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# Assisted Fertilization of Mouse Oocytes and Preliminary Results for Human Oocytes Using Zona Drilling

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*The zona-drilling procedure was investigated in mouse oocytes prior to a study on human oocytes. The procedure involved the injection of 5-nl volumes of acidic Hepes-buffered medium at pH 2.5 using a microinjection instrument. Zona-drilled mouse oocytes had significantly higher rates of fertilization (60/99; 61%) than zona-intact oocytes (6/103; 6%) at an insemination concentration of 1 × $10^4$ sperm/ml (P < 0.001). The procedure did not induce parthenogenetic activation of oocytes and more than 97% of zygotes developed to the blastocyst stage. A similar rate of live progeny was observed when zona-drilled (38.0%) and control embryos (38.5%) were transferred to pseudopregnant recipients. Chromosome analyses were performed on zona-intact, zona-free, and zona-drilled oocytes inseminated with varying concentrations of sperm and analysed at the first cleavage division. Zona-free oocytes had high rates of polyploidy (≥40%) with varying insemination numbers but the zona-drilled oocytes did not reveal significant increases in the rate of polyploidy or aneuploidy when compared to controls. In the human studies, zona-drilled oocytes achieved higher rates of fertilization than zona-intact oocytes, with sperm numbers as low as 1 × $10^4$/ml (6/8; 75%). Polyspermic fertilization was observed in 1/2 and 2/6 of fertilized oocytes inseminated with 1 × $10^5$ and 1 × $10^4$ sperm/ml, respectively. With the low sperm concentration 2/4 of those which were normally fertilized developed to healthy blastocysts. These studies suggest that the zona-drilling technique as described can be performed without apparent harm to oocytes and generate normal embryos. Micromanipulation by zona drilling should be explored further as a potential treatment mode for cases of oligo/asthenospermic infertility.*

KEY WORDS: chromosomes; in vitro fertilization (IVF); micromanipulation; oligospermia; zona drilling.

## INTRODUCTION

Improved fertilization of human and mammalian oocytes has been achieved by micromanipulative techniques such as sperm microinjection (1–6), zona drilling (7,8), zona cutting or zona cracking (9,10), and partial zona dissection (11). These techniques may prove to be useful in treating infertility associated with oligo/asthenospermia, as pregnancies have been reported with subzonal spermatozoal microinjection (12) and zona splitting (13,14). However, invasive procedures such as spermatozoal microinjection causes damage to many oocytes (1,14). Furthermore, there is uncertainty about selecting normal sperm for microinjection, as acrosome-defective sperm injected subzonally can fertilize eggs (15).

Zona drilling, on the other hand, is a simple technique which involves making an opening in the zona pellucida by the use of acidic Tyrode's medium. This allows free access of sperm to the oocyte surface (7). Encouraging results have been obtained with mouse oocytes which have been zona drilled and inseminated with low numbers of sperm, and healthy progeny have been born following transfer of resulting embryos to pseudopregnant recipients (7,9). In humans, zona drilling has resulted in fertilization and cleavage of oocytes from patients who had a previous history of failed fertilization (8). However, no pregnancies have yet been reported in women where only zona-drilled embryos have been transferred.

Cytogenetic studies performed on oocytes which failed to fertilize in in vitro fertilization programs have indicated chromosomal aberrations of the or-

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der of 22% (16), 22.2% (17), 11% (18), 22%, and 47.7% (19). The high prevalence of chromosomal abnormality in these oocytes could result from the regimens for ovarian stimulation, age of the woman, methods of oocyte retrieval, or techniques of in vitro culture used (20). The introduction of micromanipulative procedures to enhance fertilization might also have the potential to increase the incidence of chromosomal abnormalities in the embryo.

In the present study, using the mouse as a model, zona drilling was carried out and fertilization was attempted with low numbers of sperm. Checks on the viability of resulting embryos were made both in vitro and in vivo and the effects of zona drilling on the chromosomal makeup of resulting embryos was also studied. The study addresses the safety and effectiveness of the technique and its implications for treating human infertility.

## MATERIALS AND METHODS

### Superovulation and Collection of Mouse Oocytes

Female $F_1$ hybrids (CBA × C57 BL/6) 5–6 weeks of age were superovulated using intraperitoneal injections of 5 IU of pregnant mare serum gonadotropin (PMSG) (Folligon, Intervet, Australia) followed by 5 IU of human chorionic gonadotropin (hCG) 52 hr later. Oocytes were collected 13–14 hr after hCG injection. Cumulus cells were removed with 0.1% hyaluronidase (Sigma Chemical Co., St Louis, MO). Hepes-buffered human tubal fluid medium (HTFM) (21) supplemented with 3 mg/ml bovine serum albumin (BSA; fraction V, Commonwealth Serum Laboratories, Melbourne, Australia) was used for the collection, enzyme treatment, and washing of oocytes. The oocytes were further washed twice in bicarbonate-buffered HTFM containing 3 mg/ml BSA and incubated in the same medium at 37°C under 5% $O_2$:5% $CO_2$:90% $N_2$ until zona drilling was completed (45–60 min).

### Preparation of Epididymal Spermatozoa

Epididymides were collected from mature $F_1$ hybrid males and the spermatozoa were recovered through the vas deferens by pressing each epididymis with a pair of forceps. The spermatozoa were allowed to disperse in the HTFM without BSA and aliquots containing $1 \times 10^4$, $1 \times 10^5$, or $5 \times 10^5$ sperm were added to 1 ml of insemination medium (HTFM + 5 mg/ml BSA).

### Zona Drilling of Mouse Oocytes

Five or six oocytes at a time were placed in the micromanipulation chamber, which consisted of a glass cavity dish with low-density paraffin oil (Merck E, Darmstadt, FRD) covering a droplet of Hepes-buffered medium. For zona drilling, two micromanipulators (MO-102N; Narishige Sientific Instrument Laboratory, Tokyo) mounted via MM33 manipulators on an inverted microscope (IMT-2; Olympus Optical Co., Tokyo) were used. Glass holding micropipettes (drawn to 30–40 μm and polished to 5–10 μm) and injection needles (5 μm) were made with a glass microelectrode puller (PN-3; Narishige Scientific Instrument Laboratory) and a microforge (MF-79; Narishige Scientific Instrument Laboratory). Oocytes were held one at a time on the holding micropipette and a small area (approximately 5- to 10-μm diameter) of the zona pellucida was dissolved by injecting acidic HTFM (pH 2.5) through the injection microneedle. Injection of acidic medium was effected by positive pressure generated using a microinjection instrument (Picospritzer, General Valve Corporation, Fairfield, NJ). The hole in the zona pellucida was completely drilled by opening the pressure control valve of the microinjector two or three times for 5–6 msec. This allowed injection of a very small volume (approximately 5 nl) of acidic medium, thus minimizing the change in pH in the perivitelline space. Following micromanipulation, the oocytes were washed and placed in 1 ml of insemination medium containing $1 \times 10^4$ or $1 \times 10^5$ spermatozoa.

A similar number of zona-intact oocytes was inseminated with $5 \times 10^5$, $1 \times 10^5$, or $1 \times 10^4$ sperm/ml to form the control group. Some zona-intact and zona-drilled oocytes were not inseminated in order to check for parthenogenetic development.

### Fertilization and Blastocyst Formation

Five hours after insemination, oocytes were collected, washed, and placed in 1 ml of HTFM containing 3 mg/ml BSA for further culture in vitro. On the following day, the numbers of oocytes which had reached the two-cell cleavage stage were recorded. These two-cell embryos were further cultured for 3 days and the rate of blastocyst formation was determined.

### Chromosomal Analysis

In a separate experiment chromosomal spreads of zona-intact, zona-free, and zona-drilled embryos at first cleavage division were prepared and analyzed. Zona-intact and zona-free oocytes formed the control groups. Zona-free oocytes were obtained by placing oocytes in acidic medium at pH 2.5 (same as that used for zona drilling) for 5–10 sec (22) and washed prior to insemination. Zona-intact, zona-drilled, and zona-free oocytes were inseminated with $5 \times 10^3$, $1 \times 10^4$, or $1 \times 10^5$ sperm/ml. At the end of the insemination period (5 hr) the oocytes were washed and colcemid (0.5 μg/ml) was added. The oocytes/zygotes were incubated for 15–16 hr prior to the preparation of chromosomal spreads.

Chromosomal spreads were prepared according to the method described by Wramsby and Liedholm (23). In brief, oocytes or zygotes were exposed to 0.1% Na citrate for 10 min. They were then placed in fixative A (5 water:1 acetic acid:4 ethanol) for 30 to 40 sec. When the samples appeared clear, they were placed on grease-free microscope slides in a minimum of fixature A and three drops of fixature B (3 ethanol:1 acetic acid) was added. The slides were dried on a warming stage and stained with 10% Giemsa in phosphate buffer (pH 6.8). The chromosomes were viewed using a 100× oil-immersion objective.

### Zona Drilling of Human Oocytes, Fertilization, and Development to Blastocyst Stage

Four patients who had oocytes recovered for IVF-related procedures consented to donate their excess oocytes to research and these were used for zona-drilling experiments. In brief, oocytes were incubated for 4 hr after oocyte recovery and cumulus cells were removed with 0.1% hyaluronidase. Some cumulus-free oocytes were used as controls and the others were subjected to zona drilling. The culture medium used was Hepes- or bicarbonate-buffered HTFM supplemented with 10% heat-inactivated human serum.

The sperm from normospermic samples, i.e., $>10 \times 10^6$/ml motile sperm (24), were used and were prepared by the overlay technique (25). The micromanipulative procedure for human oocytes was similar to that described in the mouse model. One oocyte at a time was placed in the micromanipulation chamber and zona drilling was carried out with acidic Hepes-buffered HTFM (pH 2.5). After washing twice, the oocytes were placed individually in 1 ml of medium within 5-ml Falcon tubes. Aliquots of the sperm overlay were added to control and zona-drilled oocytes to obtain final concentrations of $1 \times 10^4$ or $1 \times 10^5$ sperm/ml. Following the addition of sperm, the tubes were incubated under 5% $O_2$:5% $CO_2$:90% $N_2$ at 37°C for 16–18 hr and the oocytes then checked for the presence of two pronuclei (2 PN). The zygotes were further cultured in vitro for 4–5 days to blastocysts.

### Statistical Analyses

The chi-square test was used to determine the statistical significance between treatments for various observations made. Where the analysis indicated significant heterogeneity between replicates within treatments, this was taken into account in assessing the significance of the main effects.

## RESULTS

The rates of fertilization and blastocyst formation of control and zona-drilled mouse oocytes inseminated with different concentrations of sperm are given in Table I. Significantly more oocytes were fertilized after zona drilling when both high and low numbers of sperm were used (35 and 6% for control and 85 and 61% for zona-drilled oocytes with $1 \times 10^5$ and $1 \times 10^4$ sperm/ml, respectively). As the concentration of sperm was reduced from $5 \times 10^5$ to $1 \times 10^5$ to $1 \times 10^4$/ml, a significant reduction in fertilization rate was observed in control oocytes (81 to 35 to 6%). No significant difference was observed between the fertilization rate of control oocytes inseminated with $5 \times 10^5$ sperm/ml and that of zona-drilled oocytes inseminated with either $1 \times 10^5$/ml or $1 \times 10^4$/ml, although there was a small but statistically significant difference ($P < 0.05$) between the latter two treatments. None of the zona-intact or zona-drilled oocytes incubated without sperm showed parthenogenetic activation. More than 97% of zygotes obtained developed to the blastocyst stage in control as well as zona-drilled groups.

Data obtained for live young born following transfer of control and zona-drilled embryos at morula/early blastocyst stage to recipients at day 3 of pseudopregnancy are given in Table II. No significant difference was noted in live birth rates or sex distribution between control and zona-drilled groups.

**Table I.** Fertilization Rate and Blastocyst Formation of Zona-Intact and Zona-Drilled Mouse Oocytes Inseminated with Different Concentrations of Sperm

| Treatment | Sperm number/ml | No. replicates | Fertilization rate* | Blastocyst formation |
|---|---|---|---|---|
| Zona intact | $5 \times 10^5$ | 5 | 71/88 = 81%[a] | 71/71 = 100% |
| Zona intact | $1 \times 10^5$ | 7 | 35/101 = 35%[b] | 35/35 = 100% |
| Zona intact | $1 \times 10^4$ | 7 | 6/103 = 6%[c] | 6/6 = 100% |
| Zona intact | — | 5 | 0/35 = 0% | |
| Zona drilled | $1 \times 10^5$ | 7 | 81/95 = 85%[a,d] | 81/81 = 100% |
| Zona drilled | $1 \times 10^4$ | 7 | 60/99 = 61%[a,e] | 58/60 = 97% |
| Zona drilled | — | 5 | 0/33 = 0% | |

* Fertilization rates differ significantly as per the superscript comparisons. a vs b, $P < 0.001$; b vs c, $P < 0.025$; b vs d, $P < 0.001$; c vs e, $P < 0.001$; d vs e, $P < 0.05$.

Chromosome numbers for zona-intact, zona-free, and zona-drilled oocytes inseminated with different concentrations of sperm and analyzed at first cleavage division are given in Table III. A total of 135 oocytes was used for the study. Seventy-four oocytes (54.8%) had metaphase chromosomes from which fertilization rates could be calculated. Of these metaphase plates, 57 (42.2%) were spread suitably enough to allow an accurate count. Metaphase plates with excess scattering were not used, and for the assessment of aneuploidy, only chromosomes that were in discrete groups were included in these data.

Fertilization rates, as determined by the proportion of oocytes with diploid (see Fig. 1) or polyploid chromosome numbers out of the total metaphase spreads counted (Table III), were similar to those observed in Table I, for both zona-intact and zona-drilled oocytes inseminated with different sperm concentrations.

A high rate of polyploidy (≥40%) was observed with zona-free oocytes inseminated with all three concentrations of sperm, being 43% for $1 \times 10^5$, 40% for $1 \times 10^4$, and 45% for $5 \times 10^3$ sperm/ml. The rate of polyploidy for zona-intact oocytes was 12.5 and 0% using $5 \times 10^5$ and $1 \times 10^5$ sperm/ml, respectively. For zona-drilled oocytes the polyploidy rates were 29 and 0% using $1 \times 10^5$ and $1 \times 10^4$ sperm/ml, respectively. Even though the rate of polyploidy for zona-drilled oocytes inseminated with $1 \times 10^5$ sperm/ml was greater than that for zona-intact oocytes inseminated with the same or five times the number of sperm, the differences did not reach the level of statistical significance.

Of the readable chromosomes, no significant change in aneuploidy was noted between the treatment groups when pooled data were compared (aneuploidy rate of 12, 9, and 6% for zona-intact, zona-free, and zona-drilled oocytes, respectively).

In the experiments with human oocytes, similar fertilization rates were observed with zona-intact oocytes inseminated with $1 \times 10^4$ sperm/ml and zona-drilled oocytes inseminated with the same or one-tenth the number of sperm (Table IV). No fertilization was obtained with three control oocytes inseminated with $1 \times 10^4$ sperm/ml. Polyspermic fertilization was noted in zona-drilled oocytes inseminated with different concentrations of sperm and the incidence was 1/2 and 2/6 of fertilized oocytes for $1 \times 10^5$ and $1 \times 10^4$ sperm/ml, respectively. Of normally fertilized oocytes (2 PN stage recognized) in the zona-drilled group, 1/1 and 2/4 developed into blastocysts following fertilization with $1 \times 10^5$ and $1 \times 10^4$ sperm/ml, respectively.

**Table II.** Live Young Born Following Transfer of Zona-Intact and Zona-Drilled Mouse Embryos at Morula/Early Blastocyst Stage into Day 3 Pseudopregnant Recipients

| Treatment | No. pseudopregnant recipients | Live birth rate[a] | Sex of young | |
|---|---|---|---|---|
| | | | F | M |
| Control | 5 | 15/39 = 38.5% | 3 | 5[b] |
| Zona drilled | 4 | 16/42 = 38.0%[c] | 9 | 5 |

[a] No. of young born per No. of embryos transferred.
[b] Sex of seven young in control group was not recorded.
[c] Two young died 1–2 days after delivery.

## DISCUSSION

Zona drilling improved the fertilization rate of mouse oocytes markedly, thus enabling the use of fewer sperm numbers by a factor of 10 or 50 times less than those required to obtain maximum results with zona-intact oocytes. The fertilization rate obtained with the lowest concentration of sperm (61%) in the present study for zona-drilled oocytes is

**Table III.** Chromosome Makeup of Zona-Intact, Zona-Free, and Zona-Drilled Mouse Oocytes/Zygotes[a]

| Treatment (sperm number/ml) | Diploid or polyploid/metaphase spreads counted (% fertilized) | No.[b] | Haploid, 1N | Diploid, 2N | Polyploid | | Aneuploid ($n$)[c] |
|---|---|---|---|---|---|---|---|
| | | | | | 3N | 4N | |
| Zona intact (5 × $10^5$) | 9/16 (56) | 8 | 4 | 2 | 1 | — | 1 (39) |
| Zona intact (1 × $10^5$) | 2/10 (20) | 9 | 6 | 2 | — | — | 1 (18) |
| Zona free (1 × $10^5$) | 4/7 (57) | 7 | 2 | 1 | 2 | 1 | 1 (17) |
| Zona free (1 × $10^4$) | 7/8 (88) | 5 | 1 | 2 | 2 | — | — |
| Zona free (5 × $10^3$) | 10/11 (91) | 11 | 1 | 4 | 4 | 1 | 1 (39) |
| Zona drilled (1 × $10^5$) | 9/11 (82) | 7 | 2 | 3 | 2 | — | — |
| Zona drilled (1 × $10^4$) | 7/11 (64) | 10 | 3 | 6 | — | — | 1 (21) |

[a] For *Mus musculus* 2N = 40 chromosomes.
[b] No. of oocytes/zygotes with readable metaphases.
[c] No. of chromosomes.

higher than that observed by Gordon and Talansky (7) (15%) or by Depypere *et al.* (9) (22%). Such variation could result from differences in the zona-drilling technique (extent of the lesion made in the zona pellucida), the culture conditions involved (1-ml volume vs 30-μl microdrops), or the strain of mice used (C57 BL/6 × CBA vs CD-1).

For a micromanipulative technique to be acceptable, it should not damage the gametes, cause parthenogenetic activation, or generate chromosomally abnormal embryos. A loss of oocytes following spermatozoal microinjection has been reported,

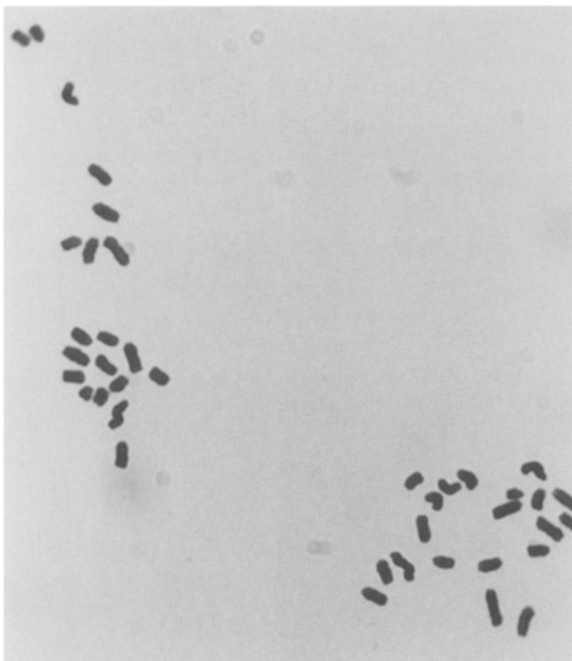

**Fig. 1.** A metaphase spread of a mouse zygote at first cleavage division showing diploid number of chromosomes (40, *Mus musculus*).

with the degree of damage related to the size of the injecting needle used (1). Furthermore, spermatozoa prior to microinjection are subjected to harsh treatments in order to obtain the acrosome reaction and to make them immobile for ease of handling (1,26). These sperm treatments can lead to increased chromosomal aberrations (27). In contrast, zona drilling, which involves the dissolution of a small area of the zona using acid Tyrode's medium, is a simple technique. Even though some oocyte damage has been reported in relation to the use of the acidic medium (7,9), no loss of oocytes associated with the zona drilling was noted in the present study. This may be due to the use of a minimum amount of acidic medium injected using the fine control of the microinjection instrument as described here. However, electron microscopic studies of zona-drilled oocytes have indicated the disappearance of microvilli on the oocyte surface near the site drilled (28). The significance of such morphological changes on the development of the resulting embryo is not known. However, in the light of the high rate of fertilization and embryo development both in vitro and in vivo observed after this treatment (7; the present study), it seems unlikely that the treatment has a detrimental effect on the oocyte, at least in mice.

**Table IV.** Fertilization Rate of Zona-Intact and Zona-Drilled Human Oocytes Inseminated with Different Concentrations of Sperm

| Treatment | Sperm number/ml | Fertilization rate (%) | Polyploidy rate |
|---|---|---|---|
| Zona intact | 1 × $10^5$ | 10/12 (83) | 0/10 |
| Zona intact | 1 × $10^4$ | 2/6 (33) | 0/2 |
| Zona drilled | 1 × $10^5$ | 2/3 (67) | 1/2 |
| Zona drilled | 1 × $10^4$ | 6/8 (75) | 2/6 |

In the present study no parthenogenetic activation of zona-drilled oocytes was observed in any of the five replicates of the experiment. Even when zona-free oocytes (generated by exposure to acidic medium) were cultured without sperm, no parthenogenetic activation was noted. However, these data may not be fully conclusive of the lack of parthenogenetic development following insemination of zona-drilled oocytes. It has been reported, for example (22), that in the presence of sperm a small proportion of oocytes (18.8%) undergoes parthenogenetic activation due to the combination of zona removal and sperm interaction at the oocyte surface. Furthermore, a recent report indicates that acid Tyrode's solution can induce parthenogenetic activation of mouse and human oocytes (29). However, their technique of exposing oocytes to acid Tyrode's differs from the micromanipulation method described here where the volume of acid medium applied is carefully controlled by microinjection instrument.

Gordon *et al.* (8) reported developmental arrest of the majority of human zona-drilled zygotes. However, this could be due to the possible detrimental effects of excess acidic medium rather than the drilling technique per se. When excess acidic medium is used to remove a large area of the zona of mouse oocytes ["aggressive drilling" (30)], the resulting embryos showed developmental abnormalities in vitro, forming giant composite morulae or blastocysts due to aggregation of embryos. This finding may further highlight the importance of keeping the hole in the zona small by controlling the amount of acidic medium injected during the drilling procedure. In the present experiments using controlled release of acidic medium, the majority of embryos (>97%) cleaved and developed to apparently healthy blastocysts in vitro. Furthermore, it was observed that zona drilling caused the blastocysts to hatch earlier than the control blastocysts, a finding similar to the observations made by Depypere *et al.* (9).

Comparable live birth rates were obtained for zona-drilled and control (zona-intact) embryos transferred at the morula/early blastocyst stage to day 3 pseudopregnant recipients. The rates obtained in this study are similar to those reported by Gordon and Talansky (7) for zona-drillled mouse embryos transferred into the oviducts. The developmental anomalies and reduced viability described by Nichols and Gardner (31) on transfer of zona-slit zygotes and two-cell embryos to the oviduct were not observed in these experiments. Perhaps slit zonas undergo more distortion during muscular contraction than drilled zonas and this leads to the observed abnormalities with the first technique. The physical protective nature of the zona pellucida on the cleaving embryo in vitro (32) is clearly shown when the dispersion of blastomeres (33), adherence of embryos to the oviductal epithelium (34), and reduction in birth rate of zona-free embryos (22) are considered. Damage due to bacterial invasion (35) or invasion by leukocytes of eggs with a zona lesion (32) can be of importance, at least for certain species. Malter and Cohen (11) suggested that administration of immunosuppressive corticosteroids or antibiotics may improve the implantation of micromanipulated embryos under such circumstances. Recently Cohen *et al.* (36) have reported improved implantation rates in IVF patients treated with corticosteroids and antibiotics when cleaved embryos were transferred following partial zona dissection and described a further potential use of micromanipulation to improve embryo hatching and implantation rates.

Analysis of chromosomes further confirmed the improvement in fertilization following zona drilling. The overall prevalence of aneuploidy among the treatment groups (average of 8.8%) was slightly higher than the figure of 3.8% reported by Kaufman (37) and the figure of 1.8% given by Donahue (38). Such differences in results may be due to the dose of PMS used (39) or to variables in culture techniques (40). Acidic drilling or the even harsher treatment of total zona removal did not increase the incidence of chromosomal aberrations. Indeed, if any damage to the oolemma or ooplasm was caused by the acidic medium, it must have been transient and certainly did not seem to interfere with binding, fusion, penetration, or decondensation of spermatozoa.

Complete zona removal eliminated the barrier to polyspermy and resulted in higher rates of polyploidy among zona-free oocytes similar to those found by Thadani (22). However, Thadani (22) also reported that when the sperm number in culture was drastically reduced (e.g., to 5–10 sperm/oocyte), normal fertilization and embryo development could be achieved with zona-free oocytes. In the case of zona-drilled oocytes, polyspermy did not appear to be a problem, even with 2000 sperm/oocyte.

The results given for zona-drilled human oocytes show an improvement in fertilization rate even

when the sperm concentration is reduced to one-tenth that used in standard IVF ($1 \times 10^5$ sperm/ml). Improvement of fertilization through zona drilling in infertile patients with a history of failed fertilization has been reported (8) but with no pregnancies resulting from embryo transfers. The incidence of polyspermy with zona-drilled human oocytes is high (8; present data). Therefore, careful control over the number of sperm used for insemination and over the extent of the zona opening is an important consideration for reducing this rate of polyspermy. Controlled zona drilling, performed with a minimum amount of acidic medium, may be beneficial to many patients who wish not to use gametes other than their own for the treatment of their infertility.

In conclusion, zona-drilled mouse and human oocytes gave significantly improved fertilization rates with lower sperm numbers than those required in routine human IVF. Embryo survival both in vitro and in vivo, comparable to that of control mouse embryos and the lack of chromosomal anomalies in zona-drilled mouse embryos indicate that zona drilling has a great potential in treating infertility associated with oligo/asthenospermia. However, when this technique is applied primarily to the oligo/asthenospermic group, the possibility of an abnormal spermatozoon fertilizing an oocyte and generating a genetically abnormal embryo may be increased. Therefore, detailed cytogenetic studies on micromanipulated human oocytes/embryos are a prerequisite before clinical trials are carried out.

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