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Research Article

SOME COTWINS OFFER EVIDENCE FOR THE POSSIBILITY OF MONO-OVULAR DISPERMIC DIZYGOTIC TWINS IN HUMANS

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ABSTRACT
This study demonstrates that the hormonal component of oral contraceptive pill can stimulate amitotic division in the nuclei of plant cells as well as in cultured human lymphocytes.
Based on this experimental evidence another possibility is also discussed that the ovum released may undergo a division/ cleavage thus producing two ova of exactly same genetic constitution, which on fertilization by two sperms would develop in two cotwins having the same maternal genes but differing in paternal genes. Such a combination of cotwins in a twin pair or in a triplet would be referred as mono-ovular (monovular) dispermic dizygotic twins/ triplets. These maternally similar cotwins in a twin pair or in a triplet can be best detected by sorting out exclusively visible maternal traits and scoring their concordance/discordance among cotwins and parents. A new approach is suggested to enable identification of such MDDZ cotwins.

Key Words: *Monoovular Dispermic Dizygotic Twins; Cotwin Control Study; Amitotic Division Induced By Oral Contraceptive Hormone; Cotwins with Common Maternal Genes*

INTRODUCTION
Human Twins have always been very fascinating objects of curiosity both from social as well as biological points of view. Twins from the point of view of origin are of two types : monozygotic or Identical twins who develop after the cleavage of a single zygote and dizygotic twins (fraternal), who develop from two different zygotes. Developmentally, it is the zygote which decides the zygosity because cotwins of a twin pair or a triplet derived from the same zygote will share common genes while cotwins of a twin pair or a triplet developing from more than one zygote will differ in sharing genes. Therefore, this becomes always a matter of investigation of the zygosity of a twin pair or triplets/ multiple births.
Genetically, MZ cotwins should share exactly the same genes and often be of the same sex while DZ cotwins share 50% genes, and can be of the same or different sex. Yet another possibility of the origin of two ova by one ovum due to its cleavage before fertilization was expressed which always has been a matter of detailed investigation. In this context Gedda (1961) has had presented in detail about the formulation of a concept by Danforth (1916) that there existed a possibility of third type of twins by assuming that the two cells produced by the division of the secondary oocyte may be fertilized by separate spermatozoa. Fisher (1919) further elaborated that the degree of resemblance characteristics of this third group would be of the same order as could be predicted if the twins were to decide the hereditary material of one gamete, but not the other. This condition would be met if after maturation, the ovum (secondary oocyte) could be induced to divide in to identical halves which in turn, could be fertilized by different spermatozoa. The two zygotes thus produced will be genetically dissimilar. Such twins may be called dizygotic, but can not be regarded as biovular since they are derived from a single ovum rather than two different ova. Obviously, the production of two genetically dissimilar twins or triplets, with one dissimilar and two similar members becomes a rare and complicated event because the situation can also be simulated by pluriovulation.
Bulmer (1970) had also summarized studies indicating that an ovum may mitotically divide into two ova, before getting fertilized. Such twins are referred to be mono ovular dispermic dizygotic twins (third category of twins). Obviously, these cotwins will be more biased for maternal traits, be of same or different sex because of different sperms and may be differing in expression of paternal genes. There has

not been any unanimity on the third category of twins for two reasons, viz. there is no any particular demonstration of a mitotically dividing ovum and secondly, this has always been difficult to prove that such cotwins have some traits due to exclusive dominant expression of maternal genes. Thus, by and large, mono ovular dispermic DZ twins have been more of theoretical significance because reliable proofs that an ovum can divide have not been extended so far. Obviously, this is a fascinating area in human genetics (Golubovsky, 2006).

As far as known, this paper offers a first ever study on triplets by a unique find of cotwin control study as a possible proof that rarely though, an ovum can cleave in to two ova before fertilization. This is supported also by indirect experimentation on induced cell divisions by oral hormone intake on plant cells and cultured human lymphocytes. An hypothesis that an ovum could have greater possibility of undergoing a amitotic division under the influence of extra dose of oral hormone (contraceptive pill) was tested on plant cells (Goswami,1978). These experiments were extended to several trials with standard protocols on *Allium* test using *Allium sativum* (garlic) cells and also, on human lymphocyte cultures. These observations are presented here as indirect evidences in favour of the hypothesis that amitotic division may be more likely an event by which an ovum may get equally divided in to two.

This short communication also presents a long term follow up study on certain cotwins of triplets in particular, and brings out information that the best proof for the existence, (may be rare, though), comes out from the same sex female triplets where two cotwins share 100% concordance with maternal traits and the third cotwin differs remarkably with the other two; in two cases even in blood groups. Follow up studies for nearly two decades on families of triplets along with laboratory experiments on induced amitotic divisions by oral contraceptive hormonal doses are presented below to retain the possibility as above.

MATERIALS AND METHODS

While studying twins and twinning from families and health center/hospital surveys conducted during 1964-1980s this was clearly demonstrated that the mothers who took extra and irregular doses of oral contraceptive pills showed greater proportion of twin births (Goswami,1983,1990; Goswam & Goswami, 1993)), particularly different sexed twins. This was suspected that quite likely, extra hormonal dose might be resulting in to either extra ovulation (so well known a phenomenon) and or possibly, a cleavage of ovum before fertilization. This was tested by simple experiments as mentioned below. Observations had then triggered a follow up study on twins/ triplets and their families.

(A) Allium test

Three repetitions were made for this experiment; each time the same solution was used for *Allium* test as was used for lymphocyte cultures. The OpS (oral pill solution) was prepared by the following method: Ten tablets (any make recommended for oral use) were finely powdered and mixed in 10 ml luke warm sterilized distilled water (each tablet contained Lynesternol BP 1.0mg + ethinyloestradiol, 0.05mg). OpS from another tablet "Triquilar (German Remedies, Ltd. Bombay; Levonorgestrael and Ethinylestradiol Tab IP) prepared the same way, was cooled by shaking for about 5 min and filtered. One ml was used in lymphocyte cultures and remaining was used for *Allium* test (garlic) with adequate controls. Garlic cloves with newly generated roots were kept as half sunk in the turbid solution so as to dip all roots for 4 hours. Root tips were squashed and stained in acetic orcein (1.5%). Photographs (Fig. 1) were taken using automatic camera attached to the microscope.

(B) Lymphocyte cultures

Lymphocytes were routinely cultured in various media during our investigations on chromosome profiles of many a kind of persons (Goswami,1986; Goswami et al, 1992). Heparinised whole blood (0.3ml) was taken and placed in a sterile tube containing 5ml culture medium (RPMI 1640 or McCoy's or M1990 supplemented with 10% fetal calf serum, 200units /ml penicillin, 100 mug/ml streptomycin and 40mug/ml phytophaemagglutin. Cultures were then incubated at 37 C for 68 h. 1 ml colchicine (0.2%) was added to each tube which was incubated further for a period of 2 h. In order to test the effect of

compound used in oral pills, sterilized 1ml of the above oral pill solution filtrate was simultaneously added with the colchicine and processed in the same way. The regular cultures with only colchicine thus acted as control and helped in comparison. The culture suspension was immediately centrifuged for 8 min at 1000-1200 rpm, and processed using standard procedures (prewarmed KCl, incubation and centrifugation). Extra cold fixative (ratio: 1part glacial acetic acid+ 3 parts methanol) was added and slides were prepared by the air drying or the flame drying method. Prepared differently coded slides were stained with Giemsa (10% pH 6.8) for 20 min, washed in distilled water, dried and sometimes mounted in euparol or DPX. Likewise G banding was followed by usual procedures (Goswami, *et al.*,1992).

(C) Zygosity Diagnosis

Polysymptomatic diagnosis is chiefly based on observations to be recorded on all possible physical, anthropometric as well as dermatoglyphic features for comparative analysis among parents and cotwins of twins and triplets (Goswami,1967).

There are more than 30 traits from hairs on the head to the nails on the toes which are convenient to study and are genetically controlled (Stern, 1960; Lewis, 1997). Their concordance and discordance has been scored on a sheet as shown in Table 1. Table 2 presents two categories: Investigational and personalized features. Personalized information has been provided only in confidence (under our working conditions) by female parent in particular. During investigations the major objective was to ascertain the zygosity of triplets (Figs 3 & 4).

Repeated attempts have often been made sometimes even with a gap of 5 to 7 years depending upon the mutual convenience of family members. The gap of years has helped in recording appearance of those traits which are sensitive to "age of onset". This is worthy of remark that only those features which are autosomal and are not sex biased have been carefully chosen for scoring. However, if all three cotwins are males or all cotwins are females then only sex biased or sex linked traits may be considered (Tables 1-2) and added for comparative assessment. The number of triplets studied has exceeded 20 ranging from 2 to 28 years of age but greater majority could never yield data on all parameters. Most families do not want to share much information and reveal, otherwise valuable information. Also the follow up investigations were planned on only those triplets where two cotwins resembled most of the maternal traits but the third one differed significantly. So, among these, only 07 triplets have yielded follow up data for the present note, with the help of their family members.

(D) Data Evaluation

Table1 gives tentatively 30 points for scoring concordane (++) or discordance for comparative evaluation. The traits have been chosen, added or modified after looking at parents; but at least mother has always been reported. Table 2 offers for 20 score points. Entry of scores was based on allotment of points and the traits in question were given 03 points for concordance and 01 point for discordance. Atleast 10 traits form Table 1 and 6 to 7 traits from Table 2 were always studied and overall percentage of concordance is based on 15 to 17 traits giving the maximum range of 45 to 51 score points . On this basis, scores were sorted out and Table 3 presents nutshell result of scores on those 07 triplets which had shown greater bias for maternal inheritance. However as seen in the table, only first four (1-4) triplets could be finally decided to have more than 98% bias for dominant maternal alleles (traits).

RESULTS

Effect of Hormone on Cell Dividion

Allium sativum root cells have clearly exhibited amitotic divisions in different trials (Fig.1)). Exactly same pictures were obtained in lymphocyte cultures (Fig.2) thus confirming earlier hypothesis that extra dose of oral hormone is capable of inducing amitotic division in an active cell. Since our epidemiological studies had earlier indicated a positive affiliation of irregular use of oral contraceptives with DZ twinning, this is plausible to infer that an ovum might divide amitotically (why not mitotically ? ; see discussion) into two identical halves.

Cotwins among Triplets

Out of nearly 20 triplets we have tried to study over these years only 7 triplets could be ensured to have two concordant and one discordant individuals (not on sex or sex dependent traits). These concordant individuals were exactly similar to many glaring and exclusive features of the female parent. The underlying hypothesis was now taken as, " in a triplet, if we find two similar cotwins resembling with each other on traits which are dominantly expressed in only mother and not seen on male parent, make a sensitive case for detailed reinvestigations. May be two such maternally concordant cotwins be from the same ovum after the cleavage ?"" Reinvestigations and follow up studies have taken many years, mainly due to non availability of all needed members at mutually convenient time. Nevertheless, the traits which are taken up as deciding factors are not influenced by external factors (Table 1-2).In the absence of detailed serological and genomic DNA studies, comparative assessment of concordance and discordance for physical, anthropometric and a few common blood genetic traits (OAB and Rh; globin chains and HPFH estimations by electrophoresis, etc) constitute a very reliable package of items for investigations of zygosity. These traits (Tables 1 &2) are autosomal and are controlled by more than one gene thus offering definite meaning to concordance and discordance approach. Entry of scores was based on allotment of points and the traits in question were given 03 points for concordance and 01 point for discordance. The total distribution of scores among 07 triplets is shown in Table 3 along with those special features which became prominent reasons for investigation. Only those traits which were concordant in two cotwins and the mother and discordant in the third cotwin have been presented in Table 3. All features designated as prominent features are alarming differences including very rare ones.

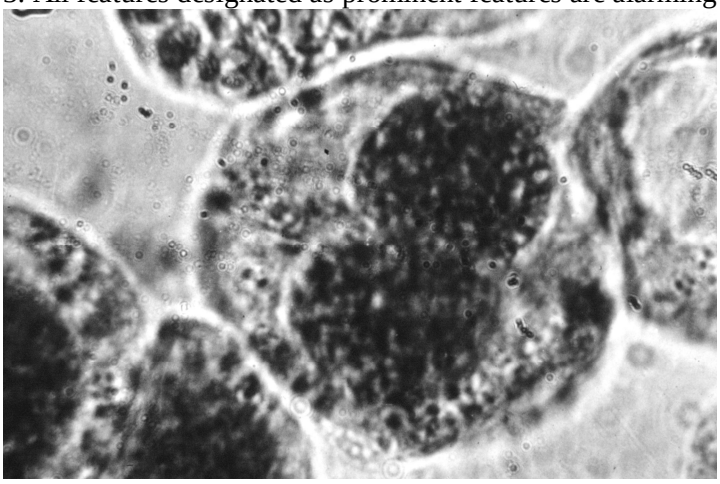

Figure 1: Classical amitotic division of the nucleus in the hormone induced root tip cell
(After Goswami, 1999-2000)

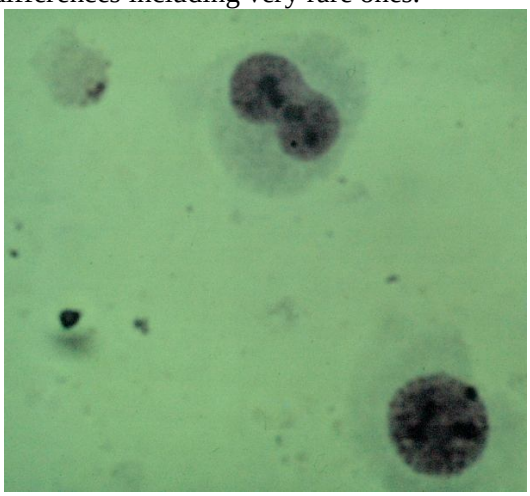

Figure 2: Lymphocyte in culture under the influence of oral contraceptive hormone showing Amitotic division

DISCUSSION

Triplets can arise either due to fertilization of three ova released or early removal (death) of one developing embryo from a quadruplet gestation, (which may be plurizygotic, monozygotic or Mz + DZ; Gedda, 1961). There is yet another possibility, that two ova might have been released, one of them underwent a cleavage and so formed three ova were fertilized to mature in a triplet pregnancy. In such an event, two monoovular twins must express almost all maternal genes while the third cotwin may not do so for all traits (Danforth, 1916; Gedda, 1961; Bulmer, 1970). In order to search for such a possibility twins and triplets have been investigated over three decades with a caution that any pair wherein two cotwins revealed excessive presence of dominant traits expressed by the mother and very few from the father were

noticed and followed up. Based on these comparative scoring of points (3 points for concordance and 1 for discordance) as many as 15 to 17 traits have been compared among father, mother, and each cotwin of a triplet. Table 3 computes overall percentage of scores and also shows some of some of those prominent features which appeared alarmingly similar in two cotwins and the mother but not in the third cotwin. However further detailed revised scrutiny revealed that out of these 07 pairs only triplets serial number 1 to 4 fall in that category while other three triplets revealed discordance for some features.

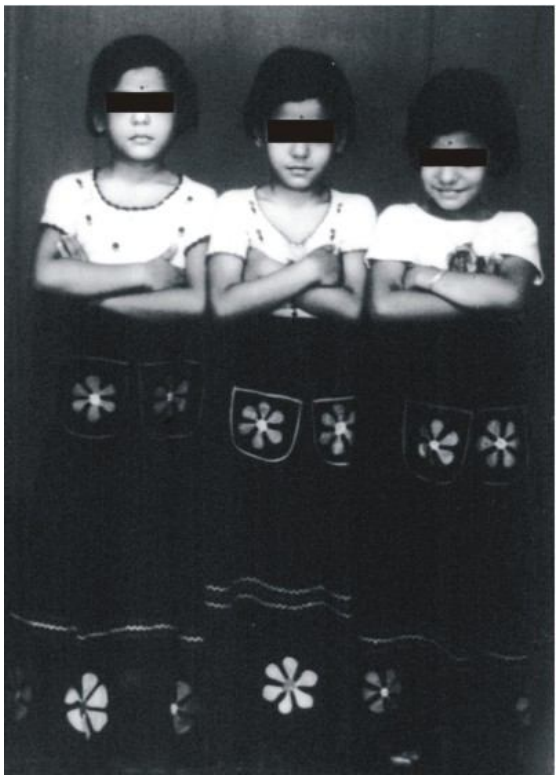

Figure 3: Triplet no 27/1983 ; one of the cotwin differs in blood group

Figure 4: Triplet 37/1988; one cotwin differs in hair colour etc

Indisputably, alarming differences in one cotwin and resemblance of the same traits in the mother and two cotwins of a triplet, or even in a twin pair, in between two cotwins; do indicate the inherited bias for the expression of maternal traits. Obviously then, such cotwins with visibly mother's exact genotype might have arisen after the cleavage of an ovum before fertilization under the influence of some extra hormonal impact.

Table 1: General features investigated for scoring concordance and discordance (30 points) among : (i) Female parent. (ii) Male parent. (iii) First Cotwin.(iv) Second CoTwin.(V) Third Cotwin.

(1). Head
(A)Shape/size
(B)Anthropometry (Frontal lobe)
(2). Hairs
(A) On head-colour
(B) Curly /plain or any other
(C) Unusual presence of hairs
(D) On any part of the body
(3). Skin
(A) colour
(B) unusual skin patches/ lesions
(C) on back./abdomen or thigh
(4). Eyes
(A).Colour
(B) Any feature
(5). Oral cavity
(A) Teeth pattern
(B) Tongue rolling
(C) Tongue twisting
(D) Tongue gliding
(6). Ears
(A). Free or joint ear lobes
(B) Shape and size of ear lobes
(C) Any feature on ear lobes (?)
(*e.g.* hairy pinnae)
(7). Nose
Shape of the nose
(8). Hands
(A). Polydactyly
(B). Syndactyly
(C). Brachydactyly
(9). Feet
(A). Polydactyly
(B). Syndactyly
(C) Short-mid metatarsal
(D) Any rare/abnormal feature ?
(eg. Incurved great toe)
(10). Any pathology
(of genetic origin) ?

Table 2: Special features of comparative search (20 points)

1. Investigational
(Biochemical)
(A) OAB Blood groups
(B) Any other blood group
(C) Percentage of Foetal haemoglobin
Analysis for HPFH (higher range of foetal haemoglobin in the mother can also be due to hereditary transmission)
(D) Tasting of mannose sugar solutions
(E) Tasting pf PTC (Phenylthiocarbamate)

2. (Dermatoglyphic)
(A) Thumb ridge count
(B)Total ridge count
(C)Special feature (if any) on the palm

(3). Personalized traits
(A) Presence of any mole or thick red skin patch
On any part of the body (These are often, due to chromosomal mosaicism)
(B) Mother may reveal a feature of importance (?)

Table 3: Serial number of triplets, among 07 studied, exhibiting Prominent features present in two cotwins and the mother but absent in the third cotwin

Feature/ Trait	**Pair No. of triplet showing concordance as above**						
	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**
(A) Total scores of all traits (15 to 17 traits)	(97%)	(98%)	(97%)	(99%)	(88%)	(90%)	(85%)
(B) Prominent- Features							
1. Blood group difference	--	++	--	++	--	--	--
2. Short third metatarsal	++	--	--	++	--	--	--
3. Hair type and colour	++	++	--	++	++	++	--
4. Skin lesion on abdomen	--	++	++	--	--	--	--
5. Eyes, shape and colour	++	++	++	++	--	--	--
6. Shape of the nose	--	--	++	++	--	--	++

Note: Since these are autosomal features it does not matter what combination of sex is a triplet.

That such an event might have been due to amitotic division and possibly not by mitosis is based on experimental observations of dividing cells under the influence of oral hormone (Goswami, 1978; Figs 1-

2). Such a treatment has never resulted in to increment in mitotic index, but even a few cells were seen to have disrupted chromatid movements during anaphasic separations. However, lot many cells were seen dividing in two equal halves by amitotic divisions. Even otherwise, amitotic divisions are not rare in various organs (liver etc) of the human body.

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REFERENCES
Bulmer MG (1970). The Biology of Twinning in Man. (Oxford, Clarendon Press**).**
Danforth CH (1916). Is twinning hereditary ?. **5** 195-202 (cited from Gedda,1961).
Fisher R (1919). Cited from Gedda, L. 1961.
Gedda L (1961). Twins in history and Science. (Charles C. Thomas Publication, Illinois, USA) 1-240.
Golubovsky M (2006). Mosaic/Chimeras and twinning in the current reproductive genetics perspective. *Human Reproduction* **21**(9) 2458-2460.
Goswami HK (1967). Studies on Twins.I: Typhoid favours cotwins. *Acta Geneticae Medicae et Gemellologiae (Roma)* **16 (4)** 402-409.
Goswami HK (1978). Effect of oral contraceptive on plant chromosomes. *Current Science* **47:** 515-516.
Goswami HK (1983). Genetics and Public Health: Some considerations in Indian context. In: *Genetics and Public Health.* (Catholic Press, Ranchi) 1-14.
Goswami HK (1986). Cytogenetic effects of methyl isocyanate exposure in Bhopal. *Human Genetics* **74** 81-84.
Goswami HK (1990). Sociobiological implications of genetic demography. In, Demography of Tribal development edited by Bose A, Sinha UP, Tyagi RP (B R Publishing Corporation New Delhi) 143-149.
Goswami HK, Rangnekar GV, Varshney S, Gandhi P, Jain B, Joshi A (1992). Crossed renal ectopia with pelvic lipomatosis: a new syndrome involving chromosome 1. *Human Genetics* **89** 666-670.
Goswami R, Goswami HK (1993). Changing trends in twinning. *Acta Geneticae Medica et Gemellologiae (Roma)* **42**(3) 289-294.
Lewis R (1997). Human Genetics: Concepts and Applications (WCB/McGraw-Hill. New York).
Stern C (1960). Principles of Human Genetics. San Francisco.