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Expansion of the Sperm Nucleus and Association of the Maternal and Paternal Genomes in Fertilized *Mulinia lateralis* Eggs

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Abstract. Sperm nuclear expansion, meiotic maturation of the maternal chromatin, and events involving the association of the male and female pronuclei leading to the two-cell stage were observed in *Mulinia* zygotes using the fluorochromes DAPI and Hoechst. The effects of ultraviolet irradiation on the fertilizing sperm were also examined. Incorporated sperm nuclei underwent changes in diameter that were temporally correlated with meiotic processes of the maternal chromatin. Following its entry, the sperm nucleus underwent a rapid, initial enlargement, which was correlated with germinal vesicle breakdown. Sperm nuclear expansion ceased during the period in which the egg was engaged in polar body formation and was re-initiated with formation and enlargement of the female pronucleus. The rates of enlargement of the male and female pronuclei were 0.59 and 0.65 μm/min, respectively. Following their migration into apposition with one another, the male and female pronuclei synchronously underwent events characteristic of prophase as separate structures; *i.e.*, chromosome condensation, and nuclear envelope breakdown. The two groups of chromosomes that formed became organized on the metaphase plate in preparation of the first cleavage division; hence, there was no fusion of pronuclei. Ultraviolet irradiation of fertilizing sperm had no apparent affect on sperm nuclear transformations leading to the development of a male pronucleus or on female pronuclear development. However, events subsequent to the apposition of the pronuclei were affected and included asynchrony of prophase and the nondisjunction of chromosomes at anaphase. These observations are discussed in relationship to events regulating transformations of the sperm nucleus and experiments to generate gynogenetic bivalve embryos.

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Introduction

For the eggs of most animals insemination occurs at an arrested stage of meiosis; *i.e.*, meiotic prophase (the germinal vesicle stage), metaphase I, or metaphase II (Longo, 1987a). Representatives of these three stages include the eggs of annelids, mollusks, and chordates, respectively. In comparison, the eggs of relatively few organisms are fertilized following the completion of meiotic maturation (the pronuclear stage). The most notable example of the latter group are eggs of echinoids. Although processes of fertilization are fundamentally the same in eggs inseminated at different stages of meiotic maturation, there are prominent differences, particularly during the transformation of the sperm nucleus into a male pronucleus and pronuclear association (Wilson, 1925; Longo, 1985).

In eggs inseminated at the completion of meiotic maturation, the female pronucleus is already present and "waiting" for the entry and transformation of the sperm nucleus into a pronucleus. In contrast, in eggs inseminated at an arrested stage of meiotic maturation, the sperm nucleus, following its entry into the egg cytoplasm, must "wait" for the maternal chromatin to complete its meiotic maturation. Observations carried out with the gametes of a variety of organisms (sea urchin, surf clam, mussel, hamster, rabbit, and mouse) have shown that both the kinetics of sperm nuclear enlargement into a male pronucleus and events attending pronuclear association are correlated with the stage of meiosis at which the egg is inseminated and the length of time the sperm nucleus spends in the egg cytoplasm before pronuclear association

(Wilson, 1925; Longo, 1985). For example, in eggs inseminated at the pronuclear stage, the rate of sperm nuclear expansion is uniform, whereas in eggs inseminated at an arrested stage of meiosis, the rate of expansion is much more complex and shows different phases that are correlated with stages of meiotic maturation of the maternal chromatin (Luttmer and Longo, 1987, 1988; Wright and Longo, 1988; Longo, 1989).

In eggs inseminated at an arrested stage of meiosis, pronuclear fusion does not occur as in eggs fertilized at the pronuclear stage. The paternally and maternally derived chromatin do not become associated with one another until prophase of the first cleavage division when chromosomes derived from the male and female pronuclei intermix and become aligned on the metaphase plate of the mitotic spindle (Longo, 1985). Evidence suggests that differences in the kinetics of sperm nuclear expansion and pronuclear association are related to cell cycle events associated with meiotic maturation and mitosis of the first mitotic division (Luttmer and Longo, 1988; Wright and Longo, 1988; Longo, 1989).

Because analyses of sperm nuclear expansion and its relationship to meiotic events of the maternal chromatin have been carried out in relatively few organisms (see Longo, 1989), and to further explore possible relationships between these processes of fertilization and cell cycle phenomena, we have initiated studies with a variety of organisms, the eggs of which are inseminated at an arrested stage of meiosis. Here we describe the course of meiotic maturation of the maternal chromatin, corresponding events of sperm nuclear enlargement, and association of the male and female pronuclei in *Mulinia lateralis* (dwarf surf clam or coot clam) eggs, which are inseminated at meiotic prophase. The effects of ultraviolet irradiation on sperm nuclear transformations and events involving and subsequent to male and female pronuclear association are also presented.

Material and Methods

Sexually mature individuals of *Mulinia lateralis,* collected from Massey's Landing, Delaware, were kept at 15°C in a recirculating seawater system. Spawning was induced by placing individual animals into 100 ml beakers containing seawater at 30°C. Eggs from 1 to 3 spawned females were pooled, washed in fresh seawater, inseminated and permitted to develop at 20°C. Unfertilized eggs and samples of fertilized ova, taken at 5 min intervals up to 1 to 1.5 h after the addition of sperm, were fixed in 1% formalin in seawater. In some experiments, eggs and sperm were incubated with 10 μM Hoechst 33342 (Hoechst) in seawater, washed in fresh seawater, and used for insemination (Luttmer and Longo, 1986).

To examine the effects of ultraviolet irradiation on sperm nuclear transformations leading to pronuclear development, sperm were irradiated with ultraviolet light as previously described (Nace *et al.,* 1970; Chourrout and Quillet, 1982; Scarpa and Bolton, 1988). Sperm suspended in a plastic Petri dish were exposed for 20 min, at a distance of 20 cm, to a 15-watt tube generating ultraviolet light ranging from 200 nm to 295 nm, with 60% of the ultraviolet light concentrated at 254 nm. Irradiated sperm were mixed with a suspension of eggs; samples were taken and fixed as described above for non-irradiated specimens.

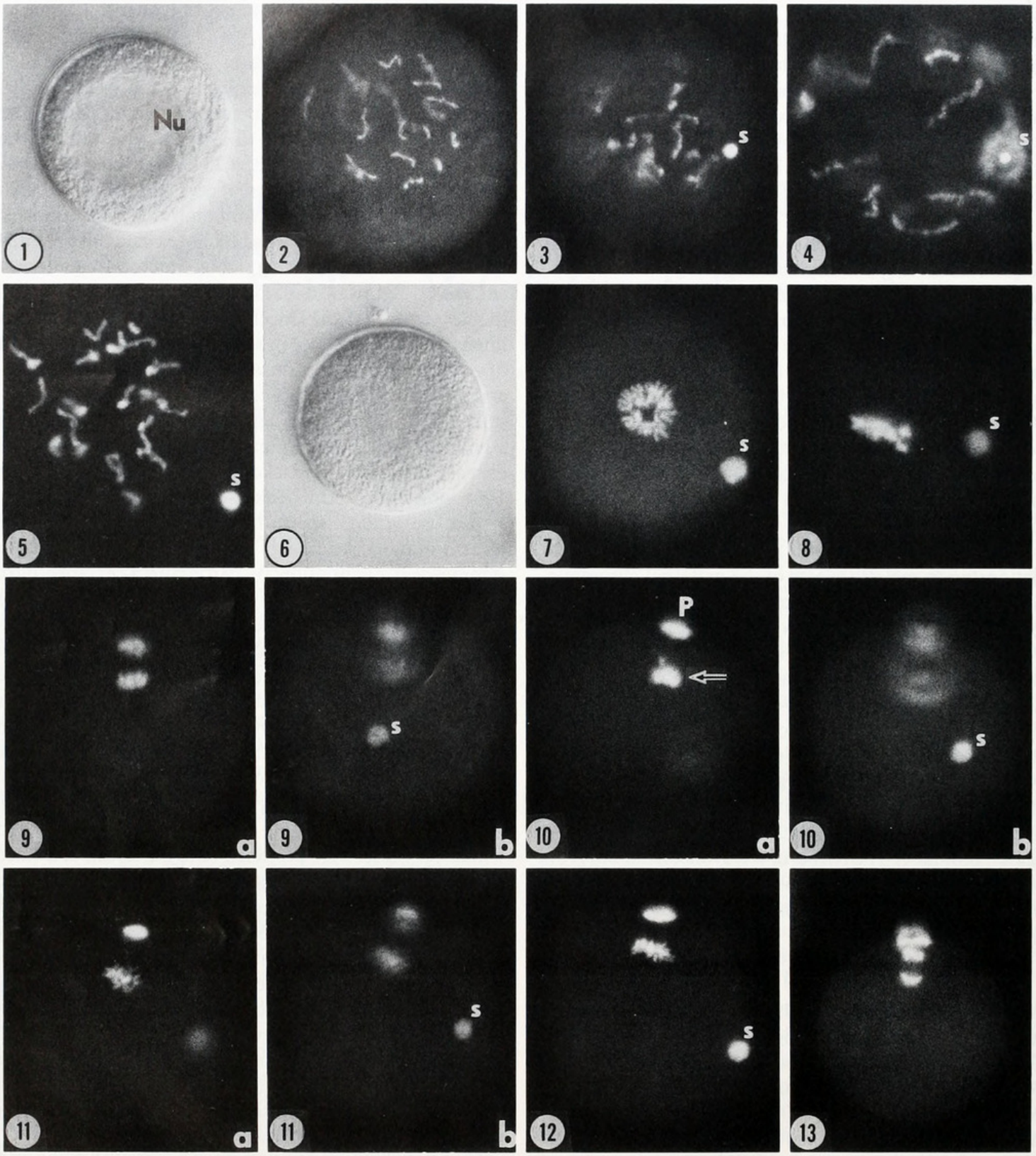
Fixed specimens were washed in seawater and stained with one of the following DNA intercalating fluorochromes: 1 μg/mL 4',6-diamidino-2-phenylindole (DAPI) in seawater, or 10 μM Hoechst in seawater. Stained specimens were washed once in seawater, placed into a droplet of glycerol on a glass slide, and covered with a glass coverslip. To improve microscopic observation, some specimens were compressed. Specimens were observed with a Nikon Diaphot microscope equipped with epifluorescence. Photographs were taken of specimens using 40× or 100× objectives and Kodak T-Max film.

Because *Mulinia* sperm nuclei are spheroid, and their transformations leading to male pronuclei produced a symmetrical distribution of chromatin (*i.e.,* spheroid), changes in the size of incorporated sperm nuclei were measured throughout the period of fertilization. To measure incorporated sperm nuclei at different periods after insemination, as well as the developing spheroid female pronucleus, stained specimens were placed into droplets of glycerol as described above. A coverslip, bearing a thin layer of Vaseline along its edges, was lowered over the droplets such that the eggs or zygotes were suspended between the slide and coverslip. Images of the cross-sectional diameters of transforming sperm nuclei and male and female pronuclei were projected onto the screen of a video monitor, checked for linearity, and traced onto plastic sheets with a felt tip pen. The traced images were analyzed with a Micro-plan II Image Analysis System (Laboratory Computer Systems, Cambridge, Massachusetts). Diameters and maximum cross-sectional areas of transforming sperm nuclei and male and female pronuclei were measured (mean ± standard deviation) at 5-min intervals following insemination and temporally correlated with the progression of meiotic maturation, female pronuclear development, and first mitosis. Twenty to forty specimens were measured at each time point.

Results

Structure of the unfertilized egg and spermatozoon

Unfertilized *Mulinia* eggs measured 46.4 ± 0.4 μm in diameter. When viewed with phase or Nomarski optics they were seen to possess a large, meiotic prophase nucleus (29.7 ± 1.4 μm in diameter), the germinal vesicle, which usually contained a single, spheroid nucleolus (10.4 ± 0.8



Figures 1 and 2. Nomarski (Fig. 1) and fluorescent (Fig. 2) preparations of unfertilized *Mulinia* eggs showing germinal vesicles, nucleoli (Nu) and meiotic chromosomes. Figure 1, ×760; Figure 2, ×960.

Figures 3 and 4. Fertilized *Mulinia* eggs depicting incorporated sperm nuclei (S) and meiotic chromosomes which are distributed throughout the germinal vesicle (5 min pi). Figure 3, ×960; Figure 4, ×1500.

Figure 5. Zygote (10 min pi) in which the meiotic chromosomes are condensing and the sperm nucleus (S) is dispersing. ×1800.

Figures 6–8. Zygotes (15 min pi) in which the meiotic chromosomes have become condensed and organized on the same optical plane (Fig. 7). The chromosomes move as a group to the cortex in preparation for polar body formation (Fig. 8). Figure 6 is a Nomarski preparation in which the meiotic chromosomes and incorporated sperm nucleus are difficult to discern; these structures are intensely stained in Hoechst- or DAPI-prepared specimens. Figure 6, ×760; Figures 7 and 8, ×960.

μm in diameter) suspended in a nucleoplasm (Fig. 1). Occasionally, specimens containing two large nucleoli were observed. Unfertilized eggs prepared with DAPI (fixed eggs) or Hoechst (fixed or unfixed eggs) observed with epi-fluorescence were essentially identical. Two features were apparent with both methods: (1) a low background staining of the cytoplasm, and (2) a relatively intense staining of the maternal tetrad chromosomes. Tetrads were distributed throughout the interior of the germinal vesicle such that chromosome number and individual chromosomal features (*e.g.*, chiasma) could be ascertained (Fig. 2). Examination of whole mounts and compressed specimens revealed that the number of meiotic chromosomes in *Mulinia* eggs; *i.e.*, the haploid number, was 19 (see also Menzel, 1968; Scarpa and Bolton, 1988; Wada *et al.*, 1990).

The structure of *Mulinia* sperm as examined by light microscopy was similar to that of other pelecypods (Franzen, 1955). The sperm nucleus was spheroidal, 1.7 ± 0.15 μm in diameter, and contained a uniform distribution of DNA as determined in fluorochrome stained preparations.

As was found for the surf clam, *Spisula* (Luttmer and Longo, 1986), living *Mulinia* sperm or eggs treated with Hoechst 33342 could inseminate and develop with no apparent ill-effects. In living *Mulinia* zygotes in which only one of the gametes was treated with Hoechst dye prior to insemination, staining of both the maternal and paternal genomes was found consistently after fertilization, indicating that the dye was not remaining confined to the nucleus of one gamete. Unlike the situation in *Spisula* (Luttmer and Longo, 1986), we were unable to achieve exclusive staining of only one genome in *Mulinia* zygotes. The following account is based on experiments employing fixed and unfixed, stained specimens.

Meiotic maturation of the maternal chromatin leading to development of the female pronucleus

The interaction of the sperm with the egg initiated the resumption of meiotic maturation and development of the female pronucleus in *Mulinia*. Resumption of meiotic maturation was heralded by the breakdown of the nuclear envelope of the germinal vesicle and the disappearance of the nucleolus (Figs. 3–6). These characteristic features of germinal vesicle breakdown were readily apparent with Nomarski and phase contrast optics (Fig. 6), but changes in the structure and location of the tetrads were much more difficult to ascertain. Meiotic events of the maternal chromosomes and transformations of the sperm nucleus were readily apparent with fluorochrome stained *Mulinia* preparations and epi-fluorescence microscopy (Figs. 3, 4, 5, 7). Concomitant with germinal vesicle breakdown was the condensation of the tetrads (Figs. 5, 7). The tetrads formed a cluster within the center of the egg; eventually they were organized on the metaphase plate of the first meiotic spindle (Figs. 7, 8). The spindle and tetrads then moved to one pole of the egg where completion of meiosis and polar body formation occurred (Fig. 9). In almost all cases examined, more than 90% of the specimens were in synchrony and had developed to metaphase I by 15 min postinsemination (pi).

Anaphase I followed localization of the meiotic spindle to the egg cortex and was seen as the separation of two fluorescent masses of chromosomes (Figs. 9, 10). With the completion of anaphase I, the chromosomes emitted within the first polar body formed a compact mass; those within the egg became reorganized on a metaphase plate in preparation for second polar body formation (Figs. 11, 12).

Anaphase II quickly followed formation of the first polar body (Fig. 13) and appeared as the separation of two fluorescent masses that were of less intensity than the chromosomal masses that formed at anaphase I, reflecting the decrease in DNA. After anaphase II, chromosomes within the second polar body formed a densely stained cluster (Fig. 14). The first and the second polar bodies became positioned side by side and remained at the pole of the egg where the meiotic divisions took place.

By 35 min pi, more than 95% of the specimens examined had completed polar body formation and were engaged in the formation of a female pronucleus (Figs. 14–16). The maternal chromosomes remaining in the egg dispersed, forming an irregularly shaped nucleus that eventually expanded to become a spheroidal female pronucleus. Measurements of the female pronucleus at different times following its formation (35 to 45 min pi) indicated that its rate of expansion was 0.65 μm/min (Fig. 23). Its average maximal size, measured at 45 min pi,

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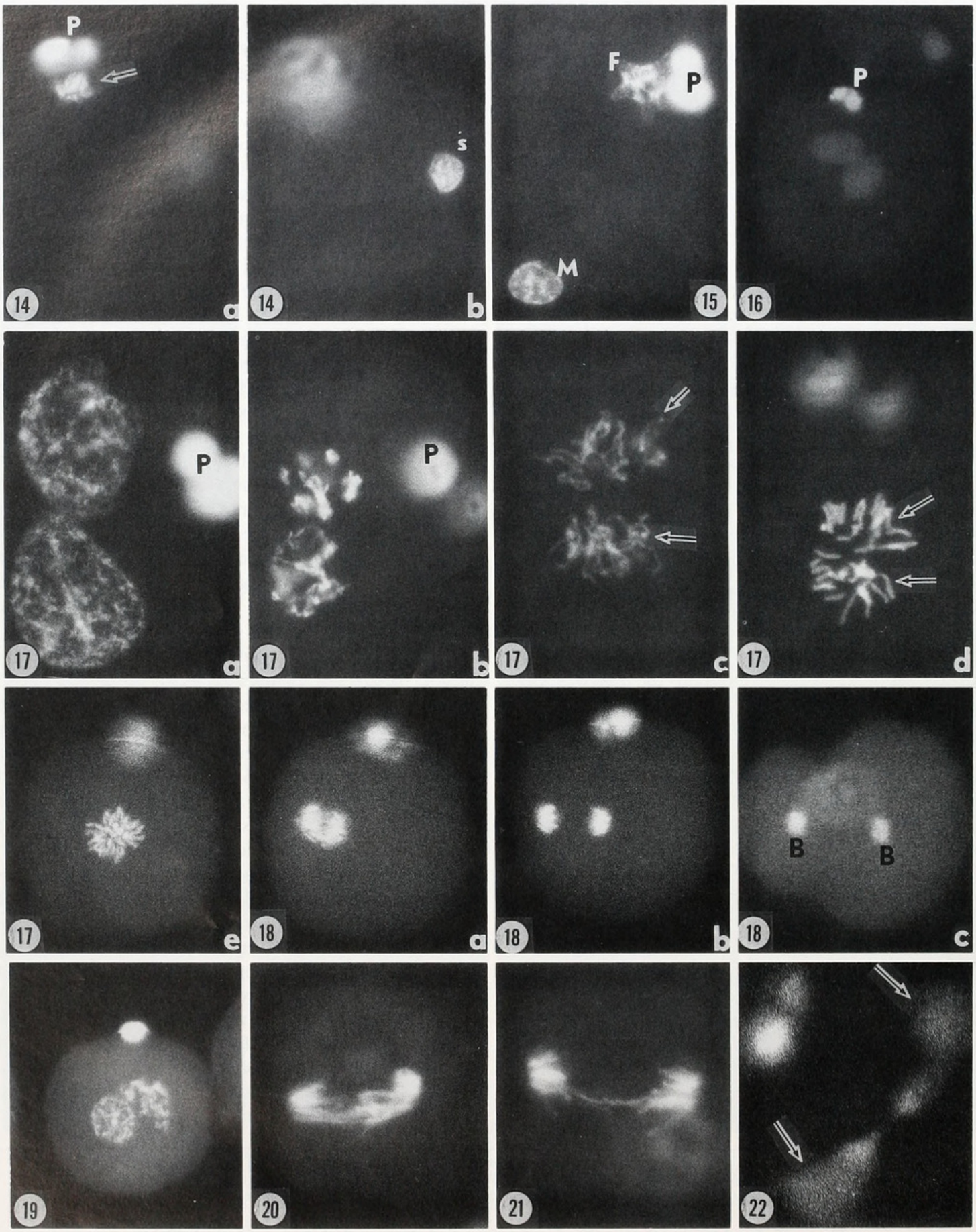
Figures 9a, b. Zygote (25 min pi) at two optical planes depicting anaphase I (Fig. 9a) and the incorporated sperm chromatin (S). Note that the latter has ceased dispersion and is smaller than the sperm nucleus depicted in Figure 7. ×960.

Figures 10a,b and 11a, b. Zygotes (30 min pi) at two optical planes in which the first polar body (P) has formed and the chromosomes remaining in the zygote are preparing for the second meiotic division (Figs. 10a, 11a). Figures 10b and 11b are at the level of the incorporated sperm nucleus which has ceased its enlargement. ×960.

Figures 12 and 13. Zygotes (35 min pi) at metaphase II (Fig. 12) and anaphase II (Fig. 13). S, sperm nucleus. ×820.



Figures 14a, b. Zygote (35 min pi) at two optical planes depicting the maternal chromosomes (arrow) that have just completed their second meiotic division and are dispersing to form the female pronucleus (Fig. 14a). Figure 14b shows the sperm nucleus (S), which is enlarging P, first and second polar bodies. ×1500.

Figure 15. Zygote (40 min pi) at a slightly later stage of pronuclear development than the egg depicted in Figure 14 in which the male (M) and female (F) pronuclei are expanding. P, first and second polar bodies. ×1900.

was 9.8 ± 1.4 μm. By 50 min pi the female pronucleus had decreased in diameter (9.4 ± 1.1 μm) and was engaged in prophase of the first mitotic (cleavage) division (Figs. 17, 23).

Transformations of incorporated sperm nuclei leading to the development of male pronuclei

Upon its incorporation into the egg cytoplasm, the sperm nucleus underwent an expansion from 1.7 ± 0.15 to 3.7 ± 0.28 μm in diameter (Figs. 3–5, 7, 23). This initial expansion occurred symmetrically while the maternal tetrads were condensing and becoming aligned on the metaphase plate of the first meiotic spindle (Figs. 5, 7). From 20 to 35 min pi, coincident with the period in which the maternal chromosomes were engaged in polar body formation, the incorporated sperm nucleus did not expand and, in fact, decreased slightly in size to 3.5 ± 0.28 μm in diameter (Figs. 9–12, 23). With the completion of meiosis and the development of the female pronucleus there was a dramatic enlargement in the sperm nucleus (rate = 0.59 μm/min; Figs. 14–16, 23). At the completion of its expansion (45 min pi), the male pronucleus measured 9.7 ± 1.4 μm in diameter. Subsequent changes in the male pronucleus included its reduction in size (9.6 ± 0.8 μm) as a part of prophase of the first cleavage division.

Morphogenesis of the male and female pronuclei leading to the first cleavage division

By 45 min pi, both the male and female pronuclei had reached their maximal sizes (Fig. 23) and individually and synchronously undergone prophase events leading to the first cleavage division (Fig. 17). By 50 min pi condensing chromosomes appeared in the two pronuclei. As the chromosomes condensed, the nuclear envelopes broke down, forming two distinct groups of chromosomes in the midregion of the zygote, one derived from the female pronucleus and the other from the male (Fig. 17). The two groups of chromosomes moved together, intermixed, and became positioned on the metaphase plate of the first mitotic spindle (Fig. 17). Subsequent morphogenesis of the maternally and paternally derived chromosomes involved their participation in the first cleavage division, which was asymmetric with respect to cytokinesis (Fig. 18). That is, the metaphase plate was displaced from the center of the zygote and, as a consequence, two unequally sized blastomeres formed upon cleavage.

Effects of ultraviolet irradiation on male pronuclear development and morphogenesis

Effects of ultraviolet irradiation were not apparent during transformation of the sperm nucleus into a male pronucleus, nor was there any apparent effect on meiotic maturation and development of the female pronucleus. Irradiated sperm nuclei expanded into pronuclei of a size comparable to those of control preparations. Effects of ultraviolet irradiation on sperm nuclei were not observed until the male pronucleus was engaged in prophase events of the first cleavage division. In eggs inseminated with ultraviolet irradiated sperm, mitotic prophase events in the two pronuclei were asynchronous (Fig. 19). The maternally and paternally derived chromosomes eventually became aligned on a metaphase plate, but anaphase of mitosis was abnormal as evidenced by chromosomal nondisjunction (Figs. 20, 21). The number of chromosomes that failed to move to the spindle poles was not constant. Consequently, material of varying fluorescent intensity was seen between the spindle poles at telophase, and between interconnecting blastomere nuclei at subsequent stages of development (Fig. 22).

Discussion

The results presented here demonstrate nuclear changes that occur in fertilized *Mulinia* eggs and lead to the two-

Figure 16. Expanded male and female pronuclei that have become associated with one another in the center of a zygote (45 min pi). P, polar bodies. ×820.

Figures 17a–e. Morphogensis of the male and female pronuclei following their apposition. Initiation of prophase in each pronucleus is evident by chromosome condensation (Fig. 17a, b). Two groups of chromosomes are produced (arrows, Fig. 17c, d) which become closely associated and positioned on the metaphase plate of the first mitotic spindle (Fig. 17e). P, polar bodies. Figure 17a, c, and d, ×2000; Figure 17b, ×1830; Figure 17e, ×870.

Figures 18a–c. First cleavage division of *Mulinia* leading to unequal size blastomers (Fig. 18c). Figures 18a and b depict early and late anaphase. P, polar bodies; B, developing blastomere nuclei. ×870.

Figure 19. Asynchronous pronuclear morphogenesis in an egg fertilized with an ultraviolet irradiated sperm. ×750.

Figure 20. Nondisjunction of mitotic chromosomes in an egg inseminated with an ultraviolet irradiated sperm. ×1400.

Figure 21. Cleaving egg which was inseminated with an ultraviolet irradiated sperm. Chromatin is spread between the two developing blastomere nuclei. ×1400.

Figure 22. Cleaved zygote that was fertilized by an ultraviolet irradiated sperm. DAPI staining material (*i.e.*, DNA) connects the two blastomere nuclei (arrows). ×1700.

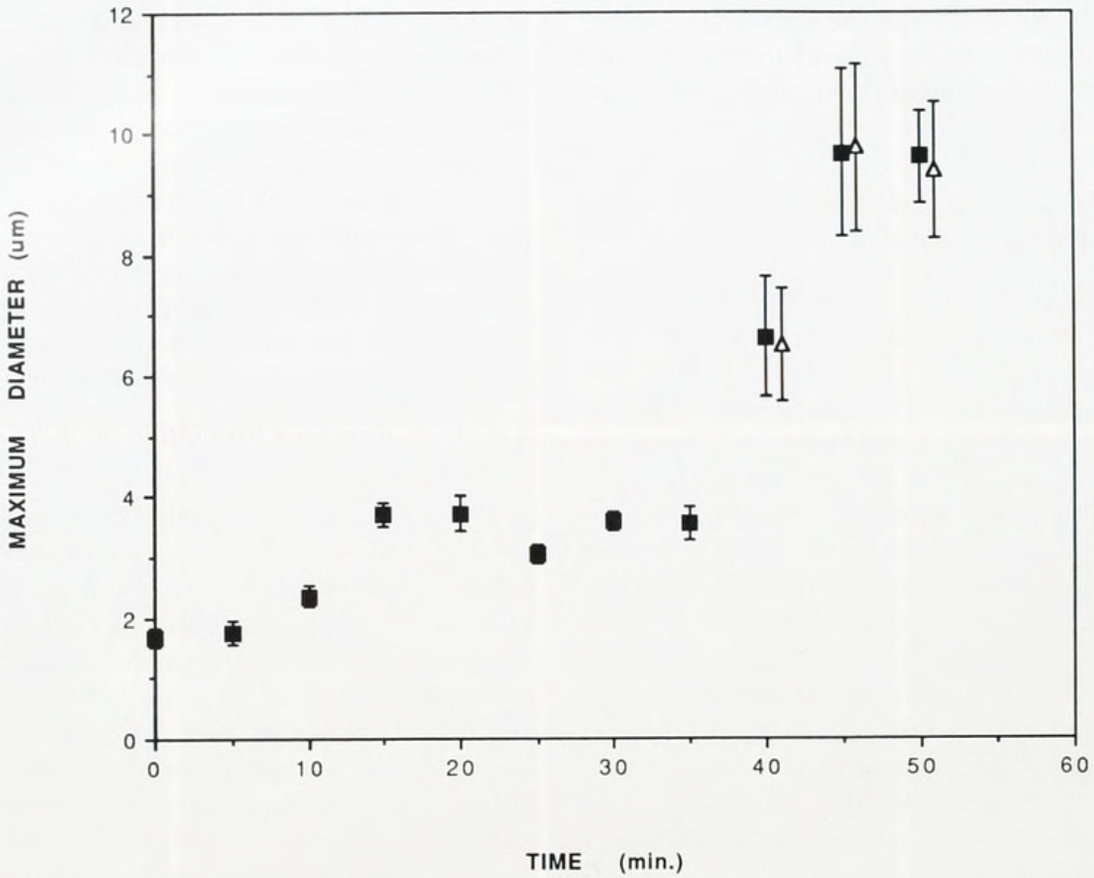


Figure 23. Expansion (mean ± S.D.) of incorporated sperm nuclei (■) and female pronuclei (△) of *Mulinia* zygotes. The sperm nucleus shows three periods of transformation: 0 to 15, 15 to 35, and 35 to 45 min pi corresponding to periods encompassing germinal vesicle breakdown, polar body formation, and female pronuclear development, respectively. The decrease in size of the male and female pronuclei from 45 to 50 min pi is correlated with the onset of mitotic prophase in both pronuclei.

cell stage. Meiosis of the maternal chromatin, transformations of the sperm nucleus, and pronuclear development and association are readily amenable to analysis in specimens prepared with the DNA intercalating dyes DAPI and Hoechst. This suitability is due to a combination of factors, such as low background of the egg cytoplasm, and chromosome size, number and structure (Wada *et al.,* 1990).

Meiotic maturation of the maternal chromatin of *Mulinia* eggs is similar to that previously described for other mollusks (Longo, 1983; Luttmer and Longo, 1988). Interaction of the sperm with the egg induces germinal vesicle breakdown. The chromosomes become organized on the metaphase plate of the first meiotic spindle apparatus which then moves to, and becomes positioned within, the egg's cortex. The mechanism by which this movement takes place has not been established, although investigations demonstrating that cytochalasin B inhibits the cortical localization of the meiotic spindle suggests that it may be an actin-mediated process (Longo, 1987b).

Anaphase I and II, as well as the formation of the first and second polar bodies, followed in quick succession, as occurs in the surf clam *Spisula* (see Longo, 1983). Formation of the female pronucleus was evident subsequent to the formation of the second polar body by the formation of an expanding mass of material staining with either DAPI or Hoechst. The rate of expansion of the forming female pronucleus was comparable to that of the male pronucleus, suggesting that the two chromatin masses may be regulated by similar mechanisms. A corresponding relationship has also been demonstrated in polygynic and polyspermic *Spisula* zygotes (Luttmer and Longo, 1988).

The kinetics of sperm nuclear expansion in fertilized *Mulinia* eggs is in agreement with previous studies demonstrating that sperm nuclear transformations share a temporal relationship with changes of the maternal chromatin (Das and Barker, 1976; Da-Yuan and Longo, 1983; Yamashita, 1985; Luttmer and Longo, 1987, 1988; Wright and Longo, 1988; Longo, 1989). Measurements of sperm nuclear expansion in *Mulinia* zygotes indicates that this process takes place in three distinct phases temporally correlated with meiotic maturation of the maternal chromatin. In previous studies, as well as in the one reported here, the incorporated sperm nucleus undergoes a period of rapid expansion followed by one of no enlargement or condensation. This is succeeded by a

dramatic expansion of the sperm nucleus leading to a male pronucleus similar in size to that of the female. The three phases of sperm nuclear enlargement in *Mulinia* correlate with germinal vesicle breakdown, polar body formation, and female pronuclear development, respectively. The kinetics of sperm nuclear expansion is similar to that described in the surf clam, *Spisula solidisima,* where four phases were observed based on closer sampling times than those taken during the course of the present study (Luttmer and Longo, 1988). In *Spisula,* the sperm nucleus, upon incorporation, underwent little change in size until germinal vesicle breakdown. Additionally, during the period of polar body formation, the expanded sperm nucleus of *Spisula* underwent a significant reduction in size; *i.e.,* it condensed. A reduction (one time point) in size of the expanded sperm nucleus of *Mulinia* zygotes was observed during polar body formation. We suspect that with closer sampling times, this reduction, as well as the status of the incorporated sperm nucleus prior to germinal vesicle breakdown, would become apparent in *Mulinia* zygotes.

Expansion of the sperm nucleus following germinal vesicle breakdown is consistent with other studies demonstrating that mixing of germinal vesicle substances with the cytoplasm precedes sperm nuclear changes (Masui and Clarke, 1979; Longo, 1981; Schuetz and Longo, 1981; Hirai *et al.,* 1981; Yamada and Hirai, 1984). This change in the sperm nucleus may be a manifestation of sperm basic protein replacement by histones present in the oocyte cytoplasm. Histone changes that occur with the early onset of sperm chromatin dispersion have been demonstrated (Poccia *et al.,* 1978, 1981; see Poccia, 1986). Because agents affecting meiotic maturation of the maternal chromatin also affect the kinetics of sperm nuclear expansion (Luttmer and Longo, 1988; Wright and Longo, 1988), factors regulating the status of the maternal chromatin during polar body formation probably act on the transformed sperm nucleus such that it ceases expansion and in some instances condenses; *e.g.*, surf clam, hamster, and starfish (Luttmer and Longo, 1988; Wright and Longo, 1988; Longo, 1989).

The second expansion of the sperm nucleus, which is correlated with enlargement of the maternal chromatin and female pronuclear formation (Zirkin *et al.,* 1989), is set into motion as a result of cell cycle changes within the fertilized egg that affect both the maternally and paternally derived chromation (Longo, 1989). In the case of *Mulinia,* as well as other species that have been studied to date, both chromatin masses undergo dramatic rates of expansion to form enlarged pronuclei (Luttmer and Longo, 1988; Wright and Longo, 1988). Unlike the situation seen in mammalian zygotes (Wright and Longo, 1988), expansion of the maternally and paternally derived chromatin resulted in pronuclei of nearly equal size (see also Luttmer and Longo, 1989).

Results presented here demonstrate that pronuclear fusion in *Mulinia* does not occur as in sea urchins (fertilized at the completion of meiotic maturation) or as in other cellular systems (Longo and Anderson, 1968). Rather, both the male and female pronuclei, as separate bodies, synchronously undergo prophase events in preparation for first mitosis. The chromosomes from each pronucleus, which replicated during the period following polar body formation, become aligned on the metaphase plate of the mitotic spindle and separate at anaphase into two masses consisting of both maternally and paternally derived chromosomes. Hence, maternal and paternally derived chromosomes do not become enclosed within the same nucleus until formation of the two-cell stage.

The effects of ultraviolet irradiation on sperm transformations that lead to the development of male pronuclei in *Mulinia* are consistent with what has been shown in other systems (Onozato and Yamaha, 1983; Arai *et al.,* 1984). Ultraviolet irradiation disrupts the DNA helix and thus interferes with the proper duplication of chromosomes prior to first cleavage (Strickberger, 1976). We anticipated that the effects of ultraviolet irradiation might be manifested at two periods during fertilization: (1) during transformation of the sperm nucleus into a male pronucleus, indicative of gross DNA disruption; and (2) subsequent to DNA replication, during the period in which the paternally derived chromosomes were engaged in mitosis. Alterations were not apparent during any of the stages leading to a male pronucleus, possibly due to an insensitivity of the method of analysis, or more likely to an inability to achieve concomitant high levels of irradiation and fertilization. (Higher doses of ultraviolet irradiation were tested but resulted in an inhibition of fertilization.) Radiation effects were seen only after pronuclear association—*i.e.,* during prophase and anaphase of the first cleavage division—and involved variable numbers of chromosomes. The manner in which the male and female pronuclei become associated in *Mulinia* and other molluscan eggs (see Longo, 1983), as well as parameters affecting both the quantity and quality of ultraviolet irradiation, call into question the effectiveness of using ultraviolet irradiation to form gynogenetic molluscan embryos. Induction of gynogenesis with variable results has been achieved by a variety of techniques, including irradiation of sperm with ultraviolet light (Chourrout, 1980; Streisinger *et al.,* 1981; Onozato and Yamaha, 1983; Lou and Purdom, 1984; Onozato, 1984; Suzuki *et al.,* 1985). Variability in cases employing ultraviolet irradiation (Chourrout, 1980; Onozato and Yamaha, 1983; Arai *et al.,* 1984) appeared to be due to difficulties in controlling parameters associated with the exposure of sperm to ultraviolet rays

and an inability to uniformly and effectively destroy all of the paternally derived DNA.

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