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Research Article

Population Cytogenetics of Abortions and Vanishing Twin

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ABSTRACT
This paper for the first time suggests that the mother who displays vanishing twin is often characterized by the presence of some gross chromosomal aberrations like, acrocentric associations (involving more than 2 acrocentric chromosomes among 13, 14, 15, 21 and 22), some chromosomes showing premature centromeric divisions (PCDs), hypoploid or hyperploid chromosome counts (missing or adding a few chromosomes) etc in at least her 10% lymphocytes (male parents also show such variations). Mothers exhibiting spontaneous abortions (more than two abortions) contain much heavier "load" of chromosomal aberrations including these as well as specific chromosomal translocations and hyperploid counts in their lymphocytes. This partly offers plausible explanation that in a twin gestation the foetus lost has gross aberrations while the other one continuing to maturity may not be having such aberrations. May be, such a twin gestation be a DZ pregnancy?

Key Words: Cytogenetics of abortions, Chromosome aberrations and vanishing twin, Sporadic bleeding during pregnancy.

INTRODUCTION
Every species reproduces to survive and survives to reproduce (Goswami,1990). Our species with the unicornuate uterus and two nipples is phylogenetically destined and anatomically predisposed to deliver single births (Bulmer, 1970). However, twin and multiple births are universal and vary from 08 per thousand to 15 or more per thousand births ; triplets and multiple births vary from 1 to 2 per 100,000 births (Bulmer, 1970; Eriksson et al, 1988; Das Chaudhary, *et al*., 1993;Goswami and Goswami, 1993 ; Imaizumi, 1998).Hereditary predisposition of the mother plays a major role as evidenced by pedigree studies (Goswami,1970; Goswami and Wagh, 1975; Parisi *et al.,* 1983). More so, it is the mother who has to have all anatomical, immunogenetic , physiological and biological loads in order to defy many of the environmental factors to sustain the pregnancy. Extensive field work and family studies in Central India had also revealed that mothers with modest inbreeding coefficient (up to F= 0.009) have higher twinning rates but high inbreeding levels (F=0.03 or so) exhibit increased proportions of spontaneous abortions (Goswami, 1987; Goswami and Goswami, 1993). Similar results were also known from chromosomal studies which had indicated that there are certain couples in the general population who transmit some or the other chromosomal aberration through the gamete which result in early termination of pregnancy (Kajii and Ferrier, 1978; Joseph and Thomas, 1987). Our studies on recurrent abortions have also been based on protocols of lymphocyte cultures on referred couples (Mandal, et al, 1993; Rangnekar et al, 2004) and the present report is a follow up study undertaken by selective repetition on those mothers who have had mentioned sporadic bleeding during first trimester of the pregnancy, but continued pregnancy and delivered a full term single birth. Such events were recorded during our earlier surveys in families and the mothers were identified as SBDP (sporadic bleeding during pregnancy; Goswami,1983). Later, some of such mothers actually revealed "the vanishing twin" on confirmation by ultrasound reports.

Many workers have been concentrating on incidences of vanishing twin and their ascertainment by embryonal sacs under ultrasound examinations (Malinowski, 1998; Rydhstroem and Walles, 1993) but as far as we know nothing significant has been done on cytogenetic studies on mothers exhibiting vanishing twin. Chromosome study on such women is being presented hereunder with an emphasis that early termination of one foetus out of a twin pregnancy is monitored by the same chromosomal mechanisms as is done on regular events of spontaneous abortions but with a little intra pair difference.

A question then emerges as to why one foetus aborts while the other matures full term.? Certain chromosomal features often associated with incident of abortions are also mentioned. This is hypothesized here that the foetus

which goes on developing as a full term child may be actually due to that gamete which may not contain many gross aberrations. There appear to be some difference in between mothers terminating single pregnancy (abortion) and those terminating one foetus out of a twin pregnancy (vanishing twin) in the first trimester. Also, quite likely, most vanishing twin might be a DZ pregnancy outcome (?) meaning thereby, that either gamete (male or female) in the vanishing twin carried some chromosomal aberration (s) while the other one, leading to embryo maturing to full term might not be loaded with these aberrations.

MATERIALS AND METHODS

Routine Chromosome investigations: Mothers with earlier history of abortions (more than one abortion) and or having a child with any defect or apparent abnormality were referred to our genetic counseling clinic (during 1990-2000) for chromosomal studies, at the department of genetics, Barkatullah University, Bhopal, Central India. While filling up information (personalized questionnaire) we also had included an important question to the mother: *"Did you experience sporadic bleeding during pregnancy (now or in earlier one) and even after that, you had continued pregnancy to full term ?*. If the answer was yes, we made a separate note of that case and chromosome slides were also offered duplicate codes. Lymphocyte cultures were routinely carried out as per standard protocols and G banding technique on a large number of couples (Goswami,1986,2001) and persons randomly referred to our laboratory for chromosomal investigations. Many of them were repeated for specific diagnosis. Involvement of chromosomes in early termination of pregnancies have been published by a large number of workers (see reviews, Qumsiyeh, et al, 2000; Borovik, 2008; Mandal et al, 1993; Goswami, 2001; Rangnekar et al, 2004).

Giemsa stained and G-banded slides were blindly scored on a minimum of 40 metaphases and major gross features were recorded. Only those features which are present in more than 10% metaphases have been given importance in this study. Based on repeated studies now we have categorically selected chromosomal features (Table 1; Figs. 1-2) whose presence in chromosome preparations can indicate or fore-warn for recurrent /spontaneous abortion

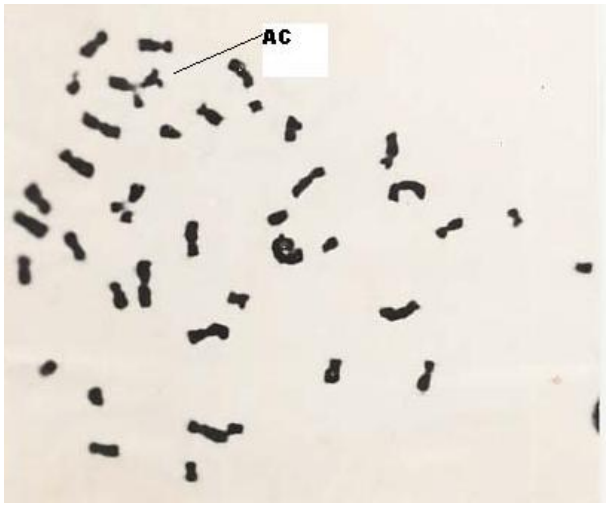
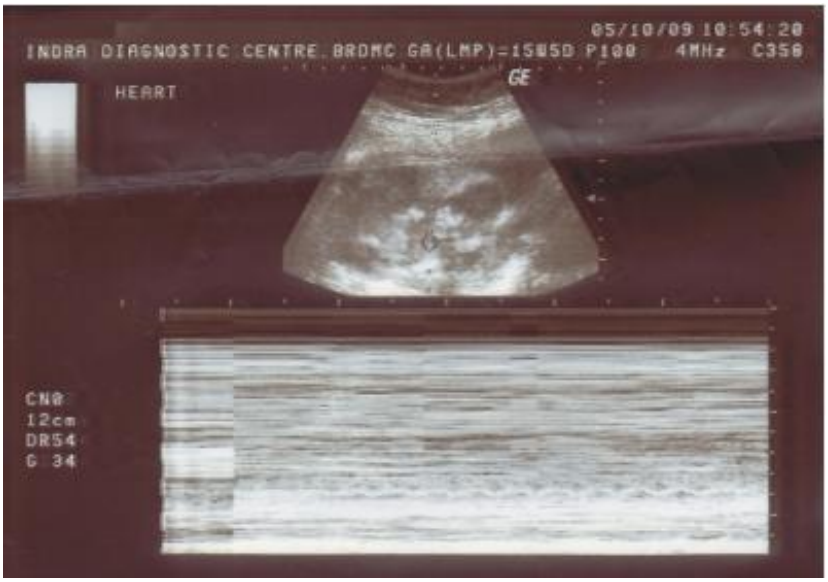
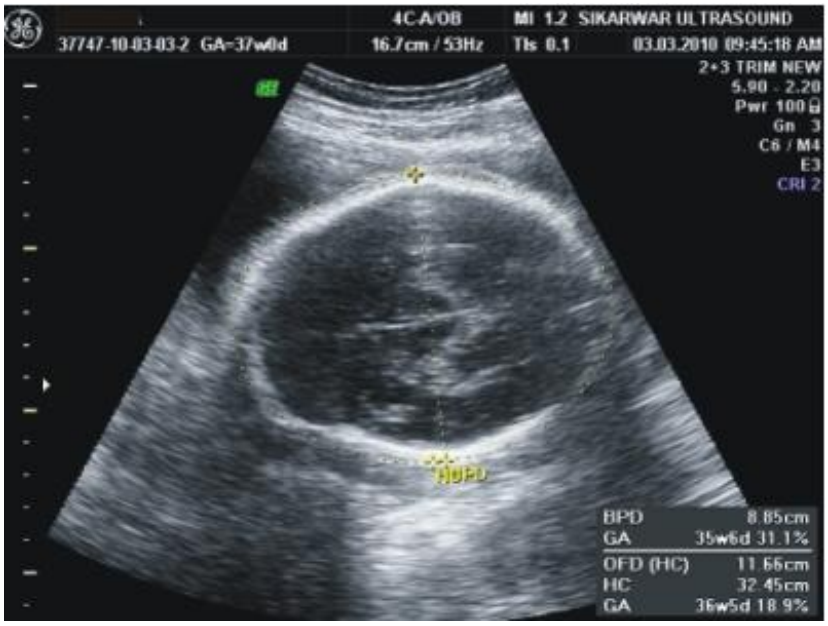


Fig. 1. A major part of the Giemsa stained metaphase in a lymphocyte from the female parent showing triangular association of acrocentric chromosomes (labeled as **AC:** Pseudo-Robertsonaian translocation) and also below, a fibrillar association of acrocentric chromosomes. (X 1600)

Fig.2. A Giemsa banded metaphase plate showing emanating dots from Ch.3 and Ch.8 so typically found with abortions. Another marker dot is indicated by a line but this is difficult to assign any specific chromosome,. A small deletion is labeled as **del**. The mother had shown two abortions and the third pregnancy resulted in a vanishing twin but also delivered a normal child. The repeated trials, after the vanishing twin, revealed presence of acrocentric associations and hyperploid counts in 10% metaphases while marker dots were in less than 4% cells(X 1600).



A



B

Figure 3: (Case No. VT/22-ALLD) Ultrasonography. **A** Ultrasonography shows the gradual loss of one embryonal sac at 7th week of pregnancy. Endometrial cavity at fundal region is having two gestational sacs with foetal node in it. Cardiac activity is seen only in one foetal node. **B** The surviving foetus has been growing alright.

Table 1: Gross chromosomal features among 18 Female Parents (FP) and 14 Male Parents (MP)

S. No.	DEL. (1)		TRANS (2)		ACR-ASS (3)		PCD (4)		HY-HPE (5)		MAR-DOT (6)	
	FP	**MP**	**FP**	**MP**	**FP**	**MP**	**FP**	**MP**	**FP**	**MP**	**FP**	**MP**
1	+	+	+	--	+	--	+	+	+	--	+	--
2.	--	--	--	--	--	+	+	+	+	--	+	+
3.	+	--	--	--	+	+	+	+	+	--	+	--
4.	--	--	--	+	--	--	+	+	+	+	+	--
5.	--	+	--	+	+	--	--	--	+	--	+	--
6.	+	--	+	--	+	+	--	--	--	--	--	+
7.	--	--	--	--	--	+	+	+	--	--	--	+
8.	+	--	+	--	+	+	+	+	+	--	+	--
9.	--	--	--	--	+	--	+	+	--	--	--	+
10.	--	--	--	--	--	+	--	--	--	+	--	--
11.	+	+	--	+	--	+	--	+	+	--	+	--
12.	--	--	--	+	+	+	+	--	+	--	+	+
13.	--	--	--	--	+	+	+	+	--	+	--	--
14.	+	+	--	--	+	+	+	--	--	--	+	--
15.	--	NA	--	NA	--	NA	+	NA	--	NA	+	NA
16.	--	NA	--	NA	+	NA	+	NA	+	NA	--	NA
17.	+	NA	--	NA	--	NA	+	NA	--	NA	+	NA
18	--	NA	--	NA	+	NA	+	NA	--	NA	--	NA
Total												
++	07	04	03	04	11	10	14	09	09	03	11	05
--	11	10	14	10	07	04	04	05	09	11	07	09

RESULTS

Vanishing Twin

During our efforts to collect personal data /questionnaire some of the mothers responded that they have had sporadic bleeding during 6 to 8 weeks of pregnancy. Also, those mothers who had indicated a possibility of vanishing twin by way of ultrasound examination were asked to definitely get examined by ultrasound expert /gynaecologist on the next pregnancy. The same request was firmly placed and often repeated to many private practicing personnel to take note of " bleeding during pregnancy, yet continuing full term delivery" . We have been able to have good cases of ultrasound records of vanishing twin on the basis of question on abortions (Fig 3 A & B). However, we could list only 34 cases of vanishing twin during 1986-1999 period, which reflected too less a number of cases referred to us. This may be primarily due to very restricted use of ultrasound investigations on pregnancy in semi urban parts of Central India by way of law, and also due to ignorance of most mothers to go for it because in most cases, a foetus is lost without much trouble and the pregnancy continues. Out of these cases, 18 mothers (female parents) and 14 male parents, were chromosomally investigated. Unfortunately, 4 male parents could not be available for investigation. Distribution of these persons to show specific chromosomal features is collectively shown in the Table 1. Table 2 presents those specific aberrations which are seen in all couples showing incidence of vanishing twin.

Gross Chromosomal Features

A large number of specific chromosomal features and somatic translocations have been variously described in the literature (see, Mandal et al, 1993; Qumsiyeh, et al, 2000) to be associated with abortions but as already mentioned, we have selected screening for following six categories of gross chromosomal features (Table 1 & 2) to be specifically prevalent in atleast 10% metaphases of lymphocyte cultures of each person with complaints of vanishing twin. These are as follows:

Acrocentric Associations: The 5 pairs of human acrocentric chromosomes (13,14,15, 21 and 22) have been found to be attached terminally among patients with abortions. The attached ends do not permanently translocate which might result in to Robertsonian translocations but are temporarily bondages (pseudo-Robertsonian translocations – AC in Fig.1; see below, three acrocentrics attach with fine threads) probably influencing segregation of chromatids in subsequent mitotic cell divisions. Female parents show 61% while male parents show very high 71% metaphases with such figures (Figs. 1 &2).

Deletion: A small break at any terminal or interstial part of a chromatid can be easily located (Fig. 2) resulting in the loss of chromatin .

Translocations: Various kinds of translocations have been observed; 16.66% among female and 28..57% among male parents.

Premature centromeric division (PCD): This is a very sensitive derailment in timing of longitudinal division of centromere of each chromosome in a cell, which otherwise is perfectly synchronized, resulting thereby in to many chromosomes already longitudinally split while other chromosomes remain intact.. We observe this feature to be more prevalent among nearly 78% female parents and 64% male parents.

Hyper/ Hypoploid counts: Instead of having 46 chromosomes, certain somatic cells show little higher or lower number of chromosomes even under normal conditions in less than 4% metaphases (Goswami, 1986). These hyperploid and hypoploid counts observed under massive screening have been found to be of very little significance but if frequency of such variant metaphases (to be strictly ascertained and counted in intact cells) increases, we will have to look for the onset of chromosomal mosiacism among cells and related consequences.

Markerdot (MD): Eversince discovered, these emanating chromatin dots (Figs. 1 & 2; measuring 1.5 to -2.5 mu) have been oobserved among various patients including cases of spontaneous abortions (Goswami, 1986; Goswami and Chang, 2001; Rangnekar et al, 2004). Table 2 shows presence of marker dots among 61% female parents and 35 % male parents. Significance of all these variations have been discussed elsewhere (Goswami, 2001). Briefly, marker dots are not small breaks or preparation artifacts but are definite chromatin bodies which have been observed repeatedly emanating from specific chromosomes(Fig.2, Ch 3 and ch. 8 clearly show such marker dots).

DISCUSSION

Vanishing twin is a selective termination of a foetus from a twin or multiple pregnancy about which the mother may not be aware of, immediately. Field work questionnaire revealed that in most cases, sporadic bleeding during pregnancy (Goswami, 1983) in the first trimester among rural folk of women might be actually the vanishing twin. Such reports were investigated by ultrasonography. We have gathered evidences from various ultrasound centres that bleeding during twin pregnancy within 6-8 weeks may often be an incidence of vanishing twin.

Table 2: Frequency distribution of gross chromosomal features among parents exhibiting vanishing twin

Gross Chromosomal Features	Female Parents (18)		Male Parents (14)	
	N	f	N	f
1. Deletion	07	0.3888	04	0.2857
2. Translocation	03	0.1666	04	0.2857
3. Acrocentric association	11	0.6111	10	0.7142
4. Premature centromeric Division (PCD)	14	0.7777	09	0.6428
5. Hyperploid/ Hypoploid Count	09	0.5000	03	0.2142
6. Marker Dot (MD)	11	0.6111	05	0. 3571

Since the pregnancy continues, the prospective mother, unless advised, does not get examined by ultrasonography. Obviously, a large number of cases of vanishing twins remain unrecorded in most parts of India, more so in smaller townships and semi urban areas. Since our population survey (1984-2000) also included random chromosomal investigations on couples with history of abortions (Mandal *et al.,* 1993; Goswami and Chang, 2001; Rangnekar *et al.,* 2004) we could select six gross chromosomal features to be comparatively screened on repeated chromosome preparations among couples presenting a case of vanishing twin. We could then plan to investigate but only on 18 female parents and 14 male parents; the results are shown in Tables 1 and 2.

Distribution of chromosomal aberrations in lymphocytes of male and female parents suggest that such aberrations may also influence to result in chromosomal irregularities, defective segregations and structural deficiencies among many cells even in the dividing gonad cells.

A large number of papers have impressed upon that chromosomal aberrations (Mameli *et al.,* 1984; Qumsiyeh, *et al.,* 2000) in somatic cells are positively associated with abortions, but this is unfortunate that almost all of them concentrate only on looking at karyotypes (Borovick et al, 2008) and their studies totally ignore basic chromosomal features (Table 2) which may not have ignorable effects. Our careful scrutiny however lays special emphasis mainly on deletions, acrocentric associations (pseudo-Robertsonian translocations; Fig.1) and other chromosomal features (Table 1).

A serious question therefore emerges as to why the other foetus goes on developing full term while one gives-up journey as a vanishing twin. According to earlier theories (Ahiron & Blickstein 1993) one of the embryos is discordant in growth patterns and or, one of the embryonal sacs has abnormal (Malinowski, 1998) yolk sac. The alternative hypothesis which appears plausible here is that the twin pregnancy, which exhibits vanishing twin may actually be a dizygotic (DZ) pregnancy thereby both the embryos must not be having same genotypic configuration and the foetus which departs away must have been loaded with defective chromosomal features while still, the karyotypic configuration of the foetus as well as the mother be the same.

REFERENCES

Ahiron R, Blickstein I (1993). Persistent Discordant Twin Growth following IVF-ET Acta Geneticae Medicae et Gemellogiae.(Roma) 42:41-44

Borovik CL, Perez ABA, da Silva, RJ, Krepischi-Santos CV, Costa SS, Rosenberg C (2008). Array-CGH testing in spontaneous abortions with normal karyotypes. Genetics and Molecular Biology. 31: 416-422.

Bulmer MG (1970). The Biology of Twinning in Man. Oxford, Clarendon Press.

Das Chaudhary AB Basu S, Chakraborty S (1993). Twinning rate in the Muslim population of West Bengal. Acta Geneticae Medicae et Gemellolgeae (Roma) 42;36-39

Eriksson AW, Bressers, W M A , Kostene, P.J. , Pitchen, K.J., Mieke,J.H., Jorde, L.B. ,Tas R.F.J., Fellman, J. O. (1988). Twinning rates in Scandinavia, Germany and The Netherlands during years of privation. Acta Geneticae Medicae et Gemellologiae (Roma). 37: 277-297

Goswami HK (1970). Studies on Twins. IV. Twinning in Madhya Pradesh. Acta.Genecae Medicae et Gemellologiae (Roma). 19: 465-471

Goswami HK (1983). Genetics and Public health. Catholic Press Ranchi

Goswami HK (1986). Cytogenetic effects of methylisocyanate exposure in Bhopal.
Human Genetics. 74: 81-84

Goswami HK (1987). Twinning and Inbreeding in India: The Fraternal component
Acta Geneticae Medicae et Gemellologiae (Roma) : 36: 343-347

Goswami HK (1990). Socio-biological implications of Genetic Demography. Pp-143-149. In, Demography of Tribal Development edited by: A.Bose, U.P.Sinha, R.P.Tyagi., B.R.Publishing Corporation .Delhi.

Goswami HK (2001). Genetic significance of Marker dots. In, Perspectives in Cytology and Genetics.10: 265-269 edts: G.K.Manna and S.C. Roy. All India Congress of Cytology and Genetics. Kalyani.

Goswami HK, Wagh KV (1975). Twinning in India . Acta Geneticae Medicae et . Gemellologiae (Roma) 24: 347-350.

Goswami R, Goswami, HK (1993). Changing trends in twinning. Acta Genicae Medicae et Gemellologiae (Roma). 42: 289-294.

Goswami HK, Chang SI (2001). Marker dots are expelled by attenuation in heterochromatin of a chromatid. Bionature; 21: 41-48.

Imaizumi Y (1998). A comparative study of Twinning and triplet rates in 17 countries, 1972-1996. Acta Geneticae Medicae et Gemellologiae (Roma). 47: 101-114.

Joseph AM, Thomas IM (1987). Paternal translocation (1:7) associated with reproductive failure (A case report) J. Postgraduate. Medicine. 33: 143-145.

Kajii T, Ferrier A (1978). Cytogenetics of aborteres and abortuses. American. Journal of . obstetrics and . Gynaecologiae. 131: 33-38.

Mameli M, Cardia S, Milla A, Aste A, Santucci S, Genazzani AR (1984). Cytogenetic study in fifty couples with recurrent abortions. Gynaecology Obstetetrics Investigations 17 : 84-88

Mandal P K, Rangnekar A, Sengupta, LK, Goswami, HK. (1993). Chromosomal Involvement in human reproductive wastage: An Overview. In, J.B.S. Haldane and Evolution (Bionature Vol 13): 297-317

Malinowski W (1998). Yolk sacs in twin pregnancy Acta Geneticae Medicae et Gemellologiae (Roma). 47:177-181

Parisi P, Gatti M, Prinzi G, Caperna G (1983). Familial inheritance of twinning. Nature 304:626-628

Qumsiyeh MB, Kim KR, Ahmed MN Bradford W 2000. Cytogenetics and mechanism of spontaneous abortions: increased apoptosis and decreased cell proliferation in chromosomally abnormal villi. Cytogenetics and Cell Genetics. 88: 230-235.

Rangnekar A, Goswami HK, Goswami R (2004). Recurrent abortions are associated with cancer history. Bombay Hospital Journal. 40: 381-383

Rydhstroem H, Walles B (1993). Ultra sound examination in Twin pregnanacy and late fetal death. Acta Genicae Medicae et Gemellologiae (Roma). 46: 199-207