Bovine reproduction

**DEVELOPMENT OF AN IN VITRO MODEL TO STUDY THE EXPOSURE TO NUTRIENT IMBALANCE IN FEMALE BLASTOCYSTS**

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**BACKGROUND-AIM**

According to the Developmental Origins of Health and Disease (DOHaD), an imbalanced maternal diet has long-term effects on the offspring's health and epigenetic events are involved. Major epigenetic remodeling, such as DNA de-methylation/re-methylation waves, occurs during preimplantation embryo development in a sex-specific manner. Here we describe the set up of experimental conditions to investigate the impact of energetic imbalance on the epigenetic remodeling of bovine embryos. We conducted experiments aimed at: 1) reducing the sex-related variability in DNA methylation patterns by using X-sorted semen for in vitro fertilization (IVF); 2) defining a serum-free culture medium to manipulate the concentration of energetic substrates without affecting the overall medium composition.

**METHODS**

COCs were recovered from 2-8 mm follicles of abattoir-derived bovine ovaries, in vitro matured, fertilized and the presumptive zygotes were fixed or cultured according to the experimental design. Embryos were cultured in synthetic oviductal fluid (SOF) with 5% serum or bovine serum albumin (BSA) plus glucose. Two discontinuous Percoll gradients, G1: 90%-45% and G2: 78.7%-67.5%, were compared to isolate of motile sperm from commercial X-sorted semen. Experimental endpoints were: spermatozoa concentration, amount, motility, and pronuclei (PN) formation. Finally, different IVF timing were tested, from 10 to 18 hours. Data were analyzed with Fisher's exact test or T-test. $P<0.05$ was considered statistically significant.

**RESULTS**

Replacing serum with BSA plus glucose did not affect blastocyst yield (43±7.56 and 35±9.51%, respectively, N=6). G1 and G2 sperm concentration, amount, and motility were similar. However PN formation was higher for G2 (G1: 9/34 (26%), G2: 27/31 (87%); $p<0.0001$). Reducing the IVF timing to 10 h improved the blastocyst rate, although not significantly (10 h: 12/46 (26%); 14 h: 5/46 (11%); 18 h: 5/47 (11%), $P=0.06$).

**CONCLUSIONS**

We reached the goal of successfully producing in vitro female bovine embryos with a serum-free medium, needed for investigating the effects of energetic imbalance during preimplantation embryo development. The limited span of IVF might prove crucial to improving the developmental competence of embryos fertilized with X-sorted semen.