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POSSIBILITIES AND IMPORTANCE OF HUMAN MEIOTIC STUDIES

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Abstract

In order to understand the mechanism by which various chromosomal abnormalities are brought about, the study of meiotic process is necessary.

The study of spermiogenesis is a rather neglected field although meiotic errors may lead to infertility. Appropriate methods are available to study meiosis by which chromosomal preparations can be produced from the testicular tissue, from nature sperms and ova to reveal chromosomal anomalies.

The most common alterations in the meiotic process are translocations. These can be traced back to breaks and abnormal chromosomal pairing.

The mechanisms of autosomal and sex-chromosomal alterations with regard to their formation can be distinguished. In some cases the study of meiosis can give a clue regarding the cause of aneuploidies.

It seems to be of special importance whether the aneuploidy is produced in the first or the second cleavage of the meiotic process.

Key words: meiosis, spermiogenesis, genetics, non-disjunction, translocation

Introduction

Study into the human meiosis is an important, but hitherto relatively neglected field of genetics. The importance of such studies are given by the fact that failures in the meiotic process many lead to various abnormalities in the fetus.

The main details of the meiotic process are well known. Our own contribution to the meiotic process were to reveal the possibilities to model the production of chromosomal abnormalities that are known in the human being — in experimental animals (SZEMERE and ZSIBRITA, 1978; YANAGIMACHI, 1976).

There have been virtually no meiotic studies so far. The reason for that is that meiotic studies are more difficult to carry out than those from lymphocytes, amniotic cells or somatic samples. But still, in order to get to a better understanding of the mechanism behind the chromosomal aberrations, deeper insight into meiotic process is of utmost importance (BIGGERS et al., 1971; CHANDLY, 1981; MARTIN, 1980).

Material and method

The human semen sample is collected in a sterile container and processed as soon as liquefaction has occurred (10 to 20 min at 37 °C). Total sperm count, number of sperm/ml, motility, forward progression, the ratio of live to dead sperm, and the morphology of the sperm are determined on a small portion of the sample. Approximately 10 ml of BWW working pollution at 37 °C is added to the semen. The semen is centrifuged at 600 g for 6 min, the supernatant is decanted, and the pellet is resuspended in 10 ml BWW. The sperm are washed two more times. The final pellet is resuspended in 5 ml of BWW solution.

The resuspended sample is put into a thermostat of 37 °C for 18 hours. The sediment is put into 0,1% trypsin for two minutes. The sample is hypotonised in KCL and dropped onto warmed slides. After two days the specimens are stained and examined under the light microscope.

Results

Human meiosis

The meiotic process differs in the males and in the females. In females it starts during embryonic development and the eggs mature in a cyclic way from puberty, while in the male the production of germ cells is continuous.

Meiosis starts from diploid (2n) stem cells in each case, and — as a result — haploid (n) mature germ cells are produced. The haploid chromosomal number is the consequence of two subsequent divisions during meiosis. One of the most striking difference from mitosis is that the prophase meiosis I is carried out in four stages: leptotene, zygotene, pachytene and diplotene. After the first division haploid cells of two chromatids are produced when the chromosomes of maternal and paternal origin are randomly distributed in the daughter-cells.

The first meiotic division is followed by an extremely short interphase in which there is no DNA replication, thus leading to the production of haploid germ cells in meiosis II, because in this process the chromatids of the two chromosomes are separated, thus producing four germ cells.

The importance of meiosis is that it ensures the maximal conservatism, i.e. the maintenance of the chromosome number characteristic of the species on the one hand and through recombination to genetic variability, on the other.

Eventual meiotic errors may lead to serious anomalies in the offspring. In this respect the most striking phenomenon is non-disjunction that brings about trisomies and nullisomies. Non-disjunction may occur both in meiosis I and meiosis II (SZEMERE and CHANDLY, 1976).

Examination of the meiotic process

There has been a breakthrough in the human and mammal meiotic studies from 1964, when the air-drying method of the spermatocytes had been introduced. This was followed by combination with other specific techniques, like G-banding, fluorescent techniques, etc. A new era has appeared in the field of the investigation of meiotic chro-

mosomes when COUNCE and MEYER, (EVANS et al., 1964; MARTIN, 1980) introduced the quick and simple 'micro-spreading' technique in 1973. The method had been improved by (MARTIN, 1963). These methods made the investigation of specific proteins that are produced in different stages of prophase, like e.g. the study of the synaptonemal complex possible. These proteins join the homologous chromosomes together during the pairing in the zygotene prophase meiosis I, through pachytene towards the diplotene, when the separation of the bivalents begin. The full separation of the chromosomes ends in diakinesis.

Study of meiotic process may occur from preparations of testicular material, mature sperm, ovaries and mature eggs.

Preparation of human sperm

Maturation of sperm is continuous in men from puberty, thus meiotic chromosomes can be analysed after the preparation procedure. The methodology had been described by BIGGERS and MARTIN (1971, 1988) and modified by ourselves (MARTIN, 1963).

Detection of translocations and non-disjunctions in the meiotic process

What are the factors that indicate the meiotic studies ?

In cases of healthy, fertile men virtually no biopsies are taken, thus the main source of studying the meiotic process is the clinical study of infertile men. The indication of the cytogenetic study of meiosis is mainly oligospermy or azoospermy. Infertility is often caused by chromosomal abnormality that can be proved by cytogenetic analysis. Infertile men are screened world-wide and cytogenetic studies on the germ-cells should be part of this screening. The target is to fully reveal the disturbances of the genome thus ensuring the identification of the spermatogenetic block in the process of spermiogenesis (AURIAS and BALKAN, 1978, 1983).

Damage of the germ-cells, lack or failure of chromosome pairing as well as translocation phenomena are connected with one another (ROBEZ, 1986; HASSOLD and MATSUYAMA,1979).

Failures may occur both in spermiogenesis and oogenesis (CHANDLY, 1976; FOREJT, 1981; MOSES, 1975).

Abnormalities during the process of germ-cell production appear in a different way in male and female offspring. In women the heterozygote carrier status does not necessarily prevent fertility, thus damage in the process of oogenesis may remain without consequences.

There is also a remarkable difference in the maturation mechanism of spermiogenesis and oogenesis. While — according to the widely accepted hypothesis — the XX bivalents are active during prophase meiosis I, the XY bivalents are not.

There is a tendency in the oocytes to failure or lack of chromosome pairing, which is explained by the transcriptional activity of the X-chromosomes (SANGER, 1971, 1977). It is highly probable that oocytes with a mispairing of chromosomes cannot mature in the adult ovaries and this is also the factor leading to the atresia of the

follicles during embryonic development. Damage in the process of spermiogenesis can be explained, among others, by the breakage of the distal part of the long arm of the Y chromosome, since the gene controlling spermiogenesis can be found in this region. Earlier, this gene was thought to be the TDF (Testicular Determining Factor), but it turned out that there are two separate genes in question. According to our present knowledge, TDF can be found in the short arm of the Y chromosome in a "pseudosomatic" domain. The spermiogenesis controlling gene is responsible - beside the formation of germ cells - for the migration of them, thus the failure of this gene may also lead to migration disturbances.

Study of meiotic processes may give an explanation of the causes of the most striking abnormalities, like aneuploidies and translocations.

It has been shown by RUSSEL and MONTGOMERY in 1974 that different types of aneuploid fetuses are born if non-disjunction of the chromosomes occurs in the first or in the second meiotic division of the spermiogenesis or oogenesis. This conclusion has also been drawn by SANGER (1971, 1977), LAURITSEN and FRIDRICH (1976), NIIKAWA (1977), HASSOLD (1980) as well as RACE and SANGER (1969, 1977). According to their studies, meiotic non-disjunction may affect both the somatic and the sex-chromosomes.

Phenomena described above indicate that meiosis may be damaged both in the maternal and paternal organism. Both may lead to infertility, this is why the study of meiotic processes is so important.

In our future studies we would like to concentrate on the study of spermiogenesis, thus making a contribution to the prevention of the consequences caused by infertility.

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