Fertilization of Heat-Stressed Oocytes

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A Research Paper Describing Efforts Towards
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Abstract

Heat stress may accelerate aging in the oocyte, which may contribute to an inferior oocyte-sperm interaction. The objective of this study was to evaluate sperm penetration and monospermy in heat-stressed oocytes. Bovine oocytes were matured for 24 h at 38.5°C or 41.0°C (first 12 h). Penetration and monospermy were assessed 9 hours post insemination (hpi). Penetration was defined as the presence of sperm in the oocyte and monospermy by the presence of just one sperm in the oocyte. Heat stress decreased the proportion of oocytes penetrated by sperm. However, monospermy of the penetrated oocytes was similar to controls. Results indicate that some of the negative effects observed in heat-stressed matured oocytes may be due to the inability of sperm to penetrate the oocyte.

Introduction

Dairy cattle exposed to heat stress exhibit reduced fertility (Hansen et al., 1999; Rensis et al., 2003) and remain not pregnant for longer periods of time than non-heat-stressed cows (Fuquay, 1981). A producer may lose up to $4.00/day/cow for every day a cow is not pregnant beyond 90 days after giving birth (Britt, 1975). Reductions in fertility are reciprocally related to elevated body temperatures (Dunlap et al., 1971).

The oocyte is most susceptible to elevated body temperature while the cow is in estrus (receptive to breeding and the oocyte is preparing for fertilization; Putney et al., 1989). Unfortunately, *in vivo* studies do not allow for effectively differentiating between maternal and direct effects of elevated temperature on

the developmental competence of maturing oocytes. However, *in vitro* studies have proven beneficial to identify direct effects of heat stress on the oocyte. These direct effects of elevated temperature (41°C) alter constituents within the oocyte cytoplasm (Edwards et al., 1996; Payton et al., 2003a) and decrease blastocyst development after fertilization (Edwards et al., 1996, 1997; Lawrence et al., 2004).

During oocyte maturation the cytoplasm and nucleus must undergo changes for continued development. Previous studies have demonstrated that an increase in temperature may modify the kinetics of maturation. Research has revealed that accelerated cytoplasmic maturation (Payton et al. (2003b) and nuclear maturation (Edwards et al., unpublished) may occur in heat-stressed eggs. Taken together, these studies suggest heat stress may induce premature aging of the oocyte, thereby reducing its fertile lifespan.

Recent results from Edwards' laboratory indicated that chemical oocyte activation may eliminate reductions in development observed in heat-stressed oocytes (Edwards et al., unpublished). Removing sperm resulted in similar development between control and heat-stressed oocytes. This indicates that the reduced development seen with heat-stressed oocytes may be due to 1) an inability of sperm to penetrate the heat-stressed oocyte or 2) an inability of heat-stressed oocytes to remodel the sperm after fertilization to continue in development. The objective of the study was to assess fertilization of heat-stressed oocytes by evaluating the number of oocytes penetrated by sperm.

Materials And Methods

Unless otherwise indicated, all chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tissue culture medium-199 (TCM-199), gentamicin, and penicillin-streptomycin were purchased from Specialty Media (Phillipsburg, NJ, USA). Bovine ovaries were purchased from a commercial abattoir (Gaffney, SC, USA). Fetal bovine serum (FBS) was purchased from BioWhittaker (Walkersville, MD, USA). Follicle stimulating hormone was obtained from Vetrepharm Canada Inc. (London, Ontario, Canada). Media for *in vitro* production of embryos were prepared as previously described (HEPES-TALP, IVF-TALP, and Sperm-TALP; Parrish et al., 1988).

Effects of Elevated Temperature on Fertilization of Oocytes

In vitro maturation (IVM) and *in vitro* fertilization (IVF) were performed as previously described (Edwards et al., 1996; Lawrence et al., 2004). Cumulus-oocyte complexes (COC) were collected from antral follicles (3-8 mm) and matured for 24 h at 38.5°C (control) or 41.0°C (heat stress) for the first 12 h of maturation and 38.5°C for the duration. After 24 h, COC were fertilized (500,000 motile sperm/mL; pool of two different bulls) with Percoll-prepared frozen-thawed semen.

Penetration of the oocyte by sperm and monospermy were evaluated 9 hpi by removing cumulus and associated spermatozoa from the oocyte. The oocytes were fixed, stained, and viewed with fluorescence microscopy (experimental schematic; Figure 1).

Statistical Analysis

Data were analyzed as a randomized block after testing for normality (Shapiro-Wilk test; W≥0.90). The main effect of temperature was evaluated (SAS Inc., 2003).

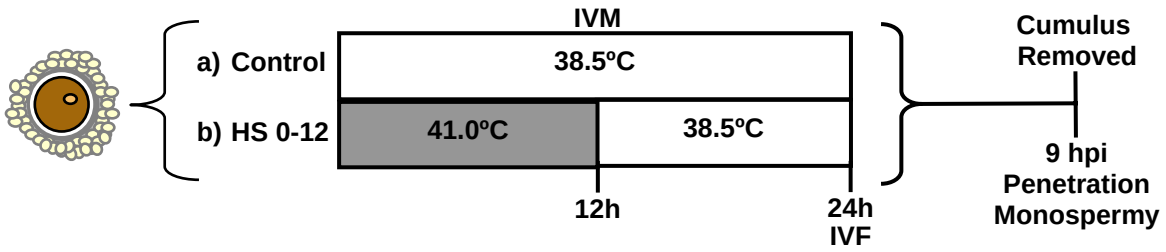

Figure 1 Experimental Design. Oocytes were cultured at either 38.5ºC or 41.0ºC (first 12h) for 24 h, fertilized, 9 hpi cumulus removed, oocytes fixed, and stained to assess penetration and monospermy.

Results and Discussion

The culture of oocytes at 41.0°C (heat stress) did not affect the proportion recovered after removal of associated cumulus and spermatozoa at 9 hpi (100 and 92.9% for 38.5 and 41.0°C, respectively; SEM=2.7; P=0.58) or the proportion that were visibly lysed. Recovery and lysis serve as indicators for a competent oocyte membrane, which is imperative for oocytes to continue in development. These results agree with previous findings that heat stress does not compromise membrane integrity (Edwards et al., 1997; Payton et al., 2003b; Lawrence et al., 2004).

The ability of sperm to penetrate oocytes was reduced by heat stress (Figure 2). These results imply that fertilization of heat-stressed oocytes is reduced. However, these results are being interpreted with caution as the

experiment was only replicated twice and data was conflicting between experimental replicates. In particular, in one of the experimental replicates, none of the heat-stressed oocytes were fertilized. In the second replication, penetration rates were similar to controls. Additionally, the observed effects conflict with preliminary data generated in Edwards' laboratory, which suggest that elevated temperature does not alter the ability of sperm to penetrate the oocyte (6 hpi; 42.4 and 37.7% oocytes penetrated by sperm; P>0.7; n=63/treatment; Edwards et al., unpublished). More research is needed to examine this further because absence of penetration in one experimental replicate may have been due to inexperience of the investigator.

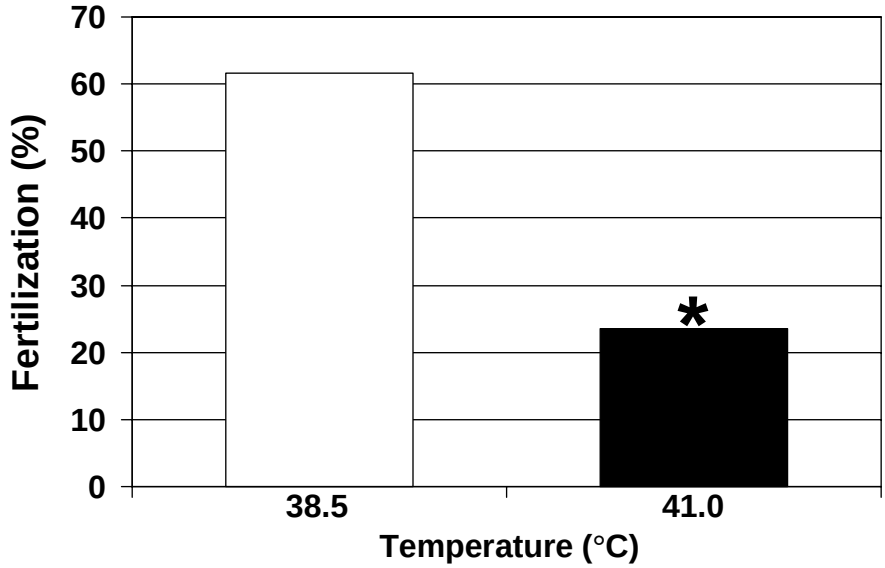

Figure 2 Proportion (%) of oocytes penetrated by sperm 9 hpi. Oocytes were matured at 38.5ºC (control, □) or 41.0 ºC (heat shock first 12 h, ■) for 24 h. (* P<0.05).

Next, the proportion of monospermic embryos within penetrated oocytes was examined. Monospermy is the number of oocytes penetrated by only one sperm. With a limited number of penetrated oocytes, there was no numerical

effect of elevated temperature on monospermy (Figure 3). Preliminary data from Edwards' laboratory also imply that monospermy is not altered at 6 hpi (82.4 and 95.6% monospermy in oocytes penetrated by sperm; $P<0.2$, Edwards et al., unpublished) or 18-20 hpi (66.7 and 75.9% monospermy in oocytes penetrated by sperm; $P<0.2$, Edwards et al., unpublished) by elevated temperature.

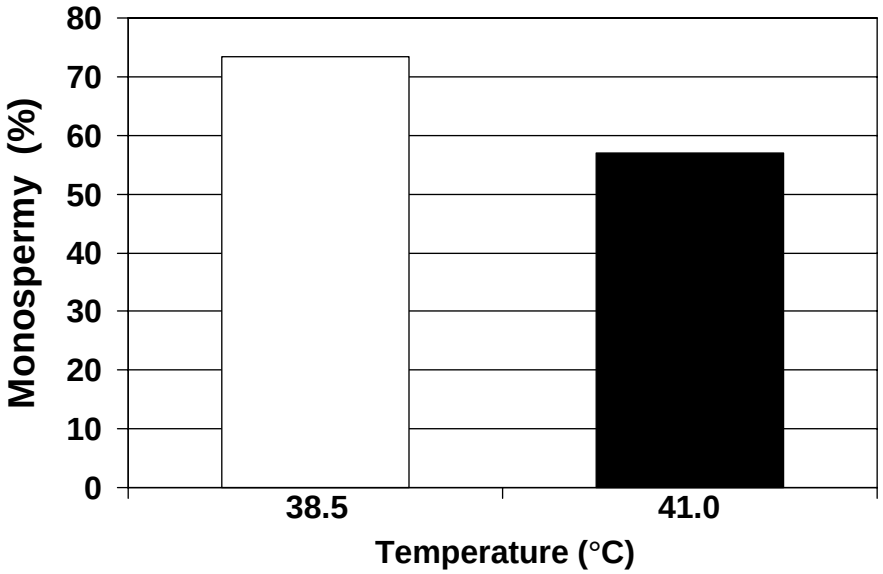

Figure 3 Proportion (%) of monospermy in penetrated oocytes 9 hpi. Oocytes were matured at 38.5ºC (control, □) or 41.0 ºC (heat stress first 12 h, ■) for 24 h.

Although numbers of oocytes were limiting in the experiment, the data obtained does warrant future studies to examine the influence of culture temperature on the ability of sperm to penetrate oocytes. Only two replicates were performed because of the time and effort needed to learn the laboratory techniques in performing the experiment. Several preliminary replications were performed to improve fertilization and culture of oocytes to a standard level. Throughout the process of completing the experiment, I worked independently with guidance from Dr. Edwards and her graduate students. Not only has this

project allowed me to gain experience with *in vitro* production of embryos, but also prompted me to pursue graduate studies in Dr. Edwards' laboratory to continue my research experience.

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