Large offspring syndrome in cattle and sheep

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Bovine and ovine embryos exposed to a variety of unusual environments prior to the blastocyst stage have resulted in the development of unusually large offspring which can also exhibit a number of organ defects. In these animals, the increased incidence of difficult parturition and of fetal and neonatal losses has limited the large-scale use of in vitro embryo production technologies commonly used in humans and other species. Four different situations have been identified that result in the syndrome: in vitro embryo culture, asynchronous embryo transfer into an advanced uterine environment, nuclear transfer and maternal exposure to excessively high urea diets. However, programming of the syndrome by all of these situations is unpredictable and not all of the symptoms described have been observed universally. Neither the environmental factors inducing the large offspring syndrome nor the mechanisms of perturbation occurring in the early embryo and manifesting themselves in the fetus have been identified.

In recent years considerable numbers of abnormally large calves and lambs have been born after various manipulations of the embryo before the hatched blastocyst stage. This review summarizes current knowledge of what is known as the large offspring syndrome and presents some hypotheses on the nature of the perturbing factors and underlying mechanisms, which are unknown at present (Fig. 1). Only a limited amount of data from experimental studies of this syndrome exists. However, there are many anecdotal reports of large offspring, derived from commercial embryo transfer companies. Although fetal overgrowth syndromes have been reported in other species (see Hastie, 1997), these have arisen from chromosomal abnormalities, spontaneous mutations or experimental genetic manipulation. So far, overgrowth phenotypes have been described specifically only as a result of insults to the early embryo in cattle and sheep. Large offspring syndrome has been initiated in bovine and ovine embryos after exposure to unusual environments both in vivo and in vitro. In most cases embryos have been exposed to these environments for about a week, during the period between fertilization and development to the blastocyst stage (Fig. 2). Commercially, livestock embryos are routinely transferred to recipients at the blastocyst stage (at days 5–6 after fertilization in sheep and days 7–8 in cattle) before hatching from the zona pellucida. In ruminants, the outer trophoderm layer of the blastocyst then rapidly proliferates in the recipient uterus to form a ‘tapeworm-like’ elongated conceptus which is free living in the uterus until attachment begins.

Although exposure of ovine and bovine pre-elongation embryos to several unusual environments has initiated the large offspring syndrome, the number and nature of the perturbing environmental factors have not been identified. In vitro, exposure to support cells co-cultured with embryos and exposure to serum are commonly associated with the syndrome. Perturbing environments in vivo may also expose the embryo to factors at unusual concentrations for a particular stage of development, but it remains to be established whether common factors perturb development in vivo and in vitro. Furthermore, it has not been established whether there is a critical phase in pre-elongation development when the perturbation occurs or whether exposure at any time is sufficient to induce the change. Current research is focussing on identification of measurable changes in the early embryo indicative of perturbed growth and development and elucidation of the underlying molecular mechanisms. Such a diagnostic correlate may expedite identification of the causative environmental factors and critical times of exposure, allowing development of new protocols that can avoid the syndrome.

Large offspring syndrome phenotype

The most striking feature of the syndrome is large size at birth, although fetal overgrowth has been detected as early as day 21 of ovine gestation (Young et al., 1996). Increases in birthweight vary widely; twice the normal birthweight is not uncommon, and a lamb of five times mean birthweight for the breed has been reported (Walker et al., 1996). Dystocia associated with increased birthweight often requires delivery by Caesarean section (Kruip and den Daas, 1997). Gestation is frequently extended, although this is insufficient to account for the increased birthweight (Walker et al., 1992). Other features apparently associated with the syndrome at birth include breathing difficulties, reluctance to suckle and sudden perinatal death (for examples see Walker et al., 1996; Garry et al., 1996). Increased prenatal losses, particularly in the first half of pregnancy (Ranilla et al., 1998), are also associated with the syndrome (Walker et al., 1992; Wilmut et al., 1997a). Such losses may represent more severe phenotypes.

Physiological studies on cloned calves which are susceptible to the large offspring syndrome suggest that altered energy metabolism is associated with postnatal weakness and mortality, although clinical data were compared with previously established ranges for newborn beef calves and not to measurements from non-cloned calves from the same population.
(Garry et al., 1996). At birth, some calves were hypothermic, some were hypoglycaemic and some had severe metabolic acidosis and hypoxia. However, there were no significant correlations with birthweight. Plasma concentrations of insulin at birth were increased fourfold and plasma glucagon concentrations were significantly correlated with birthweight. Insulin from the fetal pancreas stimulates glucose uptake across the placenta and normally decreases at birth. Garry et al. (1996) suggest that such differences in energy supply and utilization in utero would promote fetal growth and predispose the neonate to difficulty in adapting to extrauterine life, as in human babies with gestational diabetes. However, unlike human gestational diabetes, the large offspring syndrome appears to be intrinsic to the fetus rather than a response to maternal malfunction. The syndrome is associated with specific treatments to the early embryo rather than to recipient dams.

Gross abnormalities of several organs have been described, including increased muscle mass and alterations in muscle fibre composition (Maxfield et al., 1997), cerebellar dysplasia (Schmidt et al., 1996) and skeletal and facial malformations (Walker et al., 1996). Farin and Farin (1995) observed proportional changes in organs in cattle fetuses. However, these may have been directly related to the change in their size since organ:fetal weight ratios change as the fetus grows (Robinson and McDonald, 1979). On a more fundamental basis, Sinclair et al. (1997, 1998) have shown that allometric growth coefficients for fetal liver, heart, kidney and plantaris muscle are greater in oversized sheep fetuses. These observations suggest that not only is the overall growth rate altered, but that development of key organs is fundamentally perturbed. Placental abnormalities such as polyhydramnios are widely reported (Hasler et al., 1995; Kruip and den Daas., 1997; Sinclair et al., 1998), but increased fetal weights were not associated with larger placentae (Sinclair et al., 1997, 1998). Thus the overgrowth appears to be driven by the fetus and not the placenta, although placental function, rather than size, may be affected. Garry et al. (1996) suggest that disturbances in feto–placental energy regulation may account for the weakness at birth and may also be involved in deregulated growth.

It is not yet clear whether the features described are due to one or more phenotypes since they do not always occur. The incidence of large offspring born subsequent to embryo manipulations varies from none to almost 100%. It occurs inconsistently when similar protocols have been used in different studies and when identical protocols have been used at different times in the same location. The reasons for this are not understood. Worldwide, thousands of normal calves and lambs have been born as a result of embryo production in vitro (see Wilmut et al., 1997a for a description of procedures in vitro). However, in vitro produced embryos result in reduced pregnancy rate (for example 45% cattle pregnancies carried to term compared with 55% after artificial insemination or 60% after transfer of in vivo embryos) and this may be associated with pregnancy losses and other manifestations of the syndrome (Kruip and den Daas, 1997). The sporadic nature of the syndrome undoubtedly hinders understanding of causal agents

![Fig. 1. Summary of the major questions and possible approaches.](image-url)
and mechanisms. Both sexes appear to be affected (Wilson et al., 1995; Yazawa et al., 1997; Goodhand et al., 1997) and large offspring have been observed in a variety of breeds (Kruip and den Daas, 1997). Despite oversize at birth, perturbed offspring appear to achieve similar weights to control animals at about 1 year after birth (Wilson et al., 1995; Walker et al., 1996), although abnormally large hearts have been reported at this time (McEvoy et al., 1998).

Treatments known to cause large offspring syndrome

The phenomenon of ‘large calves’ was first described by Willadsen et al. (1991) after cloning by nuclear transfer. Since then, several manipulations of the pre-elongation embryo and its environment have been reported to result in fetal and neonatal oversize in both cattle and sheep (Table 1).

There is now considerable evidence that production of bovine and ovine embryos in vitro can result in the large offspring syndrome (reviewed by Walker et al., 1996; Kruip and den Daas, 1997). Exposure to conditions in vitro in the culture phase (Fig. 2) alone is enough to perturb the embryo, as indicated by the fact that in vivo matured and fertilized eggs recovered from superovulated sheep donors and cultured in vitro for 6 days showed an 18–36% increase in mean birthweight at day 125 of gestation, depending on the culture system used (Sinclair et al., 1998). However, a comparable effect also occurs with zygotes.
generated from in vitro matured oocytes (Thompson et al., 1995). It is less clear whether the period of oocyte maturation can also be perturbed, although a recent study has indicated that the syndrome may have arisen from oocytes matured and fertilized in vitro and then replaced into recipient ewes for the embryo culture period and the remainder of gestation (Holm et al., 1996).

Asynchrony in gestational stage between embryo and recipient dam has been shown to increase fetal weight in first trimester ovine fetuses when day 3 embryos were placed in a day 6 uterus for a period of 3 days and then transferred into another recipient synchronous with the embryo (Wilmut and Sales, 1981; Young et al., 1996). It is likely that the embryo in an advanced uterine environment is receiving one or more secretory signals to which a day 3 embryo would not normally be exposed and this may be the initiating factor for accelerated growth. Increasing the maternal concentration of progesterone in the first 6 days of pregnancy also increases fetal growth and accelerates conceptus development (Kleeman et al., 1994; Garrett et al., 1998; Walker et al., 1996). This premature increase in progesterone presumably mimics asynchronous transfer and exposes the embryo to inappropriate developmental signals (Garrett et al., 1988), perhaps both in the oviduct and in the uterus.

Recent advances in cloning technology have highlighted the occurrence of large offspring after nuclear transfer protocols that used both cultured adult or fetal cells (Wilmut et al., 1997b; Schnieke et al., 1997) and blastomere cells from 16 to 32 cell embryos (Wilson et al., 1995; Kruip and den Daas, 1997) as the donor nuclei. The use of older or more differentiated donor nuclei may be expected to increase the incidence of abnormal reprogramming (Jaenisch, 1997), but the limited evidence available does not support this supposition. The incidence of large nuclear transfer offspring using blastomeres appears as high as that from more differentiated cells in some studies (I. Wilmut, unpublished). Furthermore, nuclear transfer per se may not be the cause of the large offspring syndrome in reconstructed embryos. Most nuclear transfer embryos are either cultured subsequently in vitro using protocols known to perturb growth and development (for example Yazawa et al., 1997), or ligated in a sheep oviduct with no regard to synchrony of the recipient animal with the developmental stage of the reconstructed embryo (for example Willadsen et al., 1991). In addition to the possible asynchrony, culture in a ligated oviduct prevents migration of the embryo into the uterus. During the period of development to the blastocyst stage, embryos would naturally be in the uterus for 3–4 days and exposed to uterine secretions. Ligation of the oviduct may, therefore, expose the embryo to unusual secretions which provide a perturbing milieu. Experimental studies are necessary to separate the effects of nuclear transfer protocols and unusual culture environments and to determine whether there is an interaction between them. A worldwide survey that assessed the effects of embryo production in vitro and nuclear transfer on calf birthweight showed that the range of weights of both groups was similar (Kruip and den Daas, 1997). Thus so far there is no evidence to indicate that nuclear transfer per se is responsible for increasing birthweight.

Maternal dietary manipulation has also been shown to result in large offspring. Ewes fed excess amounts of non-protein nitrogen in the form of urea from 21 days before mating to day 63 of gestation resulted in oversized lambs at birth (McEvoy et al., 1997). It is not known whether either the oocyte or embryo (or both) were perturbed due to the long period of exposure to urea.

There have been no direct comparisons of the different embryo manipulations that result in the syndrome and so their relative contributions to the frequency and extent of perturbation may not be equal. One of the fundamental unknowns about the large offspring syndrome is whether all embryos subjected to a particular perturbing treatment are affected, albeit to different degrees, or whether only a subpopulation of susceptible individuals become abnormally large. An upward shift in weight distribution appears to occur in the few studies with sufficient data sets (Wilson et al., 1995; Walker et al., 1996; Sinclair et al., 1997, 1998; Kruip and den Daas, 1998), favouring

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Table 1. Causative embryo manipulations: variation in mean birthweighta

<table>
<thead>
<tr>
<th>Species and time of gestation</th>
<th>Embryo treatment</th>
<th>Type of control</th>
<th>Increase from mean control weight (%)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Sheep (term)</td>
<td>Culture with serum</td>
<td>Natural mating</td>
<td>23</td>
<td>Thompson et al., 1995</td>
</tr>
<tr>
<td>Sheep (day 125)</td>
<td>Culture with serum</td>
<td>Embryo transfer</td>
<td>24</td>
<td>Sinclair et al., 1998</td>
</tr>
<tr>
<td>Sheep (term)</td>
<td>Culture with serum</td>
<td>Natural mating</td>
<td>43</td>
<td>Brown and Radziejewic, 1996</td>
</tr>
<tr>
<td>Sheep (day 125)</td>
<td>Co-culture with serum</td>
<td>Embryo transfer</td>
<td>36</td>
<td>Sinclair et al., 1998</td>
</tr>
<tr>
<td>Cattle (day 222)</td>
<td>Co-culture with serum</td>
<td>Embryo transfer</td>
<td>21</td>
<td>Farin and Farin, 1995</td>
</tr>
<tr>
<td>Cattle (term)</td>
<td>Co-culture with serum</td>
<td>Artificial insemination</td>
<td>42</td>
<td>Behboodi et al., 1995</td>
</tr>
<tr>
<td>Cattle (term)</td>
<td>Co-culture with serum</td>
<td>Artificial insemination</td>
<td>8</td>
<td>Kruip and den Daas, 1997</td>
</tr>
<tr>
<td>Cattle (term)</td>
<td>Nuclear transfer/ sheep oviduct culture</td>
<td>Artificial insemination</td>
<td>34</td>
<td>Wilson et al., 1995</td>
</tr>
<tr>
<td>Cattle (term)</td>
<td>Nuclear transfer/ co-culture with serum</td>
<td>Embryo transfer</td>
<td>50</td>
<td>Yazawa et al., 1997</td>
</tr>
<tr>
<td>Sheep (term)</td>
<td>Urea supplement (maternal diet)</td>
<td>No urea supplement</td>
<td>19</td>
<td>McEvoy et al., 1997</td>
</tr>
<tr>
<td>Sheep (day 74)</td>
<td>High maternal progesterone</td>
<td>Artificial insemination</td>
<td>13</td>
<td>Kleeman et al., 1994</td>
</tr>
<tr>
<td>Sheep (day 21)</td>
<td>Asynchronous transfer</td>
<td>Synchronous transfer</td>
<td>42</td>
<td>Young et al., 1996</td>
</tr>
<tr>
<td>Sheep (day 34–42)</td>
<td>Asynchronous transfer</td>
<td>Synchronous transfer</td>
<td>40</td>
<td>Wilmut and Sales, 1981</td>
</tr>
</tbody>
</table>

*aComparison between studies (and sometimes within studies) is confounded by variations in litter size, sire, recipient breed, type of control, maternal nutrition, numbers of observations and protocol used.

This list provides examples only and is not exhaustive.
the hypothesis that all embryos may be affected to some extent. The difficulty of interpretation is increased by the many ova that do not progress to the blastocyst stage (typically 70–80%) and the high incidence of pregnancy failure (typically 60% failure) after transfer of cultured or manipulated embryos (Thompson, 1997). It is interesting to note that calf size at birth varied between groups of genetically identical cloned cattle embryos (Wilson et al., 1995) and full sibs derived from cultured sheep embryos (Carolan et al., 1998), suggesting pre-disposition of the oocyte (or donor nucleus) before embryo manipulation or some influence of maternal environment subsequent to embryo transfer. Unlike in the study of Carolan et al. (1998) in sheep, the cloned cattle embryos were not transferred into recipients of the same breed, age or parity. However, statistical analysis indicated that recipient breed was not responsible for the variability of birthweight between cloned calves (Wilson et al., 1995).

**Nature of the perturbing environmental factors**

The common feature of all treatments known to cause the large offspring syndrome is exposure of the oocyte or embryo to an unusual environment. The specific factor or factors responsible are unknown at present. Perturbation could be initiated by either exposure of an embryo to a factor not normally present at a specific developmental stage or present at an inappropriate concentration.

A wide variety of *in vitro* culture systems have been associated with programming of the syndrome but virtually all involve a period of exposure to a variety of complex sera (for example human, fetal calf, steer or sheep serum), with or without co-cultured support cells (such as granulosa cells, cumulus cells and buffalo rat liver cells; see Catt, 1994; Trounson et al., 1994 for reviews). In studies exposing *in vivo* matured and fertilized sheep zygotes to different culture systems, the expression of fetal oversize was dependent on both the serum source and the presence or absence of co-cultured granulosa cells (Sinclair et al., 1997, 1998). In one study there appeared to be an interaction between the serum and the granulosa cells which produced an enhanced effect and indicated that serum supplementation acted indirectly through the support cells as well as directly on the embryo (Sinclair et al., 1998). Such differential effects may be due to different doses of the perturbing factor(s) either in the serum or by stimulated production of the same or other, interacting, factors by the co-cultured cells. In addition, the embryo may be exposed to the effective dose of the perturbing factor(s) at different developmental stages or for different periods. Further evidence for serum as a source of the perturbing factor(s) has come from transfer of embryos cultured to the blastocyst stage in defined, serum-free media. When synthetic oviduct fluid (SOF), supplemented with BSA and amino acids, was used, the mean birthweights and incidence of abnormalities were similar to that of controls derived from *in vivo* embryos (Walker et al., 1992; Thompson et al., 1995; Sinclair et al., 1997). Although this defined system may avoid the problem of large offspring, lower blastocyst yields, pregnancy rates and poorer embryo survival after freezing mean that it must be improved before it can replace the use of serum or co-culture in commercial embryo culture systems.

It is possible that the perturbing factor (or combination of factors), both *in vivo* and *in vitro*, is the same in all circumstances. Alternatively, a number of factors, differing between systems, may induce the change. This last scenario would be more likely if the characteristics of the large offspring syndrome do encompass more than one phenotype and underlying mechanism. No perturbing factors have been identified to date, but putative agents include growth factors, free radicals, ammonia and progesterone. Many other components of serum may also be candidate factors and identification of different concentrations or biological activities of these elements between perturbing and non-perturbing environments would initially help to screen for potential perturbing factors.

Growth factors that influence cell proliferation and differentiation are candidates because they are found in serum, are produced by co-cultured cells and have receptors that are expressed in preimplantation cattle and sheep embryos (Watson et al., 1992, 1994). Many experiments have shown their effects upon cell proliferation and metabolism up to the blastocyst stage of development (reviewed by Kaye, 1997). However, there are no studies in livestock of the effect of growth factors on embryo survival or fetal size. Growth factors are likely candidates for inducing different cell proliferation rates and may well relate to the different outcomes of various culture systems. Oxidative damage to cells induced by embryo culture conditions (Johnson and Nasr-Esfahani, 1994; Harvey et al., 1995) may also be limited to different degrees depending on the growth factor composition of a particular serum.

The mechanism of inducing large offspring when feeding high urea diets is hypothesized to be through embryonic exposure to high concentrations of ammonia in the reproductive tract (McEvoy et al., 1997), although changes in tubal or uterine pH may also be involved. Detrimental effects on development subsequent to exposure of cultured mouse embryos to high ammonia concentrations (Lane and Gardner, 1994) suggest that there may be a direct effect of ammonia on embryonic development, although there was no evidence in this species for increased birthweight. Ammonia accumulates in embryo culture systems after the deamination of amino acids (Gardner et al., 1994). Recently it has been suggested that the extent of fetal weight increase in sheep and cattle may be related to the amount of ammonia generated in different culture systems and the period of embryonic exposure (Negrin Pereira et al., 1997).

Progesterone is another putative factor involved in some *in vivo* and *in vitro* perturbing treatments. In the *in vitro* studies of Carolan et al. (1997) the ranking of progesterone concentrations in two bovine granulosa cell culture systems was the same as that for the weights of the day 125 fetuses arising from embryos cultured in the same two culture systems (Sinclair et al., 1998). It has been suggested that progesterone may indirectly influence embryo development *in vivo* via its effect on the reproductive tract (Wilmut et al., 1985). With an *in vitro* embryo culture, progesterone may also indirectly affect development through effects on the co-cultured granulosa cells, rather than a direct effect on the embryo.

**Changes in the early embryo**

No changes have been identified in the early embryo that have been proven to be associated with the syndrome and it is not
yet possible to determine, during the pre-elongation phase of embryonic development, whether an embryo will produce a perturbed fetus. However, there are recognized differences between in vivo and in vitro generated embryos that may prove to be correlated with large fetuses.

Blastocysts produced in vitro have fewer cells than their in vivo counterparts (Walker et al., 1992). Since blastocyst formation involves differentiation of blastomeres into two cell lineages (Fig. 2), the inner cell mass which will form the fetus (and extra-embryonic membranes other than the trophoderm) and the trophectoderm which contributes to the placenta, it has been hypothesized that altered allocation of cells to a particular lineage may alter subsequent growth (Walker et al., 1996). Some serum-containing culture systems produce blastocysts with more nuclei than do defined systems (Carolan et al., 1995; Thompson, 1997), although their allocation to particular lineages remains to be directly compared.

Growth of the embryo and fetus will depend on both the rate of cell proliferation and of cell death, and development of particular tissues may depend on the timing of cell differentiation. Both cell cycle time and degree of apoptosis (Brison and Schulz, 1997) can be influenced by embryo culture conditions. Altered timing of blastocyst formation may also be symptomatic of a perturbing mechanism. Most serum-containing embryo culture systems produce blastocysts about 12–24 h earlier than do completely defined systems (Thompson, 1997). Earlier blastocyst formation was also observed when sheep zygotes were cultured in vitro compared with in vivo (Walker et al., 1992), although development to the eight cell stage appears to occur at the same rate independently of culture system (Grisart et al., 1994). Furthermore, we have observed an association between embryo culture systems that produce blastocysts earlier and increased mean fetal weight (KD Sinclair and ME Staines, unpublished), suggesting that accelerated development at the preimplantation stage may be predictive of perturbation.

In vitro production techniques increase the incidence of chromosomal abnormalities in bovine embryos and the karyotype influences development rate in vitro (Kawarsky et al., 1996). Such techniques may also ‘rescue’ embryos with intrinsic chromosomal abnormalities, present before the period of embryo manipulation. However, Yazawa et al. (1997) observed that chromosomal abnormalities in nuclear transfer embryos were unrelated to birthweight, suggesting that such defects are not responsible for programming the large offspring syndrome.

Altered embryo metabolism may provide another mechanism whereby growth is increased. Several differences in glucose, lipid, lactate, pyruvate and amino acid metabolic rates have been described between in vivo and in vitro embryos (reviewed by Thompson, 1997). More lipid is accumulated in embryos cultured in serum-containing medium than in defined medium and increased cytoplasmic fragmentation is also observed (Walker et al., 1992). This may affect nucleocytoplasmic interactions and cell volume changes may influence the regulation of transcription (Thompson, 1997).

Although differences might be expected to occur in gene expression between in vivo and in vitro embryos, very few studies have addressed this question in ovine and bovine embryos. Wrenzycyki et al. (1996) showed that expression of the connexin 43 gene was downregulated in bovine blastocysts produced in vitro. This protein is required for the formation of gap junctions and poor cell compaction is a commonly observed feature of in vitro produced embryos (see Thompson, 1997). Several laboratories are currently using the powerful screening technique of mRNA differential display (Liang and Pardee, 1992) to identify novel genes that may be involved in the large offspring syndrome. This involves screening for differential gene expression in embryos produced in culture systems previously shown to be perturbing (serum-containing) and non-perturbing (defined system).

Progress in identifying the responsible factor(s) is likely to be slow until a diagnostic method for predicting initiation of the syndrome in the early embryo is available. At present, the only method of testing an individual candidate factor is to transfer suitably treated embryos into recipient dams and to assess fetal weight or birthweight. This is largely an impracticable approach owing to the number of large animals involved and associated welfare considerations.

**Timing of the perturbation**

The embryonic stage(s) at which development may be perturbed is not known, but there are two hypotheses. One hypothesis suggests that inappropriate stimuli at any developmental stage before blastocyst hatching can lead to fetal oversize, while the other suggests that a specific stage of development is vulnerable. If there is only one stage that is vulnerable, this may be about the time when the embryonic genome assumes control of development. The first three cell cycles in cattle and sheep embryos are largely governed by the protein and RNA produced in the oocyte before ovulation (Fig. 2). Most transcription from the embryonic genome begins at the 8–16 cell stage in sheep and cattle. A disturbance at this stage of development is consistent with the unusually large lambs born after sheep zygotes were cultured for just 3 days in serum-supplemented medium (Walker et al., 1992) and the effects of asynchronous transfer were exerted on day 3 ovine embryos (Wilmut and Sales, 1981; Young et al., 1996). However, most perturbing treatments have involved exposure to an unusual environment from the time of oocyte maturation or zygote development to the blastocyst stage and it has not been possible to define a susceptible ‘window’ more specifically.

Exposure to serum in vitro or to maternal dietary or endocrine influences during the preceding period of oocyte maturation may also induce or facilitate the perturbation during this phase. Both superovulation protocols in vivo and aspiration of oocytes in vivo or in vitro result in maturation of more oocytes than would occur naturally in any one oestrous cycle and these procedures could also ‘rescue’ abnormal oocytes. It is also possible that short-term exposure to serum during donor zygote recovery or nuclear transfer protocols is sufficient to induce a change. More information is required on the exact timing of the perturbation and any possible legacy of short-term exposure to determine whether serum and perturbing treatments in vitro are harmful even after transient exposure.

**Molecular mechanisms**

The empirical hypothesis is that all perturbing treatments act through a single mechanism in the embryo. However the possibility that there may be more than one phenotype described...
under the current term 'large offspring syndrome' implies that there may be more than one mechanism. Similar phenotypes to the syndrome that occur in humans and mice as a result of altered expression of some imprinted genes make these genes the most likely candidates to date for involvement in the livestock large offspring syndrome (for examples see Moore and Reik, 1996; Eggenschwiler et al., 1997). Imprinted genes are unusual in that their expression derives from only one parental allele. Many have important roles in regulating fetal growth and development.

Changes in the DNA methylation of imprinted genes occur during normal preimplantation embryo development in mice, at a time when virtually all other DNA in the genome is entirely demethylated (see Li, 1997). If similar changes occur in cows and sheep, exposure to perturbing treatments during this critical phase provides a plausible mechanism for altering imprinted gene expression. Different genes become imprinted and are expressed from only one allele at different stages of mouse preimplantation development (Szabo and Mann, 1995). Altered expression of different imprinted genes in different bovine and ovine embryos may account for the inconsistent phenotypes observed, as occurs in human babies with the imprinting disorder, Beckwith-Weidmann syndrome (Hastie, 1997). Genomic imprinting remains to be demonstrated in livestock species, but it is likely that it is a ubiquitous mammalian mechanism for regulating fetal size (Moore and Reik, 1996). Some imprinted genes, such as Igf2, Igf2r, H19 and Ins, are known to be expressed in the embryo and fetus of large domestic livestock, implying developmental roles.

Implications

The imprinted gene-related large offspring in humans and mice result from chromosomal abnormalities or experimental gene targeting and have not been described as a result of embryo manipulations. In vitro culture of both human and mouse embryos is commonplace, but the large offspring syndrome has not been reported in these species or in any other species in which embryo manipulations are commonplace, such as pigs, rabbits or hamsters. This may be due to intrinsic differences between these species and cattle and sheep, or to differences in protocols used. Intrinsic differences may be related to factors such as the timing of critical gene expression and, in this context, differences in the imprinting of specific genes in the early embryo have been reported between mice and humans (for example Kalscheuer et al., 1993; Lighten et al., 1997). Greater litter size in species such as rodents and pigs may also limit fetal growth to a greater extent than in cattle and sheep.

An understanding of the molecular basis of programming the syndrome in the early embryo is crucial to avoid introducing the problem in any new situations. Although livestock embryos are usually transferred to recipients as blastocysts, human embryos have historically been returned to the mother at about the four cell stage and it is possible that the embryo is not exposed to a perturbing factor(s) at a critical period. More recently, human embryos have been cultured to the blastocyst stage (Gardner and Lane, 1997; Jones et al., 1998) and close monitoring will determine whether this results in large offspring or other manifestations of the syndrome. Mouse embryos are cultured routinely to the blastocyst stage but all commonly used media are serum-free. Nuclear transfer in mice has not been associated with any reports of large offspring. If differences in the protocols used, such as short culture periods or type of culture medium, have contributed to the absence of large offspring in species other than cattle and sheep, there may be important implications in developing new protocols for human reproductive technologies.

Undesirably high birthweights cause distress to the dam and neonate. Caesarian sections and associated financial constraints, as well as high losses soon after birth, currently limit the transfer of modern reproductive technologies such as in vitro embryo production and nuclear transfer into commercial agricultural and biotechnology practice. The observations that certain manipulations of the early embryo can initiate altered fetal development may provide a novel extension to the 'Barker hypothesis', which suggests that fetal development can be altered at critical stages in response to particular environmental stimuli (Barker and Clark, 1997). If the very early stages of development from oocyte maturation to implantation can significantly reprogramme subsequent growth and development under normal circumstances, there may be major ramifications for human and animal nutrition and health. Clearly, unravelling the basis for the large offspring syndrome has important implications for understanding early mammalian development and the protocols used to manipulate this process. A diagnostic method for predicting the occurrence of fetal oversize is required urgently.

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References

Key references are indicated by asterisks.


Gardner DK, Lane M, Spitzer A and Batt PA (1994) Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage in vitro in the absence of serum and somatic cells: amino acids, vitamins and culturing embryos in groups stimulate development Biology of Reproduction 50 390–400
*Kruip TAM and den Daas JHG (1997) In vitro produced and cloned embryos: effects on pregnancy, parturition and offspring Theriogenology 47 43–52
Lighten AD, Hardy K, Winston RML and Moore GE (1997) Ig2 is parentally imprinted in human preimplantation embryos Nature Genetics 15 122–123
Maxfield EF, Sinclair KD, Dolman DF, Staines ME and Maltin CA (1997) In vitro culture of sheep embryos increases weight, primary fibre size and secondary to primary fibre ratio in fetal muscle at day 61 of gestation Theriogenology 47 376
Sinclair KD, Maxfield EK, Robinson JJ, Maltin CA, McEvoy TG, Dunne LD, Young LE and Broadbent PJ (1997) Culture of sheep zygotes can alter fetal growth and development Theriogenology 47 380
Szafe PE and Mann JR (1995) Allele-specific expression and total expression levels of imprinted genes during early mouse development: implications for imprinting mechanisms Genes and Development 9 3097–3108


Young LE, Butterwith SC and Wilmot I (1996) Increased ovine fetal weight following transient asynchronous embryo transfer is not associated with increased placental weight at day 21 of gestation Theriogenology 45 231