Embryonic mortality in cattle from the embryo’s perspective

P. J. Hansen¹

Department of Animal Sciences, University of Florida, Gainesville 32611-0910

ABSTRACT: For the embryo to successfully complete development it must be capable of executing its developmental program within a microenvironment largely established by the mother. Mortality results either because of intrinsic defects within the embryo, an inadequate maternal environment, asynchrony between embryo and mother, or failure of the mother to respond appropriately to embryonic signals. To some extent, the embryo’s fate is dictated by events before fertilization: embryos formed from incompetent oocytes have a low probability of successful development. For example, embryos have reduced developmental competence when formed from oocytes from persistent ovarian follicles or from cows during the summer in Florida. Chromosomal abnormalities, caused by incompetent gametes or other causes, and homozygous recessive genes, exacerbated by inbreeding, represent additional types of intrinsic errors responsible for embryonic loss. Alterations in the maternal environment can cause embryonic mortality, as has been shown for heat stress and feeding diets high in degradable protein. The preimplantation embryo is most susceptible to certain types of stresses (most notably, heat shock) very early in development when its genome is largely repressed. Thus, the cellular adjustments the early embryo can make in response to perturbations in its environment are limited. Some genes related to resistance to cellular stress can become activated early in development (for example, heat shock protein 70) while other responses to stress are absent. For example, the early bovine embryo cannot undergo apoptosis in response to cellular stresses that ordinarily activate this process. One possibility is that the acquisition of the capacity for apoptosis represents an important mechanism by which an embryo acquires the ability to survive cellular stress. The embryo can also modify, to some extent, an inappropriate maternal environment. Development of procedures to improve oocyte competence and to manipulate embryonic stress responses may lead to new practices for improving embryonic survival.

Key Words: Development, Embryos, Fertility, Mortality, Oocytes

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Introduction

Embryonic development is initiated by syngamy of male and female pronuclei. The fate of the embryo is uncertain at this earliest stage in its life. To complete development, the embryo must progress through a series of preprogrammed developmental steps that transform it from an undifferentiated one-cell organism into a healthy neonatal animal. The probability of successful development is already partially determined at syngamy by virtue of the genetic and nongenetic inheritance that the embryo receives from the gametes from which it was formed. Subsequent errors in the execution of its developmental program can also lead to embryonic mortality.

Development is played out in the reproductive tract and the mother has a major impact on embryonic survival. Embryonic loss is increased when physiological regulation of oviductal and uterine function is inadequate or when the mother is exposed to one or more of the many stresses that can compromise embryonic survival. Conversely, maternal inputs may minimize the nature of minor deficiencies inherent within the embryo.

The embryo has some capacity to direct the maternal system to produce a local environment that serves the needs of the embryo. The embryo also possesses cellular adaptive mechanisms to limit adverse effects of various disturbances in the maternal environment. Whether a particular maternal stress affects embryonic survival depends not only on the magnitude of the stress imposed on the embryo but also on the effectiveness of embryonic adaptive responses to that stress.

This review will address the role the embryo itself plays in determination of its survival. Two features of this problem will be addressed: intrinsic errors that predispose it to failure, particularly those that arise...
**Table 1. Characteristics of cattle that affect oocyte competence**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Percentage oocytes to blastocyst</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>42.1%</td>
<td>Salamone et al., 2001</td>
</tr>
<tr>
<td>Calf</td>
<td>3.4%</td>
<td></td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>3.9%</td>
<td>Snijders et al., 2000</td>
</tr>
<tr>
<td>Third</td>
<td>10.4%</td>
<td></td>
</tr>
<tr>
<td><strong>Genetic ability for milk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>11.4%</td>
<td>Snijders et al., 2000</td>
</tr>
<tr>
<td>High</td>
<td>6.8%</td>
<td></td>
</tr>
<tr>
<td><strong>Body condition score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.3–4.0</td>
<td>9.9%</td>
<td>Snijders et al., 2000</td>
</tr>
<tr>
<td>1.5–2.5</td>
<td>3.0%</td>
<td></td>
</tr>
<tr>
<td><strong>Level of protein feeding</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-NH₃ diet</td>
<td>23.2%</td>
<td>Sinclair et al., 2000a</td>
</tr>
<tr>
<td>High-NH₃ diet</td>
<td>8.8%</td>
<td></td>
</tr>
<tr>
<td><strong>Season, Florida</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>26.2%</td>
<td>Al-Katanani et al., 2002a</td>
</tr>
<tr>
<td>Summer</td>
<td>17.6%</td>
<td></td>
</tr>
</tbody>
</table>

*After parthenogenetic activation.*

from the gametes from which the embryo was formed, and the development of embryonic adaptive responses to stress. The review focuses largely on cattle but similar principles likely hold true for other species as well.

**Gametic Determinants of Embryonic Survival**

The newly-formed zygote is composed of both genetic and nongenetic material from the oocyte and spermatozoon that produced it. Sperm mitochondria are rapidly ubiquitinated and cleared from the zygote (Sutovsky et al., 2000) but, in many species, rodents being a major exception, the sperm-contributed centriole is involved in formation of the zygote’s centrosome (Sutovsky and Schatten, 2000). The oocyte contributes even more material than the sperm; indeed, the cytoplasm of the zygote is largely derived from the oocyte and only maternal mitochondria survive in the zygote. Until the late four-cell to eight-cell stage, when there is a major round of embryonic transcription, the early embryo undergoes only limited transcription (Memili and First, 2000) and the embryo is dependent in large part on transcripts and proteins formed in the oocyte. Given the derivation of the zygote from the gametes, it is not surprising that defects in the formation or function of oocytes and spermatozoa can decrease embryonic survival.

**The Oocyte as a Determinant of Embryonic Survival**

The term oocyte competence has been developed to describe the potential for an oocyte to give rise to a normally developing embryo following fertilization. It is becoming increasingly clear that environmental or nutritional stresses can adversely affect oocyte competence. Using in vitro fertilization, several factors have been reported to reduce oocyte competence, as measured by the reduction in the proportion of oocytes that successfully develop into blastocysts (see Table 1). These factors include diets with high amounts of degradable crude protein (Sinclair et al., 2000a; Armstrong et al., 2001), low body condition score (Snijders et al., 2000), primiparity (Snijders et al., 2000), and high genetic merit for milk yield (although not actual milk yield; Snijders et al., 2000). In addition, oocyte competence is reduced in prepubertal animals (Armstrong, 2001). Using in vitro fertilization protocols, the proportion of oocytes that develop to blastocyst is greater for oocytes from larger follicles than for oocytes recovered from smaller follicles (Longeran et al., 1994). Laboratories in Louisiana (Rocha et al., 1998), Wisconsin (Rutledge et al., 1999), Israel (Zeron et al., 2001), and Florida (Al-Katanani et al., 2002a, 2002b) have reported a reduction in oocyte competence during the summer. The summer decline in oocyte competence is presumably due to heat stress. In sheep, heat stress 12 d before estrus reduced fertilization and lambing rates (Dutt, 1964.) In addition, retrospective analysis of a large reproductive data set in lactating dairy cattle revealed a negative association between heat stress 10 d before breeding and subsequent pregnancy rate (Al-Katanani et al., 1999). Near estrus, the oocyte also appears sensitive to damage. Exposure of superovulated cows to heat stress for 10 h beginning at the onset of estrus had no effect on fertilization rate but reduced the proportion of normal embryos recovered on d 7 after estrus (Putney et al., 1989a).

It is likely that oocyte competence can be compromised by changes in follicular dynamics. This has been demonstrated to be the case following estrus synchronization schemes using progestogen-releasing devices. Use of these implants results in incomplete suppression of LH secretion and development of a persistent dominant follicle of prolonged lifespan. It has long been known that fertility following insemination of cows with persistent dominant follicles is low (Savio et al., 1993;
Oocytes from persistent dominant follicles undergo premature meiosis, probably because of the premature exposure to high LH concentrations (Revah and Butler, 1996; Mihm et al., 1999). They also exhibit various abnormalities including an enlargement of the perivitelline space, intracellular vacuoles and irregular vesicles, and increased numbers of mitochondria and lipid droplets (Mihm et al., 1999). The low fertility associated with persistent dominant follicles may also represent, at least in part, effects on the reproductive tract; cows with persistent dominant follicles had altered protein secretory patterns in the oviduct (Binelli et al., 1999).

While pharmacologically induced persistent dominant follicles are associated with reduced fertility, there is yet little evidence that fertility is reduced in cows ovulating a follicle that was produced in an estrous cycle with two follicular waves (where lifespan of the dominant follicle is extended) as compared to cows ovulating a follicle that was produced in an estrous cycle with three follicular waves. In the only experiment of this type (Ahmad et al., 1997), there was no difference in pregnancy rate between cows ovulating a two-wave follicle (36/44 cows; 84% pregnant) vs three-wave dominant follicle (6/6 cows; 100% pregnant). However, studies with additional numbers of animals will be warranted to draw definitive conclusions.

### Cellular and Molecular Basis for Oocyte Competence

A matured oocyte must complete a multiplicity of processes to give rise to an embryo capable of successful development. These processes include acquisition of the sperm receptor proteins and signaling molecules on the ooplasm to allow for fertilization, accumulation of intracellular stores of calcium for signaling events, execution of the block to polyspermy, nuclear maturation with proper segregation of chromosomes, acquisition of the reducing agents and other molecules required for pronuclear decondensation, development of the cytoskeletal apparatus necessary for syngamy, and synthesis and storage of the mRNA, proteins, and other molecules necessary to support preimplantation development. Factors that reduce oocyte competence undoubtedly act at some point in oocyte growth or maturation to make for suboptimal execution of one or more of the processes listed above.

Improper chromosomal segregation is one documented cause of oocyte incompetence. Analysis by Yadav et al. (1991) indicated that about 15% of cultured oocytes possess chromosomal abnormalities (4.2% aneuploids and 10.7% diploids) and embryos formed as a result of fertilization of such oocytes would fail to develop. Heat stress, at least in mice, can cause chromosomal abnormalities in oocytes (Baumgartner and Chrisman, 1981, 1988), and it is possible that other environmental or physiological influences on the cow can also disrupt normal karyokinesis. The incidence of chromosomal abnormalities is much greater for embryos produced by in vitro fertilization than is the case in vivo (Viuuf et al., 2001) and some causes of chromosomal defects that are expressed in culture may not occur in vivo. Indeed, culture can cause profound changes in oocyte and embryo gene expression and function (see Duranthon and Renard, 2001) and it is possible that some apparent differences in oocyte competence that have been deduced using in vitro fertilization may not be observed when the oocyte undergoes maturation and fertilization in vivo.

Until it reaches about 110 to 120 μm in diameter (2- to 3-mm follicle; Hyttel et al., 2001), the oocyte is actively involved in synthesis, packaging, and storage of mRNA that will be used by the developing embryo. The bovine embryo is dependent on these maternal mRNA for the bulk of its protein synthesis until the late four-cell or eight-cell stage, when the embryonic genome becomes activated (Memili and First, 2000). Although much of the prestored maternal mRNA degrades rapidly in the embryo, it is likely that some embryonic protein synthesis depends on maternal mRNA even after embryonic genome activation. In the mouse, maternal mRNA is still present at the blastocyst stage in trophoblast and inner cell mass (Bachvarova and Møy, 1985). Failure of the oocyte to synthesize mRNA in adequate amounts to support embryonic development could lead to embryonic failure. In addition, the mRNA that is synthesized in the oocyte for use in embryonic development is packaged in a manner that allows for its storage and coordinated translation at the proper time in development (Brevini Gandolfi and Gandolfi, 2001). Disruption of this process could also compromise oocyte competence.

Translation of mRNA is controlled in part by polyadenylation at the 3′ untranslated region of the mRNA. Using morphological evaluation of the ovary to identify oocytes classified as being of high or low competence, Brevini-Gandolfi et al. (1999) demonstrated that the length of the polyA tail on several genes was shorter for oocytes of low competence, suggesting reduced translation of these mRNA. In addition, Lonergan et al. (2000) have shown that two-cell embryos that had undergone first cleavage by 30 h after insemination were more likely to develop to the blastocyst stage and to have higher amounts of transcripts for IGF-I, glucose-6-phosphate dehydrogenase, and hypoxanthine phosphoribosyl transferase than slower-cleaving embryos with reduced developmental potential. These results also support the importance of maternally derived mRNA for oocyte competence.

Membrane composition may be an important determinant of oocyte competence, because it is within the membrane that signal transduction and transport proteins function. Membranes from oocytes collected during the summer in Israel had higher lipid-phase transition temperatures than oocytes collected during the winter (Zeron et al., 2001). In the same study, there was also a higher proportion of saturated fatty acids in the summer for granulosa cells and follicular fluid.
Specific proteins synthesized by the oocyte during maturation are also likely to be important for embryonic development. One factor that improves competence during the maturation process, at least in culture, is epidermal growth factor (EGF) (Loneragan et al., 1996; Goff et al., 2001). Culture of cumulus-oocyte complexes with EGF during maturation led to induction of two oocyte proteins and increased synthesis of an additional six oocyte proteins (Goff et al., 2001).

Factors such as heat stress and diets high in crude protein that alter oocyte competence could do so by affecting oocyte development directly or by compromising the function of follicular cells supporting oocyte development. Sirard (2001) has suggested that, after completing or ceasing its growth, the follicle sends information to the oocyte that allows it to acquire competence. Evidence for this idea comes from experiments indicating that oocyte competence is improved in superstimulated cows if harvest of oocytes is delayed until 48 h after administration of the last injection of FSH (Blondin et al., 1997; Sirard et al., 1999). Similarly, competence of oocytes collected at slaughter is increased if oocytes are allowed to reside in the ovary for 4 h before harvest (Blondin et al., 1997). Using PCR-based suppressive subtractive hybridization, Robert et al. (2001) identified 18 transcripts that were present in higher amounts in granulosa cells from follicles yielding competent oocytes (100% development to blastocyst) than from granulosa cells from follicles yielding incompetent oocytes (0% development to blastocyst). These genes included the progesterone and prostaglandin receptors and the transcription factors Egr-1 and DNA binding protein A. Other genes identified included EGF, Sprouty 2, which modulates signal transduction of fibroblast growth factor, and epieregulin, which interacts with the EGF receptor. Such transcripts are potential target genes for alteration by factors that affect oocyte competence.

An unknown question is when, in the relatively long period of its growth and development, the oocyte is susceptible to disruption. The estimated time for growth of a bovine follicle from the initiation of follicular growth at the primary follicle stage to ovulation is 84 to 85 d (Picton et al., 1998; McNatty et al., 1999), of which approximately 42 d represents the antral phase of growth (Lussier et al., 1987). A stress capable of inducing oocyte lesions in the early stages of follicular growth could affect fertility for a considerable period after removal of the stress. There is some evidence that such a phenomenon may occur for heat stress. In Israel, Roth et al. (1999) harvested oocytes from 3- to 8-mm follicles during four consecutive estrous cycles in the autumn from previously heat-stressed cows. Over time, there was an increase in oocyte quality score and development. In another study, cooling cows for 42 d before slaughter in the summer did not increase oocyte competence as determined by development to the blastocyst stage after in vitro maturation, fertilization, and culture (Al-Katanani et al., 2002b). Although the cooling may not have been adequate to eliminate heat stress, it is also possible that oocytes were already damaged by heat stress before the 42-d period of cooling was initiated.

Role of the Spermatozoa in Embryonic Mortality

Like the oocyte, the male gamete influences fertility not simply by affecting fertilization rate but also, to some extent, by imparting characteristics to the embryo that influence its ability to proceed through development. Thus, one can observe differences in embryonic mortality between bulls in vivo (Wijerante, 1973) and in vitro (Hillery et al., 1990; Shi et al., 1990). In the rabbit, embryos formed from fertilization of oocytes with spermatozoa that had been exposed to elevated temperature had reduced survival (Howarth et al., 1965; Burfening and Ulberg, 1968).

One cause of male-derived embryonic loss is induction of chromosomal abnormalities. The incidence of nondisjunction in bovine spermatogenesis has been estimated at 2.8% (Logue and Harvey, 1978). Bulls carrying the 1/29 Robertsonian translocation produce a high rate of embryos that are aneuploid (Kawarsky et al., 1996) and are accordingly of reduced fertility (Dyrendahl and Gustavsson, 1979). Defective chromatin structure can also presumably lead to failure of pronuclear decondensation or early embryonic death: bulls with stable chromatin as determined by acid denaturation are more likely to give rise to offspring in heterospermic fertilization tests than bulls with less-stable DNA (Ballachey et al., 1988).

Embryonic Determinants of Embryonic Survival

Intrinsic Determinants of Embryonic Survival

Development during the embryonic period of pregnancy (until about d 42 of pregnancy when the fetus can be delineated) is a complicated phenomenon involving, among other processes, embryonic genome activation, compaction, blastocyst formation, elongation of the trophoblast, secretion of interferon-7 to maintain the corpus luteum, differentiation of placental tissues, and development of the inner cell mass into the fetus. Any of these processes could fail because of some intrinsic defect in the embryo, whether derived originally from the oocyte or sperm or originating subsequent to zygote formation.

There is little information about embryonic loss due to intrinsic errors. Chromosomal abnormalities can continue to occur after the first cleavage division to give rise to a mixoploid embryo in which some cells are diploid and others exhibit a chromosomal abnormality. The percentage of mixoploid embryos increases during the preimplantation period both in vitro (Kawarsky et al., 1996) and in vivo (Viuff et al., 2001). The proportion of mixoploid embryos from superovulated females at d 5 after ovulation was 31% (Viuff et al., 2001). It is not
known what extent of mixoploidy is compatible with live birth.

Recessive lethal genes also contribute to embryonic mortality. About 2% of Holsteins in the United States possess an autosomal recessive form of the gene for uridine-5′-monophosphate synthase which, when present in the homozygous condition, leads to embryonic mortality before d 40 (Zavy, 1994). Given the likelihood that other lethal recessive genes exist that compromise embryonic survival, inbreeding represents a threat to the genetic potential for fertility in livestock species. This is especially true for dairy cattle in which there has been an increase in inbreeding, including for Jerseys (Thompson et al., 2000) and Holsteins (Young and Seykora, 1996; Hansen, 2000). One geneticist has suggested that the problem has reached the point at which dairy producers should consider crossbreeding schemes (Hansen, 2000).

It has long been assumed that a proportion of pregnancies in cattle fail because the embryo produces inadequate amounts of interferon-τ (IFN-τ) to block uterine prostaglandin F2α production and thereby allow for continued maintenance of the corpus luteum (Thatcher et al., 1989). There is considerable variation in the degree to which the trophoblast has undergone elongation between d 15 and 17 of pregnancy, when the embryo first secretes IFN-τ in anti-luteolytic amounts. Amounts of IFN-τ secretion increase as size of the embryo increases (Geisert et al., 1988; Leung et al., 2000). At d 14 to 17, embryos were smaller in repeat-breeder heifers than in previously virgin heifers and were less likely to extend the lifespan of the corpus luteum (Albihn et al., 1991).

Inadequate IFN-τ because of delayed or retarded trophoblast development may reflect intrinsic defects in the embryo. In addition, adverse alterations in the maternal environment could affect embryonic IFN-τ secretion. Sheep (Nephew et al., 1991) and cows (Mann et al., 1998) experiencing a delay in the rise in progesterone concentrations after ovulation contained lower concentrations of IFN-τ in uterine flushes at d 13 (sheep) or 16 of pregnancy (cow) than females experiencing earlier rises in progesterone concentrations.

It has proven difficult to increase pregnancy rate in cattle by pharmacological manipulation of the luteolytic process. Administration of IFN-α to cows after breeding, to mimic the effects of the closely-related IFN-τ, did not improve fertility in cattle, but rather caused a slight reduction in pregnancy rate (Barros et al., 1992a). Interferons are pleiotropic molecules and cows administered IFN-α experienced acute hyperthermia, disruptions in LH secretion, and a short-term reduction in circulating concentrations of progesterone (Newton et al., 1990; Barros et al., 1992b). There is some reason to warrant that doses of IFN-τ itself may be found that can extend luteal lifespan while having minimal side effects (Meyer et al., 1995).

Administration of a single injection of GnRH or its analogs around d 11 to 14 after breeding has also been evaluated as a method for increasing pregnancy rate (Peters et al., 2000). Presumably, the GnRH would work by luteinizing follicles involved in initiation of luteolysis and thereby delaying CL regression so that a retarded embryo had additional time to develop adequate IFN-τ synthetic capacity. Results with this regimen have been mixed. A recent meta-analysis has indicated an overall beneficial effect of GnRH treatment to increase pregnancy rate (Peters et al., 2000) but more work is required to understand the basis for the variation in effectiveness of the treatment.

**Responses of the Embryo to the Maternal Environment: The Heat Stress Example**

In large part, an embryo’s survival depends on the maternal environment. Embryonic loss can occur when there is disruption in the physiological regulation of oviductal and uterine function due to intrinsic errors in maternal physiology or to specific environmental stresses imposed on the mother. Whether or not an embryo can survive perturbations in the maternal environment depends on the degree to which the embryo can either 1) adjust its own internal physiology to allow for its survival and continued development or 2) act on the mother to restore to some degree the microenvironment that the embryo resides in. The extent to which the embryo is capable of self-adjustment or adjustments of the maternal unit is not well understood for most maternal factors affecting fertility. The one exception is the case for heat stress; abundant information on the ability of the embryo to maintain cellular function and development in the face of elevated temperature (i.e., heat shock) is available. For this stress, at least, the embryo acquires mechanisms to become resistant to elevated temperature as it progresses through development. The biochemical and molecular nature of the thermoprotective mechanisms that allow more advanced embryos to become more resistant to elevated temperature is still under investigation.

In cattle, as for other species, exposure of pregnant females to heat stress during the embryonic period leads to embryonic loss (Putney et al., 1988; Ealy et al., 1993). One cause of embryonic death is the disruption of embryonic development caused when the embryo is exposed to elevated temperature. Thus, exposure of embryos to heat shock by elevating culture temperature blocks or reduces subsequent development (Edwards and Hansen, 1997; Rivera and Hansen, 2001). Heat shock leads to embryonic death, at least in part, because protein synthesis is reduced (Edwards and Hansen, 1996; Edwards et al., 1997) and, at least in the mouse (Aréchiga et al., 1995), free radical metabolism is increased.

One of the features of heat stress on embryonic survival, at least in the cow (Ealy et al., 1993), as well as sheep (Dutt, 1963) and pig (Tompkins et al., 1967), is that the magnitude of the depression in embryonic survival is less when heat stress is applied later in the
subjected to embryo transfer than for artificially inseminated cows in the summer for lactating dairy cows that were in estrus. In fact, several studies have shown higher pregnancy rates during periods of heat stress. The former embryos have already progressed through the stages in development most susceptible to heat stress.

7 after estrus than for inseminated cows because the survival for recipient cows receiving an embryo at d 7 was affected by stage × time ($P = 0.05$). Data are from C. E. Krininger III and P. J. Hansen, unpublished.

Figure 1. Effect of exposure of embryos to 41°C for various times on development to the blastocyst stage. Embryos were collected at 26 to 32 h after insemination (two- to four-cell stage) or at d 5 after insemination (all embryos > 16 cells), exposed to 41°C for the times indicated, and returned to 38.5°C until determination of percentage blastocysts at d 8. Data are least squares means ± SEM and represent the results from 5 to 10 replicates per group (58 to 86 embryos per group). Development was affected by stage × time ($P = 0.05$). Data are from C. E. Krininger III and P. J. Hansen, unpublished.

preimplantation period than when applied earlier in development. In cows, for example, exposure of superovulated females to heat stress at d 1 after breeding (i.e., when embryos were at the one- or two-cell stage) reduced the proportion of embryos at d 8 of pregnancy that were at the blastocyst stage of development (Ealy et al., 1993). However, heat stress had no effect on the proportion of embryos at d 8 classified as blastocysts when heat stress was applied at d 3 (four- to eight-cell), 5 (16 cell-morula), or d 7 (morula-blastocyst stage). Similarly, in culture, exposure of embryos to elevated culture temperature caused a greater reduction in development at the two-cell stage than in embryos at the > 16 cell stage or morula stage (Edwards and Hansen, 1997; also see Figure 1 for unpublished results of Krininger and Hansen).

The fact that bovine embryos increase in resistance to heat shock as development proceeds has led to the use of embryo transfer as a method of improving pregnancy rates during periods of heat stress. One would expect that heat stress would have less effect on embryonic survival for recipient cows receiving an embryo at d 7 after estrus than for inseminated cows because the former embryos have already progressed through the stages in development most susceptible to heat stress. In fact, several studies have shown higher pregnancy rates in the summer for lactating dairy cows that were subjected to embryo transfer than for artificially insem-
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Figure 2. Time-dependent, heat-induced apoptosis in bovine embryos ≥ 16-cell stage collected at d 5 after insemination. Results are least squares means ± SEM of four replicates (342 embryos total). Exposure of embryos to heat shock of 40°C (closed circles) or 41°C (open circle) increased \( P < 0.001 \) the percentage of cells undergoing apoptosis. There was a linear \( P = 0.05 \) and a quadratic \( P < 0.001 \) effect of time at 40°C and linear \( P < 0.001 \) and quadratic \( P < 0.001 \) effect of time at 41°C. However, heat shock had no effect on total cell number/embryo. Data are from Paula-Lopes and Hansen (2002).

The ability of embryos to undergo apoptosis in response to heat shock is also developmentally regulated in bovine embryos. For embryos ≥ 16 cells in number at d 5 after fertilization, heat shock induces an increase in the proportion of cells undergoing apoptosis (Paula-Lopes and Hansen, 2002; see Figure 2). In contrast, heat shock does not increase the number of apoptotic cells at the two-, four-, or eight-cell stage. The 8- to 16-cell stage appears transitional: embryos at this stage collected at d 3 after fertilization do not undergo apoptosis, whereas such embryos collected at d 4 after fertilization are capable of heat-induced apoptosis (Paula-Lopes and Hansen, 2002).

The fact that embryonic resistance to heat shock is temporally associated with ability to undergo apoptosis in response to heat shock suggests that apoptosis may be involved in embryonic resistance to elevated temperature. Cells exposed to stress that cannot undergo apoptosis frequently undergo cell death more reminiscent of necrosis (Xiang et al., 1996; Woo et al., 1998). Such necrotic death may be more inimical to the long-term developmental capability of embryos than the orderly removal of cells by apoptosis. Perhaps a limited amount of apoptosis is beneficial to heat-shocked embryos by eliminating cells damaged by heat shock and, possibly, by preventing necrosis.

The molecular basis for developmental acquisition of the ability to undergo heat-induced apoptosis is not known. The machinery for apoptosis is present, at least at some level, since the beginning of development. Thus, the protein kinase C inhibitor, staurosporine, could induce apoptosis in mouse embryos at the one- to four-cell stage (Well et al., 1996; Exley et al., 1999) and bovine embryos at the 1- to 16-cell stage (Byrne et al., 1999). The failure of heat shock to induce apoptosis early in development, then, could possibly be caused by a predominance of anti-apoptotic modulatory proteins in the early cleavage-stage embryo and a decline in the amounts of these proteins as development proceeds. There may also be some developmental changes in levels of proteins involved in induction of apoptosis. In one study, RT-PCR was used to evaluate presence of caspase transcripts in mouse embryos (Exley et al., 1999). Although transcripts for all caspases tested could be detected in oocytes, these transcripts disappeared by the zygote stage (except for caspase-2, which remained present in zygotes). Transcripts reappeared at the two-cell stage (caspase-2, -3, and -6) or at the eight-cell stage (caspase-7) or remained undetectable through the blastocyst stage (caspase-1 and -11). Thus, as for many other oocyte mRNA, transcripts for caspases decrease coincident with fertilization and reappear at some later specific stage.

Embryonic Responses to Other Stresses

The developmental pattern in embryonic resistance to a specific stress depends on the biochemical nature of the lesion induced by the stress and the developmental changes in the corresponding cytoprotective mechanisms of the embryo. For some stresses, as for heat shock, the embryo acquires increased resistance as it advances in development. In cattle, development to the blastocyst stage was inhibited by KCN, an inhibitor of oxidative phosphorylation, when added during d 1 to 3 of culture but not when added during d 4 to 6 (Donnay et al., 2000). Embryos again became sensitive to KCN when added during blastocyst formation (d 6 to 8). Rab-
bit embryos were more sensitive to disruption by bi-
clorinated biphenyls at the morula stage than at the
blastocyst stage of development (Kuchenoff et al., 1999).
Also, resistance to changes in weak acids increases dur-
ing development in mice (Edwards et al., 1998). In con-
trast, sensitivity of embryos to other stresses appears
to change little during the period of preimplantation
development. This has been shown for bovine embryos
exposed to hydrogen peroxide (Morales et al., 1999) and
sodium arsenite (Krininger and Hansen, unpublished
observations). For other toxic stresses, such as exposure
to cadmium in the mouse (De et al., 1993) and chloram-
bucil in the rat (Giavini et al., 1984), embryonic sensi-
tivity increases during development. For cadmium, this
pattern reflects increased uptake of the heavy metal
(De et al., 1993).

**Figure 3.** Evidence that the embryo can advance a retarded uterine environment. In this experiment by Ashworth
and Bazer (1989), ovine embryos at d 6 of pregnancy were transferred into the uterus of recipients that were either
at d 4 or 6 of the cycle. Other ewes were made pregnant by natural mating. Endometrium was obtained from the
embryo transfer recipients at 4 d after transfer and at d 8 and 10 of pregnancy for naturally mated pregnant ewes. Endometrial
explants were cultured with [35-S]methionine and newly synthesized secretory proteins were identified by two-
dimensional SDS polyacrylamide gel electrophoresis and fluorography. The secretion rate of 30 specific secretory
proteins was quantified. For some proteins, the protein secretory rate in embryo transfer recipients at d 8 of pregnancy
(with a d-10 embryo) more closely matched the secretory rate at d 10 of pregnancy than the rate for naturally bred
ewes at d 8 of pregnancy. Data in panel A are from one such protein. For other proteins, the presence of an advanced
embryo in the uterus did not change secretory rate. In these proteins (see panel B for data for one such protein),
secretory rate was similar between embryo transfer recipients at d 8 of pregnancy and naturally mated pregnant ewes
at d 8.

**Can the Embryo Alter the Maternal Environment?**

Beginning at d 15 to 17 of pregnancy, the embryo
directs massive changes in endocrine and endometrial
function of the mother through secretion of IFN-τ. Be-
fore this time, however, we know little about the role of
the embryo in causing specific changes in the maternal
environment. The fact that pregnancy can be main-
tained when embryos are transferred into recipients as
late as d 16 of the estrous cycle (Betteridge et al., 1980)
means that the early embryo causes no persistent effect
on maternal physiology crucial for pregnancy success
until it must act to maintain the corpus luteum. None-
theless, the embryo is engaged in secretion of several
regulatory factors during the preimplantation period.
Indeed, the bovine embryo possesses transcripts for
EGF, transforming growth factor-α, leukemia inhibi-
tory factor, basic fibroblast growth factor, and IGF-I (Yoshida et al., 1998; Eckert and Niemann, 1998; Lonergan et al., 2000). It is likely that these molecules act, at least in part, to change the maternal environment early in pregnancy.

The fact that embryonic expression of leukemia inhibitory factor is up-regulated during culture of bovine embryos (Eckert and Niemann, 1998) implies that the embryo can modify secretion of regulatory molecules in response to changes in its environment. If so, it is conceivable that the preimplantation embryo can act on the mother to improve certain inadequate maternal environments. There is some experimental evidence that this is the case. In particular, Ashworth and Bazer (1989) demonstrated in sheep that an embryo placed in a uterus that is retarded in time relative to the embryo can alter the secretion of many endometrial secretory proteins to levels similar to those characteristic of a uterus at the same day of pregnancy as the embryo (Figure 3).

Relationship Between Events in the Preimplantation Period and Postimplantation Development

It has long been known that exposure of preimplantation embryos to various toxins can lead to various developmental defects during fetal development (Elbs et al., 1982; Iannaccone, 1984; Generoso et al., 1987; Otani et al., 1991; Lane and Gardner, 1994; Jacquet et al., 1995). More recently, the question of how the developmental process can be incorrectly programmed during the preimplantation period has been placed at the center of research concerning embryo technology because of the association between in vitro fertilization, embryo culture, and nuclear cloning with the occurrence of a host of abnormalities in the fetal and neonatal period called the large-offspring syndrome (Betteridge, 2001). Postulated mechanisms for the large offspring syndrome have been the focus of many recent reviews (for example, see Sinclair et al., 2000b; Young and Fairburn, 2000; Farin et al., 2001) and will not be discussed further. It is pertinent to ask, however, to what degree errors in postimplantation development in populations of livestock species bred via natural or artificial insemination can be attributed to errors in genetic programming early in the preimplantation period. Consistent with this idea are observations in sheep that fetal overgrowth can be induced by transfer of embryos into an advanced uterine environment (Wilmut and Sales, 1981) or premature elevation of progesterone concentrations (Kleeman et al., 1994). There is some evidence in lactating dairy cows that the rate of postimplantation embryonic failure is higher than previously thought (Lucy, 2001; Stevenson, 2001) and it will be instructive to determine the proportion of these losses that represent carryover effects of stress during embryonic development into the fetal period.

Implications

Pregnancy rate per insemination in dairy cattle has declined in the last 30 yr or so in the United States and Great Britain (Royal et al., 2000; Lucy, 2001). Although some of this decline likely reflects failures of estrus detection, improper semen deposition, and so on, such a large decrease in pregnancy rate points to an increase in embryonic mortality. Evidence gathered in this review indicates that one source of embryonic mortality is inadequate oocyte growth and maturation leading to an oocyte that is incapable of becoming a healthy embryo. Perhaps failure of the embryo to undergo cellular adaptations to stabilize embryonic function in the face of an adverse maternal environment is another source of embryonic loss. Research directed toward improving oocyte competence and toward manipulation of embryonic stress responses may lead to new methods for enhancing fertility in cattle and other species.

Literature Cited


