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In vitro embryo additives

Introduction. Hyperthermia adversely affects oocyte maturation and fertilizations rates, and retards further embryonic development and viability (1). Niacin is a potential nutritional supplement for heat stressed cattle because it induces vasodilation; this effect has been shown to occur by activation of prostaglandin D2 (PGD2) and E (PGE2) in epidermal Langerhans cells. In addition, prostaglandins (cyclopentanones) such as PGD or immediate metabolites activate heat shock factor I and induces the synthesis of heat shock proteins (HSPs) in a variety of mammalian cells(2).

Based on these findings, our objective was the modification of culture medium with selective concentrations of PGE2, PGD2, niacin and their combination to evaluate cleavage rates in bovine early embryos.

Methods. Bovine ovaries from mature cows or feedlot heifers were obtained from local abattoirs and transported to the laboratory within 2-4 h. After arriving at the laboratory, ovaries were trimmed of extraneous tissue, and rinsed once again in 0.15 M NaCl. Cumulus-oocyte complexes (COCs) were aspirated from 3- to 8-mm antral follicles with an 18-gauge needle attached to a tubing system connected to a vacuum aspiration pump with 50 mm Hg of pressure; semen preparation, fertilization and early culture was described previously by Barcelo & Seidel (3) and De La Torre-Sanchez et al. (4). We evaluated whether selected levels of prostaglandin E2 (10mM), D2 (40mM), niacin (10mM) and their combination added to the culture medium (CDM-1), alleviate heat shock effects on early embryonic development. We used a completely randomized design that included additives PGD2, PGE2, niacin, temperature (39°C vs. 41°C and 39°C vs. 42°C) and the interaction among these factors as fixed effects, and semen of 3 bulls used twice as random effect. The experiment had 3 replicates.

Results. There were no effect of treatments on 2-4 cell and eight cells cleavage rates in the 39°C vs. 41°C contrast, but we found significant differences (P < 0.05) for cleavage rate between 39°C and 41°C (17.39 \pm 2.33 and 13.30 \pm 2.33 %, respectively). We found effect (P < 0.05) of temperature and niacin (10mM), increasing 2-4 cell cleavage rate at 42° C (8.78 \pm 2.69 compared to 17.82 \pm 2.69). There were no effects of treatments on eight cell cleavage rates.

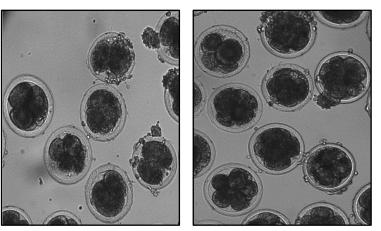


Figure.1 Bovine embryos produced by in vitro fertilization. Left panel, 2-4 cell stage. Right panel, 5-8 cell stage.

Table 1. Effect of Niacin with no heat treatment (control group) and
Niacin with heat treatment (42° C) during IVF on the number
of presumptive zygotes developing to 2-4 cell stage.

Temperature (°C)	Level of niacin (mM)	Cleavage rate % (±S.E.)
39	0	20.18±2.69 ^a
	10	14.61±2.69 ^b
42	0	8.78±2.69 ^a
	10	17.82±2.69 ^b

^{a, b} Statistical difference within temperature level (*P*<0.05)

Conclusions. The results showed that niacin may protect against heat shock at 2-4 cell stage, where embryos are more susceptible to heat stress effects.

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