CATTLE EMBRYO GROWTH, DEVELOPMENT AND VIABILITY

Authors

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1. SUMMARY

A major problem for the cattle breeding industry is the high rate of early embryo loss which compromises reproductive efficiency and genetic improvement, resulting in serious financial loss to farmers. An important part of the Teagasc research programme in this area is the investigation of basic parameters of cattle embryo growth, development and viability during the critical period when most of the embryo loss occurs. We have now characterised this period of embryo development and to our knowledge, this is the first report describing the morphology, growth rate, protein content and metabolic activity of cattle embryos during this period. The main results are summarised here and detailed results have been published in the papers listed at the end of this report.

Embryo growth rate and protein content increased exponentially between days 8 and 13 after fertilisation. Furthermore, there was a high rate of protein synthetic activity, energy and amino acid metabolism and signal transduction activity, all reaching a peak between days 8 and 13 after fertilisation. Because of the high rate of metabolic activity evident during this time it is likely that the embryos are very susceptible to environmental changes that have the potential to interfere with normal developmental mechanisms. The results arising from this project suggest that the critical period of early embryo loss in cattle may now be narrowed to a time window of day 8 to 13 rather than day 8 to 16 as presumed up to now. The main results are summarised as follows.

Embryo growth and development

Growth and development rates are important indicators of embryo viability but little information has been published to-date for cattle embryos.

- Cattle embryos undergo an exponential increase in size, from 170 µm diameter at one day old to greater than 50mm in length at 16 days old, an increase of more than 300-fold. First cell

division is completed by 2 days after fertilisation and 8 days later the cell complement is about 120 at which stage they begin to differentiate into foetal and placental tissue cells.

- Cattle embryos also undergo an exponential increase in protein content. There is a 7,500 fold-increase in protein content from day 1 to day 16.

- After day 13 and during elongation there is significant within-day variation in the size, morphology and protein content of cattle embryos with spherical, ovoid and elongated embryos of varying sizes present on any day.

- *De-novo* or "new" protein synthesis, per unit of embryo protein, increases with age up to day 13 and then begins to decrease. The indication is that protein synthetic activity reaches a peak between days 8 and 13. In contrast, phosphorylation, per unit of embryo protein, is more than 10-fold higher in 8-day old than in 13-day old embryos. *De novo* protein synthesis is associated mainly with proteins with *Mr* of 44 and 56 kDa.

Embryo Metabolic Activity

Embryo competence is positively correlated with its ability to utilise energy sources and amino acids.

- Glucose uptake and lactate production increases with embryo development up to day 16. However, 14-day old embryos tends to have a higher metabolic rate, per unit of protein, than later stage embryos. This is consistent with the protein synthetic activity.

- The amino acid requirements of cattle embryos differ widely. A striking feature was the production of alanine and glutamic acid by embryos. This may be a mechanism by which embryos get rid of ammonia, a by-product of metabolism that is embryo toxic.

Signal transduction in cattle embryos

Many hormones and growth factors involved in embryo growth and development act by controlling signal transduction within the embryo.

- Concentrations of the biochemical messengers, cAMP and cGMP decrease with embryo age from days 13 to 16. This may facilitate the rapid cell proliferation that occurs at this time.

- cAMP and cGMP are also exported by the embryos and this may be part of an embryo-maternal signaling mechanism.

2. INTRODUCTION

Reproductive wastage in the national cow herd results in fewer calves, reduced meat and milk sales, high involuntary culling rates, increased cow maintenance costs and slower genetic progress. Delayed postpartum intervals, poor heat expression, inefficient heat detection and fertilisation failure all contribute to this reproductive wastage. Following fertilisation, however, early embryo death is recognised as the major cause of reproductive wastage in cattle and results in a significant financial loss. In the dairy herd, genetic improvement and better nutrition are leading to significant increases in milk production per cow but this increased production is apparently resulting in even further increases in early embryo loss. This high increment of early embryo loss also limits the exploitation of a range of embryo-related biotechnology developments. For example, *in vitro* produced embryos are becoming a routine part of genetic improvement programmes but only about 30% reach blastocyst stage and, following transfer to recipient cows, their survival rate is low.

Results previously published from this and other research programmes suggest that the period of greatest embryo loss is between 8 and 16 days after fertilisation. During this time the embryo undergoes changes that facilitate the establishment of communication between the embryo and the uterus. For example, this communication between the embryo and the dam results in the synthesis and phosphorylation of proteins by the uterine epithelium, necessary for embryo development and the synthesis and phosphorylation of proteins by the embryo, necessary for the maternal recognition of pregnancy.

To-date, however, little is known about the development of cattle embryos during this period of embryo loss. For example little or nothing has been published on basic parameters of cattle embryo development such as size, growth rate, morphology or protein content. Similarly, there is little available information on protein synthetic activity or energy substrate metabolism of pre-implantation cattle embryos or on the presence of signal transduction molecules necessary for normal development. Such information is, however, essential in order to

understand the mechanisms involved in normal embryo growth and development and how these mechanisms may be affected by environmental factors such as nutrition.

The Animal Reproduction Department at Teagasc, Athenry has coordinated an EU funded transnational project to study cattle embryo growth, development and viability. This collaborative research project has involved Teagasc, Athenry; the National University of Ireland, Galway; the University of York, UK; INRA, France; Martin-Luther University, Halle, Germany and the commercial cattle breeding company, LTR Ltd., Italy. This was an integrated and co-ordinated study of several aspects of cattle embryo growth, development and viability. The focus of the research carried out at Teagasc, Athenry was to determine the size, growth rate, morphological characteristics, protein content and protein synthetic activity and the presence and role of embyonic signal transduction systems in *in vivo* produced cattle embryos. The results of that research form the content of this report.

Objectives

The overall objective of this project was to produce information that would ultimately lead to a reduction in the high rate of embryo loss currently sustained in cattle.

The specific objectives were to measure in pre-implantation cattle embryos:

- Size, growth rate, morphology and protein content
- Protein synthesis and phosphorylation
- Presence and role of specific signal transduction or second messenger systems
- Energy substrate and amino acid utilisation

These objectives were addressed in a series of studies, the results of which are summarised in this report. Detailed and more comprehensive results have been published in the series of papers listed at the end of this report.

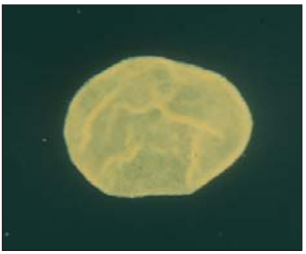
3. METHODOLOGY

Oocytes, or immature ova prior to fertilisation were collected by aspiration of follicles from cattle ovaries. *In vivo* produced embryos at various stages post fertilisation and up to 16 days old were recovered from crossbred heifers. All embryos produced were graded for normality and presumed viability on a morphological scale from 1 (excellent) to 5 (degenerate) and only grade 1 and 2 embryos were included in the study. Embryos were washed in standard media and prepared as appropriate for the various measurements and, or, assays.

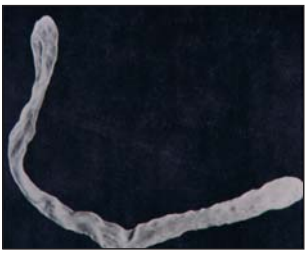
Embryo size measurements were carried out using a graticule on a stereoscopic microscope or by calipers. The morphological description of hatched embryos was based on shape in terms of spherical, ovoid or elongated and on form in terms of tubular or filamentous. Embryo protein content was measured using the standard Pierce Micro BCA assay. Incorporation of ^{35}S-methionine into embryonic protein during a 4-hour culture period was used as an index of *de novo* protein synthesis, while incorporation of ^{32}P-orthophosphate was used to measure protein phosphorylation. Glucose metabolism and amino acid uptake or efflux were determined by culturing embryos individually in synthetic oviduct fluid containing, glucose, pyruvate and a physiological mixture of 18 amino acids. Aliquots of the culture medium were analysed for glucose and lactate concentrations using a CobasBio autoanalyser and for amino acid uptake/efflux profile using HPLC following fluorimetric derivatization. Cyclic AMP and cGMP were measured by radioimmunoassay. Appropriate statistical procedures were employed in all cases.

Cattle embryo size and morphology

At day 2 the embryo is at the 2-cell stage, is enclosed in the zona pellucida and is approximately 170µm in diameter



At day 13 the embryo has hatched from the zona pellucida and is approximately 5 mm in length



At day 16 the embryo has elongated and is now approximately 50 mm in length

4. EMBRYO GROWTH AND PROTEIN CONTENT

Embryo size, morphology, and protein content are important parameters that correlate positively with embryo viability.

Embryo size and morphology

Embryo size and growth rate from the 1-cell zygote at 1-day old to the 16-day old elongated blastocyst are shown in Fig 1.

During the early development from day 1 to day 8 the cattle embryo remained within the zona pellucida, measuring approximately 170 µm in diameter. The first cleavage to the two-cell stage occured at 2 days after fertilisation. Between the 3rd and 4th day after fertilisation embryos moved from the oviduct to the uterus and at this stage contained 8-16 cells. Five and 6-day old embryos contained 16-32 cells which began to form junctions with one another resulting in a compact ball of cells termed the morula. Compaction, when cell-to-cell contact was made for the first time, followed by the formation of tight intercellular junctions to form a barrier against the extra-embryonic environment represents the first critical stage when the embryo begins to act as a unified organism. Compaction is known to be the first essential step in differentiation and is fundamental and essential for viable blastocyst formation. At 8 days the embryos formed blastocoelic cavities at which stage the cells differentiate into inner cell mass cells, destined to become the foetus, surrounded by trophectoderm cells, destined to become the placental tissues. At this stage the embryo had a total cell complement of about 120 cells with the inner cell mass comprising about 25% and the trophectoderm about 75% of the total cell number. The blastocyst, still within the zona pellucida, continued to expand by 1.5 times to reach a diameter of about 200µm with a complement of 160 cells. Between 9 and 10 days old expanded blastocysts hatched from the zona pellucida and the hatched blastocysts underwent further expansion before they started to elongate at 13 days old.

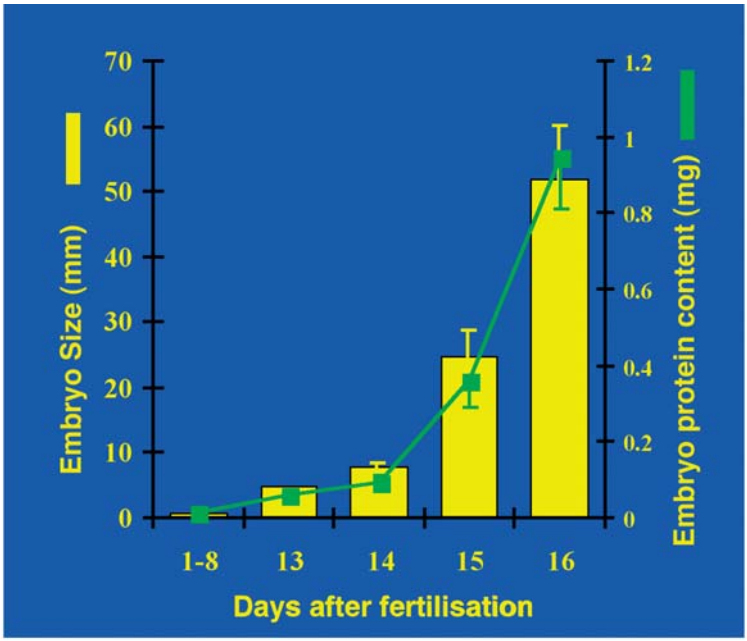


Figure 1. Cattle embryos undergo an expontential increase in size and protein content from days 1-8 through to day 16 after fertilisation.

At the onset of elongation a rapid and dramatic change in embryo size and shape was recorded (see Fig. 1 and Table 1). At 13 to 14-days old, the embryos were spherical, ovoid or elongated in shape, and embryo length and diameter increased from about 5.2 and 0.9 mm at Day 13, to about 52.0 and 1.8 mm at Day 16, respectively. The increase in embryo length over this period was both linear and quadratic while embryo diameter increased in a linear fashion.

Table 1. Elongated embryo size			
Day	No. of Embryos	Length (mm) Mean ± SEM	Diameter (mm) Mean ± SEM)
13	18	5.2 ± 0.87 (0.8 – 13.3)	0.9 ± 0.05 (0.6 – 1.3)
14	37	7.8 ± 1.80 (0.8 – 55.0)	1.1 ± 0.04 (0.7 – 1.7)
15	12	25.1 ± 3.64 (6.0 - 43.0)	1.3 ± 0.13 (0.5 – 1.8)
16	5	51.6 ± 3.82 (40.0 – 60.0)	1.8 ± 0.30 (1.0 – 2.5)
Values in parentheses signify the range			

Embryo protein content

As well as size, growth rate and morphology a knowledge of the protein content of normal pre-implantation cattle embryos is an important parameter in the assessment of embryo viability. Information on protein content is also important as a basis for the interpretation of the effects of environmental factors on embryo development and viability.

Protein content was similar for pre-fertilised oocytes and 2-cell stages; was higher at the morula and blastocyst stages, which were similar, and was higher again at the expanded blastocyst stage (see Table 2).

Table 2. Protein content of oocytes and embryos		
Stage	No. of oocytes/ Embryos	Protein (µg) per oocyte/embryo Mean ± SEM
Oocyte	115	0.126 ± 0.0059
2-cell	67	0.132 ± 0.0196
Morula	62	0.183 ± 0.0203
Blastocyst	40	0.185 ± 0.0158
Expanded blastocyst	12	0.367 ± 0.0277
Day 13	18	59.8 ± 8.19
Day 14	37	92.4 ± 21.35
Day 15	12	362.2 ± 73.16
Day 16	5	946.6 ± 135.76

From the zona pellucida enclosed expanded blastocyst stage to the hatched, day 13 blastocyst, protein content increased by 160-fold. From days 13 to 16 protein content increased exponentially, in both a linear and quadratic fashion (Fig. 1 and Table 2). There was a strong positive correlation between the product of embryo length and width and protein content (see Fig. 2).

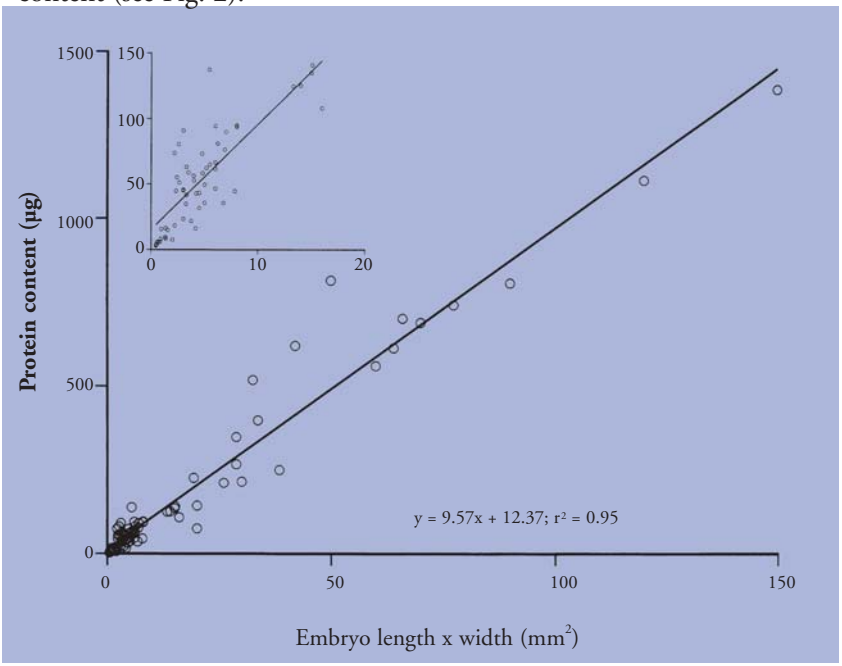


Figure 2. Protein content of hatched blastocysts recovered on days 13 – 16 expressed as a function of blastocyst length x diameter. Insert shows in detail results for smaller embryos.

The protein content of different age hatched blastocysts is shown in Table 1 and Fig. 2. There was a significant correlation between embryo size and protein content between days 13 and 16. Within days 13 and 14, however, there was a large variation in embryo size and shape and in embryo protein content and the results are shown in Table 3.

Table 3. Protein content and size of Day 13 and 14 cattle blastocysts by development stage

Day	Shape	N	Length (mm) Mean ± SEM	Protein (µg) Mean ± SEM
13	Spherical	4	0.89 ± 0.005	4.51 ± 0.661[a]
	Ovoid	12	5.48 ± 0.612	66.08 ± 4.881[c]
	Elongated	2	12.45 ± 0.851	132.71 ± 8.136[d]
14	Spherical	9	1.42 ± 0.159	12.47 ± 1.538[b]
	Ovoid	20	4.04 ± 0.348	56.98 ± 5.731[c]
	Elongated	8	24.58 ± 4.922	270.67 ± 67.884[d]

Values with different superscripts are significantly different ($P<0.05$)

The increase in protein content from day 13 to 16 reflected embryo size and morphology, increasing from spherical to ovoid to elongated and is clearly an important parameter for describing embryo growth and development. The 300-fold increase in embryo size coupled with a greater than 7500–fold increase in protein content gives an indication of the magnitude of the changes occurring in cattle embryos during this period.

The results of this study represent the first published report on the protein content of pre-implantation cattle embryos from the pre-fertilised oocytes through to the 16 day-old elongated blastocyst.

5. EMBRYO PROTEIN SYNTHESIS AND PHOSPHORYLATION

As shown earlier, cattle embryos undergo an exponential increase in size and in protein content, particularly from day 8 to day 16. Little is known, however, about the rate of protein synthesis during this period of rapid growth.

The rate of *de novo* or new protein synthesis by day 8 pre-hatched blastocysts was similar to that of day 13 hatched blastocysts. While protein content increased with embryo age (see Table 4), the rate of *de novo* protein synthesis, per unit of embryonic protein, decreased with age from day 13 to day 15, and with morphological shape from spherical to elongated (Fig. 3 and Table 5). This may reflect a decrease in the rate of protein turnover during this period. A surprising finding was the large difference in incorporation, per µg protein, recorded between the spherical, ovoid and elongated stages on day 13, with more than a 10-fold difference between the spherical and elongated stages. Surprising also was the significantly lower incorporation, per µg protein, between day 13 elongated and day 14 ovoid embryos.

Table 4 Protein content (µg) of bovine embryos collected on different days after insemination and categorised as spherical, ovoid, or elongated. The data in parentheses are those relating to intact embryos only, in the categories where both intact and fragmented embryos are represented.

Shape	Day 13	Day 14	Day 15
Spherical	21.8 ± 6.58[a]; n=11	-	-
	All intact	-	-
Ovoid	72.7 ± 7.06[a]; n=20	40.2 ± 6.38[a]; n=13	157.0 ± 9.26[b,c,d]; n=3
	(68.21 ± 5.75; n=19)	(41.7 ± 7.48; n=11)	(100.0 ± 0.00; n=1)
Elongated	203.5 ± 23.23 [b,c]; n=12	106.5 ± 14.23[d]; n=21	190.6 ± 20.40[c]; n=16
	All intact	(143.7 ± 18.16; n=12)	(172.4 ± 25.96; n=9)

Values with different superscripts are significantly different
$P<0.05$. a< b,c,d, d<c, $P<0.01$

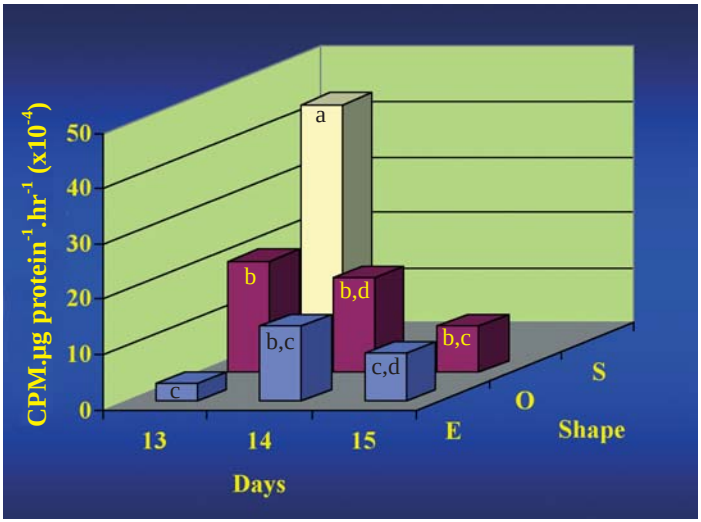


Figure 3. Protein synthesis, per unit of protein, was greatest in early stage embryos and decreased with increasing developmental stage

Table 5. Incorporation of ^{35}S methionine in cpm per µg protein into TCA insoluble material by cattle embryos collected on different days after insemination and categorised by shape as spherical, ovoid, or elongated.

	Day		
Shape	13	14	15
Spherical	43203 ± 6338^{a} n=5	-	-
Ovoid	20121 ± 2363^{b} n=12	$17059 \pm 2603^{b,d}$ n= 8	$8332 \pm 930^{b,c}$ n=3
Elongated	3105 ± 648^{c} n= 6	$13561 \pm 620^{b,c}$ n=10	$8608 \pm 1716^{c,d}$ n=10

Values with different superscripts are significantly different $P<0.05$. a> b,c,d $P<0.001$; b>c $P<0.01$.

It may be that factors that have a deleterious effect on *de novo* protein synthesis have begun to operate on or before day 13 in some embryos, resulting in embryo death at this stage. This is consistent with the fact that the rate of *de novo* protein synthesis by *in vivo* day 8 blastocysts was similar to ovoid day 13 embryos indicating that the peak synthetic activity of cattle blastocysts may occur somewhere between day 8 and day 13.

One-dimensional electrophoresis revealed little difference in the pattern of proteins expressed by 13, 14 or 15-day old embryos. *De novo* protein synthesised was associated mainly with proteins with *Mr* of 44 and 56 kDa (Fig. 4).

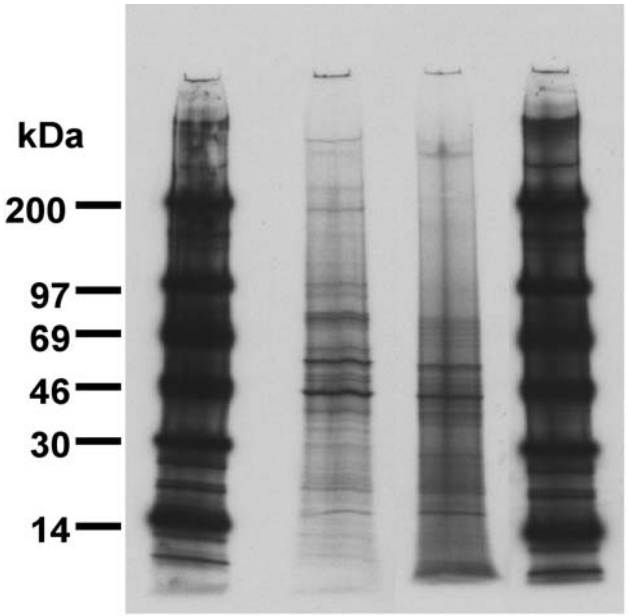


Figure 4. One-dimensional electrophoresis revealed little difference in the pattern of proteins expressed between day 13 (lane 2) and day 15 (lane 3) embryos. *De novo* protein synthesis was associated mainly with proteins with *Mr* of 44 and 56 kDa. Standard proteins are in lanes one and four.

In contrast to protein synthesis, phosphorylation per unit of embryonic protein, was over 10-fold higher in 8-day than in 13-day old blastocysts. There was a tendency for protein phosphorylation per unit of embryo protein to decrease with age from day 13 to day 15 and with morphological shape from spherical to elongated (Fig. 5 and Table 6).

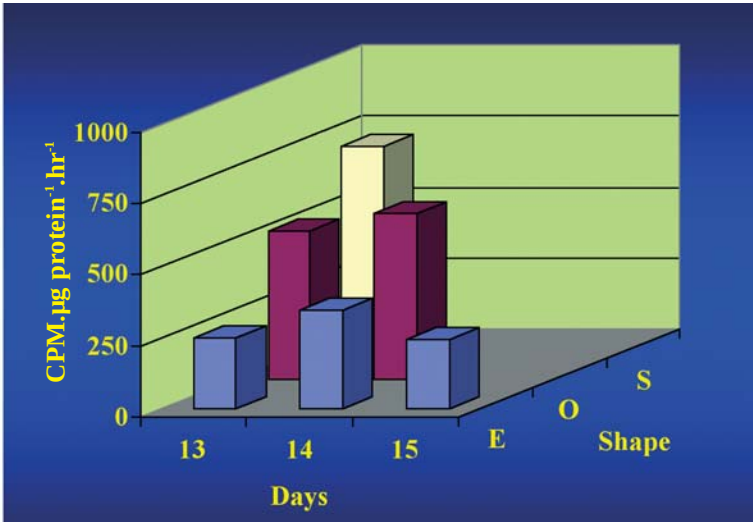


Figure 5. Protein phosphorylation, per unit of protein, tended to be greatest in early stage embryos, decreasing with increasing developmental stage

Table 6. Incorporation of ^{32}P orthophosphate in cpm per µg protein into TCA insoluble material by cattle embryos collected on different days after insemination and categorised by shape as spherical, ovoid, or elongated.

	Day		
Shape	13	14	15
Spherical	716 ± 304 n= 6	-	-
Ovoid	519± 80 n=8	581±6 n= 5	-
Elongated	245± 51 n= 6	345± 91 n=11	243±88 n=6

Overall, the results presented here show that *de novo* protein synthesis increased as the protein content of the embryo increased. *De novo* protein synthesis however, per unit of protein, tended to decrease with embryo age and with morphological stage. The increase in protein content in the face of declining synthesis is possibly a reflection of a decrease in protein turnover during this period coupled with an increasing half life of embryonic protein.

While the cattle embryo increased dramatically in size and protein content as it progresses from the spherical to elongated stage, there was a concomitant decrease in protein synthesis per unit of embryonic protein. This indicates that the earlier ovoid stage 13-day old embryo is more metabolically active than later elongated stages, and may be more susceptible to environmental stress. This is the first report describing the protein synthetic and phosphorylation activity of cattle embryos during the elongating phase from day 13 to day 15.

6. EMBRYO METABOLIC ACTIVITY

In some species embryo competence has been shown to be positively correlated with its ability to utilise energy sources and amino acids. In this study utilisation of glucose and amino acids by embryos was measured and was used as an index of metabolic activity.

Glucose metabolism

Glucose uptake and lactate production increased with embryo development from days 14 to 16. On average, 70% of glucose consumption could be accounted for by lactate appearance in the culture medium. There was considerable variation, however, between embryos within and between different days (see Fig. 6).

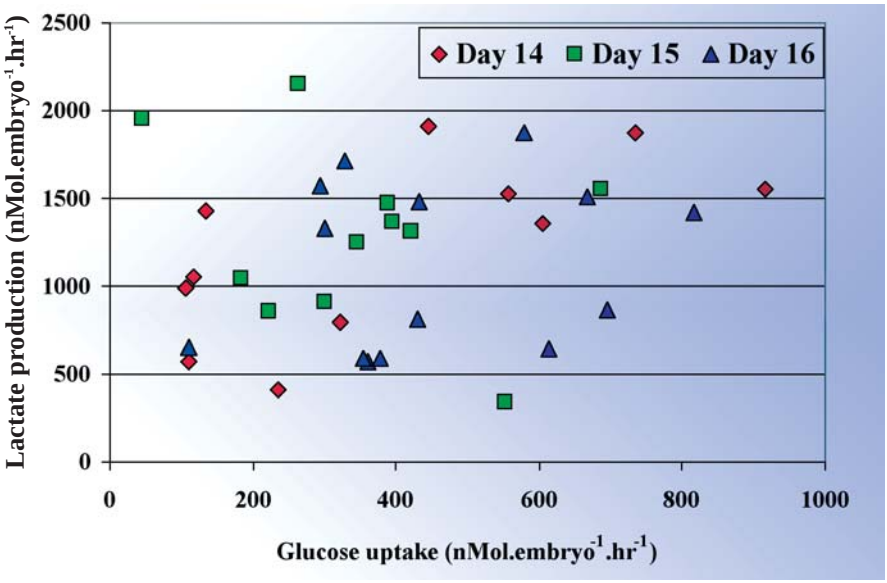
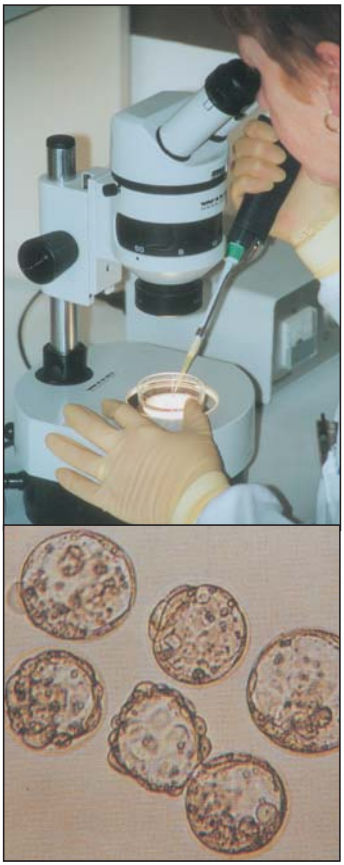


Figure 6. Lactate production accounted for 70% of glucose comsumption.

For 14-day old embryos, glucose uptake and lactate production increased as the embryo protein content increased. The pattern of glucose uptake and lactate production was less clear, however, for 15 and 16-day old embryos. However, 14-day old embryos tended to have a higher metabolic rate, when expressed per unit of embryo protein, than either 15 or 16-day old embryos (see Fig. 7). These results are consistent with the protein synthesis and phosphorylation results already shown where the earlier stage embryos also had a higher rate of protein synthetic activity. A simple non-invasive measurement such as glucose utilisation, in conjunction with other metabolic measurements such as oxygen uptake, offer the possibility of using such non-invasive measurements to establish a total energy profile for cattle embryos.



Microdrop culture allows the measurement of metabolic activity in single embryos

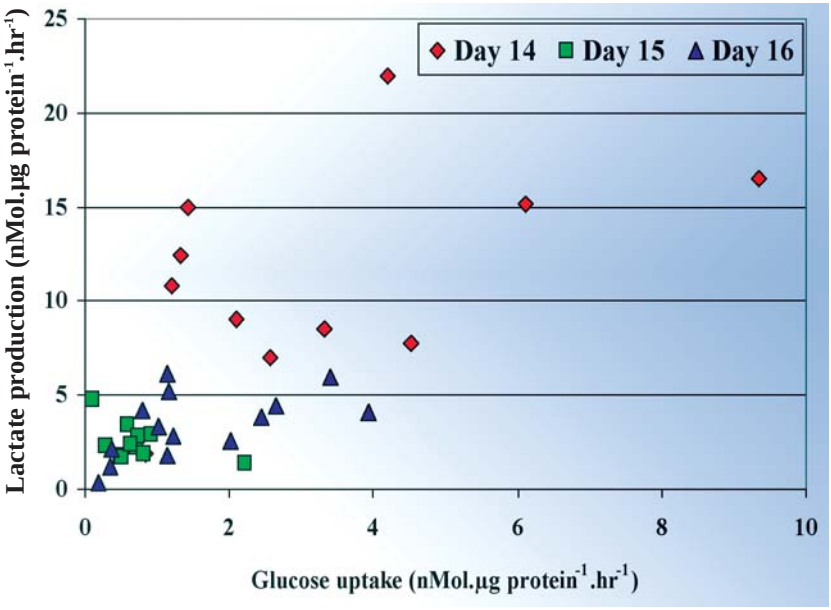


Figure 7. Earlier stage embryos were more metabolically active, per unit of protein, than later stage embryos.

Amino acid metabolism

The individual amino acid requirements of 14 to 16-day old cattle embryos differed widely. A surprising result was the output, rather than the uptake of alanine, threonine and glutamic acid which were secreted into the medium while aspartic acid, glutamine, glycine and isoleucine were taken up from the medium (see Fig. 8). Alanine or glutamic acid production or output may be a mechanism whereby embryos get rid of intracellular ammonia, a by-product of metabolism and a build up of which within the embryo would be toxic. When alanine efflux was related to embryo protein content the smaller, earlier stage embryos had

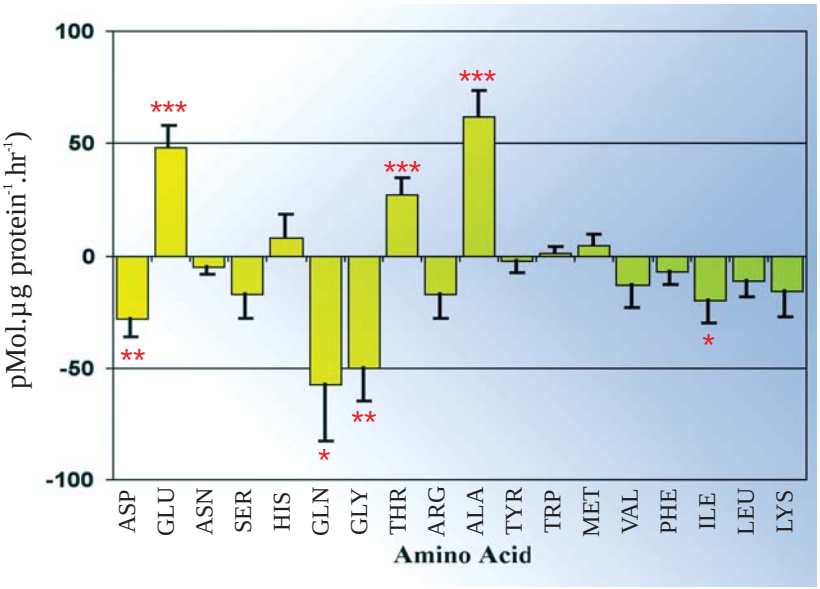


Figure 8. The individual amino acid requirement of day 14 to 16 cattle embryos differed widely.

a higher alanine output (Fig. 9) and, by inference, a higher metabolic rate than larger and later stage embryos. The pattern and magnitude of amino acid uptake or efflux could possibly be used on its own or in conjunction with other indicators of metabolic rate as an index of embryo viability.

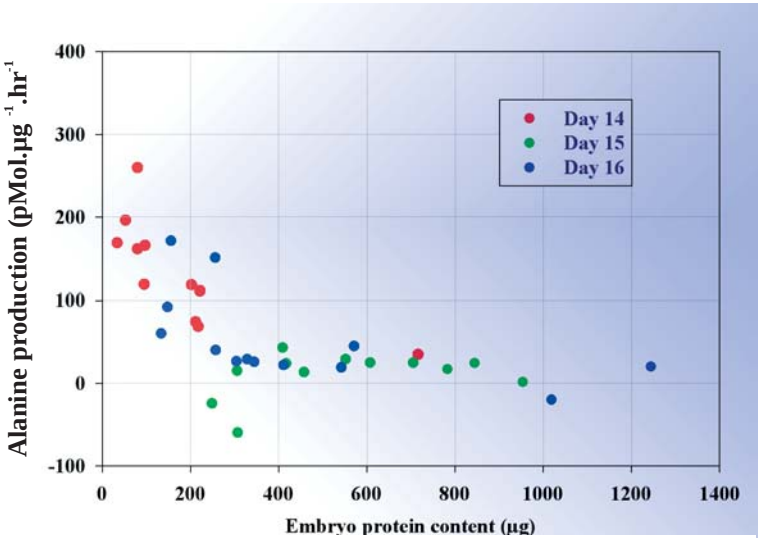


Figure 9. Production of alanine was greatest for smaller and earlier stage embryos.

Embryo metabolic activity was greatest between days 8 and 13 corresponding to the period of greatest embryo loss. It is possible that during this time cattle embryos are very susceptible to environmental effects that have the potential to interfere with the rate of metabolic activity.

7. EMBRYO SIGNAL TRANSDUCTION

Cattle embryo growth and development is characterized by cell proliferation and differentiation, gene expression and protein synthesis. The signal transduction systems, cyclic AMP (cAMP) and cyclic GMP (cGMP) are an integral part of these processes. Measurement of cAMP and cGMP activity could therefore be used as an index of synthetic activity.

Presence of cAMP and cGMP in cattle embryos

Basal intracellular cAMP increased with embryo size and protein content from day 14 to 16, but when expressed on a per unit of protein basis there was no difference in the concentrations of cAMP between days (see Table 7). Intracellular concentrations of cGMP per embryo were similar on days 14 and 15 and higher on day 16. Expressed on a per unit of protein basis, the concentrations of cGMP declined between days 14 and 15 and remained low at day 16. Basal embryonic concentrations of cAMP were 15 to 60-fold higher than those of cGMP.

Table 7. Basal embryonic concentrations of cAMP and cGMP (fmol) in cattle embryos (means ± SEM).

Day	N	cAMP. embryo^{-1}	cGMP. embryo^{-1}	cAMP. µg protein^{-1}	cGMP. µg protein^{-1}
14	7	266.9±98.65	19.2±9.83	2.30±0.212	0.14±0.028
15	5	499.3±83.33	11.0±2.38	2.52±0.082	0.06±0.009
16	4	3742.9±1089.9	68.1±16.66	2.64±0.359	0.05±0.011

Role of cAMP and cGMP in embryo development

Many hormones, neurotransmitters and other 'first messengers' act by regulating the synthesis or breakdown of cAMP or cGMP. From day 13 to day 16 intracellular and extracellular concentrations of cAMP and cGMP decreased. This is consistent with the decrease in protein synthesis, phosphorylation and metabolic activity already outlined.

Furthermore, approximately 40% of cAMP and almost 80% of cGMP was exported from the embryos between days 13 and 16. The significant export of cGMP by cattle embryos may suggest an important role for cGMP as an extracellular messenger in addition to its intracellular role.

8. CONCLUSIONS

Following fertilisation, early embryo death is recognised as the major cause of reproductive wastage in cattle, resulting in a significant financial loss to farmers. Most of the embryo loss occurs about day 16 after fertilisation. During this period embryos are undergoing changes to establish communication between the embryo and uterus. For example, this communication between the embryo and the mother results in the synthesis and phosphorylation of uterine proteins necessary for embryo development and in the synthesis and phosphorylation of proteins by the embryo, necessary for the maternal recognition of pregnancy. To-date, however, little or nothing has been published on basic parameters of embryo development at this time. This project has resulted in the characterisation of early cattle embryos in terms of their size, growth rate, morphology, protein content, protein synthesis and phosphorylation and signal transduction systems.

It is now clear that over this period from 8 – 16 days after fertilisation cattle embryos undergo an exponential increase in size and in protein content. Embryo size increases 300-fold while embryo protein content increases 7,500 fold.

Protein synthesis, per unit of embryo protein, increases with age up to day 13 and then begins to decrease. The indication is that synthetic activity in cattle embryos reaches a peak between days 8 and 13. In contrast, phosphorylation, per unit of embryo protein, is more than 10-fold higher in 8-day old than in 13-day old embryos.

Energy substrate metabolic activity, measured by the rate of uptake of glucose and the production of lactate by early stage embryos, increases with age up to day 16. However, per unit of embryo protein 14-day old embryos have a higher metabolic rate than later stage embryos.

This is again consistent with the protein synthetic activity when the earlier stage embryos have a higher rate of synthetic activity. A striking feature is the production of alanine and glutamic acid by embryos which may be a mechanism for exporting ammonia, a toxic by-product of metabolism. Cattle embryo competence may be positively correlated with its ability to utilise energy sources and amino acids.

Many hormones and growth factors involved in embryo growth and development act by controlling signal transduction within the embryo. Concentrations of the signal transduction molecules, cAMP and cGMP decrease with embryo age from days 13 to 16 which may facilitate the rapid embryonic cell proliferation that occurs at this time. cAMP and cGMP are also exported by the embryos which may form part of an embryo-maternal signaling mechanism.

Clearly the changes recorded in growth rate, protein synthetic activity, energy metabolism, amino acid metabolism and signal transduction activity, are greatest between days 8 and 13 and this corresponds to the period of peak embryo loss. It is possible that during this time embryos are more susceptible to environmental changes or events that have the potential to interfere with normal embryo development mechanisms.

9. PUBLICATIONS ARISING FROM THIS PROJECT

Morris, D.G., Diskin, M.G. and Sreenan, J.M 2001. Amino acid metabolism by elongating 13 to 15-day old cattle blastocysts. (Submitted to *Reproduction*)

Sreenan, J.M., Diskin, M.G. and Morris, D.G. 2001. Embryo survival in cattle: a major limitation to the achievement of high fertility. In: *Fertility in the high producing dairy cow BSAS Occasional Publication,* ***Vol 1,*** *pp 93-105.*

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Morris, D.G., Humpherson, P and Sreenan J.M. 2000. Amino acid metabolism by elongating cattle embryos. In:*Proceedings of the Agricultural Research Forum, University College Dublin, 14th-15th March, pp19-20,* and abstract in *Irish Journal of Agricultural and Food Research* ***39 (3):465.***

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Morris, D.G., Humpherson, P.G., Leese, H.J. and Sreenan J.M. 2000. Amino acid utilisation by elongating 14 to 16 day-old in vivo cattle blastocysts In: *Proceedings of the Society for the Study of fertility, Summer Meeting and Journal of Reproduction and Fertility Abstract Series 25, Abst. No.* ***203,*** *p 75.*

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Grealy, M., and Sreenan, J.M. 1995 Cyclic nucleotide and protein levels in preimplantation bovine embryos. *Biochemical Society Transactions.****23(1):13S.***