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An idiopathic infertility with oocytes metaphase I maturation block

Case report

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Abstract

BACKGROUND: A case of idiopathic primary infertility was attributed to a block in oocyte meiosis affecting the transition between metaphase I and metaphase II. METHODS AND RESULTS: A couple suffering unexplained primary infertility was unsuccessfully treated by various means of assisted reproductive technology. After four unsuccessful pregnancy attempts using intrauterine inseminations (IUI), IVF was attempted (all oocytes remained unfertilized), followed by an ICSI cycle. None of the retrieved oocytes expelled the polar body, and therefore were not injected. The failure of these assisted reproduction cycles was, in both cases, due to the immaturity of the oocytes recovered. Cytogenetic analysis of the oocytes retrieved for ICSI provided evidence of meiotic arrest. Using cytogenetic staging criteria we were able to show that this arrest occurred between metaphase I and anaphase I. CONCLUSIONS: Meiotic blocks affecting oocytes have already been described for various mammals. We discuss here mechanisms that might be involved in this possibly inherited disorder in humans, and ways in which our knowledge of them could be increased.

Keywords: ICSI • meiosis block • metaphase I • oocytes

Introduction

In 10% of cases, infertility remains unexplained after exploration of both the female and the male partner by standard diagnostic means (Greenhouse *et al*., 1998). Access

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to female gametes, which is possible in the course of IVF or ICSI cycles, may make it possible to understand the cause of infertility in some cases of intrinsic oocyte abnormalities.

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Patients

We report here the case of a 36 year old male and a 34 year old female with a history of 14 years of primary infertility. Routine infertility investigations (serum FSH and LH concentrations, hysterography and semen analysis) detected no signs of perturbation. Basal body temperature was biphasic, karyotypes were 46,XY and 46,XX. Hypofertility was reported for three of the four sisters of the female proponent: one had conceived two children with assisted reproduction treatments, one had had three miscarriages and no liveborn children, and one had not become pregnant in a period of 15 years.

IVF cycle

IVF was considered after the failure of four cycles of intrauterine insemination. After pituitary down-regulation, the ovaries were stimulated with 150 IU recombinant FSH daily from days 1–7 and with 225 IU FSH from days 8–12. Only the right ovary responded to hormonal stimulation, but follicle progression on ultrasound and serum oestradiol concentrations were considered satisfactory. The patient received 10 000 IU of HCG on day 13 (serum oestradiol concentration was 1138 pg/ml). Thirtysix hours later three oocytes were retrieved by ultrasound-guided follicular puncture. Attempts to achieve IVF with 90 000 motile spermatozoa per oocyte were unsuccessful. On the following day none of these oocytes was fertilized and none had a polar body. The couple was informed of the results, and were offered a second cycle with an ICSI procedure to make it possible to check the oocyte maturity and to increase the fertilization rate.

ICSI cycle

After pituitary down-regulation, the ovaries were stimulated with 225 IU FSH daily from days 1–11. Both ovaries responded and 10 000 IU HCG was administered on day 12 (serum oestradiol concentration was 3090 pg/ml). Ten follicles were aspirated and 10 oocytes of normal mean diameter were retrieved and treated with hyaluronidase (80 IU/ml). None of the oocytes had extruded the first polar body. They were incubated for a further 4 h in P1 culture medium (Irvine Scientific, Paisley, Renfrewshire, UK) and examined again, but their appearance remained unchanged. It was decided jointly by the couple, the embryologist and the gynaecologist not to inject the immature oocytes. Injection was deferred to the following day, with a new sperm sample, provided that the oocytes reached the MII stage *in vitro*. Twenty-four hours later all the oocytes were still at the MI stage, therefore ICSI was not performed. Two oocytes were incubated at 37°C in 5% CO_2 in P1 culture medium for a further 24 h but neither extruded the polar body. The other eight oocytes were fixed by the Tarkowski technique (Tarkowski, 1966) to evaluate their chromosomal constitution and for cytogenetic staging to identify the stage at which meiosis was arrested. We were able to analyse seven of the eight fixed oocytes after staining with 3% Giemsa solution. All had 46 chromosomes which appeared to be in metaphase with either: (i) replicated chromosomes in homologous pairs close together or linked by chiasmata

(chromosomes become competent to adopt this configuration at mid-pachytene stage); or (ii) replicated chromosomes already dissociated (suggestive of anaphase I initiation or of the start of chromosome pair degeneration) (Figure 1).

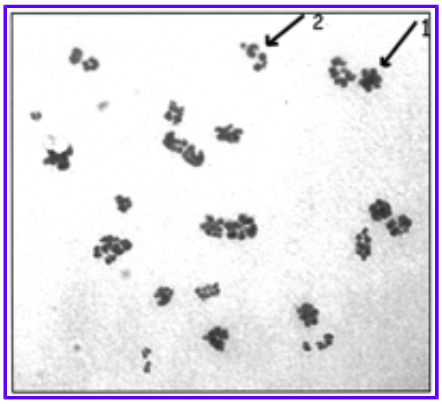

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Figure 1. Arrow 1: interlocked metaphasic I homologous chromosomes. **Arrow 2:** meiosis I late metaphase/anaphase stage, with dissociated homologous chromosomes.

Discussion

Meiosis I blocks have already been observed in the human female germ line. Few reports have dealt with this abnormality: Harrison recently reported two cases similar to our case with MI block and a lack of in-vitro maturation (Harrison *et al*., 2000). Oocytes that remained immature after culture were not subjected to further investigations. Hartshorne described a case in which all the collected oocytes initially contained a germinal vesicle and displayed germinal vesicle breakdown (GVBD), but no further maturation was observed after incubation overnight *in vitro* (Hartshorne *et al*., 1999) . These oocytes were further analysed by Hoechst 33258 staining. Fluorescence microscopy suggested that there was a block between prophase I and metaphase I, probably occurring earlier than that described here. It was found that all the oocytes observed after fixation as described by Tarkowski (Tarkowski, 1966) displayed a chromosome arrangement consistent with arrest at the end of the meiosis metaphase 1 or at the transition between metaphase I and anaphase I. Similar observations were made for immature oocytes fixed in a cohort containing both mature and immature oocytes after IVF failure (Pellestor and Sèle, 1988; Selva *et al.*, 1991). The similarity between the syndrome observed here which affected the oocytes of an entire cohort and sporadic immature oocytes in a cohort of normally maturing oocytes may indicate a critical meiotic checkpoint at this stage.

Reports of meiosis 1 arrest are more frequent for the male than the female germ line, in humans (Handel, 1997 ; Lange *et al*., 1997) and in various animal species (Baker and Plug, 1996; Zhu *et al*., 1997). Genetic

control was suspected before molecular analysis became available, on the basis of familial history and pathological examinations on the testis (Luciani, 1981). The molecular mechanisms of chromosomal desynapsis impairment in the male germ line are now partially understood (Okabe *et al*., 1998; Escalier, 1999).

In meiotic arrest of spermatocytes, both paired and unpaired homologous metaphase chromosomes are observed, due to asynapsis or the formation of fragmented synaptonemal complexes as described by Lange (Lange *et al*., 1997), or desynapsis impairment, leading to the arrest of these cells in meiosis I. Similar observations are often made, but not always investigated in testis biopsies in secretory azoospermic men, in the search for mature spermatozoa that could be used for ICSI. These cases are usually diagnosed as `meiotic arrest'.

The scarcity of observations of a similar disorder in women may be due to a recruitment and observation bias, as the number of available meiotic cells from oocytic puncture is generally lower than that available from testis biopsies. Alternatively, the frequency of this type of syndrome may be lower in the female germ line. Several molecular events regulating meiosis are known to differ in males and females; it is not possible to determine the mechanisms involved or incidence, by extrapolation (Baker *et al.*, 1996; Yuan *et al.*, 2000).

As human oocytes, whether normal or abnormal, are difficult to obtain and are not used primarily for research, the use of targeted mutagenesis in animals (Simon *et al*., 1997) seems essential to progress in this field. Indeed, the mechanisms controlling the process of meiosis are intricate and diverse, operating both in the oocyte nucleus (microtubule organizing centre, spindle, centromeric structure) and in the cytoplasm (qualitative and quantitative modifications affecting various organelles: Golgi apparatus, mitochondria, ribosomes). These mechanisms also involve the surrounding environment (culture conditions) and the cells in contact with the oocytes (paracrine intercellular interaction factors) (Fulka *et al*., 1998).

As stated previously, a block in oocyte meiosis has previously been reported in animal models, including various strains of mice. The fact that only specific recombinant inbred strains are affected is consistent with the polygenic control of meiosis (Eppig and Wigglesworth, 1994; Eppig *et al*., 1996).

From a practical point of view, the couple was informed of the results and of the likelihood that the same outcome would occur if another attempt was made. Oocyte donation and adoption were discussed.

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Notes

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