Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11088
DOI URL: http://dx.doi.org/10.21474/IJAR01/11088

RESEARCH ARTICLE

CHROMOSOMAL ABNORMALITIES IN HUMAN REPRODUCTIVE FAILURE: A RETROSPECTIVE STUDY FROM A TERTIARY CARE CENTRE

Paresh Singhal[1], Praveen Kumar[2] and Nikita Naredi[2]

1. Department of Pathology, Armed Forces Medical College, Wanowrie, Pune-411040.
2. Departments ofARTC, Command Hospital (SC),Wanowrie, Pune-411040.

Manuscript Info

Manuscript History
Received: 05 April 2020
Final Accepted: 07 May 2020
Published: June 2020

Key words:-
Human Reproductive Failure, Recurrent Pregnancy Loss, Primary Infertility, Chromosomal Abnormalities

Abstract

Chromosomal abnormalities are important cause of human reproductive failures, which may manifest as pregnancy loss or infertility. *Objectives:* To determine the prevalence of chromosomal abnormalities in couples with recurrent pregnancy loss (RPL) and primary infertility. *Methods:*Cytogenetic evaluation (karyotyping and fluorescent in situ hybridisation) of peripheral blood T-lymphocytes on 61 couples with two or more clinically proven pregnancy losses with or without history of stillbirth or children with congenital malformations; and 51 females and 63 males with primary infertility. *Results:* Overall rate of major chromosomal abnormalities in human reproductive failure was 10.2%. 61 Couples with RPL had major cytogenetic abnormality in 6.5% cases. 114 patients with female and male primary infertility had 13.7% and 14.2% as major anomalies, respectively.Structural abnormalities(4.9%) were more common in RPL group, while numerical anomalies (8.8%) were common in infertility. Polymorphic variants were seen in 6.3% of all the cases. *Conclusion:*Cytogenetic evaluation is an essential screening & diagnostic tool in reproductive failures, as appropriate therapeutic strategies along with genetic counselling can be advised to couples with chromosomal abnormalities.

Introduction:-

Human reproductive failure (HRF) is indeed a frequent event involving couples desirous of beginning or extending their family life cycle, as the reproductive system is quite vulnerable affecting large proportion of conceptions. HRF includes various entities involving primary infertility, habitual abortions, recurrent spontaneous abortions, recurrent miscarriages (RM) or recurrent pregnancy losses (RPL). These terminologies may be further confused by their synonymous usage in different context by various authors in discontiguous geographical locations.

The clinical subtype of HRF, RPL is a distinct condition defined as two or more spontaneous clinically recognised (proven by imaging or histopathology) pregnancy losses before 20 weeks of gestation [1]**.** Whereas,as per European Society for Human Reproduction and Embryology, it is defined as loss of three or more consecutive pregnancies before 12 weeks of gestation inclusive of non-visualized ones [2]. Primary infertility is defined by the World Health Organisation (WHO) as a disease of reproductive system wherein there is failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [3].

Corresponding Author:- Dr. Paresh Singhal
Address**:-** MD(Path), Post Doctoral Fellow(Cytogenetics), Armed Forces Medical College, Wanowrie, Pune, Pin: 411040.

RPL may have known causes which include immunological factors, thrombophilias, endocrine disorders, chromosomal aberrations, anatomical abnormalities, infections, drugs or chemicals [4,5]. In spite of these known factors, the etiology still remains idiopathic in remaining 50% of cases [6].

Whereas, in another subset couples presenting with primary infertility, may have one of the three major causes which include ovulatory dysfunction, tubo-peritoneal disease or multiple male factors with possible etiologies. Out of various causes documented, for male and female infertility, fractions of cases are carriers of chromosomal abnormalities which can affect gametogenesis. The rate of chromosomal aberrations in infertile group (3.9-14.3%) [7] is proportionately higher than general population (0.3-1.8%) [8]. Hence, conventional cytogenetic evaluation by karyotyping is recommended by American Urological Association (AUA) and European Academy of Andrology (EAA) in all men with total motile sperm count below 5 million/cmm [9].

Aims and Objectives:-

Cytogenetic workup for HRF is still an uncommon practice due to lack of awareness, skilled manpower as well as limited infrastructure. Cytogenetic analysis helps the treating doctor not only for establishing the genetic diagnosis, but also, it further assists in counselling the couples and in selecting appropriate therapeutic options [10]. In view of this, the aim of this retrospective study was to find the prevalence of chromosomal aberrations in individuals with reproductive failures referred to infertility clinic, of a tertiary care centre, between October 2018 to Dec 2019. The objectives were to describe the cytogenetic abnormalities in couples with following presentations: a) idiopathic RPL, as defined by American Society for Reproductive Medicine [1],b) RPL with history of any adverse pregnancy outcomes in form of stillbirths or congenital anomalies and c) Males with oligospermia/azoospermia as defined by WHO [3] and females who presented with primary infertility. Cytogenetic analysis was performed by GTG- banding of metaphase spread and further confirmation was done by Fluorescence In situ hybridisation (FISH), wherever required.

Materials and Methods:-

After obtaining approval from Institutional Ethical Committee and written formal consent from the study group, 5 ml of peripheral blood sample was withdrawn in sodium heparin anticoagulant. Conventional cytogenetic analysis (CCA) by karyotyping was performed on metaphase arrest obtained by T lymphocytes culture, stimulated by phytohemagglutinin, which is further supplemented with 15% fetal bovine serum [11]. At least 20 metaphases were analysed from each patient. When mosaicism was suspected, 30 additional metaphases were analysed in each case. Special assays like banding techniques (C (constitutive heterochromatin) and NOR (nucleolus organising region) staining) and interphase cell FISH was performed as and when required. Chromosomes analysis was done using image processor and software (Cytovision) version 7.2 build 147, Copyright Leica Microsystems (Gateshead) Ltd, Unit 7 Queens Park, Ground Floor, North Wing, Queens way North, Team Valley Trading Estate, Gateshead, NE 110Q D United Kingdom. Chromosomal abnormalities were reported according to the International System for Human Cytogenomic Nomenclature (ISCN, version: 2016) at band level of 500-500. C banding and NOR banding techniques were used for confirmation of variants. FISH analysis was done with commercially available IVD approved probes on suspicion of low level mosaicism on karyotyping for further confirmation.

The chromosomal abnormalities (CAs) were further divided into two major anomalies which include: structural abnormalities (SAs) and numerical abnormalities (NAs). SAs included balanced translocations (BTs), deletions (del), Robertsonian Translocations (RTs) and derivative chromosome (der). NAs included mosaic (mos) forms, non-mosaic forms and marker chromosomes (mar+). Another category of CAs having heteromorphic forms was classified under Polymorphic variants (PVs).

Results:-

In this retrospective study, 236 individuals with history of reproductive failure were studied for chromosomal anomalies. 61 couples presented with RPL. The mean pregnancy losses per couple were 2.7 ranging from 2 to 4. Four couples also had history of stillbirths. 63 males and 51 females had primary infertility respectively. The women in the study group ranged in age from 19-45 years (mean 25.4 years) and men from 25-47 years (mean 27.4 years). The overall prevalence of CAs in this study was 16.5%, whereas frequency of major CAs was 10.2%. The rate of major CAs in RPL couples was 6.5% and individuals with primary infertility were 14.03%. 114 patients who presented with primary infertility, females had 13.7 % (7/51) and males had 14.2% (9/63) as major CAs(Table:1).

Majority of the females (68.6%) with primary infertility cases were asymptomatic whereas non obstructive azoospermia (66.7%) was the most common clinical finding amongst male population in the present study (Table:2).

The CAs in patients with HRF are further summarised in table 3. SAs and NAs had shown similar prevalence of 5.1% (12/236) affecting 12 cases each presenting with reproductive failures. However, SAs were more frequent in RPL affecting 4.9% couples as compared to NAs (1.6%). Major SAs in RPL included BTs and RTs which were seen in 3.2% and 1.6% respectively. Females (3.25%) were more common carriers of BTs as compared to their males (1.64%) counterparts. None of the couple had CAs affecting both the partners simultaneously. SAs were common in males (6.3%) as compared to females (3.9%) with primary infertility.

NAs were the commonest chromosomal anomalies seen in males and females with primary infertility, which was commonly due to sex chromosome aneuploidies, involving 8.8% of study population (10/114). Mosaic forms of sex chromosomes were commoner NAs in both the sexes as compare to non-mosaic forms. Males showed 02 cases of mos 47 XXY/46 XY and single case of mos 47, XYY/46,XY. Whereas, Turner syndrome including mosaic form was the commonest NAs amongst infertile females affecting 7.8% (4/51) individuals. Less common NA was presence of a marker chromosome, which was seen in a female with RPL.

PVs or heteromorphisms of chromosomes, which are considered to be normal variants, were excluded from major CAs in the present study, were observed in 6.3% of all cases. These variant chromosomes were most common in infertile males.

Discussion:-

Reproductive failures are usually associated with great grief and anxiety in couples. The genesis of CAs resulting in HRF can be explained due to following phenomenon: errors in meiotic cycle during gametogenesis, errors in mitotic cycle during gametes multiplication, errors in fertilisation or post zygotic mitotic disjunction event in the embryo [12]. These abnormal mechanisms may lead to gains or losses of genetic material in gamete formations or during embryogenesis, which may result in pregnancy loss or adverse outcomes or may even cause genetic recombination in parents which may manifest as infertility. These CAs can be generated either de novo or may even run in families.

The overall prevalence of CAs in this study was 16.4%, which is significantly higher than general population [13].The frequency of major CAs was 6.5% (8/122) amongst HRF cases who presented with RPL**.** These cases had major CAs as structural anomalies affecting 4.9 % of all couples and constituted the commonest anomaly in RPL cases, which is in concurrence with other studies [14]. Further, BTs were seen in higher proportion as compared to RTs [15]. (Table- 3)

Review of literature shows that frequency of CAs with recurrent pregnancy losses varies from 1.2% to 12.0% [15-18]. These differences may be related to various inclusion criteria of study population (number of abortions, sample size, gestational age, and exclusion of already defined etiological factors) and consideration of polymorphic variant under major chromosomal abnormality [19]. The frequency of BTs in these cases was 3.3%, which involved predominantly long metacentric and submetacentric chromosomes: 2, 3 & 4 (Table-3, Fig-1 a,c&f). Single case of female patient with history RPL had an additional marker chromosome (Fig-2d). This mar + could be possibly be derived from an acrocentric chromosome, further molecular studies could not be carried out on this.

In the present study, affected females with RPL are more than their male counterparts which is in accordance Sheth et al [20]. The lower incidence of BTs in male population can be explained due to meiotic blocking of spermatogenesis which may lead to infertility, however the oogenesis is usually conserved and compatible with fertility but underlying chromosomal abnormality may results in production of defective gametes which may result in abortions or congenital malformations [21]. It has been a well-established fact that individuals who are carriers of balanced translocations (BTs and RTs) show no phenotypic abnormality, but due to the various patterns of malsegregation, (adjacent 2:2 or 3:1 or rarely 4:0) during gametogenesis they are at increased risk (1-50%) for infertility, malformed offspring or foetus with chromosomal abnormality, which is non-viable [22].

Major CAs in female infertility category was 13.7%, which is slightly lesser as compared to their male counterparts, but significantly higher than the frequency of chromosomal aberrations in general population [8]. Most of these were asymptomatic, whereas almost one third cases presented with oligomenorrhoea to amenorrhoea (Table-2). In the latter group, Turner syndrome (TS) and its variants (mosaic form and deletion of short ‘p’ arm of chromosome X)

(Fig-1d) were major abnormalities seen in 9.8% cases (5/51). Females with aneusomy of sex chromosomes are usually infertile or sub fertile, but they may also have pregnancy with RPL or even normal outcome [23]. The asymptomatic group (Table-3) had < 1% of major CAs which included a balanced reciprocal translocation involving long 'q' arm of chromosomes 11 &13,and a mos 47, XXX/46,XX (Fig-2a&4a). Monosomy of X chromosome, which may lead to TS and its variant, arise from malsegregation of sex chromosomes in post zygotic mitotic phase. Haploinsufficiency of important genes, due to absent X chromosome, results in follicular atresia and eventually ovarian dysfunction [24]. In contrast, BTs do not influence ovarian reserve and is uncommon cause for infertility, rather this group may have increased risk for adverse pregnancy outcomes as miscarriages or malformed children [21].

The frequency of CAs in males, who presented with azoospermia or total motile sperms count of less than 5 million/cmm on three separate occasions, was 14.2%, which is in agreement with other authors [25,26]. Non-obstructive azoospermia (NOA) was the commonest symptom in this category. Asymptomatic males with normal semen analysis had no major anomaly except occurrence of PVs (Table-2). Amongst major CAs, NAs (7.9%) were more common than SAs (6.3%). Klinefelter syndrome (KS) was the commonest NAs including two case of mosaic forms (>3 cells with 47,XXY chromosomal constitution) (Fig-2b), which was further confirmed on FISH analysis (Fig-4c). Apart from reproductive issues, males with KS are not only at high risk for endocrine dysfunctions and malignancies [27], but their children may also have higher risk for chromosomal disorders [28]. Therefore this further emphasizes that screening of males with NOA by cytogenetic studies, will not only explain cause of infertility and decide its management aspects, but also forewarn clinician and patient for future complications. Isolated case of mos 47,XYY/46,XY syndrome (less commonly known as Jacob's syndrome) was also seen in the study (Fig: 2c&4b). Though XYY syndrome does not manifest with infertility problems, there are few case reports in this disorder with fertility issues [29].

SAs were represented by BTs and RTs (two cases each, Table: 3 and Fig: 1b, e & g). Males who are carrier of these translocations usually are phenotypically normal as there is no loss of genetic material, but they are unfortunately at high risk for reproductive failures like infertility, RPL or may even have offspring with congenital anomalies due to major genetic imbalances in gametes (monosomy or trisomy) or even uniparental disomy (UPD) [30].

PVs were seen in 6.3% of all the cases with HRF. These variants were not included in major CAs in the present study, because still it is unclear whether these variants have any association with reproductive failures or not [31]. Most common variant in the present study was pericentric inversion of chromosome 9, inv(9) (Fig-3a). Other variants involving autosomes were prominent constitutive heterochromatin region in chromosome 16(Fig-3b); and satellite-stalk region prominence in acrocentric chromosomes, 15 and 22 (Fig- 3c&d). One male showed prominent heterochromatin in long 'q' arm of Y chromosome, which causes elongation to the extent that it resembles a group 'D' acrocentric chromosome(Fig-3e). Though the clinical significance of the consequences of these variants is still unknown, there is a need to consider the significance of future pregnancy outcomes in patients with HRF for better counselling and treatment planning [32].

Conclusion:-

According to the present study, we conclude that: (1) Frequency of major CAs was present in 10.2% of patients with reproductive failures. (2) Structural anomalies were more common in RPL, vis-a-vis numerical anomalies in primary infertility. (3) More number of metaphase analyses should be considered in diagnosing cases with low level mosaicism in primary infertility.

To further summarize, chromosomal studies still remain the corner stone for genetic evaluation of patients with reproductive failures, as this assay is the gold standard for simultaneous detection of balanced translocations and numerical abnormalities. Hence, cytogenetic analysis is important not only for genetic diagnosis of constitutional abnormalities but also may assist in appropriate management selection, so that affected couples can be counselled and advised accordingly for a happy and successful ending.

Table 1:- Prevalence of cytogenetic abnormalities in study population of HRF, RPL= Recurrent Pregnancy loss.

Reproductive failure subtype	n	Normal karyotype	Cytogenetic abnormalities (n) (%)	Polymorphic variants n (%)
Couples with RPL	61(n=122)	109	8 (6.5%)	5(4.09%)

Female Primary infertility	51	41	7 (13.7%)	3(5.8%)
Male Primary infertility	63	47	9 (14.2%)	7(11.1%)
Total	236	197	24 (10.2%)	15(6.3%)

Table 2:- Clinical profile of primary infertility cases (females and males).

Clinical Diagnosis	**n**	**Normal Karyotype (n)**	**Cytogenetic abnormality (n)**	**Polymorphic variant (n)**
Female Primary Infertility				
Asymptomatic (except infertility)	35[@]	30	2	3
Primary Amenorrhoea	12	8	4	-
Oligomenorrhoea	4	3	1	-
Total	51	41	7	3
Male Primary Infertility				
Asymptomatic [#]	9[@]	7	-	2
NOA	42	35	6	1
Oligospermia*	6	3	1	2
OAT*	5	2	1	2
Hypogonadism	1	-	1	-
Total	63	47	9	7

#Sperm count > 5 million/cmm, *Sperm count <5 million/cmm ,
@ Males with their nine asymptomatic partners.
NOA= Non obstructive azoospermia, OAT= Oligoasthenoteratoazoopermia

Table 3:- Overview of cytogenetic aberrations in patients with HRF.

Reproductive failure subtype	n	SAs	F	NAs	F	Polymorphic Variants	F
Couples with RPL	122	**(Females)** 3.25% (4/122), n=4					
		46,XX, t(2;10) (p23;q22)	1	45,X	1	46,XX,inv(9) (p11q13)	2
		46XX, t(4;6)(q21;p12)	1	47,XX, +mar	1	46,XY,16 qh+	1
		46,XX, t(3;4) (q27;q21.2)	1			46,XY, 22pstk +	2
		46,XX, t(7;14) (p15.1;q13.2)	1				
		(Males) 1.64% (2/122), n=2					
		45,XY, rob(13;21) (q10;q10)	2				
Female infertility	51	46,X,del(X) (p11.2)	1	45,X	2	46,XX,5ps+	1
		[$] 46,XX, t(11;13) (q13.3;q12.1)	1	mos 45,X[3] /46,XX[27]	2	46,XX,22 pstk +	1
				[$] mos 47,XXX [4]/ 46 , XX[26]	1	46,XX, 16qh+	1
Male infertility	63	46,XY, der(20) ins(22;20) (q11.2;q13)	1	47,XXY	2	46,XY,inv(9) (p11q13)	4
		45,XY, rob(21;22) (q10;q10)	1	mos 47,XXY[4]/ 46,XX[26]	2	46,XY, 22pstk+	1
		46,XY, t(15;17)	1	mos 47,XYY[5]/	1	46,XY,16qh+	1

		(q11.2;q24)		46,XY[25]			
		45,XY, rob(21;21) (q10;q10)	1			46,X,Yqh+	1

[$] Clinically asymptomatic, SAs= Structural abnormalities, NAs= Numerical abnormalities, F= Frequency, mos= Mosaic forms, der= derivative chromosome

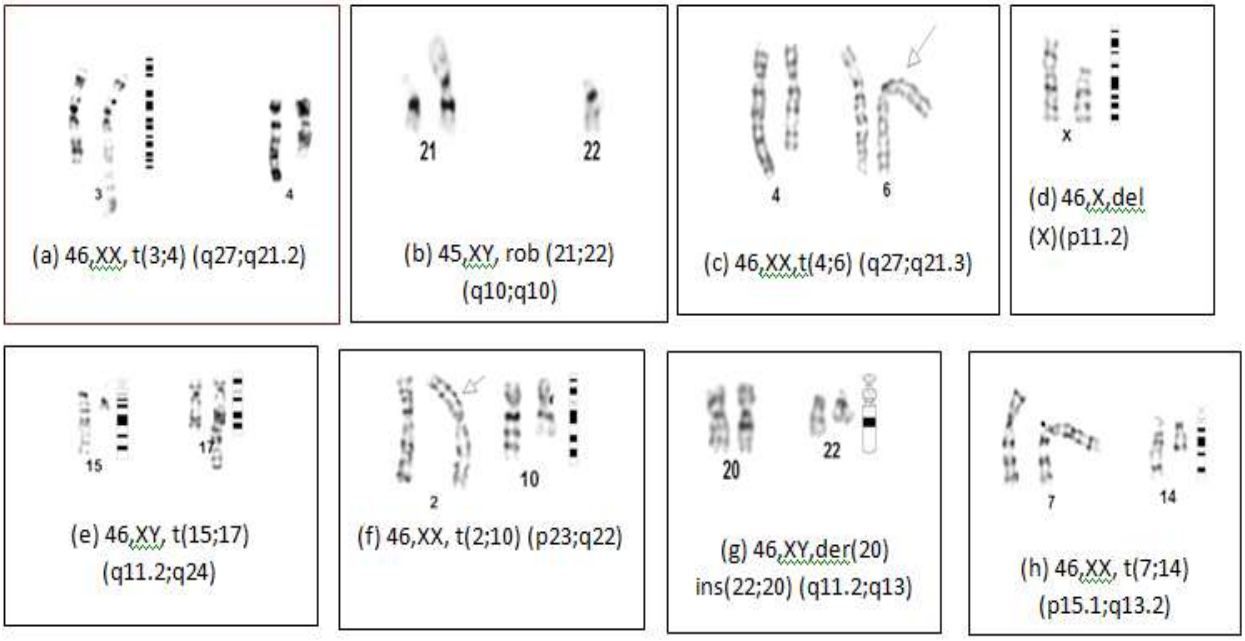


Fig 1:- Representative partial karyograms GTG banding of few uncommon structural abnormalities (SAs) with adjacent ideograms in our study.

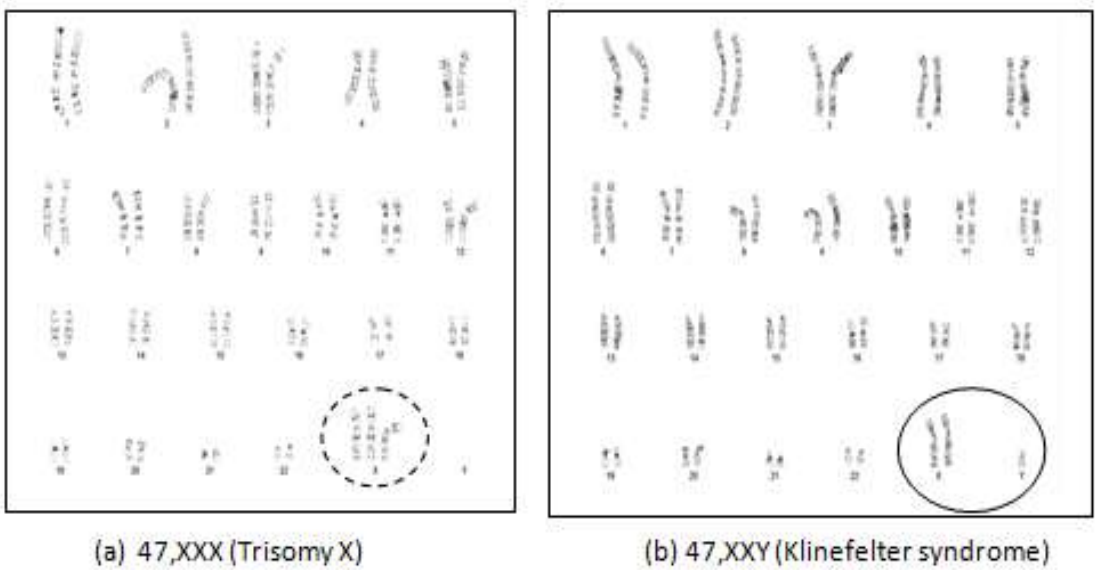
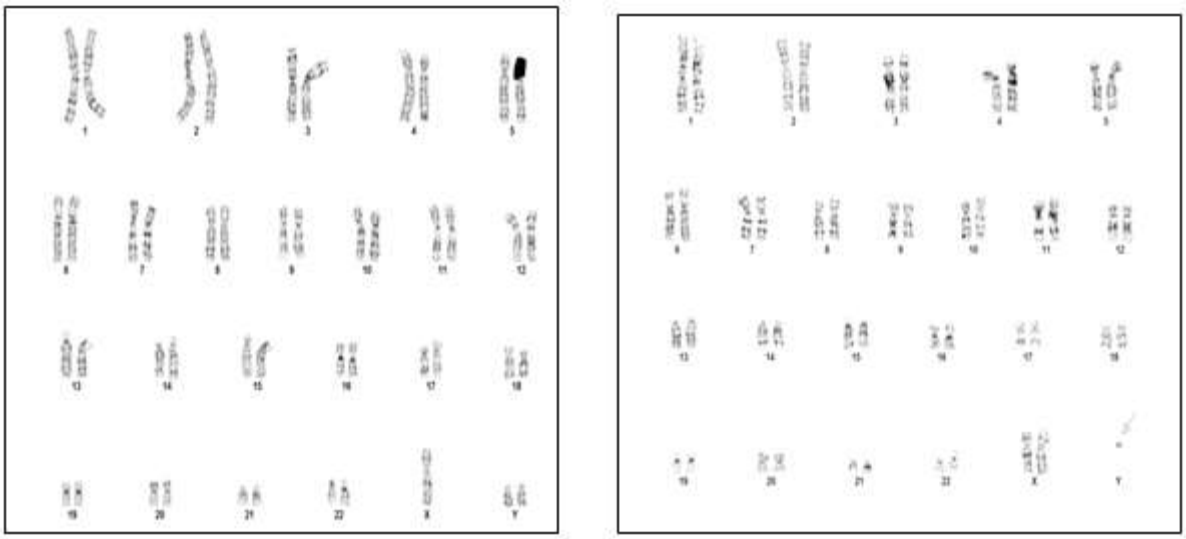
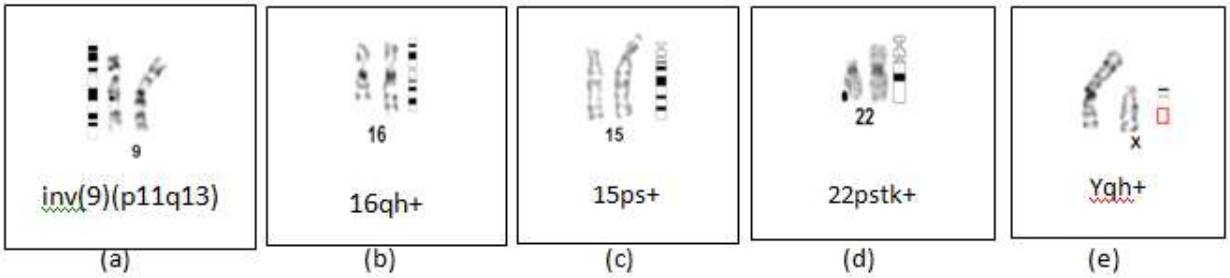


Fig 2:- Representative karyotypes GTG banding of numerical abnormalities (NAs).



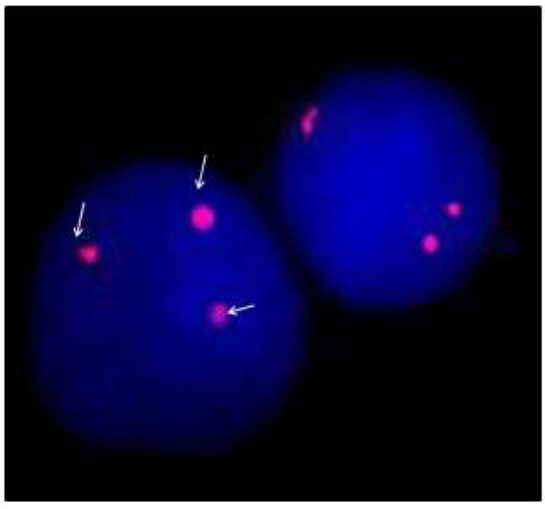
(c) 47,XYY syndrome (d) 47,XX,+mar (arrow)

Fig 3:- Representative partial karyograms of GTG banding of Polymorphic variants (PVs).

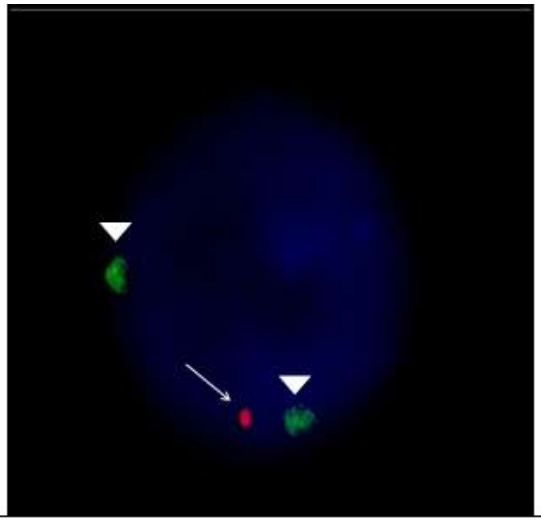


(a) (b) (c) (d) (e)

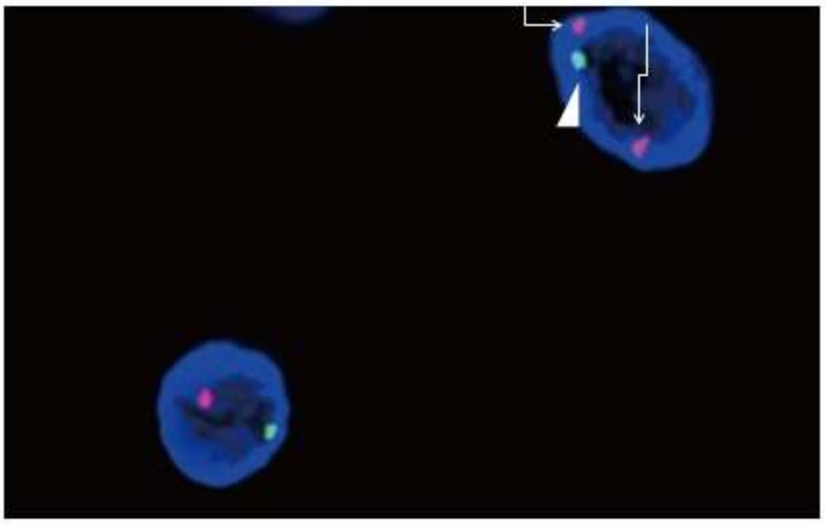
Fig 4:- Representative interphase FISH images using Chromosome Enumeration Probes for detection of sex chromosome aneuploidy.



(a) Two adjacent interphase cells showing three X chromosomes represented by red signals (arrows)



(b) Interphase cell with 01 X-chromosome and 02 Y chromosomes represented by a red signal(arrow) and two green signals(arrow heads), respectively.



(c) Two interphase cells: Top cell shows two X and one Y chromosomes represented by two red signals (arrows) and single green signal (arrow head) respectively. Bottom cell shows normal XY constitution.

References:-

1. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: A committee opinion. Fertil Steril.2012; 98:1103–11.
2. Kolte AM, Bernardi LA, Christiansen OB, et al. ESHRE Special Interest Group, Early Pregnancy Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. Hum Reprod. 2015;30(3):495–498..
3. Zegers‐Hochschild F, Adamson GD, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology. FertilSteril 2009;92:1520‐4.
4. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: current perspectives. Int J Womens Health. 2017; 9: 331-45.
5. Shahine L, Lathi R. Recurrent pregnancy loss: evaluation and treatment. ObstetGynecolClin North Am. 2015; 42 (1): 117-34.
6. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. Fert Steril.2010; 93:1234-43.
7. Kayed HF, Mansour RT, Aboulghar MA, et al. Screening for chromosomal abnormalities in 2650 infertile couples undergoing ICSI. ReprodBioMed Online. 2006;12:359–70.
8. Forabosco A, Percesepe A, Santucci S. Incidence of non-agedependent chromosomal abnormalities: a population-based study on 88965 amniocenteses. Eur J Hum Genet. 2009;17:897–903.
9. Hotaling J and Carrell DT. Clinical genetic testing for male factor infertility: current applications and future directions. Andrology 2014; 2: 339–350.
10. Mascarenhas M, Thomas S, Kamath MS et al. Prevalence of chromosomal abnormalities and Y chromosome microdeletion among men with severe semen abnormalities and its correlation with successful sperm retrieval. J Hum ReprodSci 2016; 9: 187–193.
11. Hungerford DA, Moorehead PS, Nowell PC, Mellman WJ, Battips DM. Chromosomal preparation of leucocytes cultured from human peripheral blood. Exp Cell Res 1960,20:613-16.
12. Yanowitz J. Meiosis: making a break for it. CurrOpin Cell Biol 2010; 22: 744–51.
13. Ravel C, Berthaut I, Bresson JL, Siffroi JP. Prevalence of chromosomal abnormalities in phenotypically normal and fertile adult males: large-scale survey of over 10,000 sperm donor karyotypes. Hum Reprod. 2006;21: 1484–9.

14. Fan HT, Zhang M, Zhan P, Yang X, Tian WJ, Li RW. Structural chromosomal abnormalities in couples in cases of recurrent spontaneous abortions in Jilin Province, China. Genet Mol Res. 2016; 15(1).
15. Kiss A, Rosa RF, Dibi RP, Zen PR, Pfeil JN, Graziadio C, Paskulin GA. Chromosomal abnormalities in couples with history of recurrent abortion. Rev Bras Ginecol Obstet. 2009; 31 (2): 68-74.
16. GadaSaxena S, Desai K, Shewale L, Ranjan P, Saranath D. Chromosomal aberrations in 2000 couples of Indian ethnicity with reproductive failure. Reprod Biomed Online. 2012; 25 (2): 209-18.
17. Karatas A, Eroz R, Albayrak M, Ozlu T, Cakmak B, Keskin F. Evaluation of chromosomal abnormalities and common trombophilic mutations in cases with recurrent miscarriage. Afr Health Sci. 2014; 14 (1): 216-2.
18. Kalotra V, Lall M, Saviour P, Verma IC, Kaur A. Prevalence of Cytogenetic Anomalies in Couples with Recurrent Miscarriages: A Case-control Study. J Hum Reprod Sci. 2017;10(4):302–9.
19. Fryns JP, Van Buggenhout G. Structural chromosome rearrangements in couples with recurrent fetal wastage. Eur J ObstetGynecolReprodBiol 1998; 81: 171–6.
20. Sheth FJ, Liehr T, Kumari P, Akinde R, Sheth HJ, Sheth JJ. Chromosomal abnormalities in couples with repeated fetal loss: An Indian retrospective study. Indian J Hum Genet. 2013;19(4):415–22.
21. Mau-Holzmann UA. Somatic chromosomal abnormalities in infertile men and women. Cytogenet Genome Res. 2005;111:317–36.
22. Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK. The genetic basis of infertility practice. Reproduction. 2003;126:13-25.
23. Tsui KM, Yu WL, Lo FM, Lam TS. A cytogenetic study of 514 Chinese couples with recurrent spontaneous abortion. Chin Med J (Eng) 1996;109:635-8.
24. Modi DN, Sane S, Bhartiya D. Accelerated germ cell apoptosis in sex chromosome aneuploid fetal human gonads. Mol Hum Reprod 2003;9:219–225.
25. Hofherr SE, Wiktor AE, Kipp BR et al. Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience. J Assist Reprod Genet 2011; 28: 1091–98.
26. Elghezal H, Hidar S, Braham R, et al. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. FertilSteril 2006; 86: 1792–95.
27. Sokol RZ. It's not all about the testes: medical issues in Klinefelter patients. FertilSteril 2012; 98:261–65.
28. Ron-El R, Strassburger D, Gelman-Kohan S, et al. A 47,XXY fetus conceived after ICSI of spermatozoa from a patient with nonmosaicKlinefelter's syndrome: case report. Hum Reprod 2000; 15: 1804–06.
29. El-Dahtory F, Elsheikha HM. Male infertility related to an aberrant karyotype, 47,XYY: four case reports. Cases J. 2009 Jan 8;2(1):28.
30. Tempest HG, Simpson JL. Role of preimplantation genetic diagnosis (PGD) in current infertility practice. Int J Infertil Fetal Med. 2010; 1: 1–10.
31. Hong Y, Zhou YW, Tao J, Wang SX, Zhao XM. Do polymorphic variants of chromosomes affect the outcome of in vitro fertilization and embryo transfer treatment? Hum Reprod. 2011;26:933–40.
32. Minocherhomji S, Athalye AS, Madon PF, Kulkarni D, Uttamchandani SA, Parikh FR. A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. FertilSteril. 2009;92:88–95.