Human Reproduction vol 11 no.9 pp 1942–1946, 1996

Analysis of chromosome constitution of human spermatozoa with normal and aberrant head morphologies after injection into mouse oocytes

J.D.Lee[1], Y.Kamiguchi[2] and R.Yanagimachi[1,3]

[1]Department of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, HI 96822, USA and [2]Department of Biological Sciences, Asahikawa Medical College, Asahikawa 078, Japan

[3]To whom correspondence should be addressed

The chromosome constitution of human spermatozoa was determined after injecting individual spermatozoa into mouse oocytes. Of a total 279 eggs arrested at first cleavage metaphase, 200 (71.7%) were suitable for the analysis of sperm chromosomes. Incidences of spermatozoa with numerical and structural chromosome aberrations were 1.3 and 6.9% respectively in spermatozoa with normal head morphology, showing values comparable with those found in previous studies using the hamster oocyte–human sperm fusion system. The ratio of X- to Y-bearing spermatozoa did not differ significantly from the expected 1:1 ratio. The incidence of structural chromosome aberrations was about four times higher in spermatozoa with amorphous, round and elongated heads (26.1%) than in those with morphologically normal heads, whereas the incidence of aneuploidy was not significantly different between the two groups. No increase in chromosome aberrations was found in spermatozoa with large heads. The same was true for spermatozoa with small heads. Although the sample size used in this study is rather small, the results nevertheless indicate that some morphological abnormalities in the sperm heads are associated with their chromosome defects.

Key words: chromosome analysis/human spermatozoa/intracytoplasmic sperm injection/mouse oocytes

Introduction

Humans and great apes are unusual among mammals in that their semen contains a high proportion of structurally abnormal spermatozoa (Seuanez *et al.*, 1977). It is generally assumed, but without supporting evidence, that such spermatozoa are also genetically defective. The chromosome constitution of human spermatozoa has been studied following fusion with zona-free hamster oocytes (Rudak *et al.*, 1978; Kamiguchi and Mikamo, 1986; Martin *et al.*, 1991), but that system does not permit the analysis of any specific type of abnormal spermatozoa. Such chromosome analyses can only be carried out when an individual spermatozoon is manipulated into an oocyte.

Martin *et al.* (1988) injected human spermatozoa into hamster oocytes to estimate the effects of in-vitro sperm storage and of sonication on chromosomes, but according to our preliminary experiments hamster oocytes rarely cleaved after such surgery. Thus, some of the chromosome abnormalities observed could have resulted from the adverse condition of such a system. In contrast, most mouse oocytes similarly injected with human spermatozoa cleaved, indicating that they are perhaps more 'hospitable' to human sperm chromosomes. For this reason we chose mouse oocytes to compare the chromosomes of structurally normal and abnormal human spermatozoa.

Materials and methods

Reagents

The suppliers of the following reagents were polyvinylpyrrolidone (PVP; 360 kDa) from ICN Biochemical (Costa Mesa, CA, USA); bovine testicular hyaluronidase (300 USP units/mg) from ICN Biochemical; pronase (Type XI, 110 PUK units/mg) from Calbiochem (San Diego, CA, USA); bovine serum albumin (BSA; fraction V) from Calbiochem (La Jolla, CA, USA); fetal calf serum from Gibco BRL (Grand Island, NY, USA); sodium citrate from Merck & Co Inc. (Rahway, NJ, USA), Giemsa stain from Hopkin & Williams (Santa Monica, CA, USA); and mineral oil from Squibb and Sons (Princeton, NJ, USA). All other inorganic and organic reagents, including polyvinyl alcohol (PVA; cold water-soluble, 10 kDa), were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Media

The medium used for culturing oocytes after microsurgery was CZB (Chatot *et al.*, 1989, 1990). The medium used for oocyte collection, subsequent treatments and micromanipulation was HEPES–CZB (CZB with 20 mM HEPES, 5 mM $NaHCO_3$ and 0.1 mg/ml PVA). CZB and HEPES–CZB were sterilized by filtration through 0.45 μm pore filters (Millipore Corp., Bedford, MA, USA) and used under 5% CO_2 in air and in air only respectively. Isotonic saline (0.9% NaCl) containing 12% (w/v) polyvinylpyrrolidone (PVP), called 12% PVP–saline, was used for washing the injection pipette and for the dilution (1:1) of sperm suspension.

Preparation of mouse oocytes

$B6D2F_1$ female mice, 7–12 weeks old, were induced to superovulate by an i.p. injection of 7.5 IU equine chorionic gonadotrophin, each followed by the injection of 7.5 IU human chorionic gonadotrophin (HCG) 48–54 h later. Oocytes were collected from oviducts 15–17 h after HCG injection. They were freed from cumulus cells by treatment with 0.1% bovine testicular hyaluronidase in HEPES–CZB for 3–5 min. The oocytes were rinsed thoroughly and kept in CZB for up to 5 h at 37°C under 5% CO_2 in air. Oocytes to be injected with spermatozoa were transferred to a drop of HEPES–CZB in a microinjection chamber (17–18°C) and held by a holding pipette, one by one, prior to sperm injection (see below).

© European Society for Human Reproduction and Embryology

Downloaded from https://academic.oup.com/humrep/article/11/9/1942/616037 by guest on 20 April 2024

Preparation of human spermatozoa

Fresh human semen samples were obtained from one man of proven fertility and six men from infertile couples attending a local infertility clinic Semen samples were allowed to liquefy at room temperature and analysed for semen parameters using World Health Organization (WHO) guidelines (1993).

Spermatozoa were separated from seminal plasma by either simple washing with CZB or a two-layer discontinuous Percoll gradient For simple sperm washing, semen was mixed with 6 ml CZB and centrifuged at 230 *g* for 10 min in a 15 ml sterile centrifuge tube The sperm pellet was resuspended in CZB, centrifuged again and the procedure repeated once more. In the Percoll gradient method, semen was placed gently on two 3 ml layers of 40 and 80% Percoll in CZB in a 15 ml sterile centrifuge tube. After centrifugation at 650 *g* for 30 min, spermatozoa were collected from the 40–80% Percoll interface and from the bottom of the tube. The sperm population at the 40–80% Percoll interface and that at the bottom of the tube both contained structurally abnormal spermatozoa, but the incidence of abnormal spermatozoa was higher in the former than in the latter. Two sperm populations were washed separately in 3 ml CZB by centrifugation (230 *g* for 10 min) to remove the Percoll. Washed spermatozoa were suspended in 1–2 ml CZB. A drop (20–50 µl) of the sperm suspension was added to 0 5 ml CZB which had been placed previously under mineral oil in the centre of a sterile plastic Petri dish (35×10 mm, Falcon Plastics, Oxnard, CA, USA; cat. no. 3001). The sperm suspension was kept at 37°C under 5% CO_2 in air for up to 4 days before injection. The concentration of spermatozoa in the medium was ~10^6–10^7 spermatozoa/ml. Before injection, one part of the sperm suspension was mixed thoroughly with one part of 12% PVP–saline. A drop (~6 µl) of this sperm suspension was kept under mineral oil in the microinjection chamber (see below) on the microscope stage and left there for up to 5–6 h at 17–18°C before injection into the oocytes. Spermatozoa displayed a slow flagellar movement in 6% PVP–saline, which made the classification of sperm morphology easier than when they were actively motile in CZB. Only normality and abnormality of the sperm heads were considered in this study

Sperm heads were classified according to Menkveld *et al.* (1990) (i) normal head, ~5 µm in length, ~3 µm in width, with a smooth outline, (ii) large head, >5 µm in length and >3 µm in width, (iii) small head, <3 µm in length, (iv) aberrant head, including amorphous, round and elongated heads (Figure 1) Other head abnormalities, such as double-headed, were not considered in this study

Sperm injection

The cover (10 mm in depth) of a plastic Petri dish (100×15 mm; Falcon Plastics; cat. no 1001) was used as a microinjection chamber. A row consisting of one round droplet (2 µl, 2 mm in diameter) and two elongated droplets (6 µl, 2 mm wide and 6 mm long) was placed along the centre line of the dish. The round droplet was the pipette washing medium (12% PVP–saline). The first elongated droplet was the sperm suspension in 6% PVP–saline The second elongated droplet was HEPES–CZB for oocyte storage and injection. All these droplets were covered with mineral oil and kept on the cooled (17–18°C) stage of an inverted microscope with Hoffman differential interference optics

A single motile human spermatozoon was injected into a mouse oocyte as described previously for the injection of mouse epididymal spermatozoa (Kimura and Yanagimachi, 1995) A spermatozoon was sucked, tail first, into an injection pipette attached to a piezo-electric micropipette-driving unit (model PMM-01 micromanipulator; Prima Meat Packers, Tsuchiura, Japan) When the junction between sperm midpiece and principal piece was at the opening of the pipette, two or three piezo-pulses were applied to immobilize the spermatozoon.

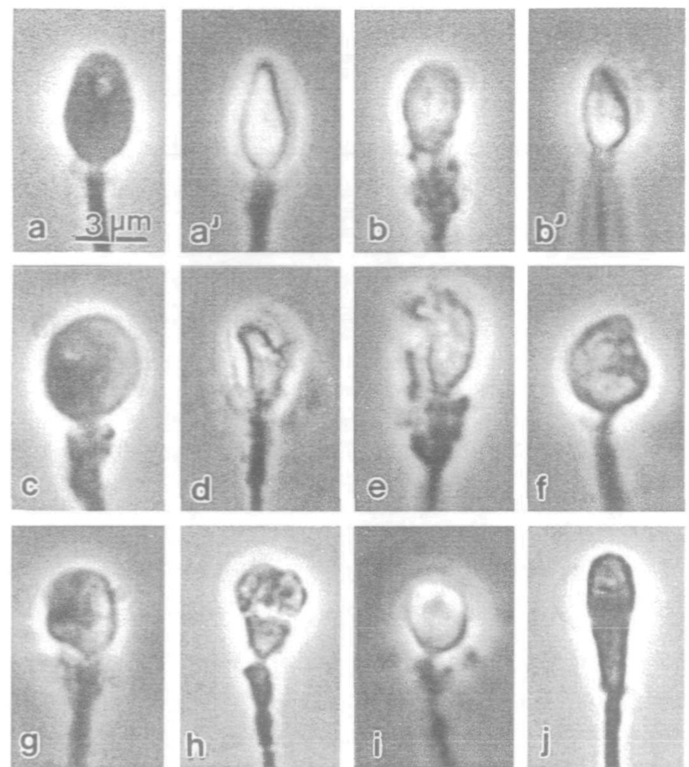

Figure 1. Phase-contrast micrographs of human sperm heads. **(a–a')** Surface and side views of spermatozoa with a normal head morphology; **(b–b')** surface and side views of small-headed spermatozoa; **(c)** surface view of a large-headed spermatozoon, **(d–h)** spermatozoa with amorphous heads, **(i)** a spermatozoon with a round sperm head; **(j)** a spermatozoon with an elongated head Magnification ×3400.

Then two or three additional piezo-pulses were applied to the sperm head region. These additional piezo-pulses were not necessary for spermatozoa from some men, but they facilitated the nuclear decondensation and transformation of spermatozoa from other men. The spermatozoon was sucked deeply into the injection pipette and the pipette tip was brought into contact with the zona pellucida of a mature oocyte on a holding pipette. The zona was drilled by piezo-pulses, the spermatozoon was pushed forward until its head was near the tip of the injection pipette and the pipette was advanced mechanically until its tip almost reached the opposite side of the oocyte's cortex. The oolemma was broken by applying one or two piezo-pulses, and the entire spermatozoon was expelled into the ooplasm with a minimum amount of accompanying medium. After as much as possible of the medium was retrieved, the pipette was gently withdrawn. All procedures were performed at 17–18°C. It usually took 20–30 min to inject morphologically normal spermatozoa into a group of five to 10 oocytes. The injection of abnormal spermatozoa usually took longer because we had to search for the spermatozoa that met our criteria for abnormalities

Incubation and block of syngamy

Sperm-injected oocytes were kept in the operation medium (HEPES–CZB) for ~20 min on the cooled (17–18°C) stage of the microscope before transfer into a dish of HEPES–CZB at room temperature (24–25°C). The oocytes were transferred 10 min later into 50 µl CZB under mineral oil in a plastic dish and incubated at 37°C under 5% CO_2 in air (Kimura and Yanagimachi, 1995). About 6–8 h after injection, the oocytes were examined with an inverted microscope and those with two pronuclei were transferred into 50 µl CZB

Downloaded from https://academic.oup.com/humrep/article/11/9/1942/616037 by guest on 20 April 2024

containing microtubule assembly inhibitors, 0.02 μg/ml podophyllotoxin and 0.02 μg/ml vinblastine About 16–20 h after injection, male and female pronuclei at the centre of the eggs disappeared in most of the eggs. Eggs were cultured for an additional 1–3 h to allow adequate condensation of the chromosomes before they were transferred to a slide.

Chromosome preparation and analysis

Spreading of the sperm chromosomes was performed according to Kamiguchi and Mikamo (1986) and Sugawara and Mikamo (1986), with slight modifications. The eggs were treated for 5 min with CZB containing 0.25% pronase and 0.3% PVA to soften the zonae pellucidae. They were then treated with a hypotonic solution (0.9% sodium citrate containing 3.0% fetal calf serum) for 15–30 min at room temperature, before being placed on slides, fixed with methanol–acetic acid and air dried. The chromosomes were stained with 4% Giemsa solution for 10 min. Some slides with chromosome spreads were C-banded.

Results

Success rate of sperm chromosome preparation

Regardless of the type of human spermatozoa injected, ~80% of the oocytes formed two pronuclei by 5–6 h. Most untreated pronuclear eggs reached the 2-cell stage by 20 h after sperm injection. Of a total 279 eggs arrested at the metaphase of the first cleavage by podophyllotoxin/vinblastine treatment, 200 (71.7%) were suitable for the analysis of sperm chromosomes; others were unsuitable because of poor chromosome spreading or overlapping of the mouse egg and human sperm chromosomes.

Morphological features of human sperm chromosomes

In hamster eggs, heterochromatic regions of human sperm chromosomes 1, 9 and 16 and the Y chromosome were commonly less condensed than other regions of chromosomes (Rudak *et al.*, 1978; Martin, 1983; Kamiguchi and Mikamo, 1986). This seldom occurred in mouse eggs. Two sister chromatids of each sperm chromosome were more tightly associated with each other in mouse eggs than in hamster eggs (Figure 2). The centromeric region was clearly detected after C-banding in all mouse chromosomes, whereas it was stained much more weakly in human sperm chromosomes except in chromosomes 1, 9 and 16 and the Y chromosome. These differences were useful in discriminating between human and mouse chromosomes.

Incidence of chromosome aberrations

Chromosome analyses were carried out with the following four types of spermatozoa: those with a normal head morphology (experiment 1), those with amorphous, round and elongated heads (experiment 2), and those with large (experiment 3) or small heads (experiment 4).

A total of 159 spermatozoa were analysed in experiment 1. Incidences of aneuploidy and structural chromosome aberrations were 1.3 and 6.9% respectively (Table I). Most of the latter were of the breakage type. A few rearrangements (one dicentric chromosome and one chromatid exchange) were also found. The ratio of X- to Y-bearing spermatozoa did not differ

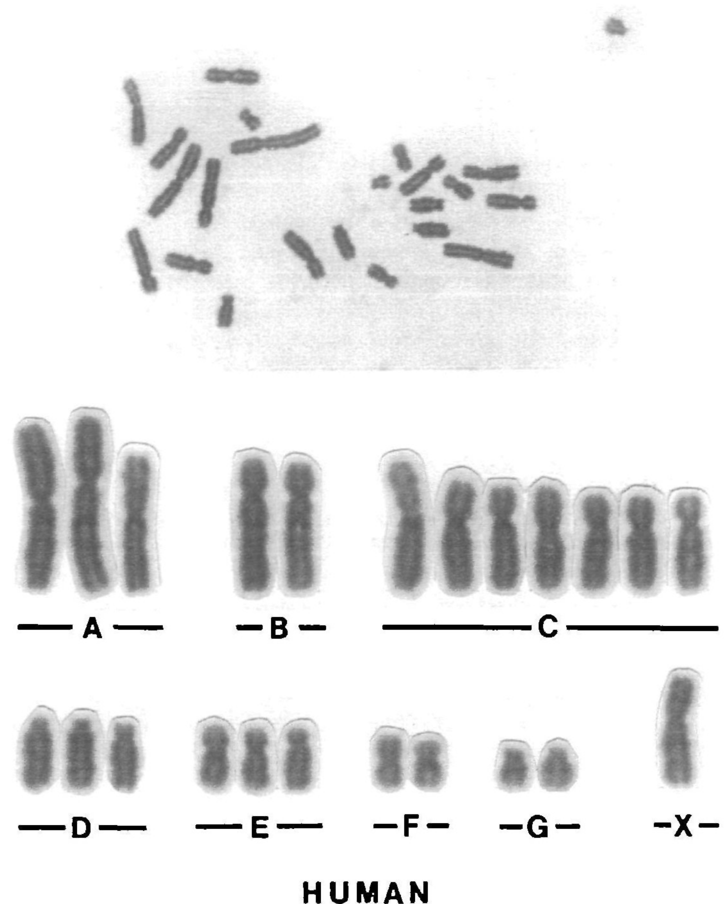

Figure 2. Chromosome preparation of a human spermatozoon microinjected into a mouse oocyte and its karyotype (23,X). Original magnification, above ×3600, below ×7000

significantly from the expected 1:1 ratio (χ^2 test, $P > 0.8$) In experiment 2, 23 spermatozoa were analysed successfully. Of these, 19 had amorphous heads, two had round heads and two had elongated heads. The incidence of spermatozoa with structural chromosome aberrations (26.1%) was significantly higher in this group than in experiment 1 ($P < 0.01$), despite the small sample size, whereas the incidence of aneuploidy (4.3%) was not significantly different from that in experiment 1. Neither aneuploidy nor structural aberration was found in experiment 3, although a chromosome analysis was successful in only nine spermatozoa. The sample size was also very small in experiment 4 (nine spermatozoa), and only one structural aberration (11.1%) was found, its incidence not being significantly different from that in experiment 1.

Discussion

Direct sperm injection into oocytes will be a powerful method of determining the possible relationships between specific sperm characteristics (e.g. head and tail morphology and flagellar motility) and the chromosome constitution of individual spermatozoa. Previous investigators examined the chromosomes of human spermatozoa after microsurgical sperm injection into hamster oocytes, but their success rate was very

Downloaded from https://academic.oup.com/humrep/article/11/9/1942/616037 by guest on 20 April 2024

Table I. Chromosome analysis of human spermatozoa with normal and aberrant head morphology

Experiment	n	Sperm head morphology	Normal		Aneuploidy		Structural anomalies	
			23,X	23,Y	N + 1	N − 1		
1	159	Normal	72	74	24,X,+E	22,X,−C	23,X, 1 csb (×3) 23,X, 1 csf 23,X, 1 ctf 23,X, 1 dic, 1 csf	23,Y, 1 csb (×3) 23,Y, 1 csb, 1 ctf 23,?, 3 csf, 1 cte
		Subtotal	146 (91 8%)		2 (1.3%)		11 (6 9%)	
2	23	Amorphous, round, elongated	6	10	0	22,Y,−E	23,X, 1 csb 23,X, 1 csf 23,X, 1 cte	23,Y, 1 csb 23,Y, 1 cte 23,?, 1 ctf, 1 r
		Subtotal	16 (69 6%)		1 (4 3%)		6 (26 1%)	
3	9	Large	6	3	0	0	0	
		Subtotal	9 (100%)		0 (0%)		0 (0%)	
4	9	Small	5	3	0	0	23, Y 1 csb	
		Subtotal	8 (88 9%)		0 (0%)		1 (11 1%)	

csb = chromosome breaks, csf = chromosome fragments, ctf = chromatid fragments, cte = chromatid exchange, dic = dicentric chromosomes, r = ring chromosomes

low; chromosomes of only three to five spermatozoa per 100 hamster oocytes were suitable for analysis (Martin *et al*, 1988; Cozzi *et al.*, 1995). In contrast, we were able to analyse sperm chromosomes in ~60% of mouse oocytes, indicating that mouse oocytes are much more suitable than hamster oocytes for the analysis of human sperm chromosomes using the direct sperm injection method. Recently, Rybouchkin *et al.* (1995) reported that human sperm chromosomes can be examined after sperm injection into mouse oocytes, but they did not mention the incidence of sperm chromosome aberrations.

In this study we found 1.3% of aneuploidy in spermatozoa with morphologically normal heads. This rate is about the same as (1.4%, Kamiguchi *et al.*, 1994) or lower than (2.1%, Brandriff *et al.*, 1990; 3.9%, Martin and Rademaker, 1990) that found in previous studies using the hamster oocyte–human sperm fusion system. The incidence of structural chromosome aberrations (6.9%) in this study was comparable with those reported in previous studies (6.9%, Brandriff *et al.*, 1990; 8.6%, Martin *et al.*, 1992).

No increase in chromosome aberrations was found in spermatozoa with large heads. The same was true for spermatozoa with small heads. These results suggest that the abnormal size of sperm heads is not associated with chromosome defects but merely with aberrant head development. However, the sample size in this study is very small. Further accumulation of experimental data is needed to draw any definitive conclusions.

In this study we found that the incidence of structural chromosome aberrations was about four times higher in spermatozoa with amorphous heads than in those with morphologically normal heads. According to Martin and Rademaker (1988), who used the hamster oocyte–human sperm fusion system, no significant relationship exists between the proportion of morphologically abnormal spermatozoa in the sperm suspension and the proportion of chromosomally abnormal spermatozoa penetrating hamster oocytes. Therefore, morphologically abnormal spermatozoa, which are frequently accompanied by structural chromosome aberrations, may be less fusion competent with zona-free hamster oocytes. If so, the findings in this study may have clinical implications. Even though not all spermatozoa with abnormal heads are chromosomally abnormal, the direct injection of such spermatozoa into human oocytes should be avoided to minimize the risk of abnormal fertilization.

Martin *et al.* (1988) have reported a considerably higher incidence of structural chromosome aberrations (39%) than ours in human spermatozoa injected into hamster oocytes. Their incidence seems to be raised by several factors. First, prior to microinjection they treated the spermatozoa with TEST-yolk buffer to reduce motility. However, they later found that this treatment caused structural chromosome aberrations in the spermatozoa (Martin *et al.*, 1992). We applied piezo-pulses to immobilize the spermatozoa. This treatment does not seem to be harmful because the aberration frequency in these spermatozoa did not differ from that in non-treated spermatozoa. Second, some morphologically abnormal spermatozoa might have been injected in the experiment of Martin *et al.* (1992), although they did not mention this. This may increase the frequency of chromosome aberrations, as shown by our study. Third, the aberration frequency may be influenced by a species-specific property of the oocytes used for microinjection. As mentioned earlier, hamster oocytes were highly vulnerable to microsurgery. Thus, some sperm chromosome aberrations may be caused by the microinjection procedure itself. This possibility was also mentioned in their own paper (Martin *et al.*, 1988). In contrast, oocytes of the hybrid mouse that we used were much more tolerant to microsurgical damage, retaining a high developmental ability (Rybouchkin *et al.*, 1995; our study). The incidence of structural defects in the mouse oocyte chromosomes in the human sperm-injected zygotes (experiments 1–4) was very low (3/207 or 1.4%), which is comparable with that (0.82%) in normally fertilized mouse zygotes (Santaló *et al.*, 1986). In other words, structural chromosome defects in our hybrid zygotes were found almost exclusively in sperm-derived chromosomes, and were probably already present prior to microinjection Aberration frequency may also be influenced by a species-specific capacity of the

Downloaded from https://academic.oup.com/humrep/article/11/9/1942/616037 by guest on 20 April 2024

oocyte to repair sperm DNA damage. The incidence of spermatozoa with structural chromosome aberrations is about three times higher in X-irradiated hamster spermatozoa (27%, Tateno *et al.*, 1996) than in mouse spermatozoa irradiated by a corresponding dose (1 Gy) of X-rays (9%, Matsuda *et al.*, 1985), both of which were examined after penetrating into homologous oocytes. This suggests that mouse oocytes have a higher ability to repair sperm DNA damage than do hamster oocytes, although this is not yet confirmed by cross-fertilization between the two species. All these factors must be studied further to elucidate the mechanisms of sperm-derived chromosome aberrations.

Acknowledgements

We thank Dr and Mrs Wesley Whitten for reading the original manuscript and giving us invaluable advice. J D.L. was on sabbatical leave from the Chang Gung Memorial Hospital, Taipei, Taiwan. This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan (no. 05278114) to Y.K.

References

Brandriff, B.F., Gordon, L.A and Carrano, A.V. (1990) Cytogenetics of human sperm structural aberrations and DNA replication In Mendelsohn, M.L and Albertini, R.J (eds), *Mutation and the Environment Part B: Metabolism, Testing Methods and Chromosomes* Wiley-Liss, New York, NY, USA, pp 425–434

Chatot, C.L., Ziomek, C.A., Bavister, B D , Lewis, J L. and Torres, I. (1989) An improved culture medium supports development of random-bred 1-cell mouse embryos *in vitro*. *J. Reprod. Fertil.*, **86**, 679–688.

Chatot, C.L , Lewis, J L , Torres, I and Ziomek, C.A (1990) Development of 1-cell embryos from different strains of mice in CZB medium. *Biol. Reprod* , **42**, 432–440

Cozzi, J , Chevret, E., Rousseaux, S , Pelletier, R. and Sele, B (1995) Human sperm chromosome analysis after microinjection into hamster oocytes *J Assist Reprod. Genet.*, **12**, 384–388

Kamiguchi, Y. and Mikamo, K. (1986) An improved, efficient method for analyzing human sperm chromosomes using zona-free hamster ova. *Am. J Hum. Genet* , **38**, 724–740.

Kamiguchi, Y , Tateno, H and Mikamo, K. (1994) Chromosomally abnormal gametes as a cause of developmental and congenital anomalies in humans *Cong Anom.*, **34**, 1–12.

Kimura, Y and Yanagimachi, R. (1995) Intracytoplasmic sperm injection in the mouse *Biol. Reprod.*, **52**, 709–720.

Martin, R.H. (1983) A detailed method for obtaining preparations of human sperm chromosomes. *Cytogenet. Cell Genet.*, **35**, 252–256

Martin, R.H. and Rademaker, A. (1988) The relationship between sperm chromosomal abnormalities and sperm morphology in humans. *Mutat. Res* , **207**, 159–164

Martin, R.H and Rademaker, A. (1990) The frequency of aneuploidy among individual chromosomes in 6,821 human sperm chromosome complements. *Cytogenet Cell Genet.*, **53**, 103–107.

Martin, R.H , Ko, E and Rademaker, A. (1988) Human sperm chromosome complements after microinjection of hamster eggs *J. Reprod. Fertil.*, **84**, 179–186.

Martin, R.H., Ko, E and Rademaker, A (1991) Distribution of aneuploidy in human gametes: comparison between human sperm and oocytes. *Am. J Med. Genet.*, **39**, 321–331

Martin, R H., Rademaker, A.W., Ko, E , Barclay, L and Hildebrand, K. (1992) A comparison of the frequency and type of chromosomal abnormalities in human sperm after different sperm capacitation conditions *Biol. Reprod.*, **47**, 268–270.

Matsuda, Y., Yamada, T and Tobari, I. (1985) Studies on chromosome aberrations in the eggs of mice fertilized *in vitro* after irradiation. I. Chromosome aberrations induced in sperm after X-irradiation *Mutat. Res* , **148**, 113–117.

Menkveld, R., Stander, F.S.H., Kotze, T.J W , Kruger, T.F and Van Zyl, J.A (1990) The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Hum. Reprod* , **5**, 586–592

Rudak, E , Jacobs, P.A. and Yanagimachi, R. (1978) Direct analysis of the chromosome constitution of human spermatozoa *Nature*, **274**, 911–913.

Rybouchkin, A , Dozortsev, D., Sutter, P.D., Qian, C. and Dhont, M. (1995) Intracytoplasmic injection of human spermatozoa into mouse oocytes. a useful model to investigate the oocyte-activating capacity and the karyotype of human spermatozoa. *Hum. Reprod.*, **10**, 1130–1135

Santaló, J., Estop, A.M. and Egozcue, J (1986) The chromosome complement of first-cleavage mouse embryos after in vitro fertilization *J. In Vitro Fertil. Embryo Transf* , **3**, 99–105.

Seuanez, H.N., Carothers, A D., Martin, D E and Short, R.V. (1977) Morphological abnormalities in spermatozoa of man and great apes. *Nature*, **270**, 345–347

Sugawara, S. and Mikamo, K. (1986) Maternal aging and nondisjunction: a comparative study of two chromosomal techniques on the formation of univalents in first meiotic metaphase oocytes of the mouse. *Chromosoma*, **93**, 321–325

Tateno, H , Kamiguchi, Y , Shimada, M and Mikamo, K. (1996) Difference in types of radiation-induced structural chromosome aberrations and their incidences between Chinese and Syrian hamster spermatozoa. *Mutat. Res.*, **350**, 339-348.

World Health Organization (1993) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.* Cambridge University Press, Cambridge, UK.

Received on March 19, 1996; accepted on June 12, 1996

Downloaded from https://academic.oup.com/humrep/article/11/9/1942/616037 by guest on 20 April 2024