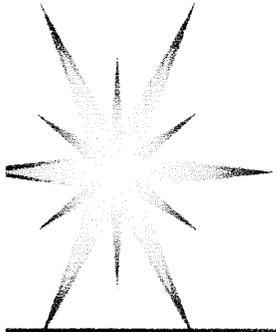
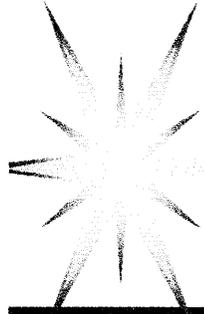


Cytoplasmic Transfer in the Human

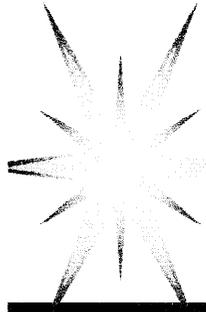


**The Jones Institute
Experience**



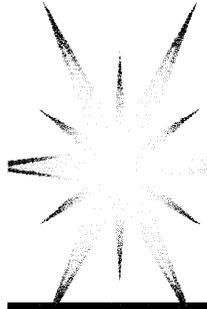
Cytoplasmic Transfer

- **In the monkey, Flood et al. (1990) found that the developmental potential of oocytes matured in vitro could be increased by injecting them with the cytoplasm of oocytes matured in vivo.**



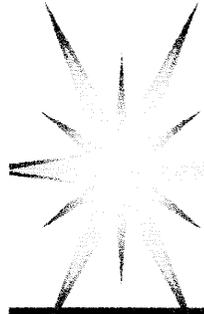
Cytoplasmic Transfer

- **13% of injected oocytes resulted in pregnancies while none of the sham injected or non-surgical controls resulted in pregnancy. Suggests that factors may be present within the cytoplasm that control genetic, maturational, and/or developmental properties.**



Cytoplasmic Transfer

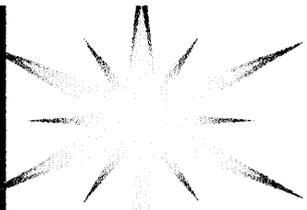
- **Cohen and coworkers (1997) reported the first human pregnancy following the transfer of cytoplasm from donor eggs in the eggs of the recipient.**
- **The goal of the procedure was to provide healthy cytoplasmic “factors” to the eggs of patients who repeatedly produce embryos of poor quality**



Cytoplasmic Transfer

The Norfolk Experience with Cytoplasmic Transfer

- Patients divided into two groups according to indication for the procedure:
 - **Wife is 40 years of age or older, or**
 - **Couple has had at least two previous IVF attempts which resulted in only poor quality embryos**
- Study approved by the IRB of EVMS and allows for the treatment of 15 consenting couples

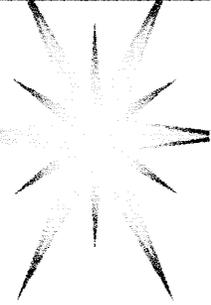


Informed Consent

RISKS TO PATIENT:

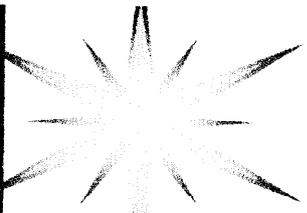
- **Effect of procedure on eggs or ability of the procedure to establish pregnancy has not been determined**
- **Unknown if the procedure will increase the risk of obstetric complications**
- **Thawed donor eggs may not survive so that procedure cannot be done**
- **Patient's eggs may not survive the procedure or may fail to fertilize or develop normally**

Informed Consent



RISKS TO OFFSPRING:

- Not known if the procedure will increase the risk of obstetric complications or fetal abnormalities
- Eggs may be damaged in some way that adversely affects the offspring
- There is a possibility that genetic material may be transferred from the egg donor to the patient's eggs and it is unknown if this could adversely affect the offspring



Informed Consent

ALSO STRESSES:

- **Because the procedure is new, there is no way to determine what the exact risks are or at what rates they would occur**
- **Amniocentesis is recommended for all patients in this study regardless of age**
- **There may be other risks that cannot be identified at this time**

The pipet, with the air stream in the tip, is inserted into donor egg.



Donor Egg

Cytoplasm, along with the sperm, is drawn up the pipette

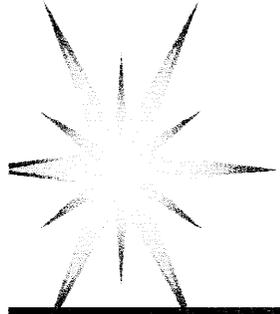


Donor Egg

The pipet is moved to the recipient egg and inserted. After aspirating a small amount of cytoplasm, the pipet is moved to the donor egg and cytoplasm is released along the pipet.



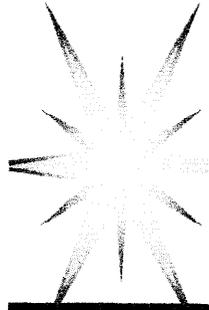
Recipient Egg



Results

Patients 40 years of age and over:

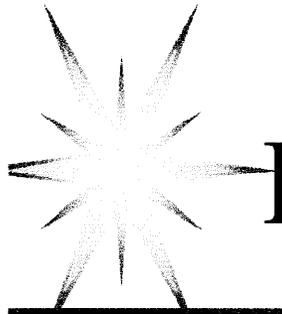
- 8 patients in 8 cycles
- Average age of 44 (range 41 to 47 yoa)
- Procedure did not appear to have an effect on embryo quality
- No pregnancies were established



Results

Patients 40 years of age and over:

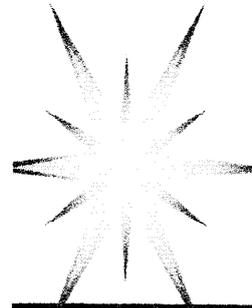
- 39 eggs retrieved (3.2, range 1 to 12)
- 21/39 (54%) 2PN fertilization
- Cytoplasm obtained from 9 donors ages ranging from 25 to 29
- 35/57 (61.4%) survived the thaw



Results

Patients with history of poor quality embryos:

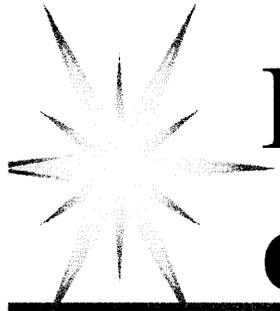
- 3 patients in 3 cycles
- Ages of patients were 35, 35 and 38
- Procedure did appear to have an effect on embryo quality
- One twin ongoing pregnancy was established



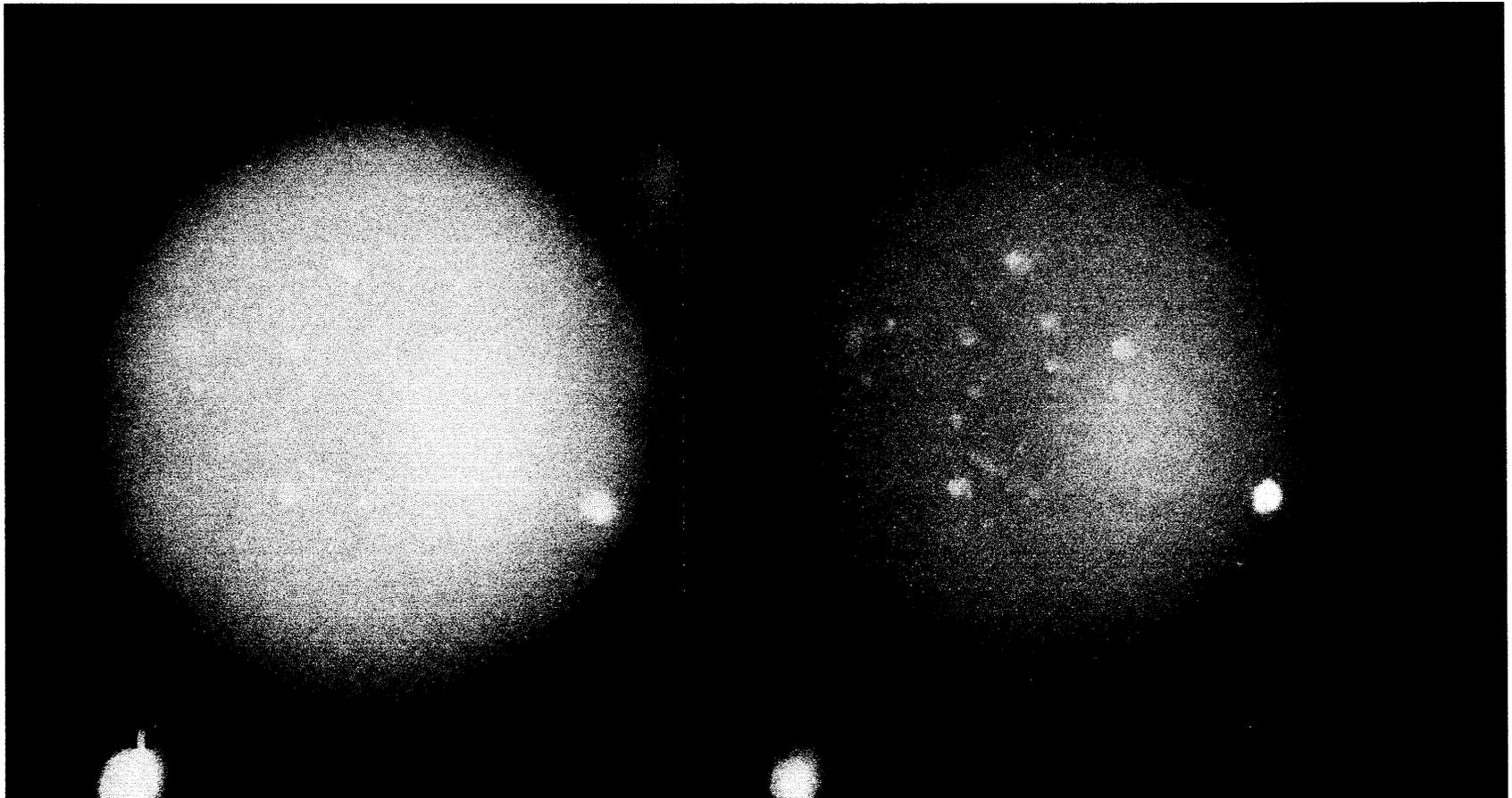
Results

Patients with history of poor quality embryos:

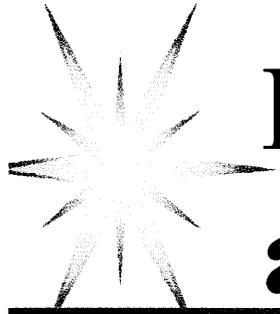
- 42 eggs retrieved (mean 14.3, range 7 to 19)
- 26/42 (62%) 2PN fertilization
- Cytoplasm obtained from 3 donors ages 30, 30 and 29
- 27/41 (66%) survived the thaw



Problem: Inadvertent Transfer of Chromosomes



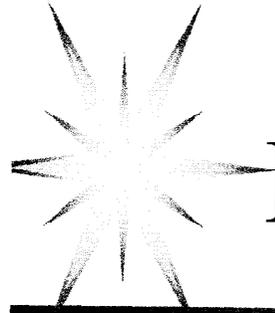
antibody



Evaluation of Meiotic Spindle and Chromosomes

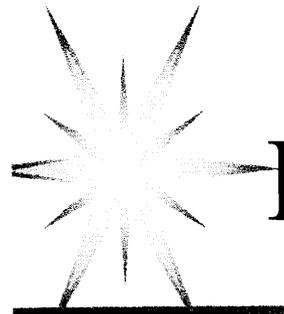
Jones et al (2001) J Assisted Reprod Genetics
18:230.

- Oocytes evaluated resulted from either clinical or research procedure for cytoplasmic transfer
- 12 oocytes were thawed but not used for transfer and served as controls
- 23 oocytes were thawed and survived the donation procedure as served as tests



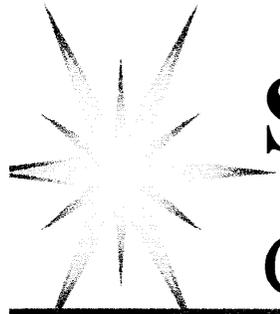
Results

- All control oocytes (12/12) demonstrated a normal meiotic spindle apparatus
- Two of the test oocytes that donated cytoplasm (2/23; 8.7%) demonstrated total dispersion of the chromosomes from the metaphase plate and complete disorganization of the spindles.



Results

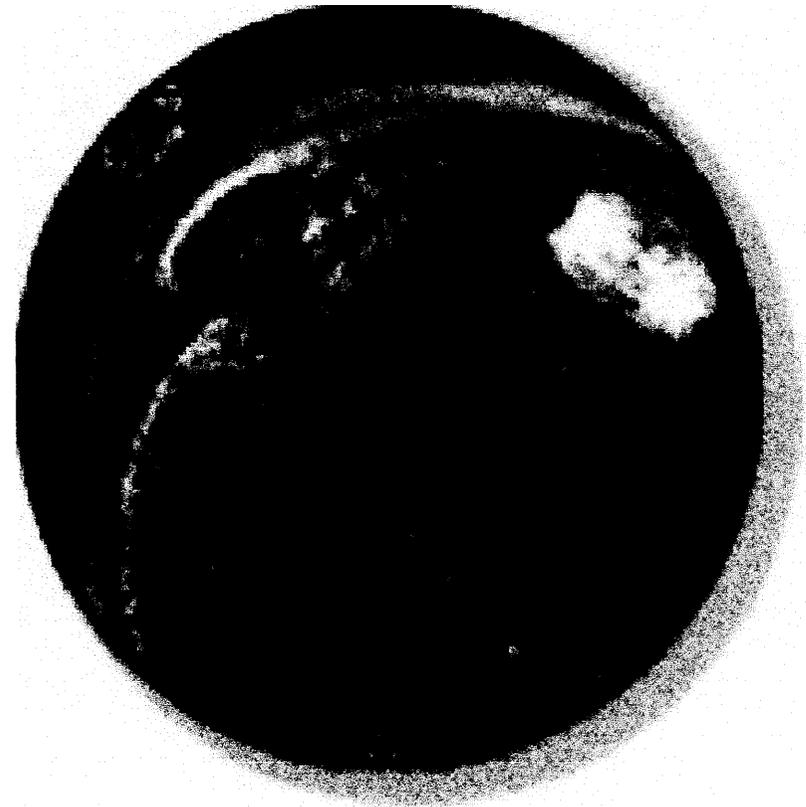
- No significant difference between the two groups
- Would this rate of meiotic spindle damage be similar to what is seen in oocytes undergo ICSI?
- Could the damage be reduced with the use of a PolScope?

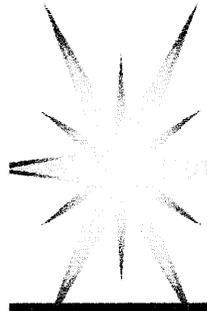


Spindle View™ Imaging System

Cambridge Research and Instrumentation, Inc.

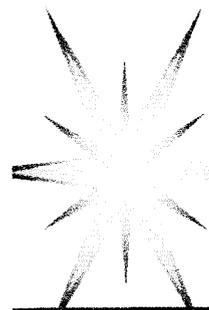
- Allows for visualization of the spindle during a procedure
- Currently used by laboratories during clinical ICSI cases and research involving enucleation





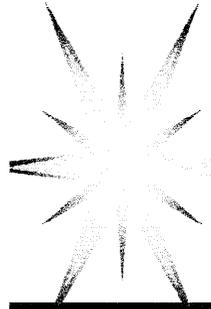
The Transfer of *In Vivo* Matured Ooplasm into Prophase I Human Oocytes before and after Maturation

- **Work performed by Samuel Brown, M.D.**
- **The developmental failure of human embryos derived from oocytes “matured” in vitro may be due to the deficiency of cytoplasmic factors.**
- **Would human prophase I oocytes become developmentally competent after microinjecting them with the ooplasm of human MII oocytes matured in vivo?**



The Transfer of *In Vivo* Matured Ooplasm into Prophase I Human Oocytes before and after Maturation

- **Hypothesized that such an injection would improve fertilization and blastocyst development of these immature eggs.**
- **If true, salvaging immature oocytes may improve pregnancy success in *in vitro* fertilization patients.**



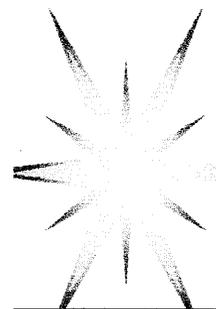
The Transfer of *In Vivo* Matured Ooplasm into Prophase I Human Oocytes before and after Maturation

The effect of cytoplasmic transfer from in vivo matured eggs into PI eggs:

- **Control eggs: 14/19 (74%) matured to MII**
- **Sham eggs: 8/16 (50%) matured to MII**
- **CT eggs: 11/19 (58%) matured to MII**

Suggests that injecting a substance into an egg may have a negative impact on maturation.

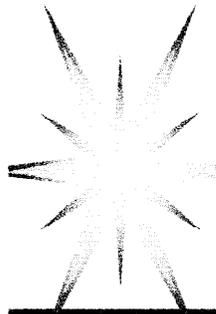
Results not significant.



The Transfer of *In Vivo* Matured Ooplasm into Prophase I Human Oocytes before and after Maturation

Fertilization results of Control, Sham and Test Groups

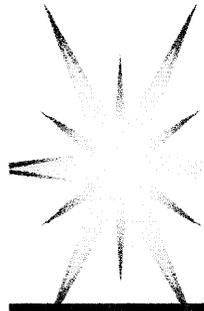
	n	2PN	0PN	3PN	Deg
Control	14	7 (50%)	1 (7%)	1 (7%)	3 (36%)
Sham	8	3 (38%)	5 (62%)	0 (0%)	0 (0%)
Cytoplasmic Transfer	8	4 (50%)	1 (13%)	3 (38%)	0 (0%)



The Transfer of *In Vivo* Matured Ooplasm into Prophase I Human Oocytes before and after Maturation

The effect of cytoplasmic transfer from in vivo matured eggs into in vitro matured eggs

	n	2PN	0PN	3PN	DEG
Control	17	9 (53%)	3 (18%)	1 (6%)	4 (23%)
Cytoplasmic Transfer	17	8 (47%)	5 (29%)	3 (18%)	1 (6%)



Conclusions

- Cytoplasmic transfer, if provided clinically, should move forward cautiously and with the full consent of the patients
- Most patients, having to choose between donor egg and cytoplasmic transfer, would not be bothered with the fact that the child may have genetic material from the mitochondria of the egg donor