

**ular Tissue.** M. A. Witt, S. E. Smith, J. R. Richard, H. I. Kort, D. L. Keenan. Reproductive Biology Associates, Atlanta, GA.

**Objectives:** Sperm extraction from testicular tissue in combination with intracytoplasmic sperm injection has provided couples with non obstructive azoospermia the opportunity to initiate a pregnancy where both partners contribute genetically to their offspring. The extraction of sperm is possible in 50 to 70% of cases. Our current technique of testicular sperm extraction involves the microdissection of tissue. This technique is time consuming and tedious taking up to 4 hours. We compare a new method of testicular tissue processing to the microdissection method to determine if it is more efficient and equally effective.

**Design:** Human testicular tissue was prepared according to two different protocols and the results analyzed.

**Materials and Methods:** Fresh human testicular tissue was obtained by open excisional biopsy from 18 testicles in patients undergoing testicular sperm extraction for non obstructive azoospermia. The specimen was weighed and divided into two equal fractions one for microdissection and one for glass glass homogenization. For the microdissection technique the tissue was minced in a petri dish using two 18 gauge needles. The specimen was then vortexed and the tissue fragments removed by slow centrifugation. The sperm were pelleted, washed and resuspended. The second method employed glass glass homogenization. Testicular tissue was placed in a grinding tube containing human tubal fluid and manually homogenized. The homogenate was subjected to slow speed centrifugation and sperm was collected from the resulting supernatant by high speed centrifugation. Sperm was washed and resuspended. The time for each procedure was noted. Sperm counts from both preparations were determined using a Makler counting chamber.

**Results:** Both methods yielded whole viable sperm adequate for intracytoplasmic sperm injection. The glass glass technique required 45 minutes versus 211 minutes for the microdissection technique. Sperm were obtained in 15 of 18 testicles (83%) using glass glass homogenization versus 10 of 18 testicles (56%) using microdissection. The slurry obtained in the glass glass technique enabled easier extraction of sperm with the aspirating pipette than in the microdissection technique.

**Conclusion:** In extracting sperm from testicular tissue we advocate the use of glass glass homogenization because it is quicker, it salvages sperm from a greater number of testicles and produces a cleaner specimen from which sperm aspiration is easier.

#### P-144

**Single Cell RT-PCR Detection of IGF-1 and IGF-1 Receptor mRNA Expression in Preimplantation Mouse Embryo: Correlation With In Vitro Development.** A. Kowalik, Z.-Y. He, C. Mele, L. Barmat. The Center for Reproductive Medicine and Infertility, New York Hospital-Cornell Medical Center, New York, NY.

**Objective:** Recent studies have demonstrated the importance of insulin like growth factors (IGF's) in murine preimplantation development. Supplementation of culture

media with IGF-1 increases the rate of blastocyst formation and the inner cell mass cell number. We studied IGF-1 gene expression in a single blastomere of an early murine embryo and correlated it with subsequent embryo development in culture.

**Materials and Methods:** Fertilized eggs and 2-cell embryos were obtained by tubal flushing in superovulated and mated B6D2F1 female mice. Embryos were placed in 1640 RPMI culture medium supplemented with 10% BSA until the time of embryo biopsy. Single cells were removed from embryos at a 4 or 5-cell stage using the standard embryo biopsy techniques. Individual blastomeres from each embryo were then assayed for the presence of IGF-1 and IGF-1 receptor mRNA using RT-PCR technique. The biopsied embryos were washed in medium and placed in co-culture with murine endometrial cells. Embryonic development in culture was assessed and blastocyst grading was performed. IGF-1 gene expression was then correlated with in vitro development.

**Results:** Fifty-five embryos at 4 and 5-cell stage were biopsied. IGF-1 receptor gene expression was detected in 54 (98.2%) embryos and IGF-1 gene expression was detected in 19 (34.5%) embryos. No correlation between IGF-1 receptor expression and subsequent embryo development was found. The table shows the correlation between IGF-1 gene expression and in vitro embryo growth. A significant association between IGF-1 expression and grade 1 blastocyst formation in vitro was found.

	Blastocyst grade	IGF-1 positive (n = 19)	IGF-1 negative (n = 36)
Blastocyst day 2 after biopsy	Grade 1	11 (57.9%)	7 (19.4%)*
	Grade 2	0	5 (13.9%)
	Total	11 (57.9%)	12 (33.3%)
Blastocyst day 3 after biopsy	Grade 2	2 (10.5%)	7 (19.4%)
	Grade 3	2 (10.5%)	6 (16.7%)
	Total	4 (21%)	13 (36.1%)

\*p = 0.004

**Conclusion:** IGF-1 receptor transcripts are almost uniformly detected at 4 and 5 cell cleavage stage murine embryos. IGF-1 expression is more variable and correlates highly with grade 1 blastocyst formation in vitro. IGF-1 gene expression could potentially be used as a marker of embryo quality.

#### P-145

**The Effect of Stages I-IV Endometriosis on IVF Pregnancy, Implantation, Spontaneous Abortion, and Cancellation Rates.** A. Saguskin, E. Wicks, D. Murray, J. Hall, M. Levy. Shady Grove Fertility Center, Rockville, MD.

**Objective:** There is conflicting data regarding whether endometriosis has a negative impact on IVF outcome. This study was undertaken to compare IVF outcome in patients with various stages of endometriosis to a control group with a diagnosis of tubal factor in a defined, limited time period.