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Cytogenetic investigation in prenatal specimens for effective prognosis of pregnancy related complications

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Abstract: Background: Alterations in chromosomal content of mother and infant are central characteristics of various complications related to pregnancy and early childhood. About 60% of the pregnancy losses, 2-3% of all the neonates and 50% of childhood deafness, blindness, mental retardation and 1 to 10 % of all the malignancies are directly due to genetic factors. Hence cytogenetic testing of pre and post natal samples can prove to be useful for discovery of non-invasive markers for prevention of such conditions beforehand. Aim of the study: The present study was carried out to detect numerical and structural abnormalities in 56 subjects with repeated miscarriages, bad obstetric history and sub fertility by analysing peripheral blood, products of conception (POC) material, and recovered cell lines from prenatal samples. Methods: Conventional cytogenetics: Peripheral blood culture (PBC) supplemented with mitogen Phytohemagglutinin (PHA), metaphase chromosomes was harvested after 72 hours for chromosome analysis. Tissue cytogenetics: Culture of solid tissue was used as a source for mitotic cells from products of conception (POC) from first trimester spontaneous abortions for aneuploidy detection; and Prenatal chromosome analysis was performed by either chorionic villus sampling (CVS), amniotic fluid and cord blood after culture. Image acquisition and analysis was performed by using automated karyotyping (IKAROS) software based on G, C and R banding. Results: Chromosomal abnormalities were located in all types of specimens but were predominantly observed in recurrent pregnancy loss (RPL) and product of conception (POC) samples. Aberrations observed were mainly translocations, satellites, additions in RPL cases like 46,XX with instances of (D/D,D/G,G/Gassociations);45,XX,rob(13;14);46,XXt(4;21);46,XX,(9qh+);46,XX,(14ps+);46,XX, t(5;6) and ploidy involving 67,XX+;64,XXX+;69,XXX;63,XXX;58,XX+ in the POC cases. Conclusion: Cytogenetic screening could provide to be a useful method for monitoring patients with abnormal pregnancies. The cytogenetic result is an independent prognostic indicator, with certain karyotypes associated with a good prognosis for the better treatment.

Keywords: Products of Conception (POC), Peripheral Blood Culture (PBC), Karyotyping (G, C and R Banding)

1. Background and Introduction

All species are affected by genetic diseases. Chromosomal manifestations are diverse and numerous, including early embryonic death, minor to major congenital defects, and infertility or sterility. Pregnancy loss is defined as the unexpected and unplanned spontaneous loss of pregnancy before the fetus becomes capable of extra uterine survival.

About 15% of all recognized pregnancies end in spontaneous abortions. The single most common reason is the presence of a chromosome abnormality in the fetus particularly if the loss occurs early in the pregnancy. Approximately 50% of such miscarriages are associated with cytogenetic abnormalities, with trisomy being the most frequent, followed by polyploidy and monosomy X [Kalousek et al., 1993]. Such miscarriages are thought to

occur on a random basis, with an increasing frequency of trisomy with advancing maternal age [Hassold and Chiu, 1985]. More than 99% of chromosomally abnormal pregnancies result in miscarriage, most of which occur prior to 10 weeks gestation [Jacobs and Hassold, 1987]. About 60% of recurrent miscarriages might be caused by chromosomal aberrations in the embryo [Carp et al., 2004]. About 3-5% of couples with recurrent miscarriage have one partner with balanced chromosomal rearrangement and carriers of reciprocal translocations are thought to have higher frequencies [Fred Kavalier 2005; Ogasawara et al., 2004].

With respect to cytogenetic studies in the above field, the most frequently applied human tissues are peripheral blood, amniotic fluid, chorionic tissue and skin fibroblasts. Amniocytes and chorionic cells are important in prenatal diagnostics [Eisenberg and Wapner 2002], and 1–15 days of cell culture are needed to obtain metaphase cells in this case [Wegner 1999]. Often, the cytogenetic results provide for definitive diagnosis and monitoring strategies.

The recent developments in molecular cytogenetic technologies, in association with conventional cytogenetic analysis, have improved the accuracy of the results and led to the finding of new chromosomal abnormalities. Hence a broad base of knowledge is necessary in order to understand, diagnose and advice patients about the complex field of pregnancy related complications.

2. Aim of the Study

The main aim of the present study was to perform cytogenetic analysis to detect numerical and structural abnormalities by using automated karyotyping (IKAROS) software in repeated miscarriage samples including products of conception (POC) material and recovered cell lines from prenatal samples

3. Materials and Methods

The present study was carried out to detect numerical and structural chromosomal abnormalities in 56 subjects (42 patients and 14 prenatal samples).

3.1. Conventional Cytogenetics

Peripheral blood culture (PBC) supplemented with mitogen Phytohemagglutinin (PHA) was setup and metaphase chromosomes were harvested after 72 hours for chromosome analysis after arrest by colchicines.

3.2. Tissue Cytogenetics

Culture of solid tissue was used as a source for mitotic cells from products of conception (POC) from first trimester spontaneous abortions for aneuploidy detection

After receiving the sample in Sodium saline, a small piece of the tissue was taken in a petridish. The tissue was washed with PBS at least 4 to 5 times and placed on a six well plate and supplemented with 3ml of DMEM with 10% FBS. The tissues were grown at 37^{o}C in CO2 incubator and later transferred to a T25 flask. When the flask was 100% confluent, culture was trypsinized. After 48 hours incubation, cells were harvested. Metaphase cells were studied for the abnormalities.

Prenatal chromosome analysis was performed by amniotic fluid, chorionic villus sampling (CVS) and cord blood after culture.

The amniotic fluid collected was spun at 800 rpm for 10 minutes. The pellet was suspendended in 5 ml of growth medium (DMEM). The primary culture was setup i.e., the collected pellet in 3ml of Amniomax medium which contains 15% FBS, and distributed in three T25 flasks

For CVS and cord blood, the tissue culture method and blood culture method mentioned above was used

3.3. High Resolution Prometaphase Chromosome Preparation

In addition to routine chromosome culture, Methotrexate, Thymidine and Ethidium bromide were added to cultures for culture and harvest for high-resolution prometaphase chromosomes [Yunis et al. 1976]. Briefly, on the day prior to harvest was added 50µl of Methotrexate to block the DNA replication followed by incubation for another 16 hours. The next day was added 50µl of Thymidine and culture further incubated for another 3 hours. Finally 50µl of Ethidium bromide solution was added and culture incubated for one hour for excessive chromosome condensation. Then 50µl colcemid solution was added to culture, incubated for 30 minutes and harvested and studied.

G,R and C banding techniques were performed as per the standard protocols and stained slides were screened for good metaphase spreads and the metaphases were captured under 100X oil immersion of the microscope (*Ziess*) attached with CCD camera. 50 metaphases were captured per sample and the karyotypes were analyzed with the help of Metasystem Ikaros software to detect any numerical or structural chromosomal abnormalities.

4. Results

Table 1 and 2 show the clinical data associated with the present study.The study included 42 patients who presented with repeated pregnancy loss, Bad obstetric history, subfertility and 14 products of conception and amniotic fluid samples. For the latter, recovered cell lines from miscarriage material and marker test positive samples were analysed (Figure 1).

It was found from the clinical data that the age groups of the patients were between 25-35 years.

Chromosomal abnormalities were located in all types of specimens using the three banding techniques namely G,R and C banding(Figure 2) but were predominantly observed in recurrent pregnancy loss (RPL) and product of conception (POC) samples(Figure 3). Aberrations observed were mainly translocations, satellites, additions in RPL

cases like 46,XX with instances of (D/D,D/G,G/G associations)(Figure 4); 46,XX,(15ps+)(Figure 5);46,XX, t(5;6) (Figure 6)45,XX,rob(13;14)(Figure 7);46,XXt(4;21);46,XX,(9qh+).46 XX (Figure 8) was predominant in amniotic fluid whereas Monosomy(Figure 9) and ploidy involving 67,XX+;64,XXX+;69,XXX(Figure 10) ;63,XXX;58,XX+ ,81 XXYY (Figure 11) were observed in the POC cases in addition to normal karyotypes.

Table 1. *Cytogenetics data of Repeated pregnancy loss, Bad Obstetric History and subfertility patients*

S.No	Age / Sex	Clinical diagnosis	Results
1.	28/F	Repeated Pregnancy Loss (RPL)	46,XX
2.	28/F	RPL	46,XX
3.	27/F	RPL	46,XX
4.	33/F	RPL	46,XX
5.	32/F	BOH	46,XX
6.	30/F	BOH	46,XX
7.	25/F	RPL	46,XX, Instances of (D/D,D/G,G/G associations)
8.	25/F	RPL	46,XX
9.	28/F	RPL	46,XX
10.	36/F	RPL	46,XX
11.	25/F	RPL	46,XX
12.	25/F	RPL	46,XX
13.	25/F	RPL	46,XX
14.	28/F	RPL	46,XX
15.	25/F	One miscarriage and infertility	45,XX,rob(13;14)
16.	28/F	BOH	46,XX, (15ps+)
17.	30/F	RPL	46,XX
18.	27/F	RPL	46,XX
19.	29/F	BOH	46,XX
20.	20/F	BOH	46,XX
21.	26/F	RPL	46,XX
22.	33/F	RPL	46,XX
23.	27/F	BOH	46,XX
24.	23/F	RPL	46,XX
25.	28/F	RPL	46,XX
26.	23/F	RPL	45,XX,rob(13;14)
27.	27/F	RPL	46,XX
28.	28/F	RPL	46,XX
29.	30/F	RPL	46,XX,t(4;21)
30.	23/F	RPL	46,XX
31.	27/F	RPL	46,XX
32.	28/F	RPL	46,XX
33.	29/F	RPL	46,XX
34.	25/F	RPL	46,XX
35.	26/F	RPL	46,XX, (9qh+)
36.	26/F	RPL	46,XX,(14ps+)
37.	25/F	RPL	46,XX
38.	28/F	BOH	46,XX
39.	23/F	RPL	46,XX,t(5;6)
40.	25/F	RPL	46,XX
41.	28/F	RPL	46,XX
42.	30/F	RPL	46,XX

Table 2. *Cytogenetic analysis for recovered cell lines from amniotic fluid, Products of conception of triple marker positive and miscarriage patients respectively*

S.No	Cell lines and Initial Karyotype	Recovered cell lines Karyotype	Source
1.	45,X	67,XX+	POC
2.	46,XY	46,XY	POC
3.	46,XY	46,XY	POC
4.	46,XX	64,XXX+	POC
5.	46,XY	46,XY	POC
6.	46,XX	46,XX	POC
7.	69,XXX	69,XXX	POC
8.	46,XX/47,XY+16	63,XXX	POC
9.	46,XX, (15ps+)	58,XX+	POC
10.	46,XX	46,XX	AF
11.	46,XY	46,XY	AF
12.	46,XX	46,XX	AF
13.	46,XX	46,XX	AF
14.	46,XX	46,XX	AF

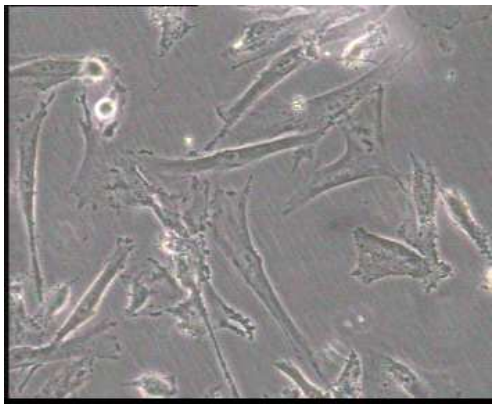

Figure 1. *Recovered cell lines from amniotic fluid sample (Fibroblast cells)*

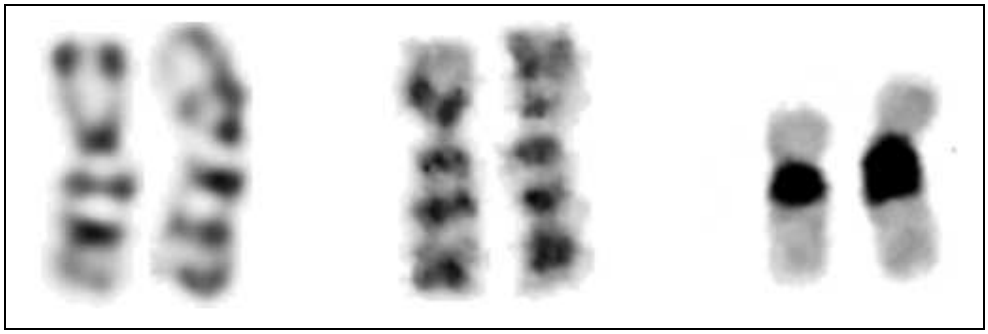

Figure 2. *Different banding techniques G banding, R banding and C banding*

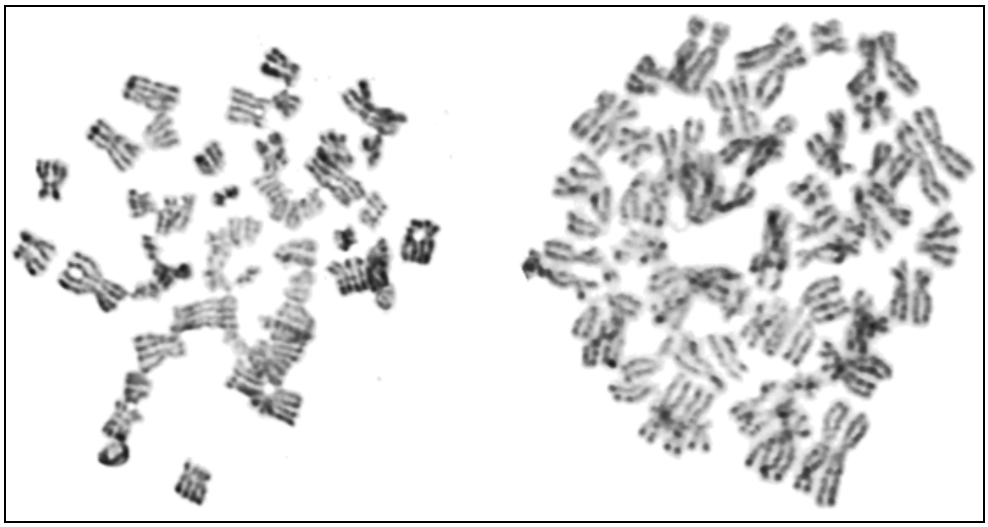

Figure 3. *A placental tissue from products of conception - Endoreduplication*

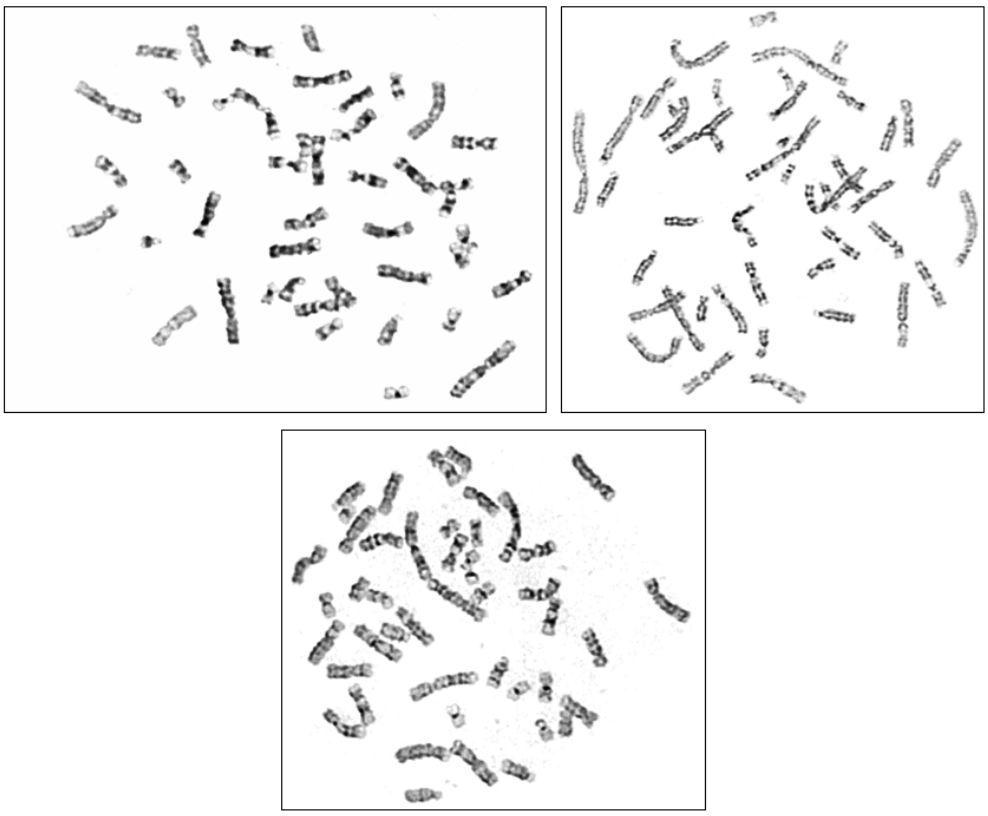

Figure 4. D*/D, D/G and G/G associations (H/o repeated miscarriages)*

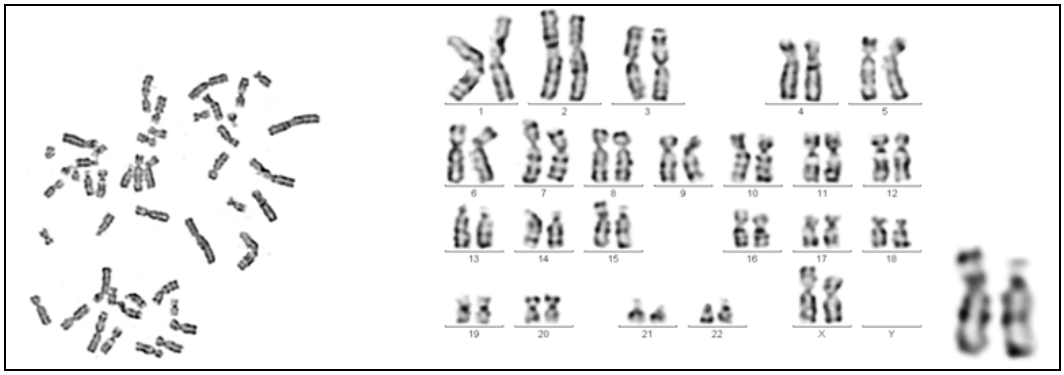

Figure 5. *46,XX,(15ps+) in a female with Bad Obstetric History*

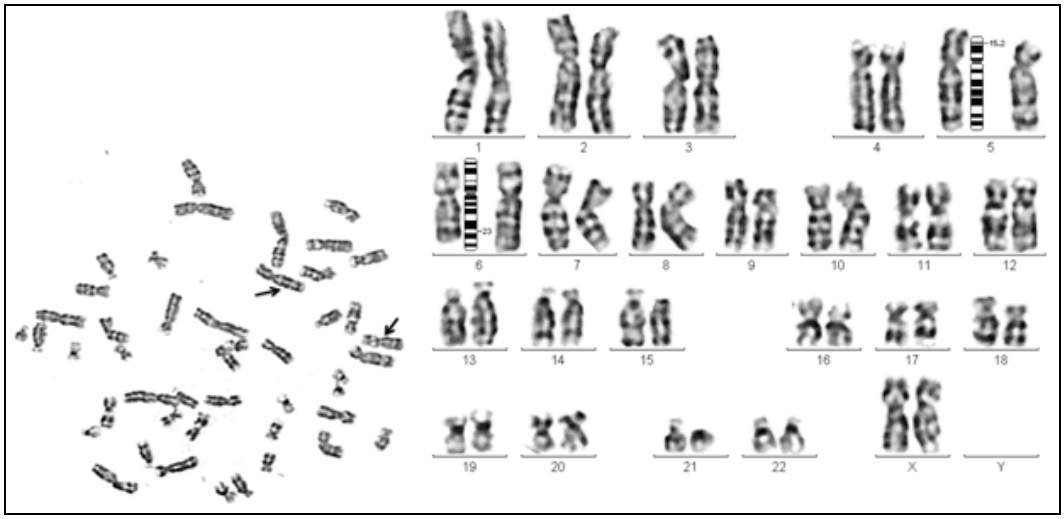

Figure 6. *Karyotype 46, XX.t (5;6) – in a patient with repeated miscarriages*

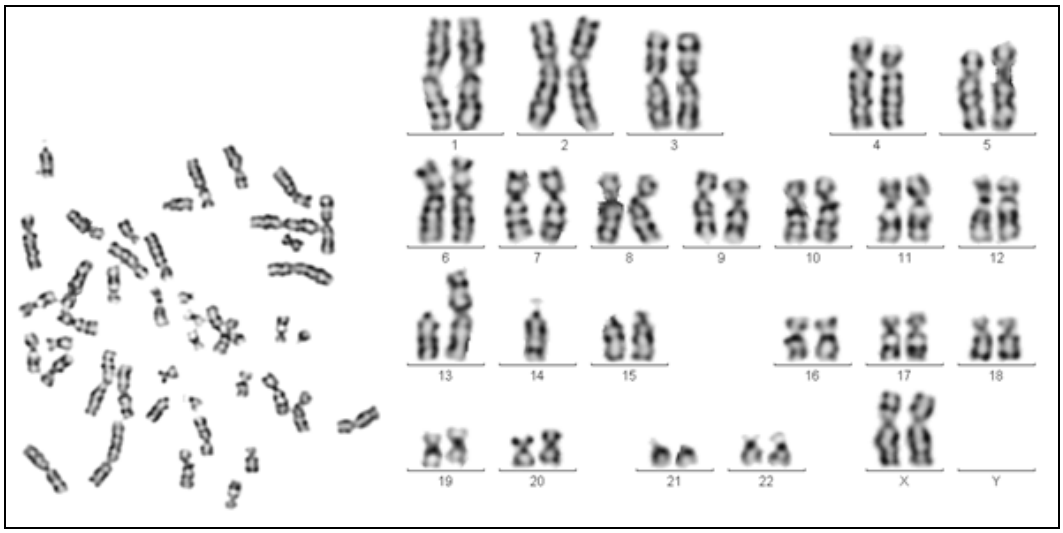

Figure 7. *45, XX, rob (13;14)) – in patient with missed abortion and subsequent infertility*

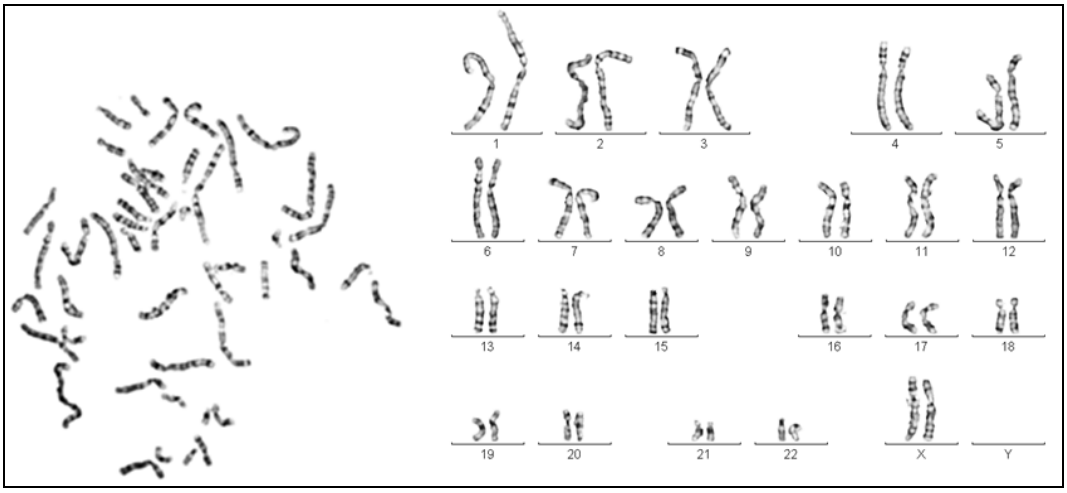

Figure 8. *46,XX karyotype (Amniotic fluid culture)*

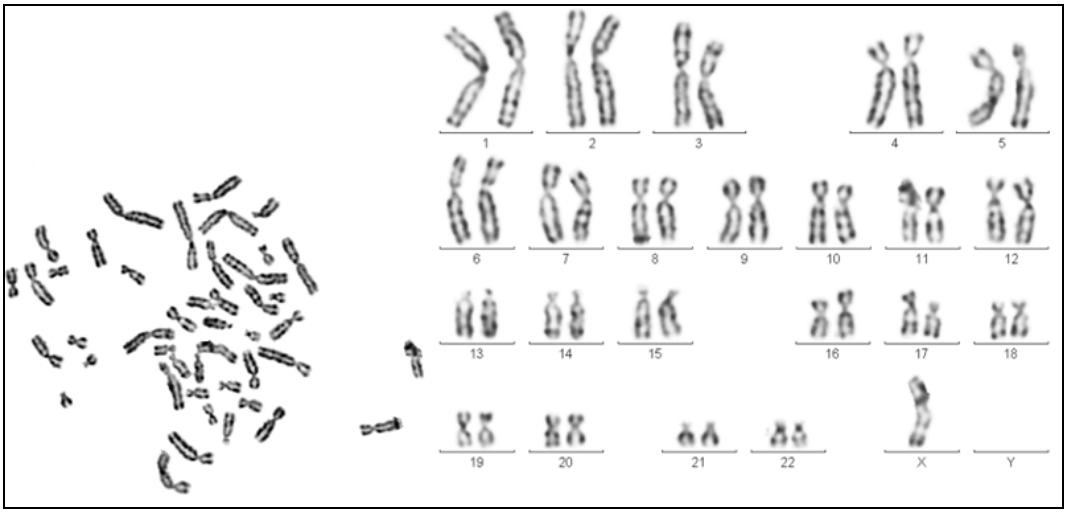

Figure 9. *Monosomy (45, X) in missed aborted material (POC)*

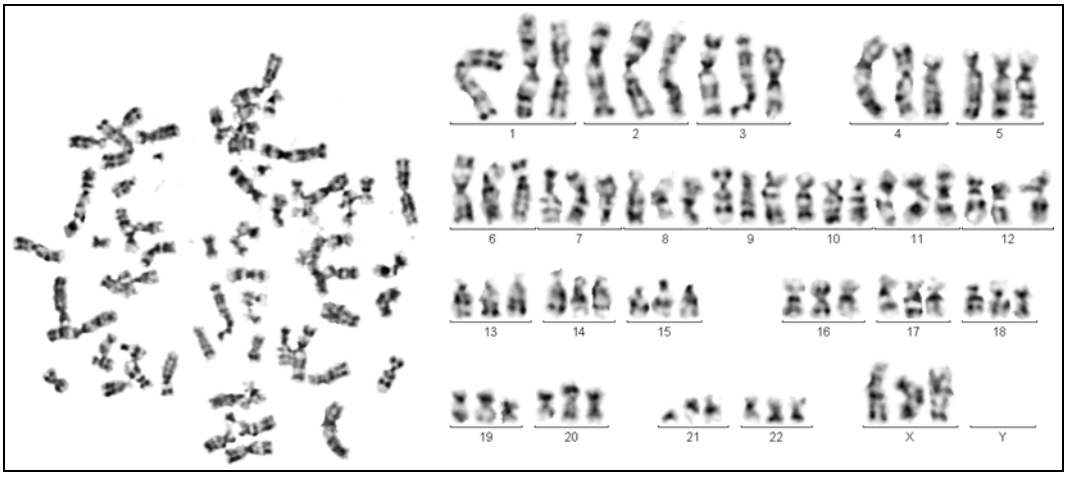

Figure 10. *Triploidy (Products of conception sample)*

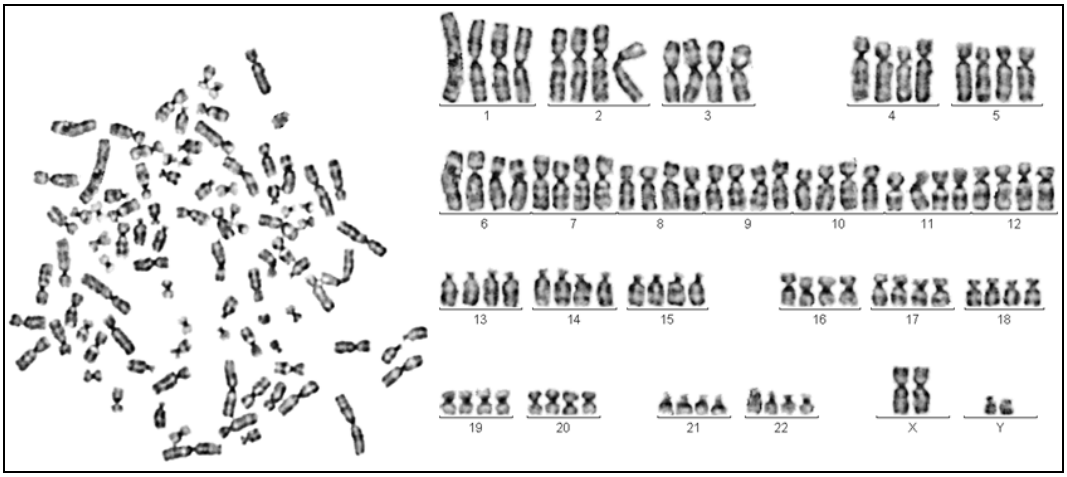

Figure 11. *Tetraploidy (Products of conception sample)*

5. Conclusion

Here we report different numerical and structural abnormalities in different prenatal specimens which could be further probed and investigated to serve as markers for pregnancy related complications. Hence cytogenetic screening could provide to be a useful method for monitoring patients with abnormal pregnancies. The cytogenetic result is an independent prognostic indicator, with certain karyotypes associated with a good prognosis for the better treatment.

Early identification of the possible cause of fetal loss can significantly reduce long term psychological distress in women with miscarriages and enables improved genetic counseling for those couples in future pregnancies.

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