ORIGINAL RESEARCH PAPER

Medical Science

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

BIOCHEMICAL AND GENETIC STUDIES ON INFERTILE SUBJECTS AND ITS RISK FOR CARDIOVASCULAR DISEASE

KEYWORDS

Infertility, Cardiovascular disease, Cytokinesis- block Micronuclei (CBMN) assay

Minsa M	Aneesh P
Department of Zoology, Mar Ivanios College, Thiruvananthapuram, Kerala, India.	Research Scholar, Meenakshi University, West K K Nagar, Chennai, Tamil Nadu, India.
Leenamma Joseph	**Lija L Raju**
Department of Zoology, Mar Ivanios College, Thiruvananthapuram, Kerala, India.	. Department of Zoology, Mar Ivanios College, Thiruvananthapuram, Kerala, India.
Krishna AP	**Dinesh Roy D**
Department of physiology, K S Hegde Medical Academy, Mangalore, Karnataka, India.	. Genetika, Centre for Advanced Genetic Studies, Pettah PO, Thiruvananthapuram, Kerala, India.

ABSTRACT Infertility is a worldwide health problem, with one in six couples suffering from this condition. Presumptive causes of infertility include anatomical, endocrinological, immunological, infections, thrombophilic disorders and other unexplained causes. Most risk factors of infertility are different in both genders and they all can contribute to a couple's inability to conceive. Common risk factors of both female and male infertility include obesity, diabetes, late marriage, alcohol consumption, smoking habits and urethral infections. Married couples who have no children had high risk of developing cardiovascular disease. The study consists of 28 infertile couples with varying risk factors were selected as study subjects and 20 healthy couples without any chronic illness were selected as control for the study. The aim of the present study was to quantify the extent of DNA damage by Cytokinesis- block Micronuclei (CBMN) assay along with evaluating the biochemical parameters in infertile subjects and to assess the risk for cardiovascular diseases. Detailed demographic, anthropometric and clinical characteristics were recorded and compared. The present study demonstrated that micronuclei frequency was significantly elevated in the study subjects than control subjects. Infertile subjects with various risk factors such as age, birth order, parental consanguinity, family history of infertility/subfertility, family history of cancer, history of CAD, history of chronic illness, hypertension and dyslipedimia etc. can lead to increased genetic instabilities and the severity of infertility. Lifestyle modifications by increasing physical exercise and dietary control will help in modifying the CVD risk factors in infertile subjects.

INTRODUCTION:

According to the International Committee for Monitoring Assisted Reproductive Technology and the World Health Organization, infertility is 'a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse' (Zegers et al., 2009). Infertility is a common medical problem that affects 5–8% of couples in developed countries and 5.8% to 44.2% in developing nations (Rutstein and Macro, 2004). It affects an estimated 15% of couples globally, amounting to 48.5 million couples (Macaluso et al., 2010). Globally, every year 60-80 million couples suffer from infertility as estimated of which India alone is probably between 15-20 million (25%) (Sharath et al., 2013).

The presumptive causes of infertility include anatomical, endocrinological factors, immunological factors, infections, thrombophilic disorders and unexplained causes (Diego et al., 2006; Manvelyan et al., 2008). In about 22-28% of couples the cause of infertility remains unexplained (Kamath and Bhattacharya, 2012). Unexplained infertility (UI) is a diagnosis when all the standard investigations such as tests for ovulation, tubal patency tests and semen analysis are normal (ASRM, 2006; Ray et al., 2012).

Most risk factors of infertility are different in both genders and they all can contribute to a couple's inability to conceive (Touqeer et al., 2014). Males are found to be solely responsible for 20-30% of infertility cases and overall contribute to 50% of cases (Ashok et al., 2015). A number of etiologies have been identified as potential causes of male infertility, which include gene mutations, aneuploidies, infectious diseases, ejaculatory duct occlusion, varicocele, radiation, chemotherapy and erectile dysfunction (Ollero et al., 2001). In females, problems regarding menstruation (ammenhorroea, polymenhorroea, menorrhagia, dysmenhorroea) along with insanitation are the major danger alarms (Maeda and Tsukamura, 2006). Any cause leading to irreversible or non-compensable damage to the genital tract, uterus, fallopian tubes or ovaries may cause inability to conceive (Touqeer et al., 2014).

Specific genotypes and karyotypes have been associated with infertility phenotypes and studies of specific genes in humans and model systems defined the nature of the polygenic and multifactorial basis of infertility (Narjes et al., 2015). Infertility phenotypes have been associated with specific genetic conditions such as mutations in the cystic fibrosis (CFTR) gene, mutations or microdeletions in specific Y chromosome genes or the presence of constitutional numerical or structural chromosomal aberrations (Shah et al., 2003). The sex chromosome aberrations and the presence of constitutional inversions, translocations or small supernumerary marker chromosomes (sSMC) can lead both to infertility and repeated

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

abortions (Liehr et al., 2004; Shah et al., 2003). The disturbances in the organization of the genomic material in sperm nuclei are negatively correlated with the fertility potential of spermatozoa, either in vivo or in vitro (Sun et al., 1997; Spano et al., 2000).

Men with lower sperm quality have been shown to have a less healthful diet, which would also be expected to lead more cardiovascular disease (CVD). Just as erectile dysfunction can indicate poor lifestyle habits and can be an important indicator of cardiovascular risk; it appears that male infertility also predicts future CVD (Sumina et al., 2014). Women with primary ovarian insufficiency (POI) may be more burdened by CVD, such as myocardial infarction and stroke (La et al., 1987; Sumina et al., 2014). Menstrual irregularity is related to increased risks for women of CVD in later life (Solomon et al., 2002). Stress and anxiety can also have an impact on both fertility and heart disease (Michael et al., 2011).

Diagnostic assessment of infertility is indicated when pregnancy has not occurred within one year of regular unprotected intercourse. Infertility can lead to distress and depression, as well as discrimination and ostracism. Global, regional and national estimates of prevalence and trends in infertility are needed to target prevention and treatment of infertility. A number of factors contribute to reproductive success; it is obvious that infertility causes are heterogeneous. Changes in genetic factors and biochemical characteristics contribute to the etiology of infertility in humans. Defects in reproductive health of a human will cause future health problems and it also predicts future cardiovascular diseases. Hence the present study was undertaken to quantify the extent of DNA damage by Cytokinesis Block Micronuclei (CBMN) assay along with evaluating the biochemical parameters in infertile subjects and to assess the risk for cardiovascular diseases.

MATERIALS AND METHODS:

Twenty eight infertile couples were selected for this study. The samples were referred from various infertility clinics of kerala to Genetika, Centre for Advanced Genetic Studies, Thiruvananthapuram, Kerala. Twenty healthy subjects without any chronic illness were also selected as control for this study. Detailed demographic, clinical and lifestyle characteristics were recorded using proforma. In this study, Cytokinesis -block Micronuclei (CBMN) assay was carried out in each subject. CBMN assay was performed by using Cytochalasin B for quantifying the extent of somatic DNA damages.

Seven ml of blood sample was collected by venepuncture. Two ml of blood was transferred into sodium heparinized vacuutainers for quantifying the extent of somatic DNA damages by Cytokinesis-block Micronuclei (CBMN) assay. The remaining five ml of blood was transferred into a plain tube. Blood was allowed to clot, serum separated immediately. Blood sugar and lipid profile were estimated using semi-automated clinical chemistry analyzer.

Two ml blood was added to a culture tube containing 10 mL RPMI 1640 supplemented with 100units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 100µg/mL phytohemagglutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) after 44th hours of initiation of cells with phytohaemagglutinin. Cells were harvested after 72 hr incubation, and they were treated with a hypotonic solution (0.075M KCl) for 1 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

RESULTS:

Study subjects include 28 infertile males and 28 infertile females with the mean CBMN frequency of 14.39 and 14.12 respectively. The control subjects include 20 males and 20 females with the mean CBMN frequency of 10.32 and 9.82 respectively. The mean CBMN frequency was statistically higher among the study subjects than the healthy control subjects.

Distribution of mean CBMN frequency according to various demographic and anthropometric characteristics of the study subjects were given in table 1.

Table 1:

Variables	Category	Male	Mean CBMN frequency	Female	Mean CBMN frequency
Age (years)	<30	3	14.23	13	14
	30 to 40	18	14.58	13	14.22
	>40	7	14.92	2	14.23
Birth Order	<3	13	14.23	14	14.10
	3 to 5	10	14.39	11	13.91
	>5	5	14.60	3	14.30
Religion	Hindu	15	14.41	15	14.01
	Christian	6	14.03	6	14.71
	Muslim	7	14.66	7	14.32
Residence	Coastal	1	14.36	1	15
	Rural	20	14.36	20	14.24
	Urban	7	14.48	7	13.67
Parental Consanguinity	Yes	3	14. 43	1	14.2
	No	25	14. 05	27	14.12
Duration of Married Life	<5	12	14. 19	12	14.07
	5 to 10	14	14. 30	14	14.15
	>10	2	14. 49	2	14.22
Economic Status	High	5	14.81	5	14.08
	Medium	19	14.34	19	14.23
	Low	4	14.07	4	13.66

Distribution of mean CBMN frequency according to various clinical characteristics of the study subjects were given in table 2.

Table 2:

Variables	Category	Male	Mean CBMN frequency	Female	Mean CBMN frequency
Family h /oInfertility/subinfertility	Yes	2	15.04	1	14.56
	No	26	14.34	27	14.11

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Family h/o Cancer	Yes	4	14.95	-	-
	No	24	14.3	28	14.12
H/o CAD	Yes	11	15.18	9	14.80
	No	17	13.88	19	13.80
H/o Chronic Illness	Yes	1	15.2	1	14.13
Diabetes	Yes	8	14. 7	6	14.13
	No	20	14. 27	22	14.12
Hypertension	Yes	5	14.39	3	14.16
	No	23	14.37	25	13.83
Dyslipedimia	Yes	11	14.49	4	14.14
	No	17	14.33	24	14.12

Biochemical assessments of all the study subjects are given in table 3.

Table 3:

Variables	Category	Male	Mean CBMN frequency	Female	Mean CBMN frequency
FBS (mg/dl)	<100	1	14.33	13	13.95
	100 to 125	9	14.41	9	14.24
	>125	18	15.2	6	14.33
Total Cholesterol (mg/dL)	<200	8	14.33	14	14.02
	200 to 240	13	14.37	10	14.14
	>240	7	14.5	4	14.35
HDL (mg/dL)	<40	15	14.44	14	14.3
	40 to 60	13	14.35	7	14.18
	>60	-	-	7	13.98
LDL (mg/dL)	<100	4	14.37	7	14
	100 to 160	21	14.39	19	14.10
	>160	3	14.42	2	14.21
TG (mg/dL)	<150	18	14.31	17	13.99
	150 to 300	8	14.5	11	14.21
	>300	2	14.54	-	-

Based on female physiological characteristics, 8 female subjects (28.57%) had irregular menstrual periods and had highest mean CBMN frequency of 14.15. Infertile women with ≥14 years of menarche age showed mean CBMN frequency was 14.22 and <14 years of menarche age showed CBMN frequency was 14.11. 2 infertile female study subjects (7.14%) had endometriosis and they showed highest mean CBMN frequency (14.13) and 3 female subjects (10.71%) had PCOS and showed highest mean CBMN frequency of 14.16. Majority of the infertile subjects were never used contraceptive drugs (n=27; 96.42%) and their mean CBMN frequency was 14. Only 1(3.57%) subject was used contraceptive drugs had the highest mean CBMN frequency of 14.13.

Male physiological characters were observed. Semen analysis showed that 23 male subjects (82.14%) were normal and had the mean CBMN frequency of 14.30. 2 male subjects (7.14%) had azoospermia with mean CBMN frequency of 15. Only 1 subject showed oligospermia with highest mean CBMN frequency of 15.04. Among the infertile male subjects none of them had the habit of chewing and drinking. 7 (25%) infertile males had the habit of smoking and they had highest mean CBMN frequency of 14.40 and others showed mean CBMN frequency of 14.39.

DISCUSSION:

Te velde and Pearson, (2002) showed that, the gradual loss of fertility becomes more dramatic in the late 30s, in spite of ovulatory cycles, ending in menopause at a mean age of 50 –51 years (Ottolenghi et al., 2004; Broekmans et al., 2007). The present study also showed that increase in the age of study subjects showed increased mean CBMN frequency (males: 14.92; females: 14.23).

According to Ajeet, (2014) demographic characteristic of the couples is one of the factors affecting fertility. The majority of cases with primary infertility were from urban area. But the present study showed, among male study subjects, men belonged to urban area had highest mean CBMN frequency (14.48) and among females, women belonged to the coastal area had highest mean CBMN frequency (15).

A study by Michael et al., (2011) found that men with no or one child seemed to show higher risks of cardiovascular disease than those with more children. The present study was also carried out to assess the risk factor for cardiovascular diseases in infertile couples. This shows that, the study subjects with family h/o CAD showed higher mean CBMN frequency (Males-15.18 & Females- 14.80) than the subjects does not have the family h/o CAD (Males: 13.88 &Females: 13.80).

Enrique et al., (2014) also indicated that higher levels of serum total cholesterol, free cholesterol and phospholipids are associated with a significantly lower percentage of sperm with intact acrosome, smaller sperm head area and perimeter. This study also showed that increase in total cholesterol may increase mean CBMN frequency. The study subjects both males and females with total cholesterol >240 mg/dL had highest mean CBMN frequency (Males- 14.5 & Females: 14.35).

Low testosterone can increase the risk of all cause mortality and heart disease. It can also cause decreased level of HDL cholesterol (Micheal et al., 2011). The present study showed that study subjects with low HDL level had higher mean CBMN frequency.

According to Enrique et al., (2014) increased serum VLDL, total triglycerides and testosterone levels were significantly correlated with decreased sperm motility in a group of infertile men. This study also showed higher mean CBMN frequency in the subjects with higher TG. Male subjects with TG >300 mg/dL had highest mean CBMN frequency of 14.5. Female subjects with TG between 15 and 300 mg/dL had highest mean CBMN frequency of 14.21.

According to Ajeet, (2014) menstrual irregularities in the form of any deviation from normality like, oligomenorrahia, hypo or hypermenorrhgia were also significant risk factors for primary infertility. The present study also showed that, the 8 (28.57%) subjects with irregular menstrual cycle showed highest mean CBMN frequency of 14.15 than the subjects with regular menstrual cycle.

Mokhtar et al., (2006) revealed that females with age of menarche more than 15 years were more risky to develop infertility than those with age of menarche less than 15 years. Study subjects with age of menarche ≥14 (n=3; 10.71%) had higher mean CBMN frequency (14.22) than the subjects with

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

age of menarche <14.

The studies by Dutta and Guha, (2007) and Mokhtar et al., (2006) found that endometriosis was considered as a risk factor for primary infertility. In this study, revealed that the mean CBMN frequency was statistically higher among women with endometriosis (14.13) women without endometriosis. Ajeet, (2014) also showed that the PCOS was a significant risk factor associated with primary infertility. The present study showed a significant relationship between PCOS and mean CBMN frequency, study subjects with PCOS showed higher mean CBMN frequency (14.16) than subjects without PCOS (13.84).

The studies have demonstrated that smoking significantly decreases the chance of conception (Hughes and Brennan, 1996; Augood et al., 1998). Cigarette smoking has been associated with adverse effects on fertility, although this is not widely recognized. Smoking negatively affects sperm production, motility and morphology and is associated with an increased risk of DNA damage (Kunzle et al., 2003). This present study showed that 25% of total male subjects had habit of smoking and they showed highest mean CBMN frequency of 14.40.

CONCLUSIONS:

In short, infertile couples showed an increased mean CBMN frequency in demographic, clinical, biochemical, physiological and lifestyle characters. The extent of DNA damages was found higher in those couples with an increased age, increased duration of married life and increased birth order and parental consanguinity. Infertile couples have higher risk of developing heart disease and CVD. These findings suggest that lifestyle changes along with increased consumption of high energy dense foods and increased physical activity leads to successful pregnancy. From this study it can be conclude that infertile subjects with DNA damages and various changes in the biochemical parameters had an increased risk for CVD.

REFERENCES:

1. Ajeet Vasant Saoji. Primary infertility problems among female have been a source of concern in india lately. innovative journal of medicaland health science 4:1 jan- feb 2014; 332-340.
2. Anderson SE, Dallal GE, Must A. infertility and its prevalence 2003; vol.132 no. 2, pp. 135-142.
3. Ashok Agarwal, Aditi Mulgund, Alaa Hamada, Michelle Renee Chyatte. A Unique view on male infertility around globe. Reprod Biol Endocrinol. 2015; 13:37.
4. ASRM. Effectiveness and treatment of unexplained infertility: the practice committee of the American Society for Reproductive Medicine. Fertil Steril 2006; 86 Suppl 4: S111–114.
5. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser BC. Female reproductive ageing: current knowledge and future trends. Trends Endocrinol Metab 2007; 18:58–65.
6. Browne RW, Bloom MS, Shelly WB, Ocque AJ, Huddleston HG, Fujimoto VY. Follicular fluid high density lipoprotein-associated micronutrient levels are associated with embryo fragmentation during IVF. J Assist Reprod Genet. 2009; 26:557–560.
7. Charlton M. Obesity, hyperlipidemia, and metabolic syndrome. Liver Transpl 2009; (Suppl 2):S83–9.
8. Crawford AG, Cote C, Couto J, Daskiran M, Cunnarsson C, Haas K, et al. Prevalence of Obesity, Type II Diabetes Mellitus, Hyperlipidemia, and Hypertension in the United States: Findings from the GE Centricity Electronic Medical Record Database. Pop Health Management 2010; 13:151–61.
9. Diego-Alvarez D, Ramos-Corrales C, Garcia-Hoyos M, Bustamante-Aragones A, et al. (). Double trisomy in spontaneous miscarriages: cytogenetics and molecular approach. Hum.Reprod 2006; 21: 958-966.
10. Dutta S, Guha R. A. Clinico- anatomical study on the ethiological factors pertaining to primary infertility in females using some common investigative procedures. J AnatSoc India 2007; 56: 14-7.
11. Enrique F. Schisterman, Sunni L. Mumford, Zhen Chen, Richard W. Browne, Dana Boyd Barr, Sungduk Kim, and Germaine M. Buck Louis. Lipid Concentrations and Semen Quality: The LIFE Study Andrology 2014; 2(3): 408–415.
12. Evenson D P, Larson K L, Jost l k. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with the other techniques. J 2002.
13. Fujimoto VY, Kane JP, Ishida BY, Bloom MS, Browne RW. High-density lipoprotein metabolism and the human embryo. Hum Reprod Update 2010; 16:20–38.
14. Hughes IA, Werner R, Bunch T, Hiort O. Androgen insensitivity syndrome. Semin Reprod Med 2012; 30: 432–442.
15. Kamath MS, Bhattacharya S: Demographics of infertility and management of unexplained infertility. Best Pract Res Clin Obstet Gynaecol 2012; 26:729-738.
16. Kunzle R, Mueller MD, Hanggi W, Birkhauser MH, Drescher H, Bersinger NA. Semen quality of male smokers and nonsmokers in infertile couples. Fertil Steril 2003;79:287–91.
17. La Vecchia C, Decarli A, Franceschi S, Gentile A, Negri E, Parazzini F. Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age. Am J Obstet Gynecol 1987; 157:1108–1112.
18. Liehr T, Claussen U, Starke H: Small supernumerary marker chromosomes (sSMC) in humans. Cytogenet Genome Res 107:55–67; 2004.
19. Lloyd-Jones DM, Hong Y, Labarthe D, et al. on behalf of the American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's Strategic Impact Goal through 2020 and beyond. Circulation. 2010; 121:586–613.
20. Macaluso M, Wright-Schnapp T, Chandra A, Johnson R, Satterwhite C, Pulver A, et al. A public health focus on infertility prevention, detection, and management. Fertil Steril 2010; 93: 16.
21. Manvelyan M, Riegel M, Santos M, Fuster C, et al. Thirty-two new cases with small supernumerary marker chromosomes detected in connection with fertility problems: detailed molecular cytogenetic characterization and review of the literature 2008; Int. J. Mol. Med. 21: 705 714.
22. Michael L. Eisenberg, et al. Fatherhood and the risk of cardiovascular mortality in the NIH- AARP Diet and Health Study Hum. Reprod 2011.
23. Mokhtar S, Hassan H A, Mahdy N, Elkhwsky F, Shehata G. Risk factors for primary and secondary infertility in Alexandria: a hospital-based case-control study. JMRI 2006; 27: 255- 261.
24. Narjes Armanet, Lucie Tosca, Sophie Brisset, Thomas Liehr, Gérard Tachdjian. Small Supernumerary Marker Chromosomes in Human Infertility. Cytogenet Genome Res 2015; 146:100–108.
25. Ollero, M., Gil-Guzman, E., Lopez, M., Sharma, R., Agarwal, A., Larson, K., Evenson, D., Thomas, A.J., Jr and Alvarez, J.GCharacterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis of male infertility 2001.
26. Ottolenghi C, Uda M, Hamatani T, Crisponi L, Garcia JE, Ko M, Pilia G, Sforza C, Schlessinger D, Forabosco A. Aging of oocyte, ovary, and human reproduction. Ann N Y Acad Sci 2004; 1034:117–131.
27. Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility 2012; 24(6):591–602.
28. Rutstein S.O, Macro O.R.C. Infecundity, Infertility, and Childlessness in developing countries. DHS Comparative Reports 2004.
29. Saad Elzanathy, Johan Malm. Effects of ejaculation- to-analysis delay on levels of markers of epididymal and accessory sex gland functions and sperm motility. Journal of Andrology 2007; 28:847-852.
30. Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK: The genetic basis of infertility. Reproduction 2003; 126:13–25.
31. Sharath KC, Najafi M, Malini SS. Association of Obesity with Male Infertility among Infertile Couples is not Significant in Mysore, South India. Advanced Studies in Biology 2013; 319 – 325.
32. Solomon C G, Hu F B, Dunaif A, Rich-Edwards J E, Stampfer M J, Willett W C, Speizer F E, Manson J E. Menstural cycle irregularity and risk for future cardiovascular disease. J Clin Endocrinol Metab 2002; 87:2013-2017.
33. Sumina Cherian, Dinesh Roy D, Pallavi JS , Jiju JS, Aswathy Sundaresh , Sansina Christy S and T Vijayakumar. Cardiovascular Risk Markers in Infertile Subjects. International Journal of Scientific & Engineering Research February-2014; Volume 5, Issue 2.
34. Sun, J G, Jurisicova A, Casper R F. Deletion of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol. Reprod 1997; 56, 602-607.
35. Te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002; 8:141–154.
36. Touqeer Hussain, Abubakar Tauseef, Asra Bari, Umber Rasheed, Jahan Ara Hassan. Awareness among general population attending Civil Hospital Karachi about risk factors associated with infertility. J Pak Med Assoc 2014; Vol. 64, No. 6.
37. World Health Organization (WHO). Infections, pregnancies and infertility: perspectives on prevention. Fertil. Steril 1987; 47:964-968.
38. Zegers-Hochschild F, Adamson GD, de MJ, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009; 92:1520–1524.
39. Zhang Hongxia, Wang Shuyu, Zhang Songwen, Wang Tao, Deng Xiaohong. 2014. Increasing trend of prevalence of infertility in Beijing. Chinese Medical Journal 2014; 127(4). Nisha I. Parikh
40. Sven Cnattingius, Murray A. Mittleman, Jonas F. Ludvigsson, and Erik Ingelsson ; Subfertility and risk of later life maternal cardiovascular disease. Hum Reprod. 2012 Feb; 27(2): 568–575.
41. Zlotogora J, Hujerat Y, Barges S, Shalev SA, Chakravarti A (2006) The fate of 12 recessive mutations in a single village. Ann Hum Genet 71:202–208.