CYTOGENETIC STUDY OF ABORTION IN EGYPT

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The aim of this study was to estimate the incidence and types of chromosomal anomalies in a sample of spontaneous and induced abortions derived from the Egyptian women. and to correlate the clinical data with the cytogenetic findings. Also to relate the findings to any possible aetiological factors which might account for the occurrence of such chromosomal abnormalities.

METHODS AND MATERIAL

Chromosomal studies were performed on 100 specimens, 60 of which were spontaneously aborted and the other 40 were products of induced abortions. Successful preparations were available from 16 cases of each group. Table 1.

The mothers were examined clinically to exclude any possible cause for abortion. Maternal ages ranged from 16-46 years. The gestational age for the abortuses ranged from 8-20 weeks.

Three methods were used for the cytogenetic study of the abortion :

First, organ culture of foetal tissues without previous trypsinisation, this technique was described by Le Jeune and modified by Yunis, 1965. The modifications used here were obtained through a personal communication with Prof. Waxman, Hawai University. (1970).

Second, direct preparations without previous culture. This simple technique was tried and developed by Dr. Badr and Dr. Abdel Aziz (1970) in the National Research Centre, Cairo.

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Third, short term whole blood culture microtechnique. This technique was described by Arakaki and Sparkes, (1963) and was applied by us with some modifications.

The sex ratio among abortuses has been studdied on basis of observation of external genetatia in well differentiated abortuses, sex chromatin analysis of extraembryonic membranes (Klinger, 1957) and on Karyotyping. The male : female ratio was 19 : 24.

RESULTS

Four spontaneously aborted conceptuses were found to have a numerical chromosomal abnormality. This gives an incidence of 25%. All induced abortions were chromosomally normal. The difference between the incidence of chromosomal anomalies in the two groups of abortions was tested statistically using the Chi-square test and found to be significant; ($X^2_1 = 4.572$; 0.02) (Table I).

No structural chromosomal abnormalities were found in both groups.

TABLE 1.

Incidence of Chromosomal abnormalities in spontaneous and induced abortions.

Type of abortion	No. of cases karyotyped	No. of cases with chromosomal abnormalities	Percentage of chromosomal abnormalities
Spontaneous	16	4	25
Induced	16	0	0

(*) Statistically, the difference was found to be significant using the chi-square test.

$$X^2(_1) = 4.572 \; ; \; 0.05 > p > 0.02$$

The first abnormal abortus has two types of cells tetraploid and diploid (tetraploid/diploid mosaicism (Fig. 1a). Tetraploid cells may occur among normal diploid amniotic cells, but their percentage does not exceed 10%. In our case 103 cells were counted and the diploid/tetraploid ratio was found to be 53 : 50.

Fig. 1b shows a tetraploid cell. Every member is represented 4 times instead of 2. The sex chromosome complement is XXYY.

FIG. 1 a.—Tetraploid diploid mosaicism

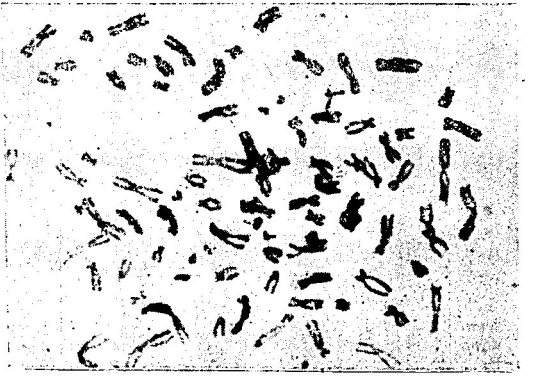

FIG. 1 b.—Tetraploid cell Case No. 3

This abnormality has not been reported in living humans. There are two previous reports of a tetraploid/diploid mosaic abortus by Thiede and Salm, 1964, and Thiede and Metcalfe (1966). Therefore our case could be considered the third tetraploid/diploid mosaic abortus on report.

Fig. 2 shows a karyotype prepared from the second abnormal case. The total number of chromosomes is 47. The extra chromosome coincides with chromosomes of group D (Group D Trisomy). The sex chromosome complement of this abortus is XY. This abnormality has been reported in newly born infants with multiple congenital malformations Patau's syndrome. These infants usually die few hours after birth.

Fig. 3 shows a karyotype prepared from the third abnormal abortus. The total number of chromosomes is 47. The extra-chromosome coincides with chromosomes of group G (Group G trisomy). The

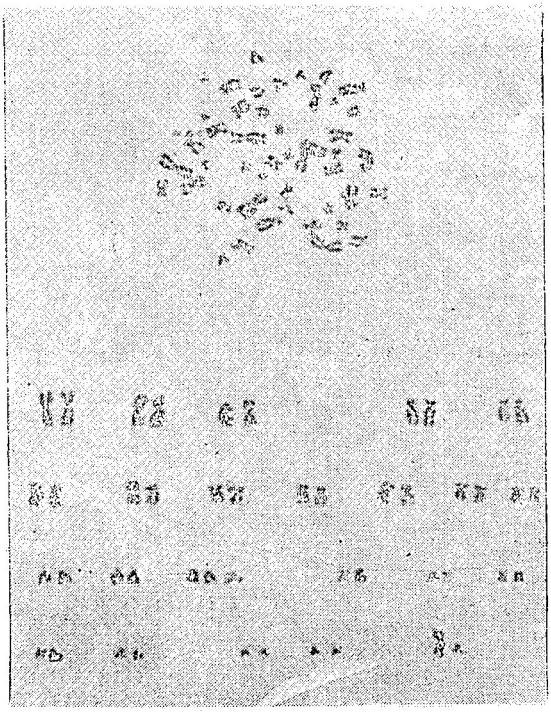

FIG. 2.—Karyotype from Case No. 13 Spontaneous abortion Group D Trisomy

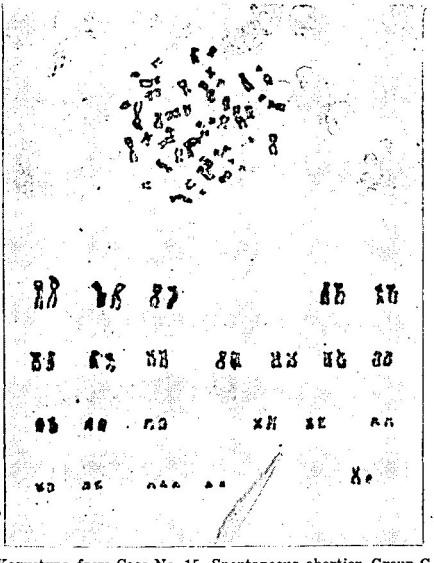

FIG. 3—Karyotype from Case No. 16. Spontaneous abortion Group G Trisomy

sex chromosome complement of this abortus is XY. This abnormality has been reported in living humans with Down' syndrome or mongolism.

Fig. 4 shows a karyotype prepared from the fourth abnormal conceptus. The total number of chromosomes is 45. The deficient chromosome is a medium sized submetacentric chromosome. The external genitalia of the foetus were of the female type. Sex chromatin studies show no chromatin bodies. The deficient element has been identified as an X chromosome. XO abortus or X monosomic abortus. This abnormality is found among most patients with Turner's syndrome.

Table 2 summarizes the clinical and cytogenetic data concerning these four abnormal abortions.

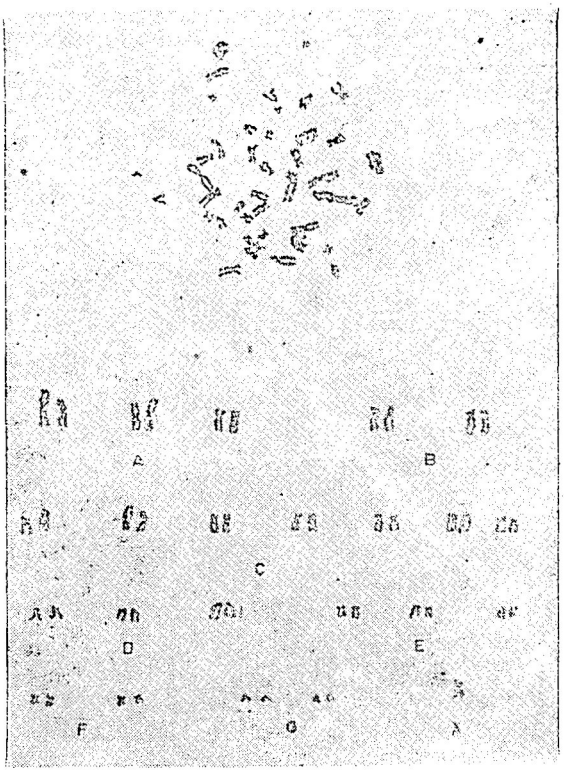

FIG. 4.—Karyotype from Case No. 16 Spontaneous abortions XO Monosomy.

Three out of the four chromosomally abnormal conceptuses were found to have gross anatomical deformities.

The three chromosomally abnormal conceptuses have been found among 12 abortions occurring before the end of the fifteenth week of pregnancy. While a single abnormality occurred among 4 spontaneous abortions rejected after the 16th week of gestation.

Table 3. shows the relation of chromosomal anomlies in spontaneous abortion to maternal age. The incidence of chromosomal anomalies among abortions obtained from mothers above 35 years is 50% stands in contrast with the incidence among the young age group, below 35 years, which is 16. 66.

TABLE 2

Clinical and Cytogenetic Data of the Four Chromosomally Abnormal Abortions.

Case No.	Maternal age years	Paternal age years	Obstetric history				Menst. history	Duration of pregnancy in weeks	Duration of bleding in days	Description of abortus	Tissues culture	Type of* abnormality
			F.T.D.	S.B.	Abortion	Total						
3	20	30	1	1	1	3	Normal	12	1	Intact empty S.C. no foetal remnants no cord stump.	Foetal membrane	Tetraplodi XXYY* diploid/XY.
13	40	50	7	—	2	9	Infrequent cycles	14	2	Normally developed foetus	F etal blood	Trisomy d* (47/XY D
15	37	40	6	—	—	6	Infrequent cycles	14	1	Growth of the foetus markedly retarded and incoordinating with the period of pregnancy, microcephally.	Foetal blood foetal memb.	Trisomy G*) (47/XY G
16	24	32	—	—	—	—	Normal	16	1	Hydropic foetus with a nuchal cystic mass and female external genitalia.	Foetal blood liver	X monosomy ** 45/X

* Abnormality unreported in living humans.

** Abnormality escribed in living humans,

TABLE 3.

Relation of chromosomal Abnormalities in Spontaneous Abortion to Maternal Age.

Maternal age in years	Chromosomally Abnormal	Chromosomally Normal	Total	Percentage.
below 35	2	10	12	16.66
35 and more	2	2	4	50
TOTAL	4	12	16	25

Table 4. shows the relation of the parity of the mother to chromosomal abnormalities in abortion. A single chromosomal abnormality was seen among 6 women giving history of less than 3 previous pregnancies. Another chromosomal abnormality was found among 4 women giving a history of 3—6 previous pregnancies. On the other hand two chromosomal abnormalities have been found in abortuses obtained from mothers giving a history of more than 6 previous pregnancies. Thus the incidence of chromosomal abnormalities increase with the parity. Usually multiparity is associated with increased maternal age.

TABLE 4

Relation of the parity of the mother to chromosomal abnormalities

No. of previous pregnancies	Spontaneous Abortions			Percentage
	Chromos. Abnormal	Normal	Total	
Below 3	1	5	6	16.66 %
3 - 6	1	3	4	25 %
6 +	2	4	6	50 %
Grand Total	4	12	16	25 %

DISCUSSION

The rate of success of tissue culture was low and found to be 10, 25, 25 per cent for foetal lung, fetal membrances and foetal blood cultures respectively.

The causes of this gross failure have been discussed by many authors and were found to be either endogenous factors affecting the variability of the specimen itself, in the form of biochemical defects and disturbed genetic constitution, or extrensic factors particularly those concerned with the laborious technique used in tissue culture e. g. infection, toxicity of the media, serum or antibiotics.

Using foetal blood culture for cytogentic study of a total of 29 abortuses in this series we put in mind the possibility of leakage of maternal lymphocytes in foetal circulation, but we could not find any evidence of lymphocyte chimeraism in the 15 abortuses with XY sex chromosome complement. Taylor and Polani (1965) reported the results of cytogenetic study of a 12 weeks abortus, 10% of foetal lymphocytes and thymus cells were XX where as cells cultured from skin fibroblasts were consistently XY. The possibility of leakage of maternal lymphocytes into the foetus was the most likely. Keer (1969) mentioned that it is impossible to say whether leak of maternal lymphocytes into the foetus is a common finding in early pregnancy.

In this study the incidence of chromononal anomalies in spontaneous abortions was found to be 25 percent. This is nearly the same as that reported by Clendenin and Benirschke, 1963; Hall and Kallen, 1964 and Carr, 1967, from United States, Sweden and Canada respectively. Carr, 1966 mentioned that 20-25% is a valid incidence for chromosomal anomalies in unselected spontaneous abortion. Analysis of published and unpublished pooled data coming from different countries, by W.H.O. experts, (Geneva Conference, 1966) show an incidence of 19%. Carr (1971), working on early spontaneous abortions mentioned a figure of element is commonly called chromosome 21, though the two pairs of neous abortions.

No chromosomal abnormalities was found among induced abortions investigated. The frequency of chromosomal anomalies in induced abortions have been studied more in countries where abortion is sanctioned by law. It has been found to vary from 1-2%, (Geneva Conference, 1966; Sasaki *et al.*, 1971). In view of that the incidence of chromosomal anomalies in spontaneous abortion is 10-12 times more frequent

than their incidence in induced abortion and 50 times more frequent than their incidence in live born infants, (Marden *et al.*, (1964).

The three types of chromosomal anomalies found in this series; tetroploid/deploid mosaicism, autosomal, trisomy and XO monosomy have been described before hand in abortion material, (Kerr and Rashad, 1966 ; Geneva Conference, 1966). The first have not been described in living subjects but the other two types are compatible with extrauterine life.

Doubling of chromosomes or tetrsploidy in man appears to be associated with abortion, it has not been reported in live born infants. Tetraploidy account for 3.5% of all chromosomal abormalities in abortion, (Szulman, 1968).

It is usually associated with gross malformation of the conceptus Thiede & Metcalfe, 1966). The abortus described here consists of an intact empty sac with no foetal remenants. Thiede and Salm (1964) described a tetraploid mosaic foetus 75 mm in length which was apparently normally developed.

Tetraploid cells may occur occasionally in culture of amnion, but the percentage does not exceed 10% (Klinger & Schwarzacher, 1960). The origin of these tetraploid cells relative to normal amniotic cells is still unknown. In our case tetraploid cells constitute 50% of the whole cells analysed. This mosaicism demonstrates a marked difference in comparison with those cases of pure tetraploidy reported by Waxman, Sasakaki and Smith (1967) and Carr (1967). Schlegel *et al.*, (1966) recommended that the laboratory diagnosis of tetraploidy, when it is made on amnion cultures, might be more convincing if based on a relatively large number of analysed metaphases than are customarily studied in cultures of other tissues. In our case a total of 103 cells have been analysed 50 of which were tetraploid.

Trisomy or the presence of an extra chromosome is the commonest chromosomal anomaly. In this study two cases, one trisomnc for D group and the other for G group were found among the four chromosomally abnormal abortuses i.e. showing 50% incidence among all types of chromosomal abnormalities. Previous reports on larger numbers of abortions showed that trisomies account for nearly half of the chromosomal abnormalities in abortion (Kerr, 1969). Trisomes affecting every group of chromosomes have been reported in abortions, (Carr, 1968) but the most commonly affected are those of group E, G and D in this order. Generally speaking the chromosome groups containing

larger chromosomes are less likely to have trisomic elements than those containing smaller chromosomes. Nevertheless, the rarity of trisomies of group F is a striking exception.

Trisomy for an extra member of group D occurs 1/14 in 500 or 1/7000 among liveborn infants but it is more commonly seen in abortions. It accounts for 7.3% of all chromosomal abnormalities in abortion or 1.8% of all spontaneous abortions (Carr, 1968). The abortus with group D trisomy in this study was apparently normal. Carr (1966) reported on 6 abortions with group D trisomy, two of which were completely normal. The absence of any morphological stigmata characteristic of Patau's syndrome or D, trisomy syndrome seen in abortions. It accounts for 7.3% of all chromosomal abnormalities in abortion or 1.8% of all spontaneous abortions (Carr, 1968). The abortus with group D trisomy in this study was apparently normal. Carr (1966) reported on 6 abortions with group D trisomy, two of which were completely normal. The absence of any morphological stigmata characteristic of Patau syndrome or D, trisomy syndrome seen in newly born infants makes it less likely that trisomy have involved the same pair affected in this syndrome.

The commonest autosomal trisomy in liveborn infants involves one of the members of group G with an incidence of 1/600. The extra 40% incidence for chromosomal anomalies in the first trimester spontachromosomes in group G cannot usually be distinguished from each other. The phenotype of patients with trisomy 21 is readily recognized (Down's Syndrome).

Group G trisomy is common among abortions. It accounts for 11.3% of all chromosomally abnormal abortions or 2.8% of all spontaneous abortions (Carr, 1968).

Group G trisomy in abortion may be in part due to the «non mongol» autosome pair. It seems that such specific trisomy has lethal effects during intra-uterine life, since the corresponding trisomy syndrome is unknown in living humans (Bartalos and Baramaki, 1967).

The foetus with G trisomy described here, was found to be retarded in growth (Crown-heel length 110 mm., Crown-rump 70 mm. Weight 90 gm.) relative to its menstrual age (14 weeks). Its head was obviously small, circumference 80 mm) (Wellis 1962). No morphological characteristics have been described for abortuses with G trisomy. Foetuses were either completely absent or grossly malformed, (Carr, 1967).

The fourth abnormal abortus in this study was karyotyped as X monosomy. The diagnosis is based on the finding of a constant number of 45 chromosomes in all the cells examined. The presence of only 15 chromosomes in Group C and 4 chromosomes in group G and the absence of sex chromatin from cells which were grown in culture and in a whole amount of amnion. The morphological characteristics of this foetus differ in no important aspect from those described by Singh and Carr (1966) and Ruchton *et al.*, 1969.

The incidence of X monosomy in this study was 25 per cent of the chromosomally abnormal abortions and 6.25 per cent of all spontaneous abortions kayotyped. XO monosomy is the most common chromosomal of abnormality found in spontaneous abortions. Carr (1967) reported in cidence of 5% for XO conceptuses. These figures of 6.25% and 5% are in contrast to the very low incidence of 1/5000 among newborn infants. (Maclean *et al.*, 1964).

Other well-known sex chromosome anomalies, XXX and XXX are virtually unknown in abortion material (Pergament and Kadotani, 1965), whereas they are relatively common in neonates. This observation supports the belief that the possession of an extra X chromosome is less harmful than the loss of one X chromosome. XXY adult males have relatively few stigmata while XXX females may be phenotypically normal.

Other chromosomal abnormalities which are known to occur among abortuses have not been observed in this study. This may be due to ; first, some chromosomal anomalies are very rare as trisomy B, F and autosomal monosomy, so that their absence is expected on account of the relatively small number of abortuses examined, second ; some chromosomal anomalies may cause early loss of the conceptus prior to the stage of clinically recognizable abortion, while others e.g. mosaicism and structural chromosomal abnormalities occur in mid trimester abortions, (Szulman 1968).

The gestational age of the conceptus is known to affect the incidence and types of chromosomal abnormalities in abortion. The mean gestational age for the 16 kayotyped spontaneous abortion is 13.62 $\pm$ 2.2 weeks which is nearly the same as that reported in most of the unselected series investigated before (Geneva Conference, 1966). Carr (1967) and Bowen and Lee (1969) reported statistically significant differences between the mean gestational ag of chromosomally normal

and abnormal abortions. However in this study the mean gestational ages for chromosomally normal and abnormal abortions are 13.62 ± 1.41 weeks and 14.0 ± 1.41 weeks respectively. The smalleness of the size of the sample studied might fail in exploration of any hidden difference in this parameter.

Analysis of the mean maternal ages for women aborting chromosomally and abnormal conceptuses, 26.33 ± 5.26 and 30.25 ± 8.44 years respectively, shows no significant difference between the two groups, (t = 0.23). The mean maternal age for the two trisomic abortions is obviously higher than the average estimated for the whole group. (38.5 ± 1.5 years). In contrast the maternal age for the tetraploid/diploid mosaic abortus and the X monosomic abortus are 20 and 24 years respectively. These findings run in parellel with the results of previous studies (Carr, 1968).

In this study 2 chromosomally abnormal conceptuses have been found among 12 karyotyped spontaneous abortions with maternal age less than 35 years giving an incidence of 17%. In contrast, 2 chromosomally abnormal abortuses (Trisomy G and Trisomy D) were found among only 4 karyotyped spontaneous abortions with maternal age above 35 years, with an incidence of 50%. It is obvious that the incidence of trisomies in abortion increases with maternal age. The same correlation was found in cases of liveborn trisomies, (Penrose, 1961). This finding agree with the notation that meiotic nondisjunction, responsible for trisomy, become more common as the maternal age increases.

The obstetric histories of women producing abortuses with and without chromosome anomalies or specimens failing to grow in culture do not differ in any obvious respect. But it was noted that the two trisomic abortions came from grand multiparous women. This was anticipated on basis of the earlier findings in Down's syndrome and the D trisomy syndrome Smith, Patau and Therman, 1961), and confirms Carr's findings (1965).

An interesting point in this study is the association of prolonged menstrual cycles or infrequent menstruation with the two trisomic abortuses (Table 2). Similar association have been previously noted by Boue *et al.* (1968). Infrequent menstruation may be due to hormonal imbalance occurring with the approaching menopause. The delay of ovum transport due to hormonal imbalance and its predisposition to chromosomal anomalies have already been discussed. (Boue *et al*, 1968).

It was noted that the paternal ages for the trisomic abortions are obviously high 40 years and 50 years for trisomy G and trisomy D respectively. In contrast to advanced maternal age, no relation had been established between the advanced paternal age and the incidence of group G and group D trisomies. Nevertheless it is not possible to postulate that there is a relationship between advanced paternal age and the occurrence of trisomy since there is a very rapid turnover of developing spermatozoa throughout reproductive life and thus little time for any aging process to have an effect. However it should be stressed that with regard to the causation in any particular trisomy there is no way at present of telling whether non-disjunction do occur during oogenesis or spermatogenesis (Carr, 1969).

Three out of the four chromosomally abnormal conceptuses were found to have gross anatomical deformities. The conceptus with tetraploid/diploid mosaicism consisted of an intact empty sac with no foetal remnants. The conceptus with trisomy G had a small head and was retarded in growth relative to its menstrual age. Finally the XO abortus was involved with oedema overall the body with a nuchal cyst. The high incidence of abnomosomal abnormalities among malformed abortuses have been noted by many authors (Szulman, 1965 and Carr 1967).

Multiple factors may be responsible for (chromosomal anomalies) e.g.; radiation, maternal aging, genetic effects and virus infections. All these factors have been proposed, singly or in combination. Witchi and Laguens (1963) have shown that aging of amphibian eggs produces a high incidence of monosomy, trisomy and occasionally polyploidy. They emphasized the fact that aging of ova can be considered in two ways. This aspect is especially relevant in mammals. There is the effect due to an ageing female in the reproductive sense whose ova have been in prophase (dictyotene) for more years than the postnatal age of the animal. On the other hand, the scarring of the overy associated with repeated ovulation and formation of corpora lutea in mammals could interfere with the bursting of mature follicles. In this case, the liberation of the oocyte could be delayed by hours and thus appreciably lengthen the time before fertilization and the completion of mieosis. This itself may interfere with normal disjunction of chromosomes.

SUMMARY

Chromosomal studies were performed on 16 cases of Spontaneous abortion and another 16 cases of induced abortion. All specimens were derived from Egyptian Women with a gestational age of 8—16 weeks. Four spontaneously aborted conceptuses were found to have numerical chromosomal abnormalities, giving an incidence of 25% of chromosomal anomalies in spontaneous abortion. The types of chromosomal anomalies found were tetraploid/diploid mosaicism, Trissomy D. Trisomy G and X-monosomy. Findings obtained were analysed, discussed in light of reports coming from other countries.

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