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Prematurely condensed human sperm chromosomes after in vitro fertilization (IVF)

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Summary. During an in vitro fertilization (IVF) program 122 inseminated eggs showing polar body extrusion, but neither formation of pronuclei nor cell cleavage were analysed cytogenetically. Nine of these eggs showed prematurely condensed sperm chromosomes of the G_1-phase (G_1-PCC) besides the haploid set of maternal metaphase II chromosomes. This phenomenon can be explained by the permanent arrest of the oocytes at metaphase II after sperm penetration and hence the continuing presence of cytoplasmic chromosome condensing factors which lead to the induction of PCC in the sperm nucleus. The overall frequency of this aberrant type of fertilization was calculated to be in the order of 3–4% of all in vitro fertilized eggs.

Introduction

In all vertebrates including man the ovulated oocytes are arrested at metaphase II of meiosis until they become fertilized. The fusion process then triggers activation of the cytoplasm which causes the completion of meiosis and the formation of both male and female pronuclei.

In his studies on normal and aberrant fertilization of the sea urchin *Paracentrotus lividus,* Brachet demonstrated in 1922 that sperms entering oocytes which were blocked at the second meiotic division formed discrete chromosomes within a few minutes. To the best of our knowledge, we show here for the first time that the same phenomenon can also be observed in human oocytes after in vitro fertilization.

Materials and methods

All oocytes analysed were obtained within one year in the course of an IVF (in vitro fertilization) program. Stimulation of multiple follicular development was induced either by clomiphene citrate and additional hMG (human menopausal gonadotropin) (Leung et al. 1984) or by hMG alone (Jones et al. 1982). Follicles were aspirated by laparoscopy 34–36 h after hCG (human chorionic gonadotropin) administration.

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A total of 612 preovulatory oocytes were preincubated in Ham's F-10 medium supplemented with 10% umbilical-cord serum for different times, dependent on the maturity stage, at 37°C in a CO_2-incubator. Mature and intermediate oocytes were inseminated after 2–6 h preincubation with 200,000 to 250,000 motile sperms per oocyte. Before insemination the semen sample was washed twice to remove the seminal plasma and incubated at 37°C for at least 30 min.

The oocytes were examined for the presence of polar bodies and pronuclei 14–18 h after insemination. Two hundred and eighty four oocytes had two or more pronuclei and were defined as being fertilized. Two hundred and sixty two of these developed regularly to the 2- to 4-cell stage and were retransferred to the patient's uterus 40–48 h after insemination. Another 22 eggs were found to be triploid and therefore were not transferred. Three hundred and twenty eight oocytes were unfertilized or failed to cleave. A random sample of 122 of these eggs were analysed cytogenetically.

The eggs were prepared without Colcemid treatment according to the method of Tarkowski (1966). The oocytes were hypotonically treated with 1% sodium citrate solution at room temperature for 8–10 min, transferred to precleaned slides, and fixed by one or two drops of cold fixative (methanol/acetic acid, 3:1). After air-drying the slides were stained with Giemsa. Some slides were destained and restained with the fluorescent dye quinacrine mustard and examined by fluorescence microscopy.

Results

Of the 122 eggs analysed showing polar body extrusion but neither formation of pronuclei nor cell cleavage, 10 were aneuploid, diploid, triploid, or were still in prophase II of the meiotic division. The remaining 112 eggs had a haploid set of metaphase II chromosomes beside the chromosomes of the first polar body. However, 9 of these 112 eggs also had a paternal set of prematurely condensed chromosomes of the G_1-phase (G_1-PCC) (Fig. 1). The chromosomes were single chromatids and of about the same length as metaphase chromosomes. In one case a Y chromosome has been identified after Q-banding confirming the paternal origin of these elements (Fig. 2). As can be seen from Table 1 these nine eggs were in a mature or intermediate stage at the time of laparoscopy and 75% of them were inseminated with sperm of nor-

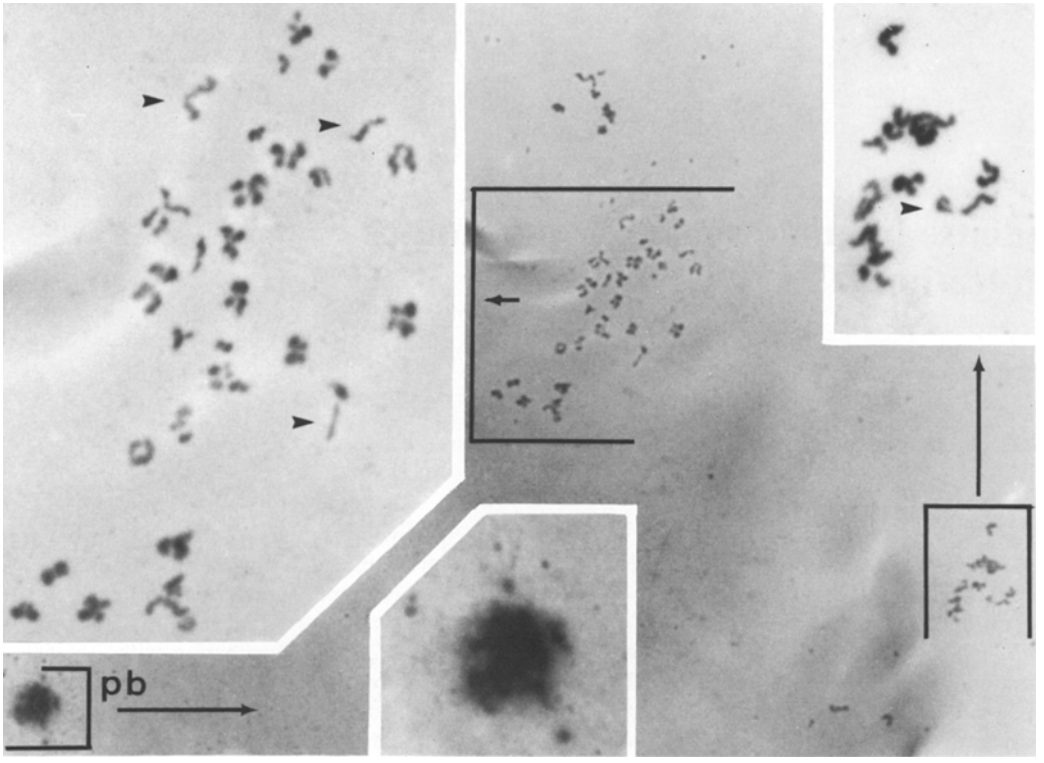

Fig. 1. Human oocyte after in vitro fertilization with prematurely condensed sperm chromosomes. Besides the polar body (*pb*) the haploid set of maternal metaphase II chromosomes is visible and the sperm chromosomes as single chromatids (G_1-PCC, *arrow heads*)

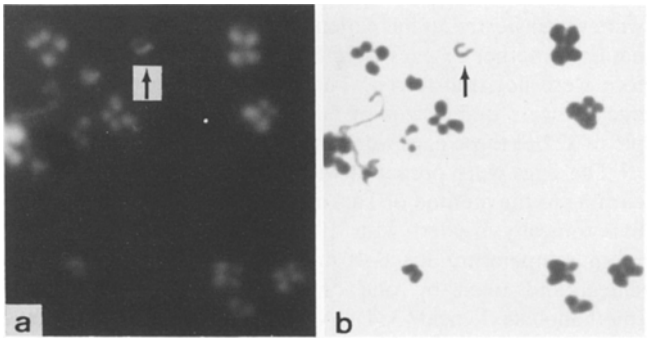

Fig. 2a, b. Human oocyte fertilized in vitro, with prematurely condensed sperm chromosomes and maternal metaphase II chromosomes and demonstrating the fluorescent Y-chromosome *(arrow)* after Q-banding (**a**). **b** Giemsa-stained complement

mal quality. They were thus not different from the retransferred eggs of the same donors or the sample in general.

Table 1. Some egg and sperm parameters of the cases showing premature chromosome condensation. n, Normozoospermy; t, teratozoospermy; o, oligozoospermy; m, mature; int, intermediate

Case no.	Sperm quality	No. of oocytes inseminated	No. of oocytes with pronuclei	No. of oocytes[a] with G_1-PCC	Maturation stage at laparoscopy (oocyte with G_1-PCC)
1	n	6	5	1	2m, 3int (int)
2	t	3	1	1	1m, 1int (int)
3	n	4	2	1	2m, 1int (m)
4	n	5	3	1	2m, 2int (int)
5	n	9	4	2	2m, 5int (m, int)
6	n	3	2	1	2int (m)
7	n	2	–	1	1m (m)
8	o	1	–	1	– (int)

[a] The inseminated oocytes were cultured for about 72 h and then prepared for chromosome analysis. The degree of maturity of preovulatory oocytes was determined using morphological parameters as described by Veeck et al. (1983)

Discussion

The cascade of events following the penetration of the sperm into the egg is regulated by the egg cytoplasm (see Beier and Lindner 1983 for review). This has been most clearly shown in the elegant experiments of Gurdon and Woodland (1968) after injection of somatic cell nuclei into both maturing and mature *Xenopus* oocytes. In the latter case the transplanted nuclei were rapidly induced to commence DNA synthesis just like sperm cells after normal fertilization. In the former case, however, the nuclei were immediately induced to condense their chromosomes which corresponds to the observation of Brachet (1922) on sperm entering oocytes arrested at metaphase II.

These observations indicate (1) that the initiation of chromosome condensation is under positive control and (2) that the inducing factors are obviously the same in meiotic and mitotic cells. This has now been directly proven since injection of extracts from either mitotic or mature meiotic cells results

in breakdown of the nuclear membrane and chromosome condensation in immature recipient oocytes and somatic interphase cells alike (Halleck et al. 1984). This phenomenon has been termed "premature chromosome condensation" (PCC) (Johnson and Rao 1970; Rao et al. 1982 for review) or "prophasing" (Matsui et al. 1972) and explains our findings (Fig. 1) that sperm cells which are in G_0-phase of the cell cycle condense into chromosomes of single chromatids (Johnson et al. 1970) which are usually as long as mitotic chromosomes (Schmiady and Sperling 1981).

An essential prerequisite for this process, however, is that after sperm penetration the oocyte does not become activated but still remains arrested at metaphase II (see Fig. 1) and hence the chromosome condensing factors are still present. Consequently, the penetrating sperm nucleus does not develop into the male pronucleus but condenses immediately into single chromosomes.

We have observed this phenomenon in 7.4% of all developmentally arrested oocytes analysed cytogenetically. Since these oocytes represent about 54% of all in vitro fertilized oocytes, one can assume that prematurely condensed sperm chromosomes can be found in about 3–4% of all in vitro fertilized eggs. If this aberrant type of fertilization also occurs in vivo at about the same frequency, it could make a significant contribution to the massive preimplantation loss of conceptuses (Leridon 1977; Edmonds et al. 1982).

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