development. Finally, these data suggest that LA does not affect aerobic metabolism, but instead affords antioxidant protection in the media.

Supported by: Vitrolife.

Monday, October 15, 2007
3:30 pm

O-96

ASSOCIATION OF CATALASE ENZYMATIC ACTIVITY IN BOVINE FOLLICULAR FLUID WITH BOTH THE PHASES OF FOLLICULOGENESIS AND THE STAGES OF THE ESTRUS CYCLE.

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OBJECTIVE: Oxidative metabolism is essential for gamete and embryo energy production and is unavoidably associated with the generation of reactive oxygen species (ROS). Catalase (CAT) is an enzymatic antioxidant expressed in the mammalian oocytes which scavenges the damaging oxygen products. The objective of this study was to correlate the CAT enzyme activity in follicular fluid with phases of folliculogenesis and stages of the estrus cycle.

DESIGN: Prospective study.

MATERIALS AND METHODS: Bovine ovaries were collected from the slaughterhouse (Champlain Beef Co., Whitehall, NY) within 30 minutes of death of the animal and placed in room temperature saline solution with antibiotic. These samples were obtained from naturally cycling cows (11/2–3 yrs). The follicular fluid collected from antral follicles of different sizes ranging from 2–25 mm was spun (7000g for 3 min) to remove all cellular contaminants, aliquoted, and frozen at −80°C. After processing of the follicular fluid, the antioxidant CAT was measured with a chemiluminescence method. The phase of folliculogenesis was assessed by examining the range of follicle sizes along with the presence of a corpus luteum (CL). Folliculogenesis was divided into four phases: 1) early (all follicles $\leq$8 mm), 2) middle (all follicles between 8 and15 mm), 3) late (largest follicle $\leq$15 mm), and 4 luteal (all follicles $\leq$8 mm with the presence of one CL). Stages of the estrus cycle were estimated based on the presence and features of the corpus luteum, with stage I corresponding to days 1–4, stage II days 5–10, stage III days 11–17 and stage IV days 18–20 of estrus cycle.

RESULTS: The average CAT level was 22.98 $\pm$ 17.97 μM. There was a statistically significant change in the levels of catalase activity throughout the different stages of the estrus cycle ($P<0.001$). The dynamics of the CAT activity change were especially significant between stage 1 vs. 2 ($P<0.001$), stage 1 vs. 3 ($P<0.004$) and stage 1 vs. 4 ($P<0.039$) by a pair wise sub group analysis with Kruskal-Wallis test. Significant differences were seen between corpus luteal stage 2 vs. 3 ($P<0.007$).

CONCLUSIONS: The above study showed significant changes in the follicular fluid levels of CAT enzyme according to the different stages of the estrus cycle and within stage II and III corpus luteal stages. Characterization of the antioxidant profiles in different compartments of follicles at stages of folliculogenesis may help characterize the antioxidant requirements needed to optimize in vitro culture media.

Supported by: None.

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3:45 pm

O-97

PICSI™ VS. ICSI: STATISTICALLY SIGNIFICANT IMPROVEMENT IN CLINICAL OUTCOMES IN 240 IN VITRO FERTILIZATION (IVF) PATIENTS.

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OBJECTIVE: The in vitro selection of sperm for intracytoplasmic sperm injection (ICSI) is critical and directly influences the paternal contribution to preimplantation embryogenesis. Hyaluronan (H), a major constituent of the cumulus matrix, may play a critical role in the selection of functionally competent sperm during in vivo fertilization. H-bound (HB) sperm carry increased levels of developmental maturity and sperm chromatin integrity. The relationship between HB-sperm and potentially enhanced levels of functional competence led to the current study examining the use of HB-sperm in the treatment of ICSI patients.

DESIGN: 240 ICSI patients were consented for the IRB-approved study. ICSI patients in Group A (control) received embryos created using standard sperm selection criteria. Group B (PICSI) patients received embryos created using HB-sperm.

MATERIALS AND METHODS: Oocytes of Group A patients received ICSI using sperm selected via the evaluation of motility and morphology. HB-sperm meeting the same criteria were used in the ICSI of Group B oocytes. The PICSI™ plate provides microdrops of H for sperm selection and was used in the study. Statistical significance was tested using the chi-squared statistic, t test and Pearson's correlation.

RESULTS: The fertilization rate carried by the PICSI oocytes (62%, n = 949) was statistically greater ($P\leq0.001$) than that of the control ICSI oocytes (61%, n = 2,562). PICSI-derived embryos demonstrated a statistically significant decrease (P=0.009) in the % fragmentation when compared to their ICSI counterparts (A: 8.4%, B: 6.8%, n = 2,150). The +beta hCG and clinical pregnancy rates (cpr) for the PICSI patients (70%, 50%, n = 66) were greater than those observed in the ICSI patients (62%, 40%, n = 174). Miscarriage rates (mr) were statistically lower ($P\leq0.05$) in the PICSI patients.

CONCLUSIONS: Oligozoospermic men requiring ICSI often carry seminal populations demonstrating increased levels of chromosomal aberrations and compromised DNA integrity. The current study suggests that the use of HB-sperm in ICSI may allow the isolation of sperm with potentially enhanced levels of functional competence, thereby exerting a positive paternal influence on preimplantation embryogenesis. The statistically significant reduced levels of fragmentation, increased cpr and decreased mr associated with the use of PICSI-derived embryos is promising. Studies are ongoing to further explore the enhanced embryonic potential associated with PICSI-derived embryos.

Supported by: PICSI dishes were provided by Biocoat, Inc.

Monday, October 15, 2007
4:00 pm

O-98

AMMONIUM BUILD UP IN CULTURE MEDIA ALTERS HUMAN EMBRYONIC METABOLISM AND GENE EXPRESSION.

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OBJECTIVE: Amino acids present in culture media, particularly glutamine, are labile at 37°C, and produce ammonium, which builds up over time. Furthermore, embryos metabolize amino acids to release more ammonium into the surrounding medium. Ammonium in embryo culture media has been reported to have a negative effect on mouse embryo physiology and gene expression, and a negative effect on human embryo development. It was therefore the aim of this study to determine the effect of an increasing ammonium gradient on the metabolism and gene expression of human embryos in culture.

DESIGN: Experimental study.

MATERIALS AND METHODS: Pronucleate oocytes were donated with consent and allocated to one of two groups (1) control; embryos cultured individually in 5 μl of medium G1 and then G2, with the medium being renewed every 24 h (n = 65), (2) the same as (1) but in increasing concentrations of ammonium (n = 65). In group 2 embryos were exposed to 75 μM, 150 μM, 225 μM, 300 μM and 375 μM on successive days of culture in order to mirror the levels of ammonium generated when 1 mM free glutamine deaminates over time. Embryo metabolism was quantitated fluorometrically on successive days of development, and global gene expression was determined in the resultant blastocysts, using Codelink Whole Genome Human Bioarray (GE Healthcare).

RESULTS: During the first 24h of development pyruvate uptake was significantly impaired by the presence of ammonium (8.7 pmol/embryo/h) compared to the control group (13.0 pmol/embryo/h; $P<0.05$). At the 8-cell stage, pyruvate uptake was also reduced in the presence of ammonium ($P<0.05$). At all other stages of development, metabolism was similar in the two groups, with a trend of higher metabolic rate in the control. Clustering analysis revealed altered gene expression profiles from ammonium exposure including both down and upregulation. Functional annotation of 154 genes identified in two considerably upregulated clusters revealed 20% of the genes to be involved in cell growth and/or maintenance, 12% cell communication, 14% development and 8% metabolism with 12% having an unknown biological process.