VITAMIN E SUPPLEMENTATION REDUCES OXIDATIVE STRESS AND IMPROVES BLASTOCYST DEVELOPMENT RATE
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Objective:
Leukocytes and abnormal spermatozoa in the semen can produce high amount of reactive oxygen species (ROS) resulting in oxidative injury to normal mature sperm. Excessive ROS production in semen results in both reduced sperm motility and the capacity for sperm-oocytes fusion. High ROS levels are also found in the peritoneal fluid of women with unexplained infertility. Our objective was to study the effect of artificial generation by activated leukocytes on the blastocyst development rate (%BDR), and to examine the protective effect of antioxidant supplementation (vitamin E) in reducing the adverse effect of ROS on mouse embryo development.

Design:
Controlled prospective study.

Materials/Methods:
Leukocytes (0.5 X 10^6/mL) separated from human anticoagulated blood using Histopaque gradient were activated with 100nM of 12-phenol 13-myristate acetate (PMA) and incubated for 30 min at 37°C to stimulate ROS production. Frozen 2-cell mouse embryos were thawed and cultured in the stimulated leukocyte supernatant for 3 and 6 hours and then transferred to fresh HTF until 72 hours. ROS production was measured by chemiluminescence assay using luminol as the probe at each time course of culture media. Blastocyst development rates (%BDR) were examined at 72h following incubation. Control group consisted of the embryos cultured in HTF medium alone (>90% BDR). In a second set, vitamin E at concentration of 100 to 600 moles was examined to study the effect on %BDR.

Results:
Median (interquartile value, 25%, 75%) value of ROS generation by leukocytes was 882.10 (837.40, 1074.3 X 10^6 cpm), and 12.46 (10.39, 14.57 X 10^6 cpm) in leukocyte supernatant. Results of BDR after incubation of mouse embryos with leukocyte supernatant are given in the table below. BDR rates decreased significantly at 6h compared to 3h (p = 0.03). Vitamin E alone at 100, 200, or 400 µM had no negative effect on the % BDR. Embryos coincubated with vitamin E at 400 µM for 6h along with ROS generating supernatant significantly improved the % BDR (p = 0.04).

Conclusions:
The presence of ROS in the culture media for extended period (6h) results in mouse embryo toxicity. Addition of vitamin E can help scavenge the excessive production of ROS in the culture media and improve embryo blastocyst development rate in-vitro.

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