Physiology and Endocrinology Symposium: The Current Status of Heat Shock in Early Embryonic Survival and Reproductive Efficiency

388 Influence of sire breed on heat stress tolerance of in vitro-produced bovine embryos. C. M. Barros* and R. A. Satrapa, *Department of Pharmacology, Institute of Biosciences, University of Sao Paulo State, Botucatu, Sao Paulo, Brazil.*

In bovine, hyperthermia reduces both quality and quantity of sperm in males and decreases fertility in females. The detrimental effects of heat stress on embryo development are critical during the first cleavage divisions, when most of the embryonic genome is inactive. However, as pregnancy advances the deleterious effects of heat stress on embryonic survival decrease. Several in vitro studies indicate that *Bos indicus* embryos are better able to survive heat stress at early stages of development and are more capable of originating pregnancies following heat stress than *Bos taurus* embryos. The evidence that embryonic genotype determines resistance to heat shock leads to the question as to whether embryos sired by *Bos indicus* breeds exhibit superior resistance to heat stress than those sired by *Bos taurus*. Contrary to previous reports, in one of our in vitro studies we found that Holstein oocytes fertilized with *Bos indicus* semen (Nelore breed) were more resistant to heat stress than those fertilized with *Bos taurus* (Angus) semen, suggesting that the sire breed influences embryo tolerance to heat stress. However, in this particular experiment, only 2 sires from each breed were used. Therefore, we suspected that the sire effect may have overshadowed the breed effect; that is, the better tolerance to heat shock was due to the bull itself and not to the breed of the bull. To minimize the bull effect, we repeated the same experiment using 6 sires from each breed. It was observed that sire breed did not influence embryo tolerance to heat stress. Overall these results indicate that the oocyte plays a more crucial role in the ability of the embryo to resist the effects of heat stress than the spermatozoa. Supported by FAPESP (Sao Paulo, Brazil).

Key Words: embryo, heat stress, IVF

389 Associations between heat shock protein 70 genetic polymorphisms and calving traits in crossbred Brahman cows. C. Rosenkrans Jr.*[1], M. Brown[2], H. Brown Jr.[1], and M. Looper[1], *[1]University of Arkansas, Fayetteville, [2]USDA-ARS, El Reno, OK.*

Stressors such as heat, cold, toxins, and oxygen deprivation are known to induce heat shock proteins. Genetic polymorphisms associated with heat shock protein genes have been associated with decreased male and female fertility. Our objectives were to 1) confirm single nucleotide polymorphisms (SNP) located in the promoter region of the bovine heat shock protein 70 (*Hsp*70) gene and 2) evaluate associations among *Hsp*70 SNP, breed, forage system, and lifetime calving rates of multiparous cows (n = 88). Angus (n = 22), Brahman (n = 30), and reciprocal crosses (n = 36) cows were assigned to and remained on their respective forage system for the duration of the experiment (8 years). Forage systems were endophyte-infected toxic tall fescue (E+) or common bermudagrass (CB). Genomic DNA was extracted from buffy coats of EDTA-treated whole blood. Primers HSP-Pro749F (GCCAGGAAACCAGAGACAGA) and HSP-Pro1268R (CCTACGCAGGAGTAGGTGGT) were used for PCR amplification of a 539-base segment of the bovine *Hsp*70 promoter (GenBank accession number M98823). Ten SNP were detected: 7 transitions (G1013A, n = 4; G1045A, n = 7; C1069T, n = 8; A1096G, n = 3; G1117A, n = 36; T1134C, n = 6; and T1204C, n = 65), 2 transversions (A1125C, n = 62; and G1128T, n = 34), and one insertion/deletion at base position 895 (C895D; n = 12). Minor alleles numerically were associated with Brahman cattle; however, G1117A was the only site associated ($P < 0.05$) with breed with minor allele frequencies of 11, 25, and 43% respectively for Angus, reciprocal crosses, and Brahman. Interaction ($P < 0.05$) between forage system and C895D genotype affected lifetime calving percentage (87, 86, 80, 85, and 20 for CC-CB, CD-CB, DD-CB, CC-E+, and DD-E+; respectively). A similar interaction ($P < 0.05$) was observed for G1117A and forage system. Homozygous guanine cows grazing E+ had lower ($P < 0.05$) lifetime calving rates when compared with GG cows grazing CB (76 vs. 90%). Our results demonstrate that Hsp70 promoter SNP will be useful in marker assisted management programs.

Key Words: cattle, *Hsp*70, tall fescue

390 Expression of heat shock protein genes and their splice variants in in vivo and in vitro bovine preimplantation embryos. H. Khatib,* *University of Wisconsin, Madison.*

Heat shock proteins (HSP) are among the first proteins produced during embryonic development and are essential to cell function. Their activities include folding, unfolding, transport, and localization of proteins and differentiation and regulation of the embryonic cell cycle. Although HSPs have been extensively studied in human and mouse, there is limited information on the roles of these genes in bovine embryos. As such, the objectives of this study were to profile the expression of HSP and their splice variants in bovine embryos (degenerates vs. blastocysts) and to carry out association analysis with fertility traits. Quantitative real time PCR analysis revealed significant differences in expression in degenerate embryos compared with blastocysts. Interestingly, all members of the HSP40 family were found to be upregulated in degenerates. Analysis of current bovine Ensembl data showed that 13 HSP genes have one transcript each, 2 genes have 2 transcripts each, and 2 have 3 transcripts each. The results indicate that some splice variants show differential expression between degenerates and blastocysts while others were not expressed at all in embryos, which implies different functions of these transcripts in embryonic development. In addition, the expression profiles of HSPs were compared between in vitro produced embryos and in vivo embryos. The most significant differentially-expressed genes were further investigated for association with fertility traits. Single nucleotide polymorphisms some HSP genes were found to be associated with blastocyst rate and fertilization rate. Collectively, results of the present study point to the important roles of HSPs in fertilization and early development of cattle embryos.

Key Words: heat shock protein, expression, alternative splicing

391 Consequences of heat shock on development of the preimplantation bovine embryo: Role of free radicals, antioxidants, apoptosis, and heat shock proteins. P. J. Hansen*[1] and M. Sakatani[2], *[1]University of Florida, Gainesville, [2]Kyushu-Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, Kumamoto, Japan.*

The preimplantation embryo is one of the most sensitive of mammalian cells to hyperthermia. Exposure to temperatures as low as 40°C can reduce competence of the bovine embryo to continue development. Temperature sensitivity in at least some species (cow, sheep, pig) is

transient, however, and the embryo acquires resistance to elevated temperature (i.e., heat shock) as it advances through development. For the bovine embryo, there is little effect of heat shock on development after the 8-cell stage. Developmental acquisition of thermotolerance can be exploited to improve fertility in heat-stressed females through transfer of embryos at advanced stages of development. Experiments with the bovine indicate that developmental changes in embryonic sensitivity to heat shock are due to changes in several molecules and processes important for thermotolerance. Perhaps most important is the change in redox balance of the embryo. Heat shock can increase free radical production and the level of at least one antioxidant, glutathione, is lowest at early cleavage stages. Moreover, some antioxidants have been reported to increase embryonic resistance to heat shock in vitro. Apoptosis, if limited in extent, can be beneficial to embryonic survival after heat shock. Inhibition of apoptosis in embryos at d 4 or 5 of development increased the magnitude of heat shock effects on development. In this regard, it is instructive that the embryo does not gain the ability to undergo apoptosis in response to heat shock until about the 8–16 cell stage. The embryonic genome is also not fully activated until the 8–16 cell stage. The lack of transcription in early embryos may contribute to their thermal sensitivity because of the inability to produce proteins that stabilize cellular function. However, heat shock protein 70 (HSP70) is not likely to be a limiting protein in determining thermosensitivity. Levels of HSP70, as determined by immunofluorescence, are similar between 2-cell embryos and d 5 embryos. Heat shock can induce changes in HSP70 synthesis as early as the 2-cell stage. Moreover, steady-state mRNA for *HSP70* is higher for 1-cell and 2-cell embryos than for d 5 embryos. To date, a suitable treatment for increasing embryonic resistance to heat shock in the pregnant cow has not been identified but experiments with mice suggest that provision of a suitable antioxidant might enhance fertility of heat-stressed females.

Key Words: heat shock, embryo, bovine