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VISUALIZATION OF BOVINE SPERM SEX CHROMOSOMES IN PENETRATED ZONA-FREE HAMSTER OVA

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To establish the optimum conditions, for the visualization of bovine sperm sex chromosomes using zona-free hamster ova, the concentration and timing of the addition of chemicals (Podophyllotoxin and Vinblastin) which interrupt karyogamy and block tubulin polymerization were investigated.

The zona-free hamster ova were inseminated in vitro with bovine spermatozoa that were pre-incubated in BO medium containing 15 mM theophylline and treated with 0.5 μ M ionophore A-23187 for 1 min. The inseminated ova were cultured in TCM 199+10% FCS.

In the first experiment, the inseminated ova were cultured in vitro without the addition of the chemicals. It was observed that pronuclear formation started 2 hrs after insemination, and the highest rate was obtained after 10 hrs. The disappearance of the pronuclei and cleavage to the 2-cell stage occurred 13 and 16 hrs after insemination, respectively.

In the second experiment, the above two chemicals were added to the culture medium, and the optimum concentration and timing of addition were examined. The highest number of metaphase plates were obtained when the concentration of both chemicals was 0.06 μ g/ml. There was no difference between the timing of addition at 10 and 13 hrs.

In the third experiment, hamster ova were inseminated with spermatozoa collected from three different individual bulls and the sperm sex chromosomes were observed. In this experiment, the chemicals were added at a concentration of 0.06 μ g/ml, 10 hrs after insemination. The ova were further cultured for 10 to 17 hrs and then subjected to either whole mount or air dried preparation to examine the frequency of ova at the first cleavage metaphase. Results from the whole mount preparation showed that 40.4–57.1% (mean 50.7%) of the inseminated ova had metaphase plates (mean 1.4). In the air dried preparation, sperm chromosomes were identified in 42.5–55.9% (mean 49.9%) of inseminated ova (number of male metaphase plates/number of ova inseminated), and 71.4–84.6% (mean 78.2%) of these metaphase plates could be analyzed for X and Y chromosomes.