Zygote 6 (February), pp 39–45. © 1998 Cambridge University Press Printed in the United Kingdom

Decondensation of the mouse sperm nucleus within the interphase nucleus

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Date submitted: 9.11.97. Date accepted: 11.12.97

Summary

Sperm nuclei incorporated into the cytoplasm (ooplasm) of fertilised mouse eggs at the pronuclear stage remain condensed, whereas those injected into male or female pronuclei decondense. Similarly, sperm nuclei injected into germinal vesicles of immature oocytes or the nuclei of 2-cell embryos decondense, while those entering the cytoplasm of these oocytes/embryos do not. These facts seem to suggest that factors necessary for the decondensation of sperm nucleus are present in interphase nuclei and are released into the ooplasm during nuclear envelope breakdown. Nucleoplasmin, which is synthesised in the cytoplasm and accumulated within the nucleus, is likely a major candidate for these factors.

Keywords: Egg, Glutathione, Nuclear decondensation, Nucleoplasmin, Oocyte, Sperm

Introduction

Sperm nuclei entering fully mature mammalian oocytes decondense rapidly before transforming into male pronuclei (Austin, 1961; Yanagimachi, 1994). The sperm nucleus-decondensing activity of the oocyte cytoplasm is cell-cycle-dependent. In the hamster and perhaps most other mammalian species, this activity appears after breakdown of the germinal vesicle (GV), reaches a maximum at metaphase of the second meiotic division, disappears soon after fertilisation, and reappears during the first cleavage (Usui & Yanagimachi, 1976). While studying the behaviour of sperm nuclei after microsurgical injection into hamster pronuclear eggs (Usui *et al.*, 1997), we found that a sperm nucleus that was accidentally injected into a pronucleus decondensed. We report here that mouse sperm nuclei injected intentionally into the nuclei of immature GV oocytes, fertilised (pronuclear) eggs or 2-cell embryos all decondensed.

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Materials and methods

Media

The medium for culturing ovarian oocytes, fertilised eggs and 2-cell embryos after microsurgery was 25 mM $NaHCO_3$-buffered CZB medium (Chatot *et al.*, 1989, 1990) supplemented with 5.56 mM D-glucose. A modified CZB with 20 mM Hepes, a reduced amount of $NaHCO_3$ (5 mM) and 0.1 mg/ml polyvinyl alcohol (30–70 kDa) (Hepes-CZB) was used for collection of oocytes, fertilised eggs and embryos as well as their subsequent treatments and micromanipulation. CZB and Hepes-CZB were kept at 37.5 °C under 5% CO_2 in air and air, respectively. Phosphate-buffered nucleus isolation medium (NIM) (Kuretake *et al.*, 1996) was used for isolation of sperm heads (nuclei), but without EDTA and PMSF. Inorganic and organic reagents for preparation of all media were purchased from Sigma (St Louis, MO) unless otherwise stated.

Collection and preparation of oocytes, fertilised eggs and 2-cell embryos

B6D2F1 female mice, 7–11 weeks old, were injected

peritoneally with 7.5 IU of pregnant mare serum gonadotropin (eCG). About 48 h later, oocytes at the germinal vesicle stage (GV oocytes) were collected from ovaries. They were freed from most surrounding cumulus cells by pipetting 10–15 min in Hepes-CZB containing 0.5% bovine testicular hyaluronidase (300 USP units/mg; ICN Biochemicals, Costa Mesa, CA) and 0.1 mM dibutyryl cAMP (dbcAMP). cAMP prevented spontaneous breakdown of GV (Downs *et al.*, 1989). The oocytes were rinsed thoroughly and kept in CZB with 0.1 mM dbcAMP for less than 2 h at 37.5 °C. Fertilised eggs at the pronuclear stage (pronuclear eggs) and 2-cell embryos were obtained as follows. Female mice were injected with 7.5 IU eCG and 7.5 IU human chorionic gonadotropin (hCG) 48 h apart. After hCG injection each female was placed in a cage with a male of proven fertility and left overnight. Fertilised eggs with two distinct pronuclei were flushed out of oviducts 20 h after hCG injection. Loosely packed cumulus cells were removed by 1–2 min treatment with 0.1% hyaluronidase in Hepes-CZB. Cumulus-free 2-cell embryos were collected from oviducts about 40 h after hC G injection.

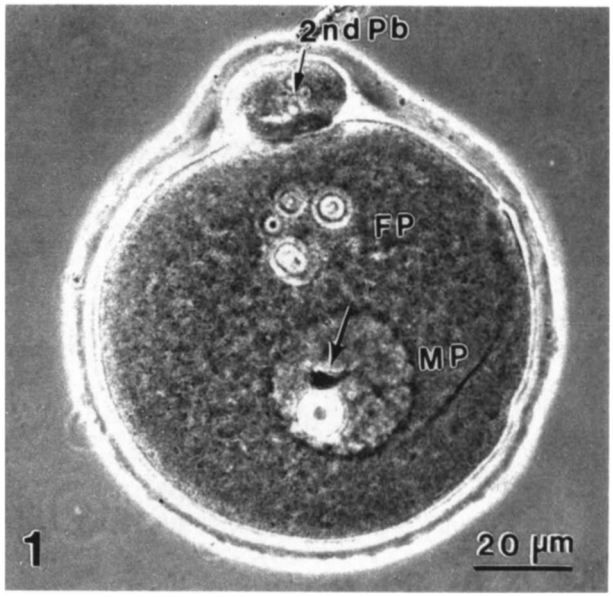

Figure 1 A pronuclear egg soon after sperm head injection into the male pronucleus. FP, female pronucleus; MP, male pronucleus with injected sperm head (arrow); 2nd Pb, the second polar body.

Preparation of sperm heads

A dense sperm mass collected from a cauda epididymis was placed in a 5 ml beaker containing 1 ml of NIM with 0.05% (v/v) Triton X-100 and sonicated for 5 s using 60% power output of an Ultrasonic sonicator (Model BP-II with 12 mm diameter horn, Bronwell Scientific, Rochester, NY). The suspension was centrifuged for 5 min at 500 *g*. The supernatant was discarded and the sperm pellet (sperm heads) resuspended in 8 ml Triton-free NIM by centrifugation for 5 min at 700 *g*. The final pellet in about 15 μl NIM was mixed thoroughly with an equal volume of NIM containing 12% (w/v) polyvinyl pyrrolidone (360 kDa). A small droplet (about 3 μl) of this suspension was kept under mineral oil (Squibb, Princeton, NJ) in the plastic Petri dish on a microscope stage (see below) for up to 2 h at room temperature before injection into oocytes/eggs/embryos.

Sperm head injection

Injection of sperm heads into fertilised mouse eggs was performed using a piezo-electric pipette-driving unit (Kimura & Yanagimachi, 1995). A single sperm head was sucked deeply into the injection pipette (5–6 μm inner diameter). The pipette tip was brought into intimate contact with the zona pellucida on a holding pipette, and the zona was drilled by applying a few piezo pulses as the pipette was advanced mechanically. The pipette tip was then brought in contact with the oolemma. After the sperm head had been pushed forward until it was near the tip of pipette, the pipette was advanced mechanically, through the ooplasm, deep into one of two pronuclei. After both the oolemma and nuclear envelope had been punctured simultaneously by one or two piezo-pulses, a sperm head was injected into the pronucleus, usually into the larger male pronucleus (Fig. 1). The volume of medium (PVP-containing NIM) introduced with a sperm head into a pronucleus was roughly 10 pl. Sperm head injection into the nucleus of a 2-cell embryo was performed in a similar manner.

To inject sperm heads into GVs, a group of immature GV oocytes were incubated at 37.5 °C in CZB supplemented with 0.1 mM dbcAMP. One to two hours later, when a small perivitelline space became distinct, a single sperm head was injected into a GV.

All injections were performed in Hepes-CZB at room temperature (25–28 °C). Oocytes/eggs/embryos were cultured in CZB at 37.5 °C after intranuclear injection of sperm heads.

Incubation and examination of oocytes, eggs and embryos

After sperm injection a group of oocytes, eggs or embryos was transferred into 50 μl CZB under mineral oil in plastic dishes and incubated at 37.5 °C. Between 0.5 and 12 h after the start of incubation, they were compressed between a slide and coverslip, fixed with glutaraldehyde, stained with aceto-orcein, and examined with a phase-contrast microscope (Yanagida *et al.*, 1991). Some sperm-injected pronuclear eggs were fixed for 1 h in 2% glutaraldehyde in

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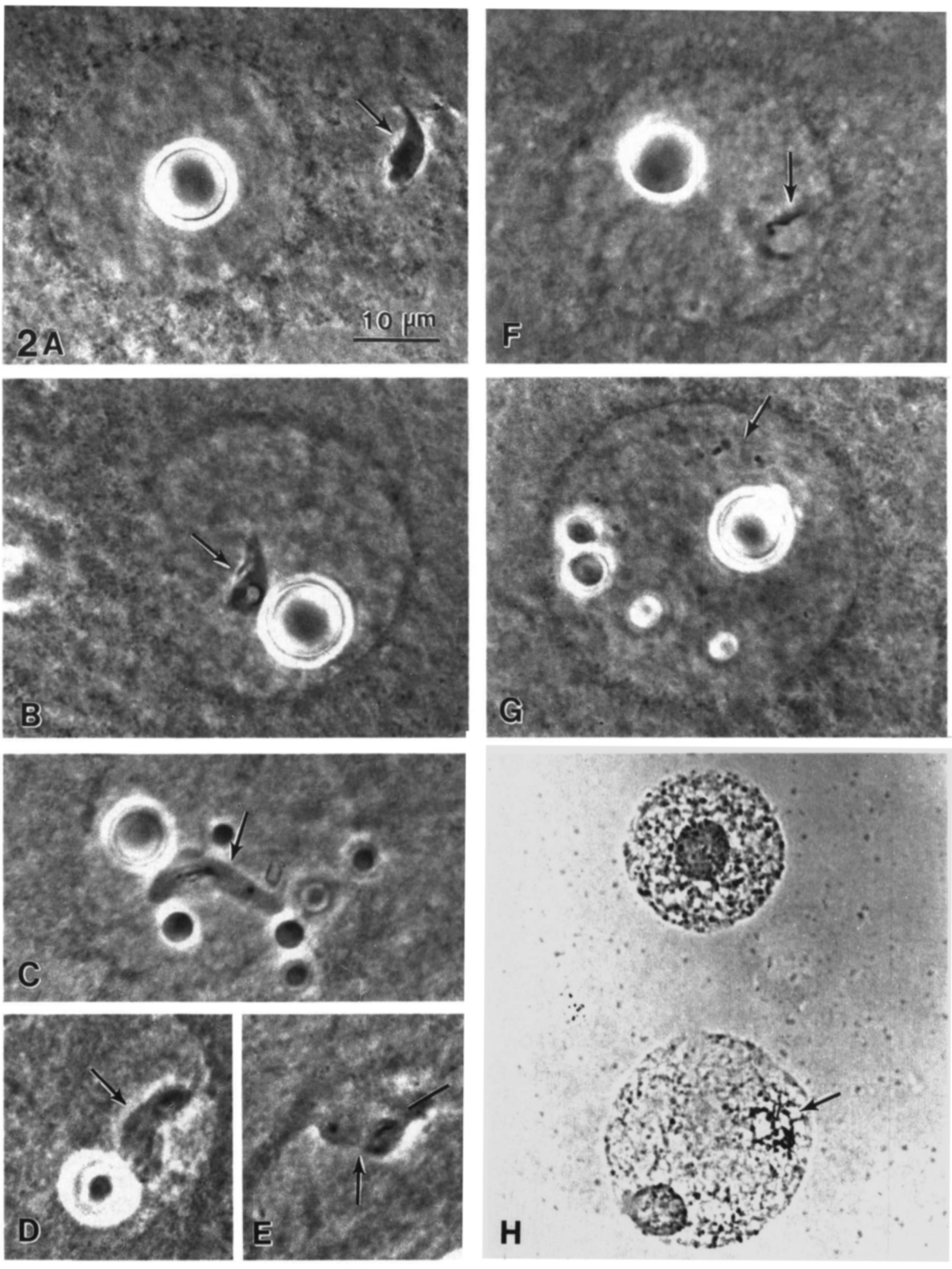

Figure 2 Behaviour of sperm heads injected into pronuclei. (*A*) This sperm head (arrow) failed to enter the pronucleus and remained condensed within the8 ooplasm. (*B–E*) Sperm heads (arrows) within pronuclei at various stages of decondensation. (*F, G*) Extensively decondensed sperm heads within pronuclei. (*H*) An air-dried preparation showing sperm chromatin mass (arrow) within the male pronuclus.

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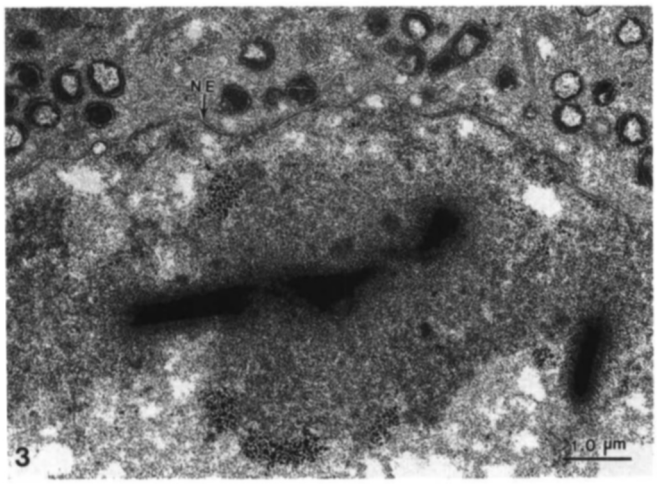
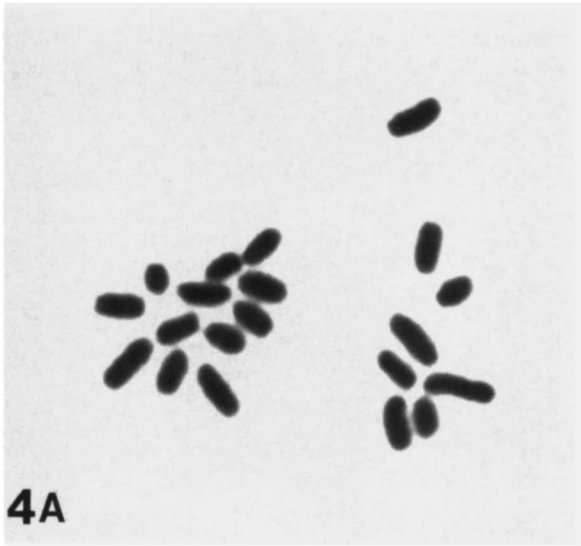


Figure 3 An electron micrograph of the pronucleus injected with a sperm head, showing decondensation of sperm chromatin, 1 h after injection. NE, nuclear envelope.

0.1 M cacodylate buffer (pH 7.2) and processed for electron microscopy. Others were incubated in CZB for 4–5 h, then in CZB containing 0.006 μg/ml vinblastin for 5–7 h. They were fixed and then air-dried on slides for examination of metaphase chromosomes in the first cleavage (Kamiguchi & Mikamo, 1986).

Results

Not all the oocytes, eggs and embryos survived sperm injection. About 10% of over 900 fertilised eggs/embryos and about 50% of over 100 immature (GV) oocytes cytolysed after microinjection. Of a total of 720 surviving fertilised (pronuclear) eggs about 20% had intact sperm heads within the ooplasm (Fig. 2*A*) between 1 and 9 h after injection. Apparently, we failed to break nuclear envelopes and therefore sperm heads were injected into the ooplasm rather than the pronucleus. In the remaining 80% of eggs between 1 and 3 h after sperm injection, sperm heads were at various stages of decondensation (Fig. 2*B–E*). Four to five hour after injection, sperm heads within pronuclei were hardly visible (Fig. 2*F*, *G*). The behaviour of sperm heads was the same in both male and female pronuclei. Air-dried preparations of pronuclear eggs (Fig. 2*H*) and electron micrographs of pronuclear eggs (Fig. 3) clearly demonstrated mingling of sperm head components with pronuclear components. Chromosome analysis of the eggs before the first cleavage revealed that sperm heads injected into pronuclei had transformed into condensed chromosomes (Fig. 4). In 30–40% of pronuclear eggs examined between 2 and 8 h after injection, the sperm heads appeared 'porous' (Fig. 5*A*), 'mini-pronucleus-like' (Fig. 5*B*), or irregularly shaped (Fig. 5C). In most



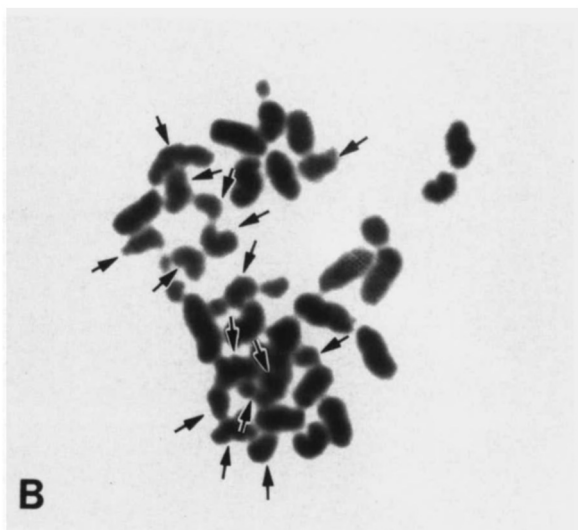


Figure 4 Chromosomes prior to the first cleavage following sperm injection into the male pronucleus. (*A*) Chromosomes from the female pronuclus; 20 chromosomes look all normal. (*B*) Chromosomes from the male pronucleus injected with a sperm head; about 40 chromosomes are seen; relatively thin chromosomes (arrows) are probably the chromosomes of an injected sperm head that failed to replicate.

of these eggs, nuclear envelopes were not clearly visible.

Sperm heads injected into the ooplasm of GV oocytes remained condensed, whereas those injected into GVs decondensed (Fig. 6*A*, *B*). Similarly, sperm heads injected into the nuclei of 2-cell embryos decondensed (Fig. 6*C*).

Discussion

A sperm head (nucleus) incorporated in the ooplasm of a mature oocyte quickly decondenses to become a

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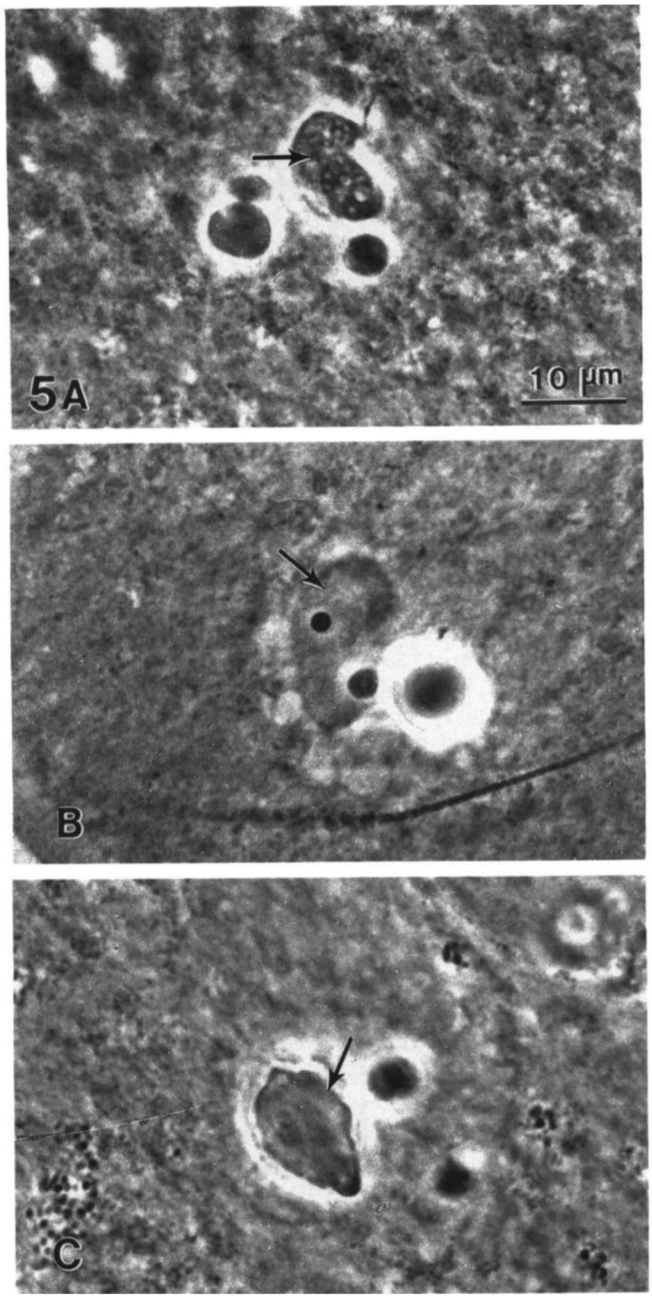

Figure 5 Aberrant decondensation of sperm heads within pronuclei. Sperm head (arrow) transformed into a 'porous' structure (*A*), a small pronucleus-like structure (*B*) and an irregularly shaped structure (*C*) structures with pronuclei.

male pronucleus. The mechanism of sperm head decondensation within ooplasm has been studied extensively in the frog (e.g. *Xenopus*), which allows investigators to manipulate sperm nuclei within a large quantity of isolated ooplasm and its fractions. A major sperm nucleus-decondensing factor is nucleoplasmin, which is released from the GV into ooplasm at GV breakdown (Philpott *et al.*, 1991). When a frog spermatozoon enters the nucleoplasmin-rich ooplasm of a mature oocyte, sperm nuclear protamines leave

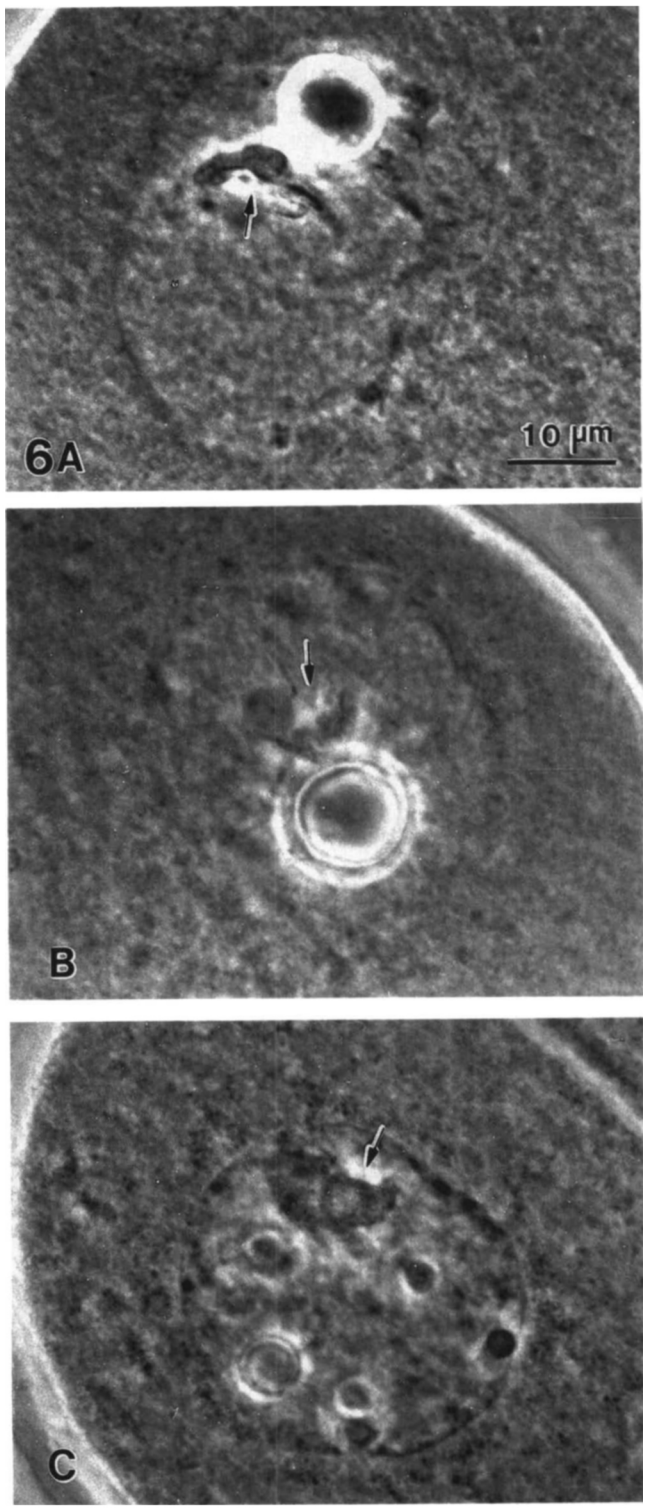

Figure 6 Sperm heads (arrows) decondensed within germinal vesicles (*A*, *B*) and the nucleus of a 2-cell embryo (*C*).

DNA to bind with nucleoplasmin, which has a very strong affinity to protamines. Free DNA then binds to histones (Philpott *et al.*, 1991; Ohsumi & Katagiri, 1991; Philpott & Leno, 1992; Ruiz-Lara *et al.*, 1996; Leno *et al.*, 1996). This protamine–histone exchange

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occurs very quickly (Ohsumi & Katagiri, 1991; Katagiri & Ohsumi, 1994).

In fully mature mammalian spermatozoa sperm protamines are extensively crosslinked by –SS– bonds that make mammalian sperm chromatin very stable (Bedford & Calvin, 1974; Ward, 1993). Reduction of these –SS– bonds by ooplasmic glutathione (GSH) is an essential preliminary to sperm nucleus decondensation in mammals (Wiesel & Schultz, 1981; Perreault *et al.*, 1984, 1988; Calvin *et al.*, 1986; Perreault & Barbee, 1991; Funahashi *et al.*, 1995; Sutovsky & Schatten, 1997). However, it is not sufficient for full decondensation of sperm nuclei (Perreault *et al.*, 1984). Perhaps, nucleoplasmin is necessary to remove protamines from DNA (Yanagimachi, 1994).

It is well established that nucleoplasmin is synthesised in the ooplasm and transported into the GV in an ATP-dependent manner during oocyte growth (Newmayer *et al.*, 1986; Paine *et al.*, 1995). As reported in the present study, sperm heads injected into GVs of immature oocytes, pronuclei of fertilised eggs or the nuclei of 2-cell embryos all decondense. This may not be surprising because these nuclei are expected to have both nucleoplasmin and GSH, although GSH concentration within the nucleus may be lower than that in the ooplasm (Heidemann & Hamborg, 1984).

In addition to its role in sperm nucleus decondensation at fertilisation, nucleoplasmin released from the GV into ooplasm during oocyte maturation may play an important role in facilitating translation of maternal RNAs by disrupting their association with ribonucleoproteins that keep them in an inactive state (Dimitrov & Wolffe, 1996; Meric *et al.*, 1997). Nucleoplasmin released from the nucleus into cytoplasm during the first and early cleavages may facilitate transcription (Burglin *et al.*, 1987; Maric *et al.*, 1997) and plays a role in cellular diversification (Litvin & King, 1988). Thus, nucleoplasmin seems to have several different functions during oogenesis through early early embryogenesis.

Acknowledgments

This study was supported by a grant from the National Institute of Child Health and Human Development (HD-03402). We thank Dr Steven Ward for his active interest in this study and for reviewing the original manuscript. We are indebted to Mrs Charlotte Oser for her assistance in the preparation of the manuscript.

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