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Heterochromatic Variations and Pregnancy Losses in Humans

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ABSTRACT A total of 440 couples (880 individuals) with history of repeated abortions were evaluated for heterochromatic variations. The present study was undertaken with the objective of investigating the role of heteromorphic variations in pregnancy losses and reproductive failures in the human population. Peripheral blood samples of the couples were cultured and processed to obtain metaphase plates. The GTG banded slides were analysed using automated karyotyping system (Cytovision) for precise analysis of the karyotype. The study revealed that the frequency of chromosomal anomalies and variations leading to Bad Obstetric History (BOH) was 17%. Chromosomal rearrangements constituted 24% of the cases while heterochromatic variations constituted 76% of the chromosomal cause for BOH. The heterochromatic variations associated with BOH included inversions and deletions. Our study revealed a correlation between BOH and heterochromatic variations of Chromosome 1 and 9.

I. INTRODUCTION

Cytogenetic studies have an important role in the evaluation of couples with repeated miscarriages and BOH. Cytogenetic studies are essential for evaluating the chromosomal abnormalities and heterochromatic variants in such couples. Contradiction exists as to the consequences of the heterochromatic variations seen in the general population with respect to its inheritance. Some studies have reported that variations in heterochromatic regions might have deleterious effects (Bhasin 1973; Halbrecht et al. 1976; Kimberling et al.1977; Lubs et al. 1977; Saby et al. 1977; Bhasin 2005). Studies by Madon et al. (2005), Sahin et al. (2008), Minocherhomji et al. (2009) showed that heteromorphisms shown by paracentric long-arm regions of chromosomes 1, 9 and 16, and inv(9) were associated with infertility. Studies by Minocherhomji et al. (2009) suggest that the increase in the long arm of the Y chromosome may affect reproductive capacity. Some studies have shown that such variations are normal and carry no implications for abnormality in the person who has such variations (Hemming and Burns 1979). Heterochromatic variations appear to have no adverse affects on outcome of *in vitro* fertilization and embryo transfer treatment (Y Hong 2010). Because of the need for more data on the knowledge of the recurrence risks involved in case of heterochromatic variations, the present study was undertaken with the objective of investigating the role of heteromorphic variations and chromosomal anomalies on spontaneous abortions and reproductive failures in the humans.

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II. METHODOLOGY

A total of 440 couples from heterogeneous population within the age group of 20 to 45 with BOH were evaluated in the study. The cases were recruited from the patient group referred by clinicians with a history of two or more pregnancy losses in the form of totally empty gestational sac, disorganized embryo, growth retarded fetus or still birth. In all the cases other causes of BOH were ruled out. The standard history of the patients was noted in a predesigned format to study

the inheritance pattern. All necessary information of the couple, their offspring and parents was noted down. The couple with BOH was subjected to chromosomal analysis from peripheral blood. Those without BOH served as control group. Control group consisted of fertile individuals with children. Primary infertility patients were not included in the control group.

The cells were cultured in RPMI culture media for 72 hours. The culture was then processed using standard protocol of leukocyte culture (Verma and Babu 1989). After processing of the culture, metaphase plates were prepared and subjected to GTG Banding technique for analysis of the chromosome variability. The chromosomes were analysed at a band resolution of 450-550. From each patient 20 metaphase plates were analysed for heterochromatic variations using automated karyotyping software (Cytovision 3.6) at 1000X magnification. The data was analysed statistically using Chi-Square test. Whenever necessary, the results were compared by two-tailed Fisher's exact test, calculated online at http://www.graphpad.com/quickcalcs/contingency 1.cfm.

III. RESULTS

Cytogenetic evaluation of 440 couples (880 individuals) with BOH revealed chromosomal anomalies and variations in 75 cases. In our control group of 200 individuals, chromosomal variations were observed in 7 individuals (3.5%). Thus, the frequency of chromosomal anomalies and variations leading to BOH was 17% which was significantly higher *(P=0.0005)* as compared to the control. Of these 75 cases, 18 chromosomal rearrangements were observed constituting 24% of the total chromosomal anomalies and variations. Heterochromatic variations were observed in 57/75 cases, constituting 76% of the chromosomal anomalies and variations. Frequency of these heterochromatic variations are depicted in Figure1.

The heterochromatic variations associated with BOH included inversions, qh+, ps+ and deletions. The most frequent of these were the qh+ (40.35%) followed by ps+ which constituted 31.58%. The inversions and deletions constituted 24.56% and 3.51% respectively. Various types of heterochromatic variations are depicted in Figure 2. Rate ofoccurence of the hetrochromatic variations as compared to the control group is depicted in Figure 3. The photographs of various heterochromatic variations are depicted in Plate 1.

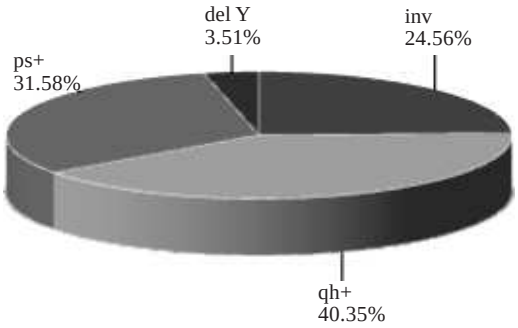

Fig. 1. Frequency of heterochromatic variations

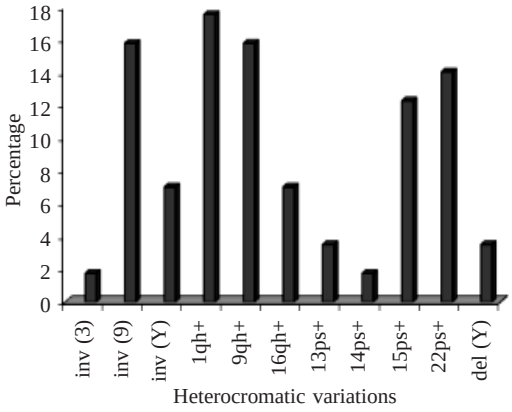

Fig. 2. Frequency of different types of heterocromatic variations

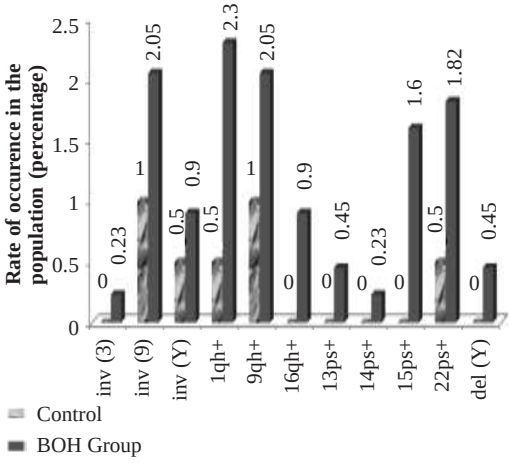

Fig. 3. Occurence of heterochromatic variations in BOH Group as compared to the control

Table 1: Frequency of hetrochromatic variations in couples with pregnancy losses

Cytogenetic evaluation: 440 couples
Chromosomal abnormalities and variations: 75 (17%)
Heterochromatic variations: 57/75 (76%)
Chromosomal rearrangement: 18/75 (24%)

Type of heterochromatic variation	*Chromosome with heterochromatic variation*	*No. of cases*	*Frequency of occurence in BOH group (%)*	*Frequency of 1st trimester pregnancy losses (%)*	*Frequency of 2nd trimester pregnancy losses (%)*
1) Inversions	Inv(3)	01	1.75		
	Inv(9)	09	15.79	88.9 #	11.10
	Inv(Y)	04	07.02	100	00
	TOTAL inversions	14	24 .56		
2) Variations of 'q' Heterochromatin (qh+)	1qh+	10	17.54	80*	20
	9qh+	09	15.79	55.6**	44.4
	16qh+	04	07.02	100	00
	TOTAL (qh+}	23	40.35		
3) Presence of Satellite on Short Arm 'P' (ps+)	13ps+	02	3.51	50	50
	14ps+	01	1.75	100	00
	15ps+	07	12.28	42.68***	28.58
	22ps+	08	14.04	62.5	37.5
	TOTAL (ps+}	18	31.58		
4) Deletion	Del(Y)	02	3.51	50	50

Fisher's exact test: * P= 0.0001, ** P=0.4788, *** P=0.395, # P= 0.0001.

1) Variations of 'q' Heterochromatin: (qh+):

The qh+ constitute 40.35% of the heterochromatic variations. The most frequently affected chromosome with qh+ was chromosome 1 (17.54%), followed by 9qh+ (15.79%) and 16qh+ (7.02%) (Table 1).

1qh+: Amongst the BOH couples, 1qh+ was

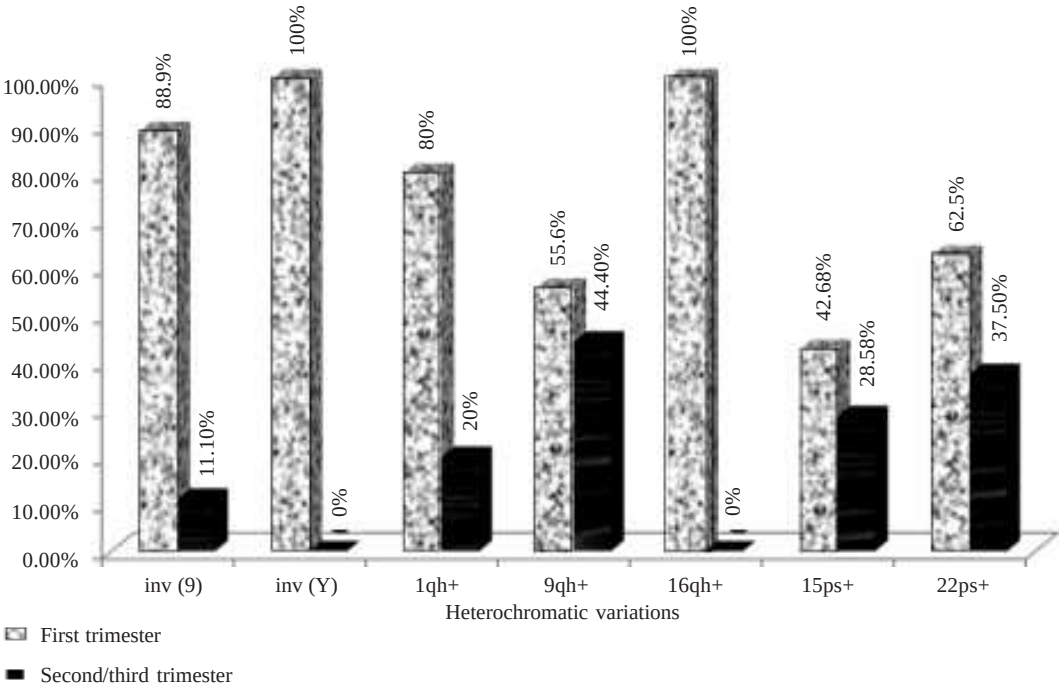

Fig. 4. Frequency of heteromatic variations and stage of pregnancy loss

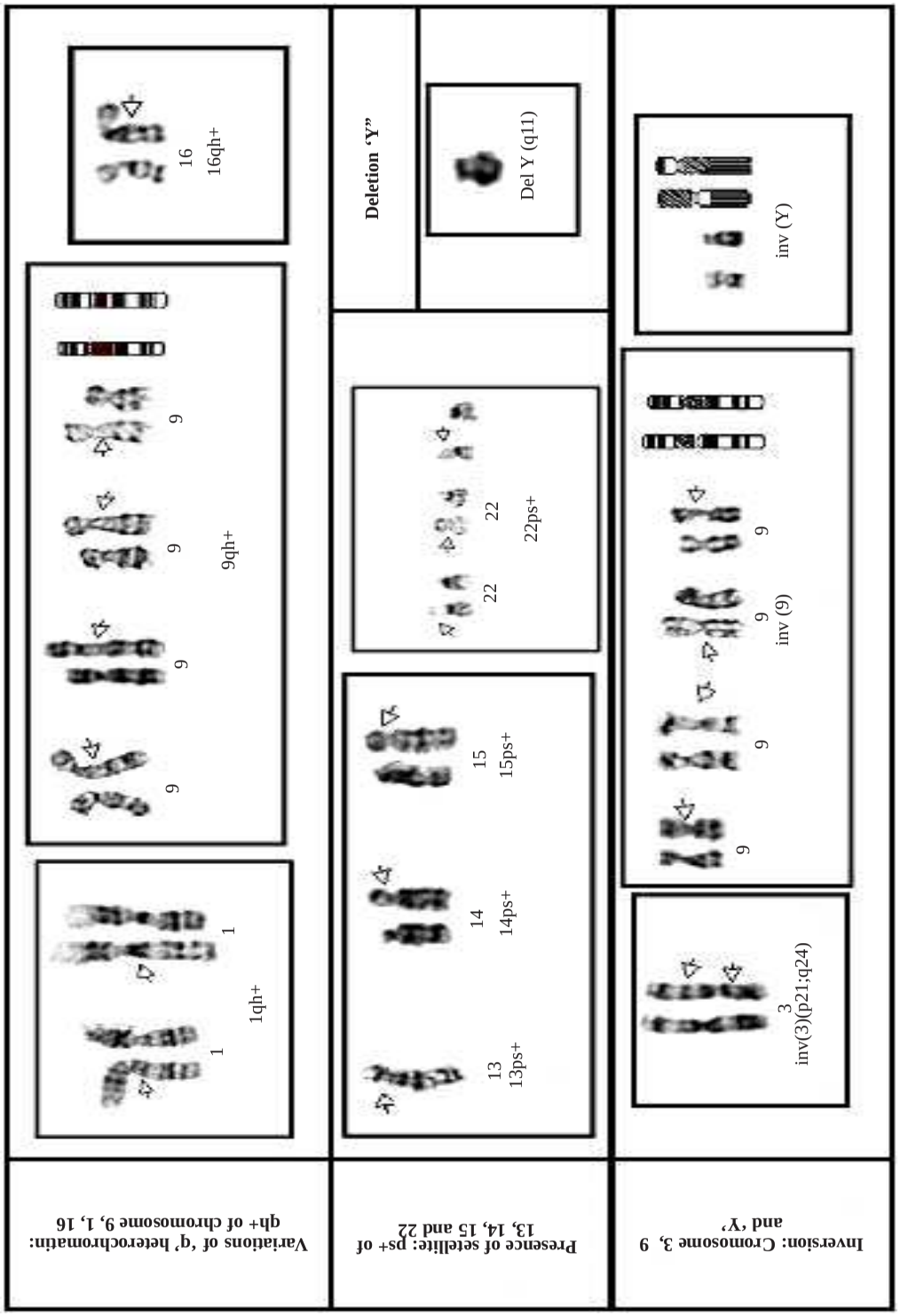

Fig. 5. Showing the heterochromatic variations in couples with BOH

significantly higher *(P=0.0059)* in the males (70%) as compared to the females (30%). BOH observed in those was first trimester pregnancy losses was significantly higher *(P= 0.0001)* at 80% while that in second trimester was 20%, with congenital defects.

9qh+: Presence of 9qh + was significantly higher *(P= 0.0001)* in the males (77.8%) as compared to the females (22.2%). The BOH observed in those with first trimester pregnancy losses was 55.6% while that in second or third trimester was 44.4% (IUFD) (Fig. 5). The difference was statistically insignificant *(P=0.4788)*.

16qh+: Heterochromatic region of 16qh+ was significantly higher *(P= 0.0001)* in the females (75%) as compared to the males (25%). BOH observed in all these cases was first trimester pregnancy losses (100%) (Fig. 4 see Fig. 5).

2) Presence of Satellite on Short Arm 'p' (ps+):

The ps+ constitute 31.58% of the heterochromatic variations. The most frequently affected chromosome with ps+ was chromosome 22 (14.04%), followed by 15ps+ (12.28%). Ps+ of chromosome 13 and 14 constituted 3.51% and 1.75% of the heterochromatic variations respectively (Table 1).

22ps+: Presence of 22ps+ was higher in the females (62.5%) as compared to the males (37.5%). The difference was statistically insignificant *(P= 0.1169)*. The BOH observed in those with first trimester pregnancy losses is 62.5% while that in second or third trimester was 37.5% (Fig. 4 see Fig. 5).

15ps+: Presence of ps+ on chromosome 15 in BOH cases was higher in the males (57.1%) as compared to the females (42.9%). The difference was not significant *(P= 0.3950)*. The BOH observed in those with first trimester pregnancy losses was 42.68% while that in second or third trimester was 28.58% (not statistically significant, *P=0.395)*. In two cases there was ectopic pregnancy (28.57%).

13ps+ and 14ps+: Amongst the acrocentric group 13ps+ and 14ps+ constituted 3.51% and 1.75% of the total heterochromatic variations respectively. Both the patients having 13ps+ had history of two missed abortion while the one with 14ps+ had an ectopic pregnancy and one had a normal child**.**

3) Inversions:

The inversions constitute 24.56% of the heterochromatic variations. The most frequently affected chromosome with inv was chromosome 9(15.79%), followed by inv(Y) (7.02%) and inv (3) (1.75%) of the heterochromatic variations (Table 1).

Inv(9): Amongst the BOH couples, frequency of inv(9) was significantly higher (15.79%, $p<0.05$) as compared to the control group (1.26%). Inv(9) was significantly higher *(P= 0.0214) in* males (66.6%) as compared to females (33.3%). The BOH observed in those with first trimester pregnancy losses was significantly higher *(P= 0.0001)* at 88.9 % while that in second or third trimester was 11.1% (Fig. 4 see Fig. 5).

Inv(Y): The BOH observed in those with first trimester pregnancy losses was 100%. The frequency of heterochromatic variations associated with BOH are depicted in Table 1.

IV. DISCUSSION

Chromosome abnormalities are responsible for at least half of spontaneous abortions or miscarriages and are an important cause of congenital malformations (Hook 1992; Tolmie 1995; Rimoni 2002; Bhasin 2005; Korteweg 2008). Several early studies suggested that the variation in size of heterochromatic regions on human chromosome might have deleterious effect (Halbrecht and Shabtay 1976; Lubs et al. 1977b; Lubs et al. 1977a; Say et al.1977; Bhasin 2005; Madon et al. 2005; Sahin et al. 2008). The length heterochromatin of 1, 9, 16 and Y and its role in reproductive wastage was examined by Rodrigues et al. (1987) in 100 couples with recurrent miscarriages. Thorough review of the heterochromatic variations in the human population and its scoring criteria is done in the studies by Bhasin (2005), wherein he stressed the importance of studying its clinical significance.

The present research finding reveals that carriers of heterochromatic variations are at higher risk of having BOH. The heterochromatic variations associated with BOH included inversions, qh+, ps+ and deletions. The chromosomal polymorphism of short arms of acrocentric chromosomes and heterochromatin variations of chromosomes 1, 9, 16 and Y also have been reported in humans by Podugolnikova et al. (1982). Ac-

cording to the present study, the most frequently affected chromosome was chromosome 1 and 9 as compared to the control group. Some workers have considered variations in heterochromatin in chromosomes 1 and 9 to be associated with fetal wastage, recurrent abortions, and abnormal phenotypes. But studies of (Hemming 1987) indicated no significant difference in the heterochromatic regions between aborting and non-aborting couples. Studies of Podugol'nikova et al. (1982) also revealed that heterochromatic regions of chromosomes 1, 9, 16 and Y in children showed signs of embryonic development disorder. Heterochromatin has a specific role and behaviour in the synapsis of human homologous chromosomes. Association of heterochromatic variation with BOH is also indicated in the studies of Maes (1983), Yuce (2007), Minocherhomji (2009).

The pericentric inversion of chromosome 9 or inv(9) is commonly seen in normal humans and the frequency estimated to be 1 to 3% in general population (Nielsen et al. 1975; Ko 1992; Teo 1995; Thomas 1999). Our present study revealed a frequency of 1.26% of inv(9) in humans with normal offspring (control group). But the frequency of inv(9) associated with BOH was significantly higher (15.79%) as compared to the control group.

Among the non-acrocentric human chromosomes, chromosome 9 represents highest degree of morphological variations. The mechanisms of origin of inversions 9 are highly complex (Verma 1996). Our studies revealed that chromosome 9 showed the maximum variations which included qh+ (15.79%) and inv(9) (15.79%). These individual with chromosome 9 abnormalities had high frequency of first trimester abortions. Inv(9) showed a frequency of 88.9% of first trimester while 9qh+ has a frequency of 55.6%. Studies of Tsvetkova (1979) also showed that individuals with reproductive failure were carriers of variants of chromosomes 9. In the present study, heterochromatic variations of chromosome 9 were significantly more frequent as compared to the control individuals. Inv(9) reported to be associated with BOH, infertility and congenital anomalies even in the studies of Thomas (1999), Davolos (2000), Madon et al. (2005), Nagvenkar (2005), Sahin et al. (2005). A high frequency of inv(9)(p12q13) was detected in children with dysmorphic features (Rao 2006).

This indicates that the inv(9) has a role in the abnormal phenotype development. During inversion event there might be loss or suppression of euchromatin chromosome region and hence detailed chromosomal break point study is important to understand the clinical significance of the pericentric inversion of chromosome 9. Apart from showing a high incidence of gaps and splits at pachytene stage in synaptonemal complex preparations, chromosome 9 has been described as being especially prone to breaks that produce asymmetric bivalents in meiotic metaphase spreads (Sarrate et al. 2004) and structural chromosome aberrations in sperm studies (Brandriff et al. 1988; Estop et al. 1995; Templado et al. 2005). The incidences of gaps and splits differed among all of these secondary heterochromatic regions, suggesting a different timing of synapsis for each region. A possible explanation for these differences in timing among heterochromatin regions could be variations in size, in composition and in molecular organization of the heterochromatic region (Tagarro et al. 1994; Strachan and Read 1999).

In the study of Rao et al. (2006), a high frequency of inv(9) was detected in children with dysmorphic features and developmental delay and about 70% were *de novo* origin. The correlation of inv(9) with clinical features of children with dysmorphic features revealed that most of the children had facial dysmorphism, abnormal phenotype and delayed milestones. This suggests that the unbalanced inversions at different break point regions might also have a role in the abnormal phenotype development.

The other most frequently affected chromosome in the present study was chromosome 1 (17.54%). Variations in the size of band 1q12 of chromosome 1 were earlier noted to occur in 1/100 to 1/1000 newborns (lubs et al. 1977). An elongated 1qh region referred to the "uncoiler" was used as a familial marker in the first successful effort to map an autosomal gene, the Duffy blood group, to chromosome 1(Donahue et al. 1968; Ying et al. 1968). Heterogeneity is evident in 1qh heterochromatin not only by C-banding but also by Giemsa -11 staining, and by lateral asymmetry (Magenis et al. 1978). C-bands of chromosome 1 have been reported to be more variable in patients with malignant disease compared to normal controls (Atkin et al. 1977 and Tesouz et al.1993). Rearrangements in the vicinity of the centromere are overrepresented in many types of human cancer (Verma et al. 1988) in-

cluding the characteristic rosette formation in a rare disease called ICF (immune deficiency, centromeric instability and facial anomalies).

Buretic-Tomljanovich et al. (1997) studied couples with two or more miscarriages, couples with a stillborn child and couple with no history of miscarriages and at least two normal children. Results showed an increase in heterochromatin of chromosome 16 in couples with a stillborn or malformed child. The frequency of chromosome 16 abnormality in the present study was 7.02%. The frequency of first trimester abortions in these cases was 100%.

Numerous studies have suggested indirect effects such as higher incidence of spontaneous abortions associated with striking chromosomal variants such as a large Y or large satellites on acrocentric chromosomes. Though pericentric inversions in Y chromosome are considered to be as normal variant with no possible effect on fertility (Shapiro et al.1984; Verma et al.1982), a few cases have been reported showing infertility (Acar et al.1999; Causio et al. 2000; Giltay et al. 2000; Tomomasa et al. 2000; Ferlin et al. 2006; Mc Elreavey et al. 2008; Ravel et al. 2009; Stouffs et al. 2009). Studies of Lemos et al. (2008) suggests that Polymorphic Y Chromosomes Harbor Cryptic Variation with Manifold Functional Consequences. Heterochromatin and the hidden epigenetic geography of the Y chromosome is highlighted in the studies of Navarro-Costa et al. (2011).

No specific functions have been reported to be associated with the satellite segments (ps+). However such variations in the couple may make the fetus susceptible to translocations which may lead to fetal wastage.

If polymorphism is observed in either of the partners, the history is viewed and the couple is called for Genetic Counseling. Invasive prenatal diagnosis is always an option discussed with the patients having previous bad obstetric history. For example, retrospective studies suggest that parents having large satellite on acrocentric G group chromosome have increased risk for having a child with Down syndrome. This is due to the non-disjunction which occurs during meiosis I, meiosis II, cellular reproduction or during mitosis phase. If the couple has at least one normal living child and history of BOH they were willing for an invasive procedure. In case of patients with BOH and or with infertility or advanced maternal age opted for non invasive procedures like maternal serum screening and ultrasound evaluation if any marker is observed invasive procedure is suggested. Cases where heteromorphism is observed in the fetus similar to parent the couple is comforted that chance of the fetus being normal are high.

V. CONCLUSION

Heterochromatic variations especially of chromosome 1 and 9 (inversions and qh+) are significantly higher in couples having BOH. Pregnancy losses were also significantly high in couples with 22ps+ and 15ps+. Therefore our studies suggests significantly higher incidence of pregnancy losses in those who are carriers of heteromorphic variations of chromosome 1, 9, 15 and 22. We consider this significant for offering genetic counseling in couples with BOH.

VI. RECOMMENDATIONS

Since heterochromatic variations are associated with BOH, the couple should be counseled for cytogenetic testing and confirmation of fetal chromosomal status by invasive procedures of cytogenetic evaluation of fetal tissue by Amniocentesis.

REFERENCES

Acar H, Cora T, Erkul I 1999. Coexistence of inverted Y, chromosome 15p+ and abnormal phenotype. *Genet Couns*, 10: 163-170.

Atkin NB, Baker MC 1977. Pricentric inversion in chromosome 1: Frequency and possible association with cancer. *Cytogenet Cell Genet*, 19: 180-184.

Bernardo Lemos, Araripe Luciana O, Hartl Daniel L 2008. Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences. *Science*, 319 (5859): 91-93. DOI: 10.1126/science.1148861.

Bhasin MK 2005. Human population cytogenetics: A review. *Int J Hum Genet*, 5(2): 83-152.

Bhasin MK, Forster W, Fuhrmann W 1973. A cytogenetic study of recurrent abortion. *Humangenetik*, 18: 139.

Brandriff BF, Gordon LA, Moore D, Carrano AV 1988. An analysis of structural aberrations in human sperm chromosomes. *Cytogenet Cell Genet*, 47: 29–36.

Buretic-Tomljanovich A, Rodojcic Badovinac A, Vlastelic I, Randic LJ 1997. Quantitative analysis of constitutive hetrochromatin in couples with fetal wastage. *Am J Reprod Immun*, 38: 201-204.

Causio F, Canale D, Schonaure LM, Fischetto R, Leonetti T, Archidiancono N 2000. Breakpoint of a Y chromosome pericentric inversion in the DAZ gene area. A case report. *J Reprod Med*, 45: 591-594.

Davolos IP, Rivas F, Ramos AL, Galaviz C, Sandoval L, Rivera H 2000. Inv(9)(p24q13) in three sterile brothers. *Ann Genet*, 43: 51-54.

Donahue RP, Bias WB, Renwick JH, McKusick VA 1968. Probable assignment of the Duffy blood group locusto chromosome 1 in man. *Proc Natl Acad Sci* (USA), 61: 949-955.

Estop AM, Márquez C, Munne S, Navarro J, Cieply K, Van Kirk V, Martorell MR, Benet J, Templado C 1995. An analysis of human sperm chromosome break-points. *Am J Hum Genet*, 56: 452–460.

Ferlin A, Arredi B, Foresta C 2006. Genetic cause of male infertility. *Reprod Toxicol*, 22: 133-141.

Giltay JC, VanGolde RJ, Kastrop PM 2000. Analysis of spermatozoa from seven ICSI males with constitutional sex chromosomal abnormalities by fluorescence in situ hybridization. *J Assist Reprod Genet*, 17: 151-155

Halbrecht I, Shabtay F 1976. Human chromosome polymorphism and congenital malformations. *Clin Genet*, 10: 113-122.

Hemming L, Burns C 1979. Heterochromatic polymorphism in spontaneous abortions. *J Med Genet*, 16(5): 358-362.

Hong Y, Zhou YW, Tao J, Wang SX, Zhao XM 2011. Do polymorphic variants of chromosomes affect the outcome of *in vitro* fertilization and embryo transfer treatment. *Hum Reprod*, 26: 6. 10.1093/humrep/deq333.

Hook EB 1992. Prevalence risks and recurrence. In: DJ Brock, CH Rodeck, M Ferguson-Smith (Eds.): *Prenatal Diagnosis and Screening*. Edinburgh: Churchill Livingstone, P. 351.

Kimberling WJ 1977. Q and C banding polymorphisms in 7 and 8 year old children: Racial difference and clinical significance. In: E B Hook, I H Porter (Eds.): *Population Cytogenetic Studies in Humans*. New York: Academic Press, pp. 133-159.

Ko TM, Hsieh FJ, Chang LS, Pan MF, Lee TY 1992. Pericentric inversions of chromosome 9 in Taiwanese fetuses. *J Forms Med Assoc*, 91: 473-474.

Korteweg FJ, Bouman K, Erwich JJHM 2008. Cytogenetic analysis after evaluation of 750 fetal deaths. Proposal for diagnostic work-up. *Obstet Gynecol*, 111: 856.

Lubs HA, Patil SR, Kimberling WJ 1977a. Q and C banding polymorphisms in 7 and 8 year old children: Racial differences and clinical significance: In: E B Hook, I H Porter (Eds.): *Population Cytogenetic Studies in Humans*. New York: Academic Press, pp. 133-159.

Lubs HA, Kimberling WJ, Hecht F 1977b. Racial differences in the frequency of Q and C chromosomal heteromorphisms. *Nature*, 268: 631-633.

Madon PF, Athalye AS, Parikh FR 2005. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online*, 11(6): 726–732.

Maes A, C Staessen, L Hens, E Vamos 1983. C heterochromatin variation in couples with recurrent early abortions. *J Med Genet*, 20: 350-356.

Magenis RE, Palmer CG, Wang L 1978. Heritability of chromosome banding variants. In: E B Hook, I H Porter (Eds.): *Population Cytogenetic Studies in Humans*. New York: Academic Press, pp. 179-188.

McElreavey K, Chantot-Bastaraud S, Ravel C, Mandelbaum J, Siffroi JP 2008. Y chromosome and male infertility: What is normal Y chromosome? *J Soc Biol*, 202: 135-141.

Minocherhomji S, Anthalye AS, Madon PF, Kulkarni D, Uttamchandani SA, Parikh FR 2009.A case–control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. *Fertil Steril*, 92: 88-95. doi:10.1016/j.fertnstert.2008.05.071.

Nagvenkar P, Desai K, Hinduja I, Zaveri K 2005. Chromosomal studies in infertile men with oligozoospermia and non-abstructive azoospermia. *Indian J Med Res*,122: 34-42.

Nielsen J, Sillesen I 1975. Incidence of chromosome aberrations among 11,148 newborn children. *Humangenetik*, 30: 1-12.

Paulo NC, Carlos EP 2011. Heterochromatin: The hidden epigenetic geography of the Y chromosome. *Hum Reprod Update*, 17: 2-11.

Podugolnikova OA, Korostelev AP 1980.The quantitative analysis of polymorphism on human chromosomes 1,9,16, and Y. IV. Heterogeneity of a normal population. *Hum Genet*, 54: 163-169.

Podugol'nikova OA, Bliumina MG 1982. Heterochromatic regions of chromosomes 1, 9, 16 and Y in children with signs of embryonic development disorder. *Genetica*, 15(10): 1870-1879.

Ramo S, Alcantara MA, Molina B, 2008. Acrocentric cryptic translocation associated with non-disjunction of chromosome 21. *Am J Med Genet*, 146A: 97.

Rao BV, Kerketta L, Korgaonkar S, Ghosh K 2006. Pericentric inversion of chromosome 9[inv(9) (p12q13)]: Its association with genetic diseases. *Indian J Hum Genet*, 12: 129-132.

Ravel C, Chantot-Bastaraud S, El Houate B 2009. Y chromosome AFZc structural architecture and relationship to male fertility. *Fertil Steril*, 92(6): 1924-1933.

Rimoni DL, Connor JM, Pyeritz RE, Korf BR 2002. *Principles and Medical Genetics*. Vol 1, 6th Edition. Edinburgh: Churchill Livingstone.

Rodriguez-Gomez MT, Martin-Sempere MJ, Abrisqueta JA 1987. C- band length variability and reproductive wastage. *Hum Genet*, 75: 56-61.

Saby B, Carpenter NJ, Lanier PR, Banez C, Jones K, Coldwell JG 1977. Chromosome variants in children with psychiatric disorders. *Am J Psychiatry*, 134: 424-426.

Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, Zeyneloglu HB 2008.Chromosome heteromorphisms: An impact on infertility. *J Assist Reprod Genet*, 25: 191-195.

Sarrate Z, Blanco J, Egozcue S, Vidal F, Egozcue J 2004. Identification of meiotic anomalies with multiplex fluorescence in situ hybridization: Preliminary results. *Fertil Steril*, 82: 712–717.

Shapiro LR, Petterson RO, Wilmot PL, Warburton D, Benn PA, Hsu LYF 1984. Pericentric inversions of the Y chromosome and prenatal diagnosis. *Prenat Diag*, 4(6): 463-465.

Stouffs K, Vandermaelen D, Tournaye H, Liebaers I, Van Steirteghem A, Lissens W 2009. Genetics and male infertility. *Hum Reprod Update*, 15(6): 623-637.

Strachan T and Read AP 1999. Organization of the human genome. In: F Kingston (Ed.): *Human Molecular Genetics 2*. Oxford, UK: BIOS Scientific Publishers.

Tagarro I, Fernandez-Peralta AM and Gonzalez-Aguilera JJ 1994. Chromosomal localization of human satellites 2 and 3 by a FISH method using oligonucleotides as probes. *Hum Genet*, 93: 383–388.

Teo SH,Tarn M, Knight L, Nag I 1995. Pericentric inversion 9-incidence and clinical significance. *Ann Acad Med Singapore*, 24: 302-304.

Templado C, Bosch M , Benet J 2005. Frequency and distribution of chromosome abnormalities in human spermatozoa. *Cytogenet Genome Res*, 111: 199–205.

Tesouz A, Kitsiou-Tzeli S, Kosmidis H, Paidouis K, Katsouyanni K, Sinaniotis C 1993. Consecutive heterochromatin polymorphisms in children with acute lymphoblastic leukemia. *Pediatr Hematol Onco*, 10: 7-11.

Thomas IM 1999. Cytogenetic basis of recurrent abortions. *Perinatology*, 1: 181-187.

Tolmie JL 1995. Chromosome disorders. In: M J Whittle, J M Conner (Eds.): *Prenatal Diagnosis in Obstetric Practice*. Oxford: Blackwell Scientific, P. 34.

Tomomasa H, Adachi Y, Iwabuchi M 2000. Pericentric inversion of the Y chromosome of an infertile male. *Arch Androl*, 45: 181-185.

Tsvetkova TG, Iankova MF 1979. Human chromosome polymorphism and disordered reproductive function. II. C-variant chromosomes *Genetika*,15(10): 1870-9.

Verma RS, Evans-McCalla M, Dosik H 1982. Human chromosomal heteromorphism in American Blacks. VI. Higher incidence of longer Y owing to non fluorescent (nf) segment. *J Med Genet*, 19: 297-301.

Verma RS, Macera MJ, Babu A 1988. The role of heterochromatin in the origin of isochromosome 1 neoplastic cells. *Eur J Cancer Clin Oncol*, 24: 821-823.

Verma RS, Babu A 1989. *Human Chromosome: Manual of Basic Techniques*. New York: Pergamon, P. 24.

Verma RS, Ramesh KH, Mathews T, Kleyman SM, Conte RA 1996. Centromeric alphoid sequences are breakage prone resulting in pericentromeric inversion heteromorphism of qh region of chromosome 1. *Ann Genet*, 39(4): 205-208.

Ying Kl, Lives EJ 1968. Asymmetry of chromosome number 1 pair in three generations of phenotypically normal family. *Canad J Genet Cytol*, 10: 575-589.

Yuce H, Tekedereli I, Elyas H 2007. Cytogenetic results of recurrent spontaneous miscarriages in Turkey. *Med Sci Monit*, 3: CR286-CR289.