The Role of Chromosomal Aberrations in Recurrent Pregnancy Loss

Sangamithra Saravanan

Abstract

Miscarriage is the loss of pregnancy before the 20-24th week of gestation. The loss of more than two pregnancies before 20 weeks of gestation is referred to as recurrent pregnancy loss (RPL), recurrent miscarriages (RM), or spontaneous abortion due to natural foetal loss (ASRM) by the European Society of Human Reproduction and Embryology (ESHRE), and by the American Society for Reproductive Medicine (ASRM). The most commonly known factors responsible for miscarriages include chromosomal aberrations, anatomical abnormalities of the uterus, endocrine dysfunction, immunological issues, congenital irregularities, infectious diseases, and psychological and environmental factors. Approximately 50% of these are unexplained recurrent pregnancy losses (URPL). The current research study focuses on Recurrent Pregnancy Loss due to Chromosomal aberrations. In this study, 24 Blood samples and 5 Products of Conception (POC) samples were harvested from individuals with RPL and were analyzed through Conventional karyotyping and Fluorescent in-situ Hybridisation (FISH), respectively. Around 16.66% of the blood samples showed an abnormal karyotype, whereas 2 out of the 5 POC samples had trisomy 21.

Keywords: Chromosomal Aberrations, Product of Conception, Conventional Karyotyping, Fluorescent In-Situ Hybridisation.

Introduction

The loss of a pregnancy before 20-24 weeks of gestation is called a miscarriage. Intrauterine miscarriages are relatively common outcomes in pregnancy with 10-20% of clinical pregnancies resulting in miscarriages. It is a relatively common event, occurring in 15% - 25% of pregnancies, and increasing prevalence with maternal age **(Barnabei et al., 2002)**. Indeed, the risk is between 9% and 12% in women aged 35 years but increases to 50% in women aged 40. This figure is likely an underestimation as it excludes miscarriages which occur before the pregnancy is detected. Recurrent Pregnancy Loss (RPL), or Recurrent Miscarriages (RM) or spontaneous abortion due to the natural loss of the fetus are the terms used to define the loss of >2 pregnancies before 20 weeks of gestation by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). There is inconsistency in the definition of RPL with controversies on whether biochemical losses and non-consecutive losses should be included in the definition which would increase the prevalence of RPL to 2-3% **(Elisabeth Clare Larsen et al., 2013)**. Miscarriages occur due to a wide range of factors. These commonly include chromosomal aberrations, anatomical abnormalities of the uterus, endocrine dysfunction, immunological issues, congenital irregularities, infectious diseases, and psychological and environmental factors. Approximately 50% of these are unexplained recurrent pregnancy losses (URPL) **(Sultana S et al., 2020).**

Couples could be normal but carriers of a balanced chromosomal abnormality. Parental chromosomal aberrations such as translocations, inversions, and abnormal copies of a chromosome (trisomy or monosomy etc.) are as common as 2-5% than in the general population

(0.7%) **(Dimitriadis et al., 2020)**. There is a 4-5% chance of recurrent miscarriages due to balanced translocation in parental chromosomes. According to **Van dyke et al., 1983 & Jacobs et al., 1992**, every 1 person in 500 has a reciprocal translocation heterozygote. Approximately, half of the total number of miscarriages arise due to an irregularity in the transfer of chromosomes to the embryo. This particular genetic issue arises accidentally. It is not brought on by any medical issue. However, the probability of this complication occurring increases with age. Typically, people carrying an abnormal karyotype, such as a translocation, do not exhibit symptoms themselves but have a higher chance of producing gametes with chromosomal aberrations. If the embryonic aneuploidy is irregular, it often leads to miscarriages **(Genetic Alliance, 2009).**

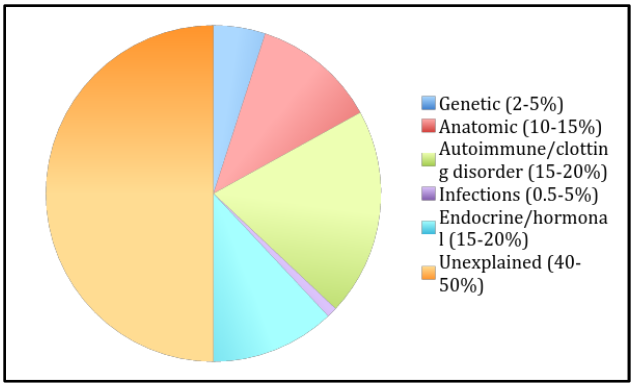


Figure 1: Causes of Recurrent Miscarriages (Source: uscfertility.org)

Figure 1. illustrates specific medical issues that could contribute to having more than one miscarriage more likely.

1. **Congenital Problems of the Uterus**–Though there are other congenital uterine diseases, a septate uterus is one of the most prevalent and linked to miscarriage. In this circumstance, a tissue wall partially separates the uterus into two portions (Figure 2).
2. **Aschermann syndrome** –This ailment results in uterine adhesions and scarring.
3. **Fibroids and polyps** –They are benign (noncancerous) uterine growths.
4. **Antiphospholipid syndrome (APS)** is an autoimmune condition that affects blood clotting and may result in several health issues. APS can manifest on its own or alongside other autoimmune conditions like lupus.
5. **Diabetes mellitus**–Diabetes can raise the chance of miscarriage, particularly if the condition is not properly managed. Lower the risk by maintaining normal blood sugar levels both before and throughout pregnancy.
6. **Thyroid disease**–If thyroid gland issues are left neglected, the risk of miscarriage may increase. Treatment for thyroid issues can reduce the risk. **(acog.org)**
7. **PCOS**–Individuals with polycystic ovarian syndrome (PCOS) have a three-fold increased risk of miscarriage during the first trimester of pregnancy **(Boomsma et al., 2008).**
8. Other hypothesized causes, such as persistent endometritis, familial thrombophilia, and high sperm DNA fragmentation levels, are also up for debate.

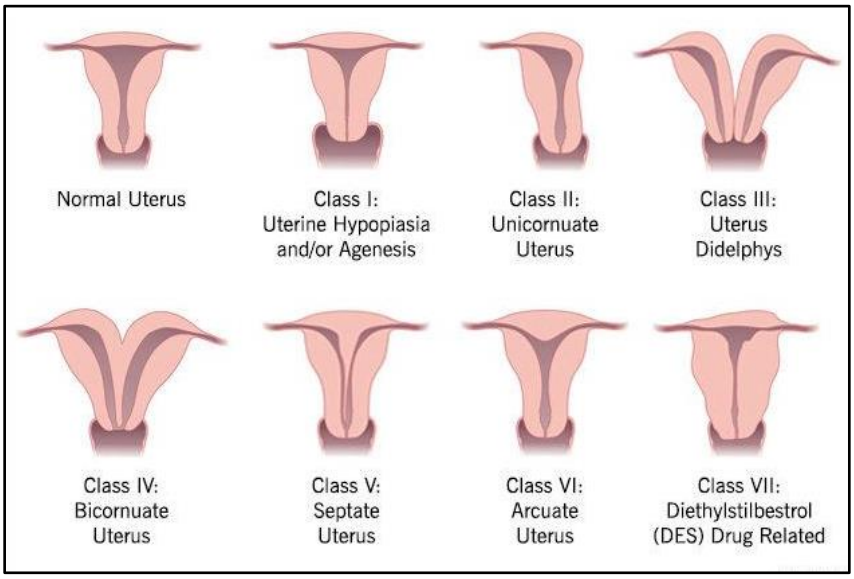


Figure 2: Anatomical Abnormalities of the Uterus (Source: New Centralized Care for Patients with Mullerian Anomalies, 2023s)

Identifying the cause: In the vast majority of instances, RPL has no identified aetiology.

1. Age, medical background and previous pregnancies.
2. A thorough physical examination including a pelvic exam.
3. Examination of the blood to look for immune system issues.
4. Genetic causes of recurrent miscarriages may be found through testing.
5. If a uterine issue is causing recurrent miscarriages, imaging testing may be considered. **(acog.org)**

Material and methods:

The study was conducted with 24 blood samples of 12 couples for Conventional Karyotyping (CK) and 5 samples were of Product of Conception (POC) for Fluorescence in situ hybridization (FISH). The samples used in the study are of couples with recurrent pregnancy loss/ frequent miscarriages/ repeated pregnancy loss (RPL) who have had two or more spontaneous abortions. For Conventional Karyotyping, the age group of the females ranged from 26 to 39 whereas the age group of males ranged from 28 to 41. Information about demographic details like age, sex, clinical indications, lifestyle factors etc. were taken from the couples. For FISH, the POC samples were obtained from females aged between 31 to 39. The POC samples were obtained by the practitioners and sent to the AGILE Lab, New Delhi, for the investigation of genetic causes of the patient's miscarriages. The Product of Conception samples mentioned above, according to the general definition, are the fetal tissues, and uterine contents like the umbilical cord, placenta etc. that are removed due to the miscarriage.

Slides for CK were prepared using Giemsa banding Technique (GTG banding) and were analyzed using the CytoVision software. At least 50 metaphases were observed from each sample. In the

case of FISH, 2.5µl of DAPI (4',6-diamidino-2-phenylindole) probe solution was applied to the Region of Interest (ROI) and analyzed under a fluorescent microscope. In the first image of the pair of images from a sample of POC, the green-coloured probe is attached to chromosome 13 and the orange is for chromosome 21. In the second image of the pair, the aqua-coloured probe is for chromosome 18 and the green is for chromosome X.

RESULTS

Table 1: Case Details and Results for Conventional Karyotyping (CK)

Case	Test	Age	Sex	Sample Type	Karyotypes
1	CK	31	Male	Blood	46, XY
	CK	34	Female	Blood	46, XX
2	CK	35	Male	Blood	46, XY
	CK	32	Female	Blood	46, XX
3	CK	37	Male	Blood	46, XY
	CK	28	Female	Blood	46, XX
4	CK	36	Male	Blood	46, XY
	CK	33	Female	Blood	46, XX
5	CK	35	Male	Blood	46, XY
	CK	29	Female	Blood	46, XX
6	CK	39	Male	Blood	46, XY

	CK	37	Female	Blood	46, XX
7	CK	38	Male	Blood	46, XY
	CK	37	Female	Blood	46, XX
8	CK	32	Male	Blood	46, XY
	CK	28	Female	Blood	46, XX
9	CK	39	Male	Blood	46, XY
	CK	35	Female	Blood	Abnormal *Female*, *45, XX,t(13;14)*
10	CK	41	Male	Blood	46, XY
	CK	39	Female	Blood	Abnormal *46, XX,t(6;15)*
11	CK	28	Male	Blood	Abnormal *46 XY,t(7;11)*
	CK	26	Female	Blood	46, XX
12	CK	37	Male	Blood	Abnormal *46, XY, Inv9*
	CK	37	Female	Blood	46, XX

Table 2: Case Details and Results of Product of Conception (POC) Samples

Case	Test	Age	Sex of the fetal tissue	Sample Type	Karyotype
1	FISH	31	Female	POC	Abnormal *Trisomy 21*
2	FISH	35	Male	POC	46, XY
3	FISH	39	Male	POC	46, XY
4	FISH	37	Female	POC	Abnormal *Trisomy 21*
5	FISH	32	Male	POC	46, XY

Conventional Karyotyping (CK)

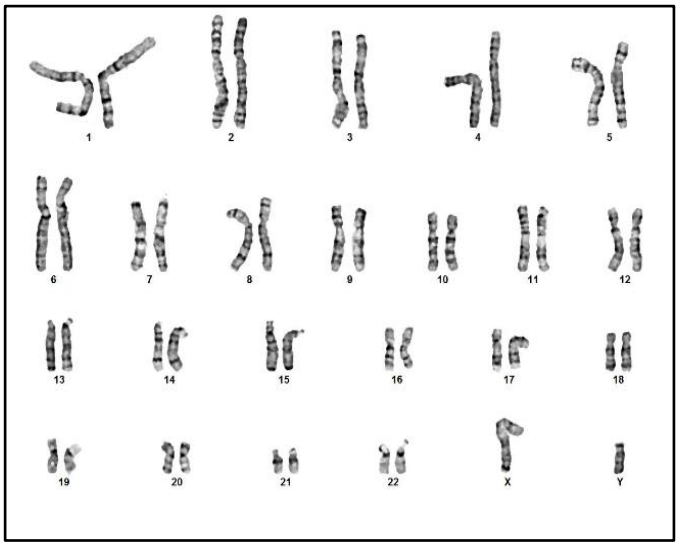


Figure a: Normal Male Karyotypes (46, XY)

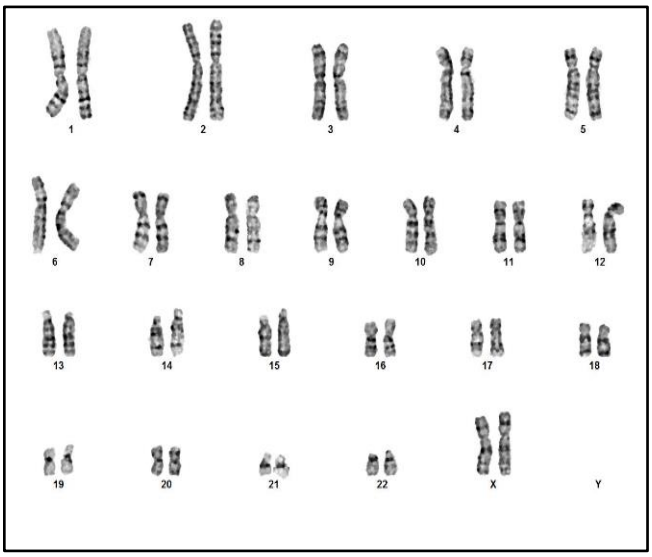


***Figure b*:** Normal Female Karyotype (46, XX)

Abnormal Karyotype Cases

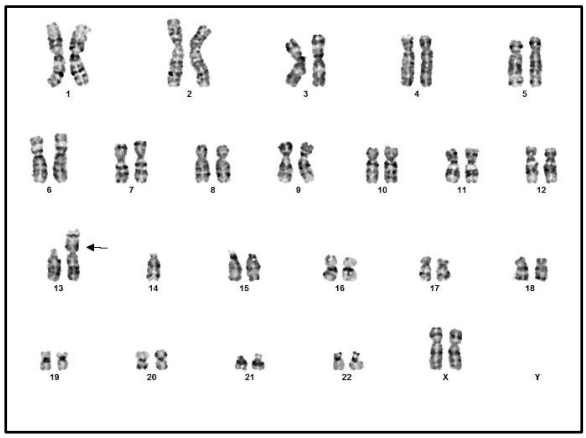


***Figure c*:** Case 9, Abnormal Female, 45, XX, t(13;14)

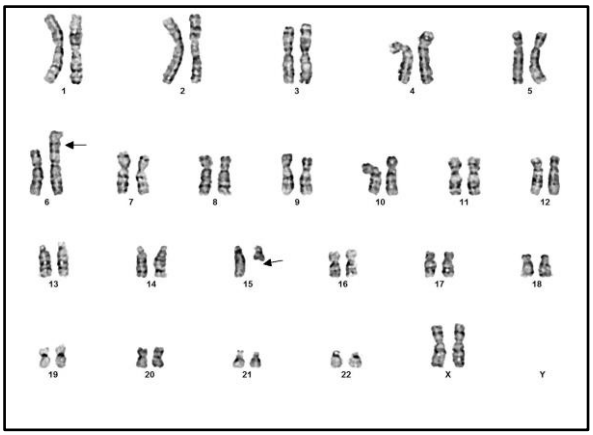
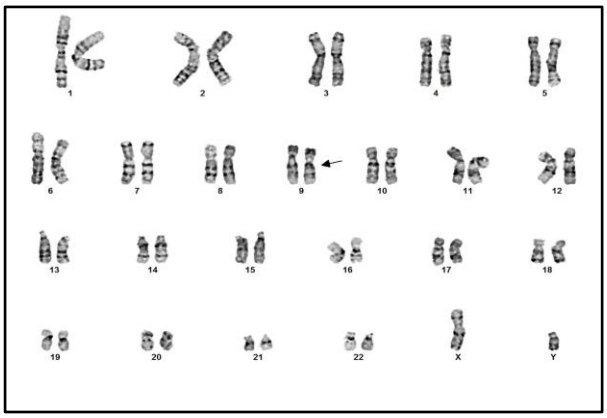


***Figure d*:** Case 10, Abnormal Female, 46, XX,t(6;15)



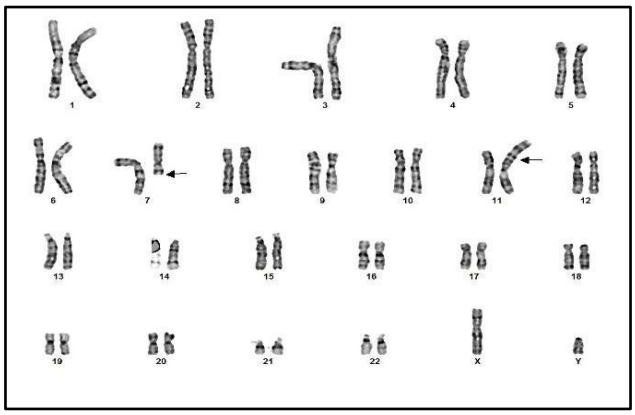


Figure e: Case 12, Abnormal Male, 46, XY, Inv9 ***Figure f:*** Case 11, Abnormal Male, *46 XY,t(7;11)*

A total of 12 couples with a history of two or more repeated miscarriages were included in this study. The mean age of the female partner was 29.83 years (range: 26-39 years), and the male partners were 35.66 years (range: 28–41 years) **(Pal et al., 2018)**. In the present study, all females were nonsmokers and nonalcoholic. However, the information on the percentage of tobacco chewing and alcohol intake in males was unidentified.

Among 12 couples (24 individuals), chromosomal abnormalities were detected in 4 couples (33.33%). Details of the chromosomal abnormalities of both partners are shown in Table 1.

Three of the four subjects (75%) displayed structural abnormality, whereas one subject (25%) exhibited numerical variations. Four cases were examined, out of them, two (50%) had balanced translocations, one (25%) had Robertsonian translocation, and one (25%) had chromosome 9 inversion. Four of the 12 couples—2 (16.66%) males and 2 (16.66%) females—exhibited abnormal karyotypes. As a result, both males and females experienced the exact prevalence of chromosomal abnormality.

Case 9 (figure c) shows the abnormal karyotype of a female with 45 chromosomes instead of 46 because there is a Robertsonian Translocation of chromosomes 14 to 13. Case 10 (Figure d) shows the abnormal karyotype of a female with 46 chromosomes. In this case, there is a balanced translocation of chromosome 15 with 6. Case 11 (Figure e) shows the case of an abnormal karyotype with 46 chromosomes, where there is a balanced translocation of chromosomes 7 to 11. Case 12 (Figure f) is a male sample with an inversion of chromosome 9.

FISH Results on Product of Conception (POC): Normal FISH

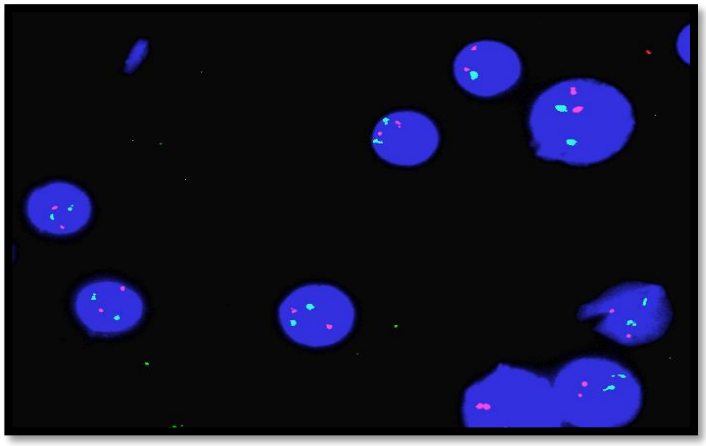


Figure g: Case 5 Normal 13, 21

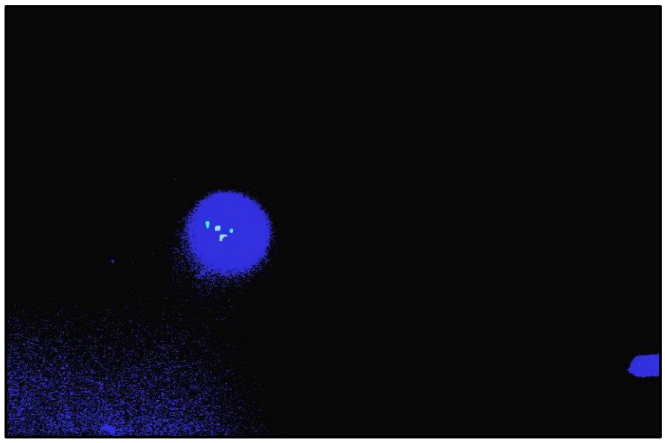


Figure h: Case 5 Normal 18, XY

Note: In figure g, the green colour probe is attached to chromosome 13 and the orange is for chromosome 21. In Figure h, aqua is for chromosome 18 and green is for chromosome X.

Abnormal Cases – FISH

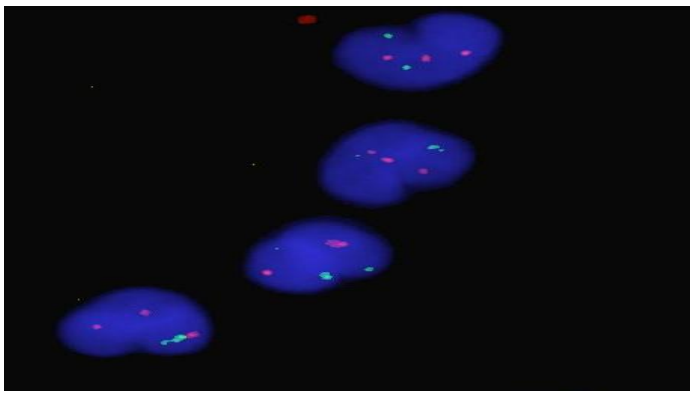


Figure i*:* Case 1, Abnormal (13, Trisomy 21)

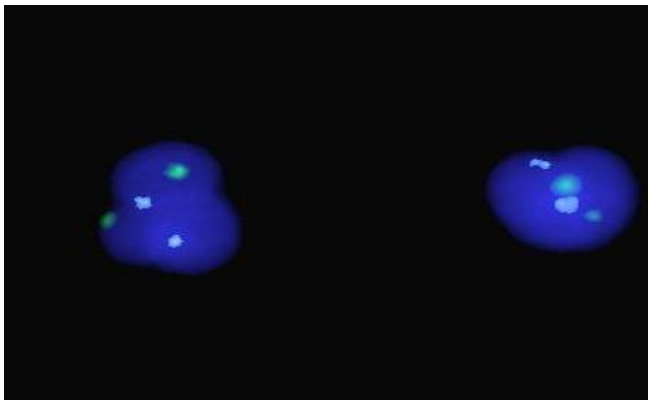


Figure j*:s* Case 1, 18, XX

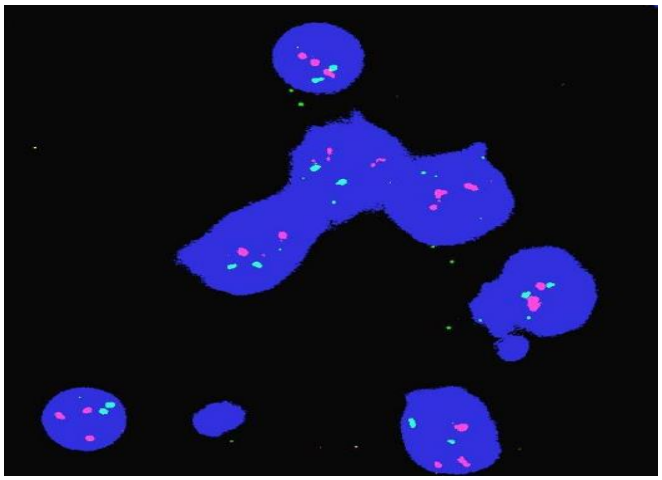


***Figure k**:* Case 4, Abnormal (13, Trisomy 21)

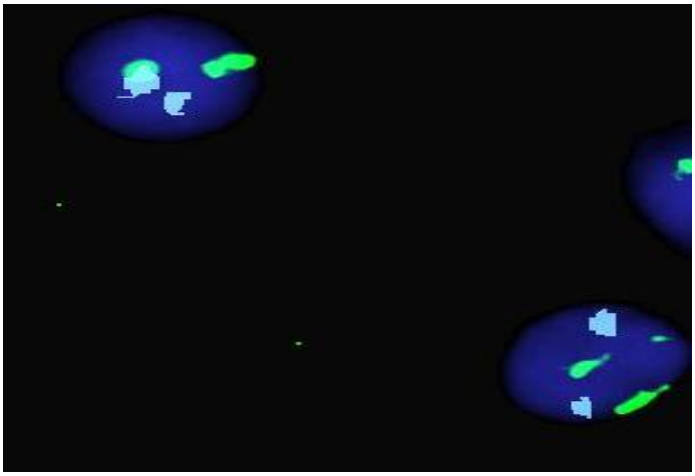


***Figure l*:** Case 4, 18, XX

In the POC samples observed using FISH, two of the five (40%) had abnormal aneuploidy. Both the abnormal cases, Case 1 (Figure i) and Case 4 (Figure k), had a trisomy of chromosome 21 which leads to Down syndrome.

Discussion:

In this study, the prevalence of chromosomal aberration in Conventional Karyotyping was 33.33 %. In couples encountering RPL but displaying chromosomal abnormalities, RPL may also be caused by anatomical abnormalities, environmental factors, genetic composition of the parents, hormonal imbalances, and autosomal disorders. More than 50% of RPLs remain unexplained. Chromosomal aberrations include balanced reciprocal translocations, Robertsonian translocations, inversions of chromosomes, and trisomies or monosomies of chromosomes.
Balanced reciprocal translocations are caused due to the exchange of DNA segments with non-homologous chromosomes. Among the chromosomal abnormalities leading to pregnancy loss, they have the highest prevalence where the risk of miscarriage is 25%-50% **(Pal et al., 2018)**. This is supported by the results of this study as 50% of the cases with chromosomal abnormalities which resulted in miscarriage had balanced reciprocal translocation.
Robertsonian translocations, on the other hand, are comparatively less prevalent as the estimated risk of miscarriage in this Robertsonian translocation is approximately 25% **(Pal et al., 2018)**. Robertsonian translocation occurs when two acrocentric chromosomes fuse. The current results of this study align with this, wherein 25% of the cases had Robertsonian translocation **(Elhady et al., 2020)**.

The greater the imbalance in the karyotype, the likelihood of a miscarriage also increases. Parents may have normal phenotypes and still be carriers of translocations. These translocations in the chromosomes are exemplified during meiosis cell division, which results in a larger imbalance in the gametes.

Down syndrome is caused due to an extra copy of chromosome 21 in the genetic composition. It's the most common intellectual disability associated with chromosomal conditions occurring in every 1 in 800 births worldwide. In 2002, it was observed that strict monitoring and treatment for their associated conditions such as congenital heart disease has increased their life expectancy from 30 years to 60 years. More research is being actively undertaken to increase the life expectancy of individuals with Down syndrome (Bull, M.J., 2020).

Conclusions:

Further research is required to understand the root causes of unexplained recurrent pregnancy loss (URPL). The current study shows that only 16.6% of the blood samples had chromosomal abnormalities. Also, of the 5 POC samples, only two had trisomies of chromosome 21. Recurrent Pregnancy Loss (RPL) is physically and mentally taxing on the parents. The parents must be well informed of their chances of delivery. Hence, Genetic Counseling should be made mandatory for couples facing RPL. Thereby helping the patients understand their situation and lay out their possibilities for a better understanding of the stressful situation. A good initiative may include making mental health care accessible to such patients. There has been significant improvement in diagnosing chromosomal aberrations via techniques such as Next Generation sequencing. Following this, a universal definition for RPL should be stated which will help in receiving accurate data, which currently has been diluted and varied due to the inconsistencies in the definition.

Acknowledgement:

I want to thank the mentors at LabAssure, AGILE, New Delhi for allowing me to use their resources. Most of all, I want to thank my mentor Dr. Ankita Dave (Ph.D.) for guiding me through this study.

References:

1. ACOG: Home. Retrieved September 3, 2023, from https://www.acog.org/
2. Barnabei, V. M., Grady, D., Stovall, D. W., Cauley, J. A., Lin, F., Stuenkel, C. A., ... & Pickar, J. H. (2002). Menopausal symptoms in older women and the effects of treatment with hormone therapy. Obstetrics & Gynecology, 100(6), 1209-1218.
3. Boomsma, C. M., Fauser, B. C., & Macklon, N. S. (2008). Pregnancy complications in women with polycystic ovary syndrome. *Seminars in Reproductive Medicine, 26*(1), 72−84.
4. Bull, M. J. (2020). Down syndrome. New England Journal of Medicine, 382(24), 2344-2352.
5. Causes of Pregnancy Loss. Retrieved August 29, 2023, from https://uscfertility.org/usc-center-pregnancy-loss/causes-recurrent-pregnancy-loss/
6. Dimitriadis, E., Menkhorst, E., Saito, S., Kutteh, W. H., & Brosens, J. J. (2020). Recurrent pregnancy loss. Nature reviews disease primers, 6(1), 98.
7. Elhady, G. M., Kholeif, S., & Nazmy, N. (2020). Chromosomal aberrations in 224 couples with recurrent pregnancy loss. Journal of Human Reproductive Sciences, 13(4), 340.
8. Genetic Alliance; The New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services. Understanding Genetics: A New York, Mid-Atlantic Guide for Patients and Health Professionals. Washington (DC): Genetic Alliance; 2009 Jul 8. APPENDIX F,

CHROMOSOMAL ABNORMALITIES. Available from: https://www.ncbi.nlm.nih.gov/books/NBK115545/

9. Jacobs PA, Browne C, Gregson N, Joyce C, White H. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. J Med Genet. 1992;29:103–8.
10. Larsen, E. C., Christiansen, O. B., Kolte, A. M., & Macklon, N. (2013). New insights into mechanisms behind miscarriage. *BMC medicine*, *11*(1), 1-10.
11. *New Centralized Care for Patients with Mullerian Anomalies*. (2022, March 3). Consult QD. Retrieved August 29, 2023, from https://consultqd.clevelandclinic.org/new-centralized-care-for-patients-with-mullerian-anomalies/
12. Pal, A. K., Ambulkar, P. S., Waghmare, J. E., Wankhede, V., Shende, M. R., & Tarnekar, A. M. (2018). Chromosomal aberrations in couples with pregnancy loss: a retrospective study. Journal of human reproductive sciences, 11(3), 247.
13. Sultana S, Nallari P, Ananthapur V. Recurrent pregnancy loss (RPL): an overview. J Women's Health Dev 2020;03:302–15.
14. Sultana S, Nallari P, Ananthapur V. Recurrent pregnancy loss (RPL): an overview. J Women's Health Dev 2020;03:302–15.
15. Van Dyke DL, Weiss L, Roberson JR, Babu VR. The frequency and mutation rate of balanced autosomal rearrangements in man estimated from prenatal genetic studies for advanced maternal age. Am J Hum Genet. 1983;35:301–8