Objective: To determine why the American Association of Bioanalysts (AAB) proficiency test gave erroneous results in our laboratory.

Design: After reporting the carefully replicated finding that neither of the two test media in the 10/1999 AAB Embryology Culture (EC) proficiency test was embryotoxic, national summary results were received that indicated EC#1 was embryotoxic as detected by most participating programs. Tests of possible variables in our mouse embryo culture conditions were conducted in an attempt to understand this failure.

Materials and Methods: Media were placed in center-well culture dishes (Falcon 3037) and allowed to equilibrate overnight (37°C, 5% CO_2) prior to addition of embryos. Two-cell stage mouse embryos were thawed, pooled and rinsed two times in human tubal fluid medium (HTF) prior to placement into test media. Dishes were examined and number of embryos developing to blastocyst was determined after 72 hr. Exp 1—inner-wells contained 1 ml of one of the EC media while the outer-wells contained 3 ml of either the same EC media or HTF. Exp 2—inner-wells contained 0.8 ml HTF while outer-wells contained 5 ml of either EC#1 or HTF.

Results: In Exp 1 embryos grown in the unadulterated media EC#2 reached the blastocysts stage regardless of outer-well contents (33/34 and 33/33). Embryos grown in EC#1 with EC#1 in the outer-well did not reach the blastocyst stage (0/25). However, embryos grown in EC#1 with HTF in the outer-well reached the blastocyst stage at a high rate (31/35). In Exp 2 embryos grown in HTF developed to blastocysts at a high rate when the outer-well contained HTF (35/37). When the outer-well contained EC#1 embryo development was arrested (0/35).

Conclusions: The adulterated AAB EC medium apparently contains a toxic additive that is volatile and water soluble. The presence of unadulterated media in the outer-well can act as a sink to remove enough of the substance from the inner-well to allow embryo development (Exp 1). Conversely, a large volume of adulterated medium in the outer-well allows the contaminant to volatilize and re-dissolve in the inner-well rendering it embryotoxic (Exp 2). Because of this volatility the AAB proficiency test is subject to false readings depending on the type of culture system used. Concerns about the possibility of contaminating other specimens contained in an incubator also need to be addressed.

P-032

Comparison of G1.2 and Ham's F10 With Fetal Cord Serum in Day 3 Transfer After IVF. J. Stevens, W. B. Schoolcraft, T. Schlenker, L. Wagley, S. Oliver, M. Baird. Colorado Center for Reproductive Medicine, Englewood, CO.

Objective: Our center has historically used Ham's F10 with 15% fetal cord serum (FCS) as culture media for IVF with day 3 embryo transfer. Upon the introduction of G1.2 and G2.2 media and their successful use in extended culture and blastocyst transfer, the effect of using G1.2 for embryo culture in IVF with day 3 transfer was explored.

Design: A prospective randomized trial comparing G1.2 to Ham's F10 with FCS in IVF with day 3 transfer.

Materials and Methods: Between January and September 1999 72 patients agreed to participate in the trial. Using a computer generated randomization table, patients were placed in either the G1.2 group or the Ham's F10 group. 34 patients were randomized into the G1.2 group and 38 patients fell into the F10 group. All embryos were cultured in their respective media until day 3 when the best quality embryos were transferred. All embryos transferred had assisted hatching performed on them. Implantation rates and pregnancy rates of the two groups are compared. An IRB was obtained to use the G media.

Results: The mean age of the patients in the G1.2 group (37.7) was not significantly different from the F10 group (37.2). The mean FSH of the patients in the G1.2 group (7.5) was not significantly different from the F10 group (7.7). In the G1.2 group, 25 of the 34 patients had positive pregnancy tests (73.5%). This was not significantly different than the F10 group where 27 of 38 patients had positive pregnancy tests (71.1%). In the G1.2 group, 67.6% of the patients had ongoing pregnancies (past the first trimester). This was not significantly different from the F10 group where 57.9% had ongoing pregnancies. The implantation rate (sacs) in the G1.2 group (33.3%) was not significantly different from the F10 group (33.9%).

Conclusions: The use of G1.2 medium for day 3 transfers is a viable alternative to other culture systems such as Ham's F10. In addition it is an excellent way for IVF laboratories to move away from culture systems containing serum. For IVF laboratories wishing to move towards blastocyst culture, the use of G1.2 for day 3 culture seems a natural transition into extended culture and the decision to perform extended culture on a given patient may be made after day 3 embryo quality is assessed.

ART: MALE FACTOR

Monday, October 23, 2000

P-034

Intracytoplasmic Sperm Injection in Men with Totally Immotile Ejaculated Sperm. A. Kamal, C. Rhodes, I. Fahmy, R. T. Mansour, G. Serour, M. A. Aboulghar. The Egyptian IVF-ET Center, Maadi, Cairo, Egypt.

Objectives: Abnormalities of sperm motility are a poorly understood cause of male infertility, with a variety of etiologies. One of these is immotile cilia syndrome. A proportion of these cases will have classic Kartagener's syndrome, with situs inversus. Chemes et al described another variant, with a study of patients with dysplasia of the fibrous sheath and missing or grossly distorted axonemas. We present a case series of 17 men with totally immotile ejaculated spermatozoa undergoing intracytoplasmic sperm injection (ICSI) in which four cases had short tail syndrome.

Design: A prospective study of seventeen couples with totally immotile spermatozoa in the ejaculates was performed.

Materials and Methods: Semen from patients with totally immotile spermatozoa were subjected to a vitality test (eosin Y 1% exclusion). When the vitality score was 0% testicular, sperm extraction (TESE) was performed (n=12). Ejaculated sperm was used in the remaining cases (n=5). A further vitality test was performed on the testicular sperm. No definite criteria were used to choose which immotile sperm were more suitable for injection. Sperm with greater tail flexibility and a more glistening head were chosen. Fertilization rates (FR) and pregnancy rates (PR) after ICSI were compared between the ejaculated and the testicular immotile spermatozoa. Electron microscopy examination (EM) of the sperm was done in 6 cases.

Results: In the twelve cases of ICSI with testicular sperm extraction (TESE) the FR was 41.8% with a PR of 33.3%. In five couples, ICSI was performed using ejaculated spermatozoa, with an FR of 51.7% and a PR of 40%. The overall FR was 45.6%, with a PR of 35.3%. The ultrastructural examination showed that in two cases sperm had no dynein arms. Short tail syndrome was diagnosed in four men; three of these cases had EM showing degenerate sperm tails with short tails. In one case, there was a 5+2 axonemal pattern with degeneration, short tails, and absent dynein arms.

Conclusion: ICSI using immotile testicular or ejaculated sperm is a reasonable treatment option in cases of totally immotile ejaculated spermatozoa. Pregnancy can be achieved using randomly selected ejaculate sperm or testicular sperm. Further studies are needed to assess the use of testicular versus ejaculated sperm, and the place of sperm selection methods such as the hypo-osmotic swelling test. Ultra-structural studies and advances in the genetics of sperm motility should clarify the etiology of this rare abnormality.

P-035

Rheologic Behavior of Spermatozoa Exposed to Hypoosmotic Conditions at Varying Oncotic Pressure Environments: Possible Protective Mechanisms Against Sperm Swelling by the Addition of Protein Supplements. [1]J. R. Correa-Pérez, [1]O. Torres-Santiago, [1]R. Fernández-Pelegrina, [2,3]P. N. Zarmakoupis-Zavos, [2,3]P. M. Zavos. [1]Centro de Fertilidad del Caribe, Río Piedras, Puerto Rico, [2]Andrology Institute of America and [3]Kentucky Center for Reproductive Medicine & IVF, Lexington, KY.

Objective: Exposure of spermatozoa to hypoosmotic conditions that cause optimal swelling for assessment of sperm membrane functional integrity causes sudden and irreversible changes in membrane structure. In almost all cases, spermatozoa cannot support viability as observed by lack of motility during and at the end of the incubation period (30 to 60 min). This behavior of human spermatozoa exposed to hypoosmotic conditions has been previously assessed, which resulted in the development of a sperm functional test (hypoosmotic swelling (HOS) test. *J Reprod Fertil* 1984;70: 219). The aim of this study was to assess possible osmoregulatory mechanisms of substances contributing to oncotic pressure (exerted by colloids in solution) such as protein supplements against exposure to hypoosmotic conditions (exerted by dissolved substances in solution).

Design: Exposure of processed spermatozoa to controlled hypoosmotic