Chromosome aberrations in human spermatozoa treated with Ca$^{2+}$ ionophore A23187

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Incorporation of A23187 ionophore into the human–hamster fertilization system clearly improves the ability of human spermatozoa to penetrate zona-free hamster oocytes. Thus, an increasing number of laboratories working in human sperm cytogenetics have substituted classical incubation with Biggers–Whitten–Whittingham (BWW) medium plus human serum albumin (HSA) by pretreatment of spermatozoa with calcium ionophore A23187 which directly induces the acrosome reaction in spermatozoa. However, there have been no formal studies on the effects of this ionophore pretreatment. To determine whether calcium ionophore could affect the cytogenetic characteristics of human spermatozoa we compared A23187-treated spermatozoa with controls (only incubated with BWW + HSA) by analysing a total of 447 sperm chromosome complements from two normal donors. Our results show that there are no statistical differences in the frequency and the types of human sperm chromosomal abnormalities between the two methods of sperm treatment. Thus, ionophore A23187 seems not to affect the cytogenetic characteristics of human spermatozoa, and the results of laboratories using either sperm capacitation in BWW + HSA or acrosome reaction by calcium ionophore can be compared.

Key words: A23187/capacitation/chromosomal abnormalities/IVF/sperm chromosomes

Introduction

Rudak et al. (1978) reported a technique to obtain human sperm chromosomes by fertilizing zona-free hamster ova in vitro with human spermatozoa. In the original technique spermatozoa were preincubated in Biggers–Whitten–Whittingham (BWW) medium (Biggers et al., 1971) supplemented with human serum albumin (HSA) to allow their capacitation. As the success rate of chromosome analysis with this technique is low, many authors have modified it in order to increase its efficiency. Increased efficiency becomes even more important when using poor quality semen samples or small aliquots of frozen semen.

In 1986, Kamiguchi and Mikamo reported an improved, more efficient method for analysing human sperm chromosomes, in which calcium ionophore A23187 was used to increase the penetration rate. Calcium ionophore has been shown to induce the acrosome reaction in human spermatozoa, thus obviating capacitation. A23187 forms a lipophilic complex with Ca$^{2+}$ which results in a rapid influx of Ca$^{2+}$ across the sperm plasma membrane (Smith et al., 1983). The result of this Ca$^{2+}$ entry is the induction of the acrosome reaction and the concurrent (or resulting) generation of a fusogenic equatorial segment.

At present, an increasing number of laboratories working in human sperm cytogenetics have substituted classical incubation with HSA by pretreatment with calcium ionophore to allow the acrosome reaction to take place (Kamiguchi and Mikamo, 1986; Wrambsy and Yanagimachi, 1986; Rosenbusch et al., 1989, 1991). However, there have been no formal studies of the effects of this ionophore treatment on the chromosomal characteristics of human spermatozoa.

It has been suggested that a different pretreatment method (incubation in a TEST yolk buffer to allow sperm capacitation; Brandriff et al., 1985) may increase the frequency of structural chromosome abnormalities in spermatozoa (Martin et al., 1990, 1992). The purpose of this study, therefore, was to determine whether calcium ionophore A23187 could similarly affect the cytogenetic characteristics of human spermatozoa, by comparing the frequencies and types of chromosomal abnormalities after sperm capacitation with BWW + HSA or acrosome reaction by calcium ionophorepretreatment.

Materials and methods

Sperm treatment

Semen samples from two control donors were used. Two series of experiments were performed for each donor: control and ionophore-treated series.

In both series of experiments, semen samples were collected in a sterile, non-toxic container and washed with BWW with 0.3% HSA to eliminate seminal plasma. Motile spermatozoa were collected by a swim-up procedure (Andolz et al., 1987). In the control series of experiments, motile spermatozoa were washed once or twice in BWW 0.3% HSA and finally resuspended in BWW medium with 3.3% HSA to achieve the required concentration (1–2 × 10$^6$ motile spermatozoa/ml). The final suspension of control spermatozoa was incubated for 4–5 h at 37°C, 5% CO$_2$ to allow capacitation of sperm
cells. In the ionophore series of experiments, motile spermatozoa were centrifuged (600 g, 6 min), and the pellet was resuspended in 10 mM calcium ionophore A23187 solution in BWW 0.3% HSA. Spermatozoa were incubated in this ionophore solution for 10 min at 37°C, 5% CO₂. The sperm suspension thus treated was centrifuged again to eliminate ionophore, and finally resuspended in BWW medium with 3.3% HSA, adjusting the concentration to 1–2 × 10⁵ motile spermatozoa/ml. The final suspension of ionophore-treated spermatozoa was incubated for 2 h before insemination.

**Hamster superovulation and oocyte processing**

Adult female Syrian hamsters were induced to superovulate by injection of gonadotrophins. Oviducts were punctured in Petri dishes containing BWW medium with 0.3% HSA to extract the cumulus masses, which were transferred to a 0.1% hyaluronidase solution to free the oocytes from cumulus cells. Finally, the oocytes were treated with a 0.1% trypsin solution to remove the zona pellucidae.

**Gamete coincubation and egg culture**

Zona-free hamster oocytes and human spermatozoa (capacitated in BWW medium with 3.3% HSA alone or previously treated with calcium ionophore) were incubated in BWW medium 3.3% HSA droplets under paraffin oil at 37°C in 5% CO₂ for 1–2 h. During coincubation of gametes, checks of penetration were carried out from time to time by examining 5–10 oocytes under a phase contrast microscope; a coverslip was placed over a slide on four wax spots and observed. As soon as the frequency of swollen sperm heads with tail per oocyte was 0.5 or higher, oocytes were washed and placed in F10 medium droplets. After 12 h of culture at 37°C in 5% CO₂, they were transferred to F10 medium droplets containing 0.4 mg/ml Colcemid (Gibco, New York) for a further 4–6 h period.

**Chromosomal preparations and cytogenetic analysis**

The eggs were fixed by the method described by Tarkowski (1966). Human sperm metaphases were analysed after sequential uniform stain-G banding. The classification of chromosome abnormalities was performed according to the International System for Cytogenetic Nomenclature (ISCN, 1985). Gaps were not scored as abnormalities.

**Results**

**Penetration rates**

Spermatozoa treated with calcium ionophore showed a greater ability to fertilize hamster oocytes than those capacitated with BWW 3.3% HSA alone. As shown in Table I, the concentration of motile spermatozoa and coincubation time needed to achieve the correct penetration rate (55–75% of hamster eggs showing a swollen sperm head) were lower when using calcium ionophore-treated spermatozoa than when using spermatozoa without calcium ionophore pretreatment.

Ionophore treatment of human spermatozoa results in a loss of motility, which is quite variable in different experiments. However, the spermatozoa recover their normal motility within 1 h of ionophore treatment.

**Cytogenetic results**

To determine whether ionophore treatment modifies the cytogenetic characteristics of human spermatozoa, we compared the results from the calcium ionophore-treated sperm series with the results from the control series. A total of 447 sperm complements were analysed after sequential uniform stain-G banding technique. In all, 183 sperm complements were from the control series and 264 sperm complements were from the calcium ionophore-treated
sperm series. Table II summarizes the results obtained from each type of sperm treatment.

When the incidences of the different types of chromosome abnormalities were compared, there were no significant differences between BWW + HSA-capacitated spermatozoa and those treated with calcium ionophore, either for structural aberrations (6.6 versus 6.1\%) \((P = 0.8448,\) two-tailed Fisher's exact test) or for aneuploidy (5.5 versus 3.8\%) \((P = 0.4866,\) two-tailed Fisher's exact test). The level of aneuploidy was conservatively calculated as twice the hyperhaploidy frequency. Taking into account this correction, the total frequencies of abnormalities (12.0 versus 9.5\%) were not significantly different \((P = 0.4340,\) two-tailed Fisher's exact test).

For both series, the most common structural abnormalities were chromosome breaks andacentric fragments (Table III). An example of a structurally abnormal sperm complement is shown in Figure 1.

**Discussion**

Ionophore A23187 has been shown to induce the acrosome reaction in human spermatozoa (Aitken et al., 1984) as well as in other mammalian spermatozoa (Green, 1978; Sharms-Bothan and Harrison, 1981; Smith et al., 1983). In our study we have shown that human spermatozoa treated with a micromolar concentration of calcium ionophore have an increased ability to fertilize hamster oocytes. Similarly, Aitken et al. (1984) and Boldt et al. (1991) demonstrated that A23187-treated mammalian spermatozoa penetrated zona-free eggs to a greater extent than control spermatozoa. More recently, Tanphaichitr and Hansen (1994) have shown that treatment of mouse spermatozoa with as low as nanomolar concentrations of calcium ionophore causes the same effect. This higher capacity of A23187-treated spermatozoa to fertilize zona-free eggs probably reflects the higher percentages of acrosome-free spermatozoa found in A23187-treated samples in comparison with control samples incubated in culture medium only (Palermo and van Steirteghem, 1991; Tanphaichitr and Hansen, 1994). In our study, since the experiments were designed to obtain sperm chromosomes, polyspermy was avoided. Thus, in the ionophore-treated series, monospermic or dispermic fertilization was ensured by inactivating the hamster oocytes with spermatozoa at a low concentration for a short time.

In our study we did not find any differences between numerical or structural chromosome abnormalities in spermatozoa treated with calcium ionophore and control spermatozoa capacitated in BWW medium containing albumin. Since numerical chromosome abnormalities present in first cleavage metaphases are generated during meioysis, differences in the frequencies of aneuploidy were not expected. With regard to structural abnormalities, those most frequently found (csb and ace) are compatible with postmeiotic damage to sperm DNA. However, their frequencies in both series are not statistically different \((P = 0.9768\) and \(P = 0.2594\) respectively, two-tailed \(t\)-test) (Table III). In fact, chromosome breaks andacentric fragments are the most frequent structural abnormalities observed in large cytogenetic studies of spermatozoa using different methods of preparation (BWW + HSA, Martin, 1984; Martin et al., 1987; ionophore A23187, Kamiguchi and Mikamo, 1986; TEST buffer, Brandriff et al., 1985, 1988; Benet et al., 1991; Martin et al., 1992).

Overall, our results indicate that the frequency of chromosome abnormalities in human spermatozoa does not appear to be affected by the method of preparation of spermatozoa, and that the results from laboratories using BWW + HSA only, or calcium ionophore treatment prior to BWW + HSA incubation, can be compared.

Incorporation of A23187 ionophore into the human–hamster fertilization system offers definitive advantages. It allows the analysis of sperm chromosomes in low quality semen samples frequently found in patients treated for cancer and infertile men, because pretreatment of spermatozoa with calcium ionophore clearly improves the ability of human spermatozoa to penetrate zona-free hamster oocytes. It shortens the protocol for obtaining human sperm chromosomes by at least \(2\ h\), since treatment of spermatozoa with calcium ionophore accelerates acrosome reaction. Finally, as already pointed out by Aitken et al. (1984) and by Kamiguchi and Mikamo (1986), another important advantage of incorporating A23187 is that, by circumventing the process of capacitation, this ionophore treatment overcomes the problems due to individual variations in the time of incubation needed for sperm capacitation.

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