techniques, endometriosis, hyperprolactinemia, Infertility, ovulatory factor.

ABSTRACT

Infertility is defined as the inability of a sexually active couple to achieve pregnancy despite unprotected intercourse for a period of greater than 12 months (World Health Organization, 2000). It is estimated that globally, 60-80 million couples suffer from infertility every year, of which probably 15-20 million are in India alone. This is an equally important national problem concerning reproductive health (Poongothai, 2009).Primary infertility is as common and distressing a problem in India as in other parts of the world. Primary infertility cases were more common than secondary infertility cases. More than half of the infertile couple's causes were from both male and female partners. The causes of male and female factors were abnormal semen parameters, endometriosis, tubal factor and ovulatory factor. The pregnancy rate and pregnancy outcome of assisted reproductive techniques (ART) such as IUI, IVF and ICSI are comparable in most reports. Semen abnormalities (22.4%), anovulation (17.2%), ovarian failure (8.8%), hyperprolactinemia (8.4%) and tubal disease (7.2%) are common causes of infertility. Overall, 30% of infertile women had primary infertility, and 70% had secondary infertility. Female age is the single most important determinant of spontaneous as well as treatment-related abortion, with a gradual decline in fertility especially after the age of 35 years (Menken *et al*., 1986; Templeton *et al.*, 1996).

INTRODUCTION

Infertility is defined as a failure to conceive in a couple trying to reproduce for a period of two years without conception. The World Health Organisation (WHO) has defined infertility as a period of two years without conception, but many couples actually seek a medical opinion after one year of infertility (Poongothai*et al*., 2009).Infertility can be due to endocrine cause, related to age, exercise, obesity or infectious disease; it can be immunological, psychological, result from surgery or blockage, or be associated with defined abnormalities in the gametes (for example aberrant semen parameters). Perhaps the most common 'cause' of infertility is simply 'unexplained' and this accounts for about 20% of couples (Uehara et al., 2001). The genetic causes of infertility seen in about 15% of male and 10% of female individuals is accounted for

by chromosomal abnormalities, single gene disorders and phenotypes with multifactorial inheritance. Chromosomal abnormalities including low level sex chromosome mosaicism were detected in 12% of male and unexpectedly high in 6% of the women (Chandley 1984). The infertility male and female definitely have an increased risk to carry a chromosomal abnormality. Detection of such an abnormality is of fundamental importance for the diagnosis of infertility, the following treatment, the evaluation of the risk for the future child and the appropriate management of the pregnancy to be obtained. Therefore cytogenetic screening of both partners is mandatory prior to any type of ART (Mau-Holzmann 2005). Approximately 5% of the couples are definitive infertility with a nearly zero chance of becoming spontaneously pregnant in the future. With age, cumulative probabilities of conception decline because heterogeneity in fecundity increases due to a higher proportion of infertile couples. In truly fertile couples cumulative probabilities of conception are probably age independent. Under appropriate circumstance a basic infertility work-up after six unsuccessful cycles with fertility-focused intercourse will identify couples with significant infertility problem to avoid both infertility under- and over-treatment, regardless of age: Couples with a reasonably good prognosis (e.g. unexplained infertility) may be encouraged to wait because even with treatment they do not have a better chance of conceiving. The other may benefit from an early resort to assisted reproduction treatment (Gnoth et al., 2005). Although there are prospects for screening of sperm, current routine clinical practice is based on the screening of peripheral blood samples. Identification of genetic factors in the infertile couple has become good practice for appropriate management of the infertile couple.An attempt was made to review in brief the several causes underlying female infertility and to investigate a few female partners of infertile couples of to rule out a chromosomal etiology.A total of 20 female partners of infertile couples were evaluated to determine their constitutional karyotype in this study.

METERIALS AND METHODS: Chromosomal analysis was performed on cultures of peripheral blood lymphocytes by the standard method. The karyotype was confirmed in all patients with the G-banding technique; C-banding, nucleolus organizing regions (NOR) and fluorescence in-situ hybridization (FISH) were also employed when necessary. In these subjects the cytogenetic screening is mandatory prior to any ART procedure (including intrauterine insemination, IUI).

RESULTS: A total of 20 female partners of infertile couples were investigated to determine the constitutional karyotype in the present study. These individuals were provisionally diagnosed to have primary infertility.

CLINICAL DATA:

The mean age of these women was 32 years (range 24 – 40 years). Consanguinity was seen in 3 cases (IF 3W, IF 17W and IF 20W). The average menorrheal age was 14 years (range 11 – 17 years). The average length of infertility was 12.5 years (range 1½ years to 25 years). The infertility was secondary in case of one patient, IF 2W. She gave a history of three first trimester spontaneous abortions and a subsequent two-year period of infertility. It was of interest to note that her cousin had also three spontaneous abortions but followed by 15 years of infertility. The maternal grandmother of the case IF 1W had three children born to her after ten years of marriage while her two maternal aunts experienced infertility having two spontaneous abortions initially. The maternal aunt of the patient IF 5W is

infertile for 25 years, while the sister of IF 10W has no issues for 10 years. Hormonal assay revealed a normal study wherever data was available. Ultrasonogram (USG) study revealed a bilateral polycystic ovarian disease in four cases (IF 8W, IF 12W, IF 14W and IF 15W). Laparoscopy has been performed for 8 cases - Polycystic Ovarian Disease (PCOD) was observed in one (IF 19W), mild salpingitis in two (IF 4W and IF 16W), bilateral tubal block in one case (IF 8W) and salpingectomy (which had been performed prior to the investigation) in one (IF 2W) (Table 3). The remaining three recorded a normal study. HSG was performed on 7 female partners and flimsy adhesion of uterine cavity was seen in one case (IF 3W). Endometrial polyposis was noticed in a woman (IF 10W) and bilateral tubal block in another (IF 18W) block in another (IF 18W).The other women were found to have normal uterus, fallopian tubes and ovaries.

CYTOGENETIC PROFILE: Analysis of GTG-banded metaphases revealed a normal karyotype 46,XXin all 20 female partners of infertile couples in this study (Table 3; Fig. 2). A normal GTG-banded karyotype depicting 46,XY pattern is given in Fig.1. A heteromorphic variant involving an extended satellite stalk on D group chromosomes was seen in two cases. The variant chromosome was a chromosome 15 in one case (IF2W; Fig. 3) while it was a chromosome 14 in the other (IF10W; Fig.4). The variants were studied using AgNOR banding technique.

CONCLUSION

A total of twenty infertile women were investigated to determine the constitutional karyotype as the primary step. The average duration of infertility was 12.5 years (range 1½ years to 25 years). Consanguinity was present in three cases. Polycystic Ovarian Disease was seen in five individuals while two women showed bilateral tubal block. Analysis of GTG-banded metaphases obtained from cultured lymphocytes using standard protocols revealed a normal chromosomal pattern in all the individuals. Heteromorphic variants such as an extended satellite stalk on chromosomes 14 and 15, 14pstk+ and 15pstk+ respectively, were noted in a single case each. An overall increased frequency of chromosomal aberrations in male and female partners of couples referred for intracytoplasmic sperm injection has been observed and they consist mostly of translocations,inversions and numerical sex chromosome aberrations (Mau *et al.*, 1997). Routine cytogenetic analysis cannot be advocated in normovulatoryinfertile women although the relatively higher frequency of abnormal karyotypes in women with secondaryinfertility indicates that this subgroup of patients might benefit from a routine karyotype analysis (Papanikolaou*et al.,* 2005). The probands IF2W and IF10W showed heteromorphic variations in chromosomes15 and 14 respectivelyin this study. AgNOR-banding confirmed that it was due to an elongation of the satellite stalk region localized at the proximal short arm in the former. Human chromosome polymorphic variants can be seen in three forms: i) heteromorphisms shown by short-arm regions of D- and G- group chromosomes, ii) heteromorphisms shown by paracentric long arm regions of chromosomes 1, 9 and 16, and iii) variation in the length of the Y chromosome. It has been known that the short arm of all five acrocentric chromosomes of both D-chromosomes and G-chromosomes are satellited and the regions namely i) satellite, ii) stalk and iii) short arm proper vary greatly in size and morphology. Short arms of acrocentrics are heterochromatic, and therefore, extensive variations are possible, usually without any detrimental effect.

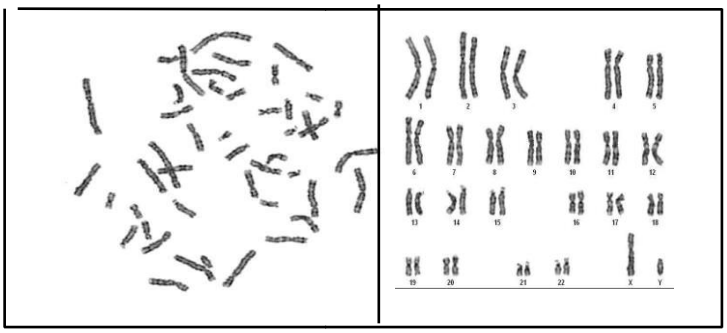

Fig. 1 - GTG-banded karyogram from a healthy individual showing a normal 46, XY Chromosome pattern. Inset shows the banded metaphase.

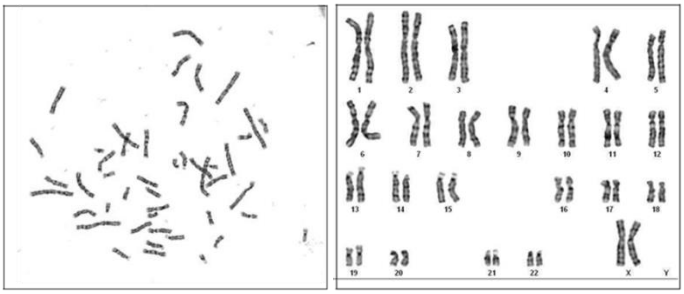

Fig. 2 - GTG-banded karyogram from the patient IF 4W showing a normal 46, XX Chromosome pattern. Inset shows the banded metaphase.

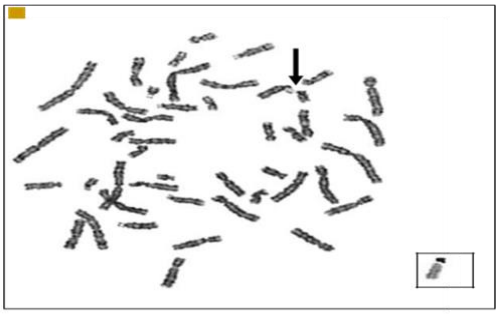

Fig. 3 - GTG-banded metaphase from the patient IF 2W showing the heteromorphic variant chromosome 15 (shown by arrow). Inset shows the AgNOR-banded variant chromosome.

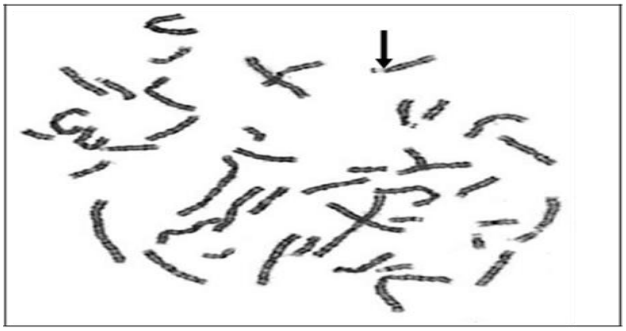

Fig. 4 - GTG-banded metaphase from the patient IF 10W showing the heteromorphic variant chromosome 14 (shown by arrow).

Patient ID	Age (y)	Duration of marriage (y)	Consan-gunity	Age at menarche (y)	Hormonal assay	USG	LP	HSG	Karyotype
IF 1W	35	9	No	13	FSH-3.51 LH-6.39 P-17.9	Uterus normal, both ovaries normal, small follicles seen in both ovaries.	Not available	Not available	46,XX
IF 2W	33	2	No	16	FSH-3.84 LH-3.92 P-24.94	Uterus normal	Salpingectomy	Not available	46,XX, 15pstk+
IF 3W	28	9	Yes	13	Not available	Not available	Not available	Uterine cavity with flimsy adhesion	46,XX
IF 4W	27	9	No	14	FSH-4.31 LH-2.24 P-14.55	Not available	Uterus normal right tube normal mild salpingitis	Ostia normal	46,XX
IF 5W	36	11	No	13	Not available	Not available	Not available	Not available	46,XX
IF 6W	40	25	No	17	Not available	Not available	Uterus ovary normal	Not available	46,XX
IF 7W	30	7	No	13	P-7.73	Not available	Not available	Not available	46,XX
IF 8W	38	3	No	15	FSH-7.36 LH-2.49 P-17.32	Both ovary PCOD	Ovary is normal, left distal tubal block, right corneal block	Cavity normal	46,XX
IF 9W	34	7	No	13	FSH-6.0 LH-3.5 P-5.8	Ovary uterus normal	Not available	Not available	46,XX
IF 10W	32	2½	No	15	Not available	Not available	Ovary is normal	Uterine cavity ostia normal, endometrial polyposis	46,XX, 14pstk+
IF 11W	42	6	No	12	Not available	Not available	Not available	Not available	46,XX
IF 12W	25	1½	No	13	P-8.14	Tubular cystic lesion both ovaries	Not available	Chronic hydrosalpinx	46,XX
IF 13W	30	8	No	13	FSH-7.1 LH-17.5 P-34.5	Uterus is normal	Ovary is normal	Not available	46,XX
IF 14W	27	7	No	11	FSH-6.38 LH-4.60 P-18.77	Polycystic ovary	Not available	Uterus normal	46,XX
IF 15W	28	5	No	14	FSH-5.07 LH-9.80 P-6.01	PCOD	Not available	Not available	46,XX
IF 16W	24	4	No	15	FSH-4.62 LH-2.31 P-27.69	Not available	Normal bended with mild salpingitis, fimbria free.	Not available	46,XX
IF 17W	24	6	Yes	13	P-18.73	Not available	Not available	Not available	46,XX
IF 18W	37	12	No	13	FSH-9.45 LH-8.45 P-34.19	Not available	Not available	Uterus normal bilateral tubal block	46,XX
IF 19W	29	12	No	13	FSH-4.27 LH-5.67 P-34.19	Not available	PCOD	Not available	46,XX
IF 20W	29	15	Yes	15	Not available	Not available	Not available	Not available	46,XX

Table 3 - Clinical and cytogenetic data on female partners of infertile couples USG – Ultrasonography; LP – Laparoscopy; HSG – Hysterosalpingogram PCOD - Polycystic ovary disease; FSH - Follicle-stimulating hormone; LH - Luteinizing hormone; P – Prolactin.

The biological significance of these variants, or heteromorphisms, is still poorly understood. Yet their use as genetic markers is a powerful tool in clinical diagnosis, paternity exclusion and population genetics (Kalz and Schwanitz, 2004; Bhasin 2007). However, this routine screening also includes a karyotype ofthe female partner though there is still no general agreementwhether such an analysis is mandatory (Papanikolaou*et al*.2005; De Braekeleer*et al*. 2006). A review of the relevantliterature concludes that 3.6% of the men and 4.2% of thewomen carry a chromosomal abnormality (De Braekeleer*etal*. 2006). In conclusion, genetic testing including chromosomal analysis in both partners of couples undergoing ICSI treatment was strongly recommended (van der Ven*et al.* (1998; Schreurs*et al.,* 2000;Rosenbusch 2010).

REFERENCES

- Akande VA, Hunt LP, Cahill DJ and Jenkins JM (2004) Differences in time to natural conception between women with unexplained infertility and infertile women with minor endometriosis. Hum Reprod 19: 96–103.
- Allaire C. Endometriosis and infertility: a review. J Reprod Med. 2006 Mar;51(3):164-8.
- Andréa T. Maciel-Guerra Infertility and marker chromosomes: Application of molecular cytogenetic techniques in a case of inv dup(15) J Appl Genet 47(1), 2006, pp. 89–91
- Badovinac R, Buretic-Tomljanovic A, Starcevic N, Kapovic M, Vlastelic I, Randic L: Chromosome studies in patients with defective reproductive success. Am J Reprod Immunol 2000; 44: 279 ± 283.
- Benagiano G, Bastianelli C, Farris M. [infertility: a global perspective] Minerva Ginecol. 2006 Dec;58(6):445-57.
- Bhattacharya S, Porter M, Harrild K, Naji A, Mollison J, van Teijlingen E,Campbell DM, Hall MH, Templeton A. Absence of conception after caesarean section: voluntary or involuntary? BJOG 2006;113: 268–275
- Brugo-Olmedo S, Chillik C, Kopelman S Definition and causes of infertility.Reprod Biomed Online. 2001;2(1):41-53.
- Butler WJ, McDonough PG: Molecular genetics and infertility; in: Seibel MA, (ed): Infertility: a comprehensive text. USA:Appleton & Longe, Boston, 1997, pp 371 ± 385..
- C.Gnoth, D.Godehardt, E.Godehardt, P.Frank-Herrmann and G.Freundl Time to pregnancy: results of the German prospective study and impact on the management of infertility Human Reproduction Vol.18, No.9 pp. 1959±1966, 2003
- C.Rubio, C.SimoÂn F.Vidal, L.Rodrigo, T.Pehlivan, J.RemohõÂ and A.Pellicer Chromosomal abnormalities and embryo development in recurrent miscarriage couples Human Reproduction Vol.18, No.1 pp. 182±188, 2003
- Chandley A.C. (1979) Chromosomal basis of human infertility. British Medical Bulletin, 35: 181-186.

- Chandley A.C. (1984) Infertility and chromosomal abnormality. In: Clark J.R (Ed.) Oxford Reviews of Reproductive Biology, Vol 6, Chapter 1, Oxford University Press, Oxford, pp. ???
- Collins JA. Unexplained infertility. In: Seibel MM (ed). Infertility: A Comprehensive Text, 2nd edn. Stamford, CT: Appleton & Lange, 1997, 323
- Dor J, Homburg R, Rabau E. An evaluation of etiologic factors and therapy in 665 infertile couples. Fertil Steril. 1977 Jul;28(7):718-22.
- E.Clementini, C.Palka, I.Iezzi, L.Stuppia, P.Guanciali-Franchi and G.M.Tiboni. Prevalence of chromosomal abnormalities in 2078 infertile couples referred for assisted reproductive techniques Human Reproduction Vol.20, No.2 pp. 437–442, 2005
- E.G.Papanikolaou, V.Vernaeve, E.Kolibianakis, E.VanAssche, M.Bonduelle, I.Liebaers A. Van Steirteghem and P.Devroey Is chromosome analysis mandatory in the initial investigation of normovulatory women seeking infertility treatment? Human Reproduction Vol.20, No.10 pp. 2899–2903, 2005
- Foresta C, Ferlin A, Gianaroli L, Dallapiccola B.Guidelines for the appropriate use of genetic tests in infertile couples. Eur J Hum Genet. 2002 May;10(5):303-12.
- Foresta C., Ferlin A., Gianaroli L., Dallapiccola B. (2002) Guidelines for the appropriate use of genetic tests in infertile couples. Eur J Hum Genet 10: 303 – 312.
- Franks S, Gherani N,McCarthyM: Candidate genes in polycystic ovary syndrome. Hum Reprod Update 2001; 7: 405- 410.
- Gekas J, Thepot F, Turleau C et al: Chromosomal factors on infertility in candidate couples for ICSI: an equal risk of constitutional aberrations in women and men. Hum Reprod 2001; 16: 82±90.
- Goto A, Yasumura S, Yabe J, Reich MR. Addressing Japan's fertility decline:influences of unintended pregnancy on child rearing. Reprod Health Matters 2006;14:191–200.
- Henry G. Burger Infertility Principles of Medical Biology Volume 12, 1998, Pages 317-332.
- Hens L, Devroey P, Van Waesberghe L, Bonduelle M, Van Steirteghem AC and Liebaers I (1989) Chromosome studies and fertility treatment in women with ovarian failure. Clin Genet 36,81–91.
- Hens, L., Bonduelle, M., Liebaers, I. et al. (1988) Chromosome aberrations in 500 couples referred for in-vitro fertilization or related fertility treatment. Hum. Reprod., 3, 451–457.
- Kao LC, Urbanek M, Driscoll D et al: The genetic basis of polycystic ovary sindrome. in Kovacs GT, ed. Polycystic ovary syndrome. Cambridge: Cambridge University Press, 2000, pp 35 ± 48.
- Karinen L, Pouta A, Hartikainen AL, Bloigu A, Paldanius M, Leinonen M, Saikku P, Jarvelin MR. Association between Chlamydia trachomatis antibodies and subfertility in the Northern Finland Birth Cohort 1966 (NFBC 1966), at the age of 31 years. Epidemiol Infect 2004; 132:977–984.
- Katrin van der Ven, Ba¨rbel Peschka,Markus Montag, Ricarda Lange, Gesa Schwanitz and Hans H.van der Ven Increased frequency of congenital chromosomal aberrations in female partners of couples undergoing
- Kalpana K., Manjuvani S.,Shoba K.Insilico Comarative Modeling of

Maturase K Protein in Cymbopogon Martinii Plant. Research & Reviws: A Journal of Bioinformatics. 2018; 5(3): 30 – 36 p.

- Peschka B, Leygraaf J, van der Ven K et al: Type and frequency of chromosome aberrations in 781 couples undergoing intracytoplasmic sperm injection. Hum Reprod 1999; 14: 2257 ± 2263.
- Plachot, M. (1997) The human oocyte. Genetic aspects. Ann. Genet., 40, 115–120.
- Shoba.k and Dr. Mazher sultana, Three - dimensional structure and motif prediction studies on collagenase protein in fiddler crab, International journal of novel trends in pharmaceutical sciences, Issn: 2277 – 2782,volume 6, issue 4, pages 79 – 83.
- Templeton A, Morris J, Parslow W. Factors that affect outcome of in-vitro fertilisation treatment. Lancet 1996;348:1402–1406
- Templeton A. Infertility-epidemiology, aetiology and effective management. Health Bull (Edinb). 1995 Sep;53(5):294-8.