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Sperm Concentration and the Fertilization of Human Eggs In Vitro

D. P. WOLF,[1] W. BYRD,[2] P. DANDEKAR[3] and M. M. QUIGLEY[4]

Departments of Obstetrics, Gynecology and Reproductive Sciences
The University of Texas Health Science Center at Houston
Houston, Texas 77030

ABSTRACT

The effect of sperm concentration on the fertilization of preovulatory and immature human eggs was studied in the context of an ongoing in vitro fertilization-embryo transfer (IVF-ET) program. Fertilization success was independent of the follicular recruitment protocol used, and with preovulatory eggs, was inversely related to sperm concentration over the range of 2.5 – 50 × 10^4 motile sperm/ml. Maximum fertilization (80.8%) occurred at a concentration of 2.5 × 10^4 motile sperm/ml. The incidence of polyspermic fertilization was directly related to the sperm concentration, decreasing from 5.5% at 10 × 10^4 to 0% at 1–2.5 × 10^4 motile sperm/ml. Immature eggs cultured in vitro, then inseminated, also demonstrated an inverse realtionship between fertilization and sperm concentration with a maximum fertilization rate of 66.6% at 5 × 10^4 motile sperm/ml. The percentage of motile sperm in the inseminating population had no influence on fertilization rates unless the value dropped below 40%. Fertilization success using sperm from oligospermic and polyzoospermic males was also examined. In contrast to males with normal semen parameters, oligospermic males demonstrated highest fertilization success at 50 × 10^4 motile sperm/ml. The IVF of preovulatory eggs using sperm from polyzoospermic males was comparable to that for males with normal semen parameters at equivalent sperm concentrations. The implications of these findings to the application of IVF-ET technology to the infertile couple is discussed.

INTRODUCTION

The fertilization of follicular eggs in vitro by washed, ejaculated sperm with subsequent transfer of the cleaving embryo to the uterus is an established treatment modality for infertility of absolute tubal etiology in the human (Edwards et al., 1980; Trounson and Wood, 1981). Clinical pregnancy rates have gradually increased so that success can be anticipated in approximately 15–20% of the patients to whom a transfer is made (Jones et al., 1982; Grobstein et al., 1983; Byrd and Wolf, 1984). An increase in the average number of embryos transferred back to each patient largely accounts for these improved rates.

An initial concern in establishing an in vitro fertilization-embryo transfer (IVF-ET) program was the high sperm concentrations (50–100 × 10^4 sperm/egg) used for the fertilization of human eggs in vitro (Lopata et al., 1980; Trounson et al., 1981; Testart et al., 1982; Edwards et al., 1983; Wortham et al., 1983). These concentrations or sperm-egg ratios are extremely high compared with the number of sperm estimated to be at the site of fertilization in vivo, i.e., on the order of a few hundred (Settlage et al., 1973). Although it is important to utilize sperm concentrations in vitro that result in high levels of fertilization, it is important also to avoid polyspermic fertilization since this outcome in mammals leads to abnormal development and fetal wastage (Edwards, 1981). Polyspermic fertilization of human eggs has been reported (Evans et al., 1980; Sathananthan and Trounson, 1982a); however, the relationship between sperm concentration and the incidence of polyspermy has not been evaluated. This relationship is complicated by the fact that IVF involves the recovery of eggs of varied maturity (Testart et al., 1983a). Immature eggs (those which have not undergone germinal vesicle breakdown) require a prolonged maturation in vitro before they are fertilizable (Veeck et al., 1983), and they may be more susceptible to

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[1] Reprint requests.

[2] Present address: Dept. of Obstetrics and Gynecology, The University of Texas Health Science Center at Dallas, Dallas, TX 75235.

[3] Present address: Dept. of Obstetrics, Gynecology and Reproductive Sciences, The University of California Medical School, San Francisco, San Francisco, CA 94143.

[4] Present address: Dept. of Gynecology, Cleveland Clinic Foundation, 9500 Euclid Avd., Cleveland, OH 44106.

polyspermic fertilization due to the incomplete maturation of cortical granules (Sathananthan and Trounson, 1982).

IVF-ET was limited originally to couples with infertility of absolute tubal etiology, however, with success has come expansion in the use of IVF-ET to treat other types of infertility problems, such as endometriosis, unexplained and immunologic infertility. This technology also seems appropriate for oligospermic males and/or males with abnormal semen parameters, since sperm-egg ratios can be controlled and the number of sperm required to effect fertilization is relatively small (Fishel and Edwards, 1982).

In the present report, we systematically evaluate fertilization outcome for preovulatory as well as immature eggs inseminated over a 50-fold range in motile sperm concentration. The incidence of polyspermy, and the fertility of males with abnormal semen analyses, is examined. Also, a brief ultrastructural description of a polyspermic human egg resulting from IVF is presented in which we attempt to confirm dispermic fertilization.

MATERIALS AND METHODS

Patient Population

Patients included in this study were undergoing IVF-ET as treatment for infertility of absolute tubal etiology and/or oligospermia. All patients ovulated predictably, as evidenced by regular menstrual periods and appropriate mid-luteal phase serum progesterone levels (>12 ng/ml) in spontaneous cycles. Males were defined as "normal" if the semen analysis showed a sperm density of $\geq 20 \times 10^6$ sperm/ml, a motility of >50%, a progression of at least 2 (scale 0–4) and the presence of at least 60% normal morphology (Amelar and Dubin, 1982). Oligospermic males treated in this program had sperm counts of less than 20×10^6 sperm/ml but at least 2.5×10^6 motile sperm/ml in the ejaculate, while polyzoospermic males had sperm densities $\geq 250 \times 10^6$/ml.

Five different follicular recruitment programs were used during the period 1981–1984: *Trial 1*--clomiphene citrate 150 mg/day, Days 5–9; *Trial 2*--clomiphene citrate 50 mg/day, Days 5–9; *Trial 3*--clomiphene citrate 50 mg/day, Days 5–9, combined with hMG (human menopausal gonadotropin; Pergonal, Serono Labs., Randolph, MA) 2 ampules (amps)/day, Days 6, 8 and 10; *Trial 4*--clomiphene citrate 50 mg/day, Days 5–9, combined with hMG 1 amp/day, Days 5–9, plus individualized levels until the day of human chorionic gonadotropin (hCG); and *Trial 5*--hMG alone, 4 amps/day from Day 3 until a minimum of two follicles reached at least 16 mm. Human hCG (Profasi HP, Serono Labs.) was given when the mean diameter of the largest follicle reached at least 20 mm (two follicles 16 mm or greater for Trial 5). The patient received 2500–5000 IU of hCG to induce preovulatory egg maturation, and follicular aspiration was performed 36 h later. Most accessible follicles greater than 10 mm were aspirated; however, aspiration was usually discontinued after the recovery of five preovulatory eggs.

In Vitro Fertilization

The culture medium employed was a modified Ham's F10 supplemented with heat-inactivated (56° for 30 min) maternal serum (Lopata et al., 1980; Sokoloski and Wolf, 1984). Eggs, recovered from follicular aspirates, were rinsed in culture media containing 7.5% maternal serum, and preincubated for 5–6 h in organ culture dishes (Falcon #3037) in a humidified atmosphere of 5% CO_2:5% O_2:90% N_2 before insemination (modified from Trounson et al., 1982). Semen was collected by masturbation, and a routine semen analysis was performed following at least 30 min of liquifaction at 37°C. Two aliquots of semen (usually 1 ml each) were diluted with 3 volumes of culture medium containing 7.5% maternal serum and centrifuged for 10 min at $500 \times g$; the supernatants were removed by aspiration before the wash was repeated. The final sperm pellet was resuspended in 1 ml of culture medium containing 7.5% maternal serum, and preincubated 5 to 6 h before the wife's eggs were inseminated. With oligospermic males, sperm pellets were reconstituted in 0.5 ml to effect a doubling in the final sperm concentration. Inseminations were conducted in organ culture dishes containing 0.9 ml of medium with $1–100 \times 10^4$ motile sperm. After approximately 16 h, the adhering cumulus and corona cells were removed mechanically, and the egg was examined for the presence of pronuclei. Two pronuclei were taken as presumptive evidence of fertilization. Eggs were transferred to growth medium (Ham's F10 containing 15% maternal serum) and examined for cleavage (2 or 4 cells) at 38–42 h postinsemination. All normally cleaving embryos were transferred to the donors uterus at approximately 40–48 h postinsemination, except in the rare instance where eggs matured in vitro were transferred at the pronuclear stage with or without cleaving embryos. Clinical pregnancies were established exclusively in patients receiving the lower dose (50 mg) of clomiphene citrate (Trial 2; Quigley et al., 1983) which was sometimes supplemented with hMG (Trials 3 and 4).

Electron Microscopy

For microscopy, the egg was fixed in 2.5% glutaraldehyde in cacodylate buffer at pH 7.2. After postfixation in 1% osmium tetroxide and dehydration in ethanol, the ovum was flat-embedded in Spurr plastic (EM Sciences, Fort Washington, PA). Serial sections were cut on a Sorvall MT II ultramicrotome. Thick sections (0.5 – 1 micron) were stained with toluidine blue and examined with a Leitz photomicroscope, while thin sections were stained with uranyl acetate, followed by lead citrate and examined with a JOEL 100B electron microscope.

Statistical Analysis

The oocyte was viewed as the unit of observation, with the outcome classified as fertilized or unfertilized.

Fertilization rates were compared among different motility levels, sperm concentrations and follicular recruitment protocols using chi-square analysis. Confidence limits were taken from tables in Beyer (1968), confidence limits for proportions (confidence coefficient 0.95).

RESULTS

Sperm Concentration, Sperm Motility and the Fertilization of Preovulatory Eggs In Vitro

Initial attempts at fertilization of human eggs in vitro were made with sperm concentrations of 50–100 x 10^4 motile cells/ml. Although concentrations of either total or motile sperm in this range have been used by most active groups and support acceptable levels of fertilization, concern over the possibility of polyspermic fertilization encouraged exploration of egg fertilizability at lower sperm concentrations. To begin these studies we confined our attention exclusively to patients from whom three or more preovulatory eggs were recovered. In these cases, two or more eggs were inseminated at the standard sperm concentration and the remainder at a lower concentration. If and when high fertilization rates were established at the lower concentration, then it became the standard. During these studies, several different follicular recruitment protocols were employed. The fertilizability of preovulatory eggs with sperm from normal males was independent of the follicular recruitment protocol used (Fig. 1). No statistically significant difference between follicular trials at any one sperm concentration was noted. Therefore, data from all patients were pooled (Table 1). Statistical analysis of sperm concentrations from 50 x 10^4 to 2.5 x 10^4 motile sperm/ml demonstrated a significant

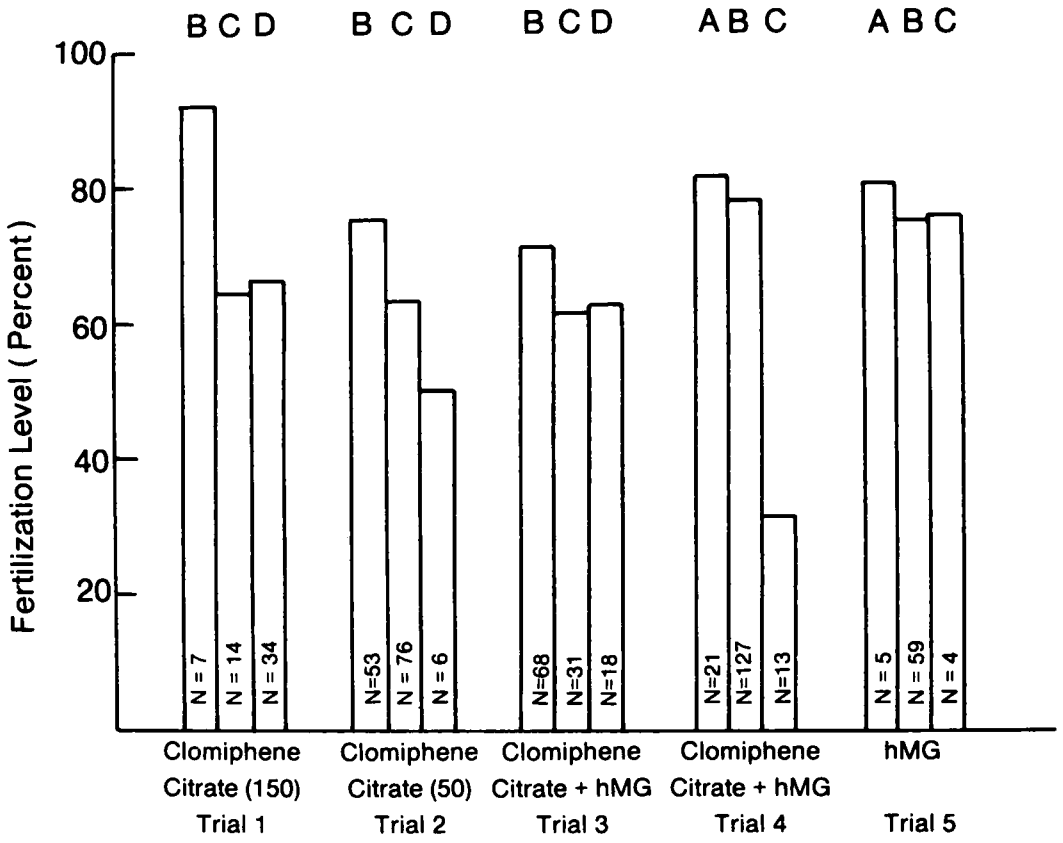

FIG. 1. Fertilization success following insemination of preovulatory eggs at different motile sperm concentrations. Five different follicular recruitment trials were utilized (see *Materials and Methods* for details). Motile sperm concentrations used to inseminate eggs: *A*) 2.5 X 10^4, *B*) 5 X 10^4, *C*) 10 X 10^4, and *D*) 50 X 10^4.

TABLE 1. Fertilization of human preovulatory eggs in vitro with sperm from males with normal semen parameters.

Sperm concentrations (motile sperm/ml)	Number of of eggs inseminated	Percentage of eggs fertilized
500,000	65	60.0 (47.0–71.9)[a]
100,000	146	60.9 (52.4–68.7)
50,000	351	75.5 (67.9–77.8)
25,000	26	80.8 (60.6–93.5)
10,000	7	28.6 (3.7–71.0)

[a]Figures in brackets represent 95% confidence limits. The confidence limits represent two times the standard error of the mean.

inverse linear trend with respect to fertilizability (chi-square for regression=12.3, P=0.001; total chi-square=13.4, P=0.04). These data also suggested that there may be a threshold below which fertilization may decline, but the sample size was too low to make a definitive conclusion. Sperm concentrations in the range of 2.5–5.0 x 10^4 motile sperm/ml appeared to be optimal for human IVF.

The incidence of polyspermy in these preovulatory eggs also showed a marked dependence on sperm concentration. At 10 x 10^4 motile sperm/ml (N=146), 5.5% of the eggs showed evidence of 3 pronuclei, while at 5 x 10^4 motile sperm/ml, only 1.4% of 351 eggs were polyspermic (P<0.01). Polyspermia has not been observed, in our experience, with inseminating concentrations below 5 x 10^4 motile sperm/ml (N=33).

Sperm motility is undoubtedly an essential requirement for the fertilization of preovulatory eggs. We selected for motile sperm by taking only sperm that remained in suspension as they underwent capacitation. The percentage of motile cells in this suspension almost always increased over the original ejaculate and at least 60% of the cells were motile in the majority (78%) of sperm suspensions (Fig. 2). A significant difference was found in fertilization between different motility levels (chi-square=21.07, P=0.002). However, this difference was due to the marked decrease in fertilization of eggs at sperm motilities below 40%. When cases below 40% were excluded from analysis, no significant differences were noted between groups (chi-square=9.27; P=0.1).

Microscopic Observations of a Trinuclear Egg

In an attempt to corroborate the diagnosis of polyspermy made by light microscopy (3 pronuclei) an ultrastructural examination was undertaken. This egg, inseminated at 50 x 10^4 sperm/ml, was surrounded by an intact zona pellucida, but was devoid of cumulus and corona cells (Fig. 3a-d). The presence of three pronuclei, two in one section (Fig. 3e) and a third in a different section was confirmed in serial thick sections of the egg (Fig. 3f). Short microvilli characterized the egg surface except near the polar bodies where they were longer

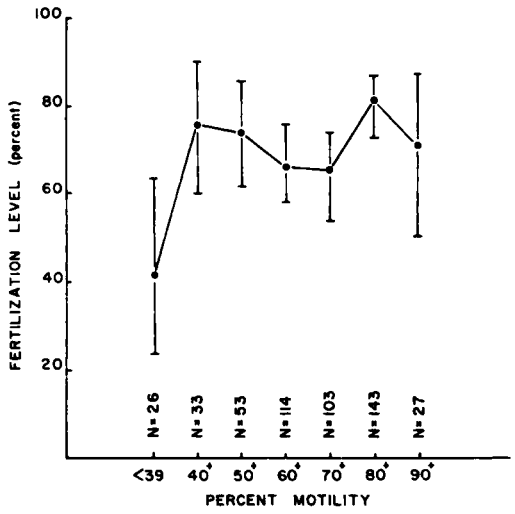

FIG. 2. Fertilization success of preovulatory eggs as a function of the percentage of motile sperm present prior to insemination. Bars represent 95% confidence limits. The limits represent two times the standard error of the mean.

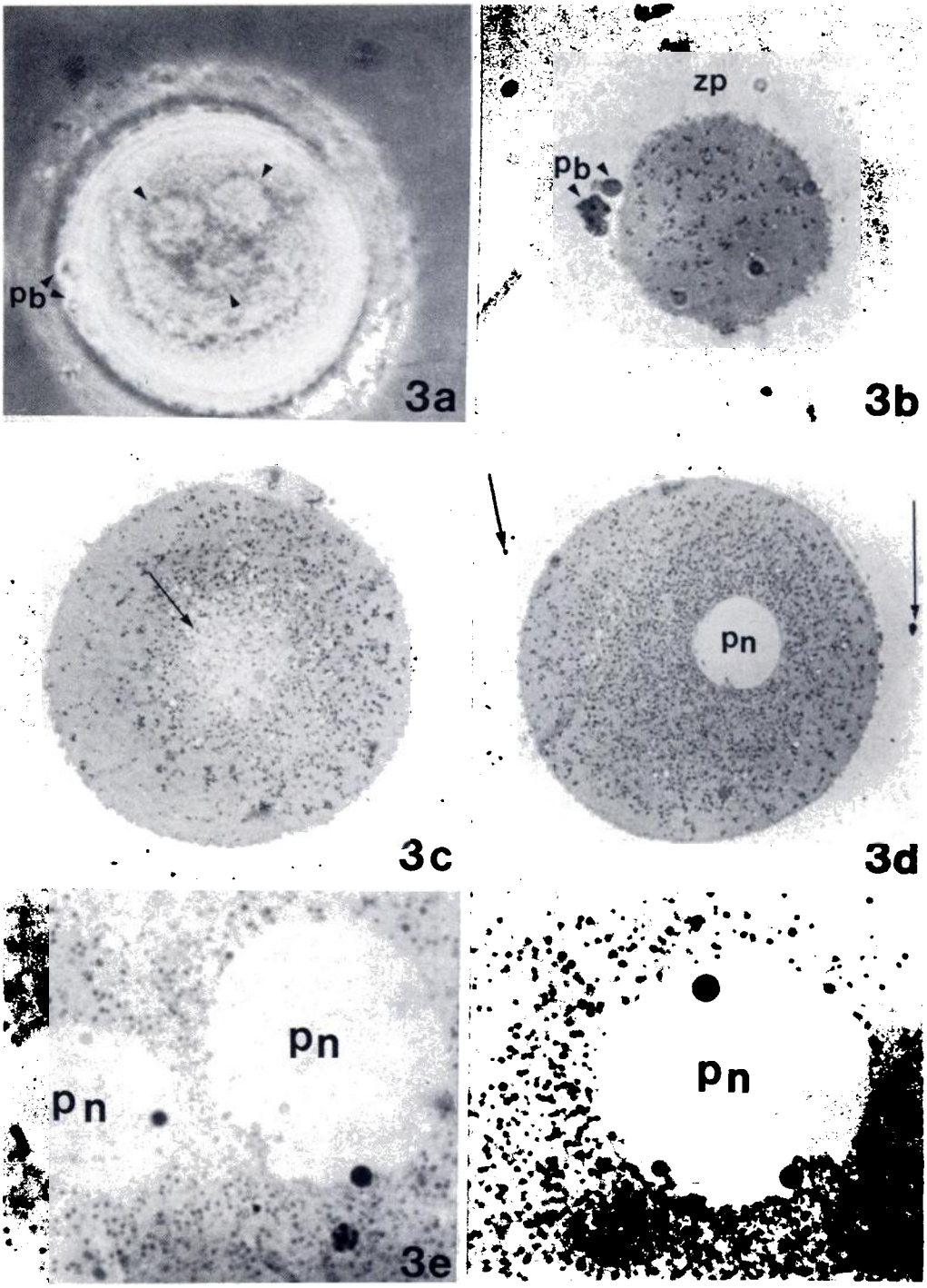

FIG. 3. Light micrographs of a human egg fertilized in vitro. *a*) Phase micrograph of a polyspermic egg, *arrows* point to three pronuclei in the egg. Polar bodies (*Pb*) are seen in the perivitelline space. X400. Serial thick section of the same egg in (*b*, c and d). The *arrow* in *c* denotes a central mesh-like area; *arrows* in *d* identify sperm adhering to the zona. Two polar bodies are seen in the pervitelline space; zona pellucida (*Zp*). X400. *e*) Two are pronuclei (*Pn*) each with one nucleolus in one section. X1000. *f*) Third pronucleus in another section with three nucleoli. X1000.

and thinner (Fig. 4a). Cortical granules, present in the cortex of unfertilized eggs, were absent, although several granules were seen undergoing exocytosis (Fig. 4a *inset*). The limiting membrane underneath these granules was electron dense, reflecting perhaps a mosaic membrane resulting from fusion of egg plasma and cortical granule membranes. At higher resolution, an irregular-shaped first polar body was observed in the perivitelline space (Fig. 4b), identified by cortical granules underneath a microvillar-rich plasma membrane and by filamentous chromatin which was not membrane limited (Zamboni, 1971; Fig. 4b). In contrast, the second polar body was ovoid in shape with a relatively smooth plasma membrane, few cortical granules in the cortex, and dense chromatin that was membrane limited (Fig. 4c).

The presence of two sperm tails would corroborate a diagnosis of dispermia and remnants of the tail(s) were observed (Fig. 5a and b). Additionally, structure resembling disintegrating sperm external dense fibers (Fig. 5c and d), as well as clusters of swollen sperm mitochondria, were present (Fig. 5e-g). It was not possible, however, to confirm unequivocally the presence of two penetrating sperm on the basis of these observations.

Fertilization of Immature Eggs

With exogenous hormonal stimulation, immature eggs were occasionally recovered, characterized by the presence of a germinal vesicle, tightly adhering granulosa cells and the absence of an expanded cumulus mass. These immature eggs were matured in vitro for 24–36 h and inseminated. Immature eggs showed significantly impaired fertilizability at high sperm concentrations. At 5×10^4 motile sperm/ml, 66.6%, of the eggs (N=18) fertilized as compared with only 25.0% at 10×10^4 (N=16), and 20% at 50×10^4 (N=5). Fertilization of immature eggs at $10-50 \times 10^4$ motile sperm/ml was significantly ($P<0.05$) reduced below that seen for preovulatory eggs. No polyspermy was observed in this limited experimental series.

Fertilization by Males with Abnormal Semen Parameters

The relationship between sperm concentration and the fertilization of preovulatory eggs with sperm from oligospermic males is shown in Table 2. Fertilization decreased significantly as the sperm concentration was lowered, from a maximum fertilization rate of 61.5% at 50×10^4 sperm/ml (N=26) to 23% at $5-10 \times 10^4$ motile cells/ml (N=13). Moreover, at $5-10 \times 10^4$, but not at 50×10^4 motile cells/ml, results for the oligospermic male were significantly reduced below males with normal semen parameters ($P<0.05$). Since the number of motile cells was the same in the insemination dish, this finding is suggestive that parameters in addition to sperm numbers were affected in oligospermic males. As additional evidence for this conclusion, we compared fertilization rates between oligospermic males and a subset of normal males showing comparable parameters in the inseminating sperm suspension (after washing and preincubation). These values were $10.4 \pm 4.3 \times 10^6$ cells/ml with a percent motility of 60.2 ± 1.5 (N=39) in the case of the oligospermic males and $9.0 \pm 1.9 \times 10^6$ sperm/ml and 67.8 ± 10.3 motility, (N=20) for the subset of males with normal semen parameters. The combined fertilization rates at all sperm concentrations was 75.5% in the subset of normal males versus only 48.7% in oligospermic males. In contrast, when abnormally high sperm concentrations were observed in the ejaculate (polyzoospermia) there were no adverse effects on the in vitro fertilization of preovulatory eggs (Table 2). These fertilization rates were not statistically different from those obtained with sperm from normal males.

DISCUSSION

A major conclusion of the present study is that in men with normal semen parameters, motile sperm concentrations of 2.5 to 5×10^4 motile cells/ml support high levels of fertilization of both immature and preovulatory eggs in vitro, and that higher sperm concentrations actually impair fertilization. This concentration range is not necessarily optimal for the in vitro fertilization of human eggs and, in fact, there are indications that concentrations as low as 1×10^4 can be used (Craft et al., 1981; Sathananthan and Trounson, 1982; present study). Although limited fertilization success has been reported with sperm/egg ratios approaching unity in the hamster (Bavister, 1979), it is of interest to note that the concentrations employed here for human IVF are considerably lower than the optimal concentrations required for the IVF of rodent and other mammalian eggs (Rogers, 1978). This relatively low sperm concentration requirement in the human may

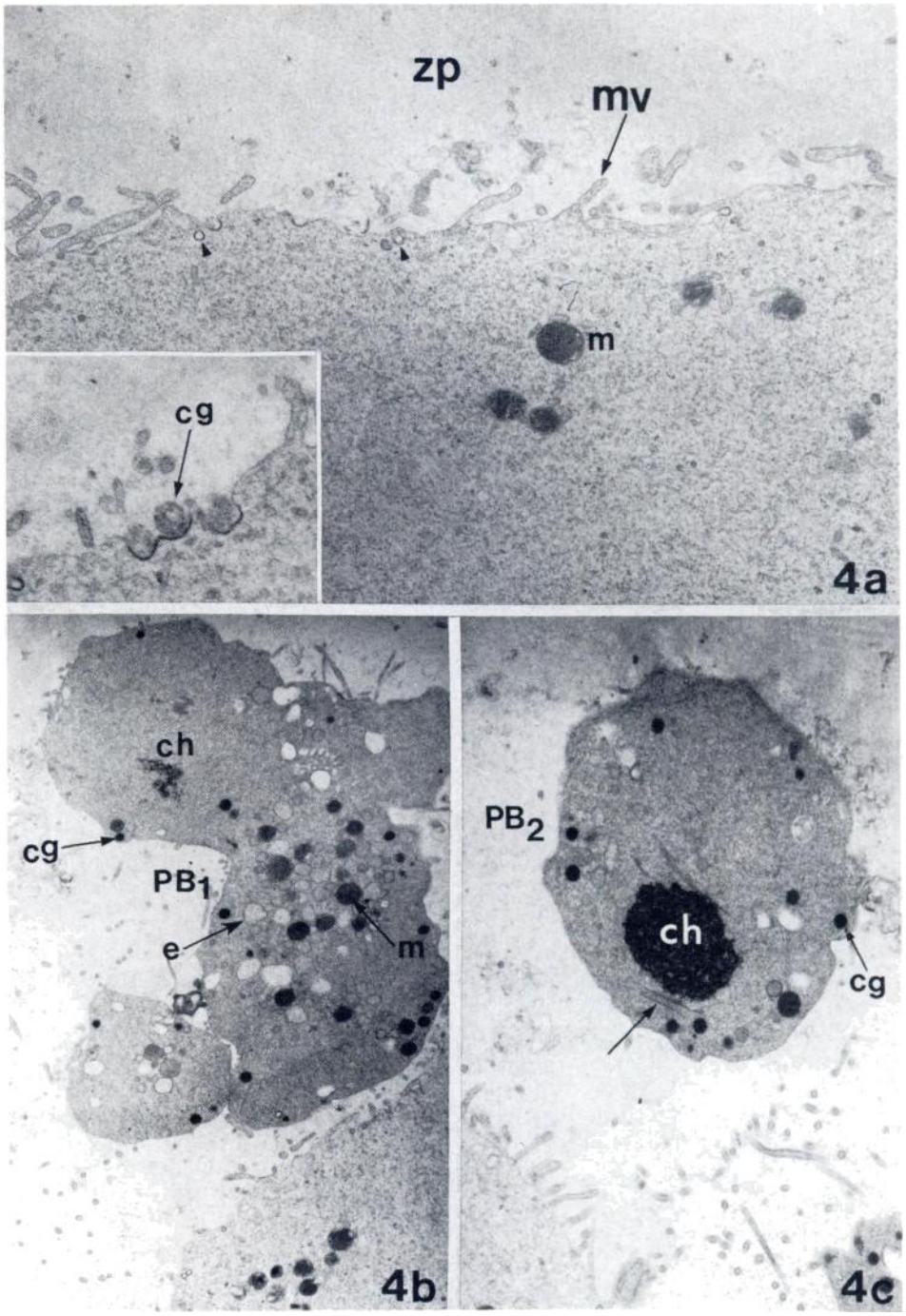

FIG. 4. *a*) Transmission electron micrographs of the polyspermic egg. Egg plasma membrane showing short microvilli (*mv*) projecting into the perivitelline space which is surrounded by the zona pellucida (*Zp*). ×14,000. Mitochondria can be seen in the section, note the absence of cortical granules in the egg cortex. Exocytosis of cortical granules (*cg*) at the egg plasma membrane can still be seen in insert. ×25,000. *b*) Irregular-shaped polar body showing cortical granules, fibrillar chromatin (*cb*), mitochondria and vesicles of smooth endoplasmic reticulum (*e*). ×11,000. *c*) Second polar body with well-developed nucleus and a few cortical granules; microtubules (*arrow*) from second polar body extrusion are present. ×14,000.

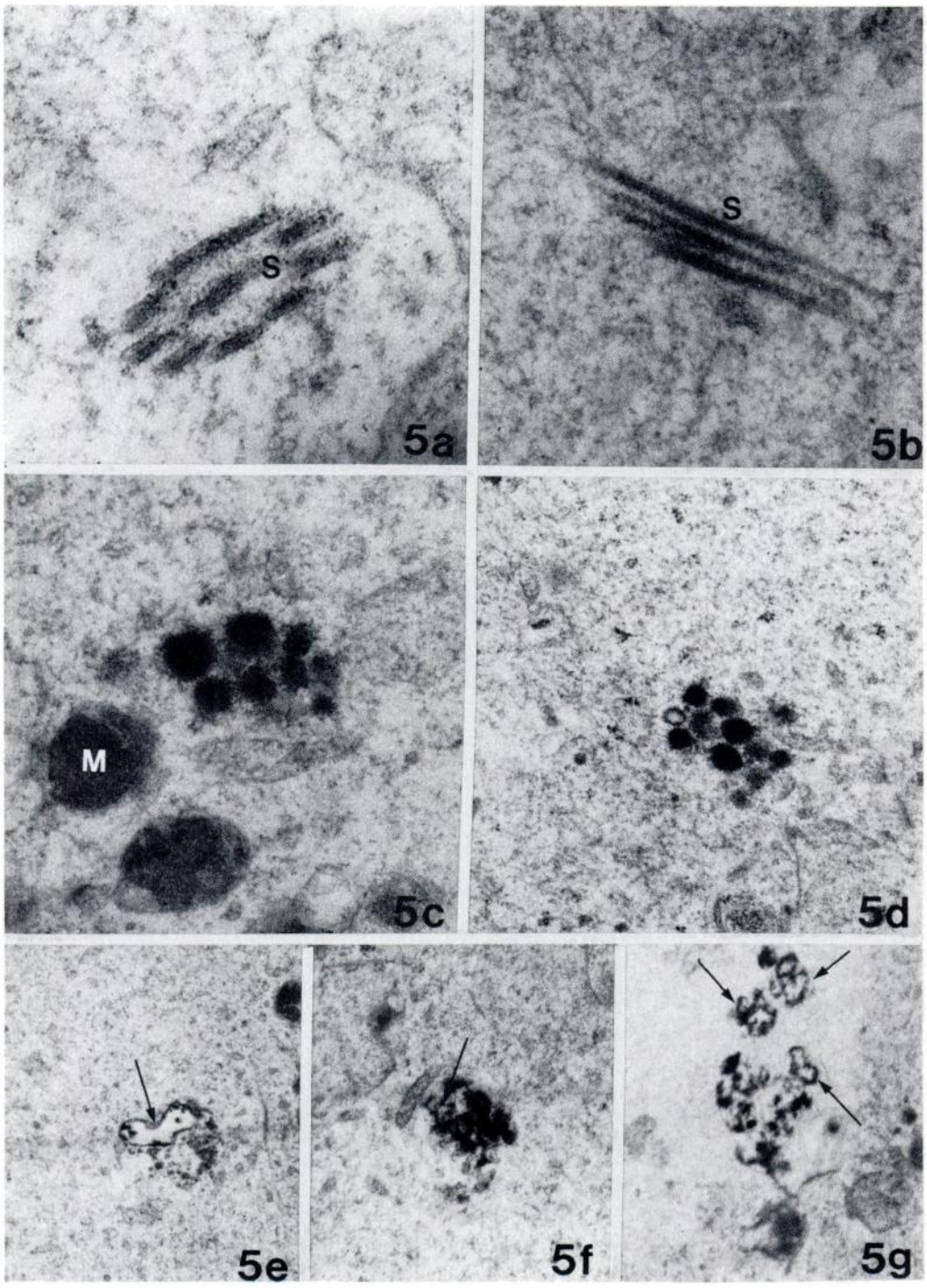

FIG. 5. Sperm tail remnants in the penetrated egg cytoplasm. *a*) Sperm tail(s) as an axial filament in oblique cross section. ×121,000. *b*) Appearing as double microtubules in a tangential section. ×76,000. *c* and *d*) Disintegrating sperm external coarse fibers. Egg mitochondria (*M*) in *c*, ×50,000; *d*, ×35,000; *e*, *f* and *g* sperm mitochondria swelling and signs of degeneration (*arrows*). *e*=×20,000; *f*=×36,000; *g*=×24,000.

TABLE 2. Fertilization of human preovulatory eggs in vitro with sperm from males with abnormal semen parameters.

	Sperm concentration (motile sperm/ml)	Number of eggs inseminated	Percentage of eggs fertilized
Oligospermic males	500,000	26	61.5 (40.5–79.8)[a]
	50,000 to 100,000	13	23.0 (5.0–53.8)
Polyzoospermic males	100,000	9	55.6 (21.2–86.3)
	50,000	57	78.9 (66.1–88.6)
	25,000	4	75.0 (19.4–99.4)

[a]Figures in brackets represent 95% confidence limits. The confidence limits represent two times the standard error of the mean.

reflect the ease with which human sperm capacitation can be effected in vitro. As observed in the present studies, even with normal semen parameters and optimal sperm numbers, some preovulatory eggs remain unfertilized after insemination. Undoubtedly, both under- and overripe eggs are involved and there is evidence that follicular fluid steroid levels may serve as a prognosticator of fertilizability (Fishel et al., 1983).

The relationship between sperm concentration and fertilization outcome for men with normal sperm parameters was in marked contrast to the results observed with oligospermic males. Maximal levels of fertilization were achieved at 50×10^4 motile sperm/ml in men who had an initial count of less than 20×10^6 sperm/ml in the ejaculate. Since inseminations are normalized to equivalent concentrations of motile sperm, the requirement for higher sperm concentrations in the oligospermic male suggests that additional, unrecognized factors must also be involved. Awareness of the unique relationship between sperm concentration and fertilization success in the oligospermic male may carry significance for the success of IVF-ET. Fishel and Edwards (1982) have reported 2 pregnancies involving oligospermic males, while Testart et al. (1983b) and Trounson and Wood (1981) report limited success with fertilization but no pregnancies. Similarly, in our experience, although eggs can be fertilized at the higher sperm concentrations, we have not yet established a clinical pregnancy in this patient category.

Polyzoospermia is an ill-defined condition that has been associated with reduced fertility and increased rates of spontaneous abortion in vivo (Joel, 1966; Barnea et al., 1980; Rehan et al., 1982). Apart from sperm density, other semen parameters in polyzoospermic males such as morphology, motility and mucus-penetrating ability appear normal (Glezerman et al., 1982), although impaired fertility has been associated with reduced seminal fructose and prolactin levels (Singer et al., 1979). In the present study, sperm from polyzoospermic males fertilized eggs in vitro at levels equivalent to those seen for normal males. This suggests that either the in vitro conditioning of sperm overcomes any inherent sperm dysfunction or else that the reduced fertility observed in vivo is associated with impairments in other processes such as embryonic development or implantation.

The high sperm/egg ratios employed in human IVF raise the possibility of an unacceptable level of polyspermy (Evans et al., 1980) as well as exposure of eggs to metabolic and degenerative by-products of sperm. Under natural conditions where the sperm to egg ratio is in the range of 100 to 1, the incidence of aneuploid conceptions may be as high as 7.5% of recognized conceptions (summarized by Golbus, 1981), and among first-trimester spontaneous abortions polyploidy occurs at a frequency of approximately 10% (Simpson et al., 1982). In a retrospective study of 1499 fetuses, Boué et al. (1975) found abnormal karyotypes in 39% of the cases of which 25% were polyploids. Cytogenetic analysis of triploid abortuses revealed that 66% were due to dispermic fertilization, 23% due to fertilization by diploid sperm, while the rest were the result of monospermic fertilization of diploid eggs (Jacobs et al., 1978). It is clear, therefore, that polyspermic fertilization is a highly undesirable outcome and must be avoided. In the present study, poly-

spermic fertilization decreased from 5.5% to 1.4% when the sperm concentration was decreased from 10×10^4 to 5×10^4 motile cells/ml. Although it is reasonable to assume that polyspermy frequency will decline even further with additional decreases in sperm concentration, a level of approximately 1% is acceptable from our present perspective. Such low levels of polyspermy have not always been observed in the IVF of human eggs. Trounson et al. (1982) demonstrated that polyspermy frequency was related to the state of maturity of eggs recovered from preovulatory follicles. Eggs that were inseminated immediately (30×10^4 sperm/ml) exhibited polyspermy levels approaching 30% while only 7% of the preovulatory eggs that had been matured in culture for 5 to 6 h before insemination became polyspermic, perhaps suggesting that cortical granule maturation was incomplete in the former group. Only one occurrence of a triploid fetus resulting from IVF-ET has been reported (Steptoe et al., 1980) but the origin of the triploidy was not ascertained.

The block to polyspermy in human eggs is primarily at the level of the zona pellucida since supplemental sperm are seldom seen in the perivitelline space of monospermic eggs (Soupart and Strong, 1974, 1975; Sathananthan et al., 1982). An essential component of a functional zona block is cortical granule exocytosis and modification of sperm receptors in the zona pellucida by cortical granule contents (Yanagimachi, 1981; Wassarman, 1983). Sathananthan and Trounson (1982) have correlated polyspermy frequency with the status of egg cortical granules in the human; immature eggs that do not have a complete complement of granules and undergo either delayed or incomplete cortical granule release may be at increased risk of multiple sperm penetration. In the egg examined in the present study, cortical granules were absent except for those seen undergoing discharge. Since this egg was fixed many hours after sperm-egg fusion, delayed exocytosis may have occurred. An alternate hypothesis is that the egg underwent an adequate exocytotic response and the polyspermic condition resulted from simultaneous penetration by two sperm. Our ultrastructural analysis, although not conclusive, was consistent with the diagnosis of polyspermy. Polygyny as a mechanism seems unlikely since both first and second polar bodies were identified in the perivitelline space. Several other authors have also described the dispermic human egg (Soupart, 1974; McMaster et al., 1978). In our experience, some polyspermic eggs resulting from in vitro fertilization can undergo cleavage, resulting in normal-appearing 4- to 6-cell embryos at approximately 44 h postinsemination. In addition, the development of triploid fetuses arising from dispermic fertilization in vivo (Boué et al., 1975) suggests that cleavage can occur in these eggs. However, based on the above observations, it is our opinion that a polyspermic embryo should not be transferred to the uterus of the egg donor, the report of Trounson (1982) that triploid embryos can revert to diploids, notwithstanding.

Sperm motility is undoubtedly an essential requirement in successful sperm/egg interaction either in vivo or in vitro. In the present study, we also examined the fertilization of preovulatory eggs as a function of the percent of motile cells in the inseminating suspension. When eggs were insemintaed with equivalent concentrations of motile sperm, fertilization levels were independent of the percentage of motile cells until that percentage dropped below 40%. This decreased fertilizability may reflect a subtle but undetected difference in sperm progression or it may be related to differences in sperm viability. We do not know exactly when actual sperm/egg fusion occurs following inseminations in vitro; presumably it involves a time frame of several hours (Lopata et al., 1978), and motility determinations at the outset of the insemination may not always be relevant to the cells present at the time of fertilization.

Fertilization of the immature egg poses a special problem in IVF-ET. Such eggs are characterized by incomplete meiotic maturation (germinal vesicle stage or prophase of the first meiotic division) and standard laboratory protocol involves culturing for 24–30 h to allow meiotic progression to metaphase II before insemination. A reduced fertilization success compared to preovulatory eggs is expected since not all immature eggs spontaneously mature in vitro (Veeck et al., 1983; Byrd and Wolf, 1984). In agreement with the present findings, Veeck et al. (1983) reported comparable fertilization rates (59%) for immature eggs and, most encouragingly, have recorded two cases in which the transfer of only immature eggs fertilized in vitro led to the establishment of a clinical pregnancy.

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