THE SOCIETY FOR MALE REPRODUCTION AND UROLOGY ABSTRACTS

Wednesday, October 17, 2007
2:30 pm

O-198


OBJECTIVE: The scarcity of gametes often represents a main hindrance to overcoming spermatogenic failure through the use of ART. Thus, the possibility to propagate a male genome would provide an alternative means through which to consistently obtain conceptuses.

DESIGN: To duplicate the sperm genome, haploid androgenotes were generated and allowed to cleave. Haploid parthenotes were obtained by activating eggs. To assess the ability of the male clones to support normal embryo development, diploid constructs were derived by transferring them into parthenotes.

MATERIALS AND METHODS: Following enucleation, mouse MII oocytes injected with a single sperm and defined as androgenic replicates, were cultured for ~20 h. To generate gynogenic counterparts, another cohort of eggs was exposed to Src12. Karyoplasts isolated from the cleaving haploid androgenotes were transferred to haploid parthenotes. The constructs were electrofused to constitute a diploid zygote and cultured for 96 h. Some of the haploid androgenotes were processed for cytogenetics. ICSI conceptuses served as control. To investigate post-implantation development and offspring wellbeing, blastocysts were transferred to pseudo-pregnant mice.

RESULTS: Of 192 MII oocytes initially manipulated 168 survived, and all came to display a single male PN. A total of 155 cleaved and maintained their haploid status in 88% analyzed. Oocyte activation was successful in 95% and once constructs were electrofused, 98% generated biparental zygotes. During the 4 day culture, 77% of the study constructs developed into blastocysts at a rate comparable to ICSI embryos (81%). However, the transfer of 64 blastocysts yielded only 11 offspring (17%), fewer than the 43% achieved in the control group (P<0.05). Moreover, of those only 4 grew normally whereas all the ICSI offspring became adults. Although 3 were cannibalized and 4 died soon after birth, no genotypic or phenotypic anomalies were identified in the study group.

CONCLUSIONS: It is possible to replicate the male genome through its injection into ooplasm. Such androgenotes maintain their genotype, ploidy, and the ability to achieve syngamy. Moreover, their propagation as cleaving pseudo-blastomeres did not alter epigenetic imprinting of the male genome. In comparison to whole genome cloning, the relatively higher reproducibility of the technique indicates that it is possible to create multiple copies of the male genome through which to gain genetic information on a particular gamete or to propagate it when scarce.

Supported by: None.

Wednesday, October 17, 2007
2:45 pm

O-199

SUBTLE CHANGES IN CULTURE ENVIRONMENTAL TEMPERATURE APPEAR TO AFFECT SPERMATOZOA PHYSIOLOGY. L. Penrose, M. Seller, S. Jabara, S. Overley, J. Copeland, S. Prien. OB/GYN, Texas Tech University Health Sciences Center, Lubbock, TX; Animal and Food Sciences, Texas Tech University, Lubbock, TX.

OBJECTIVE: It is well documented that spermatozoa are temperature sensitive. Previous studies have demonstrated that rapid shifts in temperature of 5–10°C during culture or processing can lead to activation of shock proteins, affecting on cellular physiology and activation of biochemical pathways leading to apoptosis. However, it is unclear how more subtle changes in culture environment might affect spermatozoan function. The objective of the present study was to determine the affects of subtle changes in temperature on sperm physiology and activity as a first step in creating an optimum environment for sperm culture.

DESIGN: Laboratory based study of temperature affects using a porcine model.

MATERIALS AND METHODS: Using a system developed in this laboratory, sperm cells were simultaneously presented with four temperature environments designed to be within ±2°C of gonad temperature in .5°C increments. Temperature was continuously monitored to insure the stability of the environmental relationships. Porcine ejaculates were prepared using standard techniques and placed in the system and the sperm were allowed to freely migrate over 3 hrs of incubation. At 3 hrs, the environments were sealed from each other and samples collected for measurement of standard semen parameters using CASA. Additional samples were collected and either frozen or fixed with glutaraldehyde for later biochemical analysis.

RESULTS: While overall temperature of the four environments increased at a rate of 3°C/10 minute interval, the relationship between the 4 environments remained stable in .5°C increments. Migration patterns showed a trend (P<0.13) toward migration of sperm to the chamber at approximately gonadal temperature and away from warmer or cooler environments. There was also a trend toward increased motility in this environment (P<0.20). However the forward progression of cells did not appear to be as affected by the subtle temperature differences (P>0.05). Biochemical assays are pending at this time.

CONCLUSIONS: Data from the present study appears to suggest that spermatozoa in culture would prefer cooler incubation temperatures than currently used, appearing to be in a range closer to those of the male gonad. Further study is warranted to determine the optimum culture environment to maintain cell physiology and biochemical function.

Supported by: None.

Wednesday, October 17, 2007
3:00 pm

O-200

EFFICACY OF CLomid, ARimidex, AND ANDROGEL IN NORMALIZING TESTOSTERONE IN YOUNG HYPOGONADAL MEN PRESENTING WITH INFERTILITY AND SEXUAL DYSPHORIA. D. A. Paduch, J. Kiper. Urology and Reproductive Medicine, Weill Medical College of Cornell University, New York, NY.

OBJECTIVE: Compare effectiveness of three different method of testosterone replacement in young hypogonadal men who presented with infertility or sexual dysfunction.

DESIGN: Retrospective study of 350 patients who were referred to single academic practice over 12 months. All men underwent physical examination, laboratory evaluation: at minimum: total and free testosterone (liquid chromatography -MS), FSH, LH, PRL, estradiol (chemiluminescence), thyroid studies, and PSA. Hypogonadism was defined as low testosterone using age adjusted norms for fertile men: <400 ng/dl for men younger than 40 and <350 ng/dl for men older than 40.

MATERIALS AND METHODS: Men were treated using following protocol: Androgel was a preferable method of replacement. Men who were seen for infertility or men who preferred oral method of replacement were assigned to Clomid or Arimidex treatment group using E/T ratio of 10. Men with ratio below 10 – low estradiol group were treated with Clomid, 1/2 tablet three times a week, men with E/T ratio above 10 were treated with Arimidex 1 mg once a day. T, E, FSH, LH were checked in all men 4–6 weeks after starting therapy and therapy adjusted. Only men who have been treated for minimum 3 months and who had all labs performed using the same methodology were included in the study.

RESULTS: 45 men treated for minimum of 3 months were included in the study. There were 15 men in Clomid group, 15 in Arimidex and 15 in Andro- gel group. There were no statistically significant differences in initial testosterone between groups: 291 (247–334) ng/dl for Cl, 290 (235–345) for Ar, and 292 (232–351) ng/dl for An. All three treatment methods restored testosterone to normal levels. Testosterone after treatment was: 573 ng/dl (463–683) in Clomid, 493 ng/dl (403–583) in Arimidex, and 630 ng/dl (457–803) in Androgel group (P<0.0001). Men who were taking Clomid had significantly higher estradiol level after treatment (P<0.05).

CONCLUSIONS: Each form of treatment restored testosterone to normal levels. The subtle differences in estradiol level after treatment should be considered when deciding on best treatment. It was shown that high estradiol level in young men is a better predictor of risk of atherosclerosis than LDL thus elevated estradiol may actually be harmful. It is also assuring that the changes in estradiol level in Arimidex group are less than 25% of initial estradiol level, thus making it unlikely that treatment with Arimidex will negatively affect mineral bone density in men.

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