Biological optimization, the Goldilocks principle and how much is “lagom” in the preimplantation embryo.

HENRY J LEESE\*1, FABRICE GUERIF2 VICTORIA ALLGAR1, KERSTI LUNDIN3 AND ROGER G STURMEY1

Hull York Medical School, Centre for Cardiovascular and Metabolic Research, Hertford Building, University of Hull, Hull, UK.

\*Corresponding author:

1Hull York Medical School, Hertford Building, University of Hull, Hull HU6 7RX

Email: [henry.leese@hyms.ac.uk](mailto:henry.leese@hyms.ac.uk)

2 Medecine et Biologie de la Reproduction, CHRU de Tours; UMR83 PRC, Université de Tours, Tours, France

3Reproductive Medicine, Sahlgrenska University Hospital, 413 45 Gothenburg, Sweden

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Abstract

In this paper, the quiet embryo hypothesis (Leese, 2002) is re-visited using retrospective and prospective data on the metabolic activity and kinetics of preimplantation development alongside the concept of an optimal range of such indices, and of energetic efficiency. It is concluded that these considerations may be rationalised by proposing the existence of a ‘Goldilocks zone’, or as it is known in Sweden, of ‘lagom’, meaning ‘just the right amount’ within which embryos with maximum developmental potential are located.

INTRODUCTION

Leese (2002) proposed that early embryo viability was best served by a relatively low level of metabolism; the so-called ‘quiet embryo hypothesis. The hypothesis was further developed by Baumann et al., (2007) in terms of potential molecular determinants of ‘quiet’ metabolism, by Leese et al., (2007) who introduced the idea of a *quiet range* of nutrient turnover, and by Leese et al., (2008) who considered categories of quietness, namely, (i) 'functional' quietness; the contrasting levels of intrinsic metabolic activity in different cell types (ii) inter-individual embryo/cell differences in metabolism and (iii) loss of quietness in response to environmental stress. With hindsight, the original quiet embryo hypothesis was too rigid in its distinction between ‘quiet’ and ‘active’ metabolism, when, for example, a metabolism that is too quiet most likely represents an embryo about to arrest. The aim of the present paper is to develop the hypothesis building on two aspects considered below and discussed by Johnson (2013); (a) the idea of an optimal range of metabolic activity and (b) the concept of energy efficiency. The concepts inherent in the hypothesis will also be compared with those in the ‘Goldilocks Principle’.

The *Goldilocks principle* states *‘that something must fall within certain margins, as opposed to reaching extremes’* (<https://en.wikipedia.org/wiki/Goldilocks_principle>. It is derived from the old fairy tale, *Goldilocks and the Three Bears* (<https://en.wikipedia.org/wiki/Goldilocks_and_the_Three_Bears>), largely attributed to the Victorian era British Romantic author Robert Southey, in which a little girl called Goldilocks wanders into a house owned by three bears and discovers three bowls of porridge, three chairs and three beds, in each case characterised by three options: two extremes and a middle one. Thus, the porridge was ‘too hot’, too cold’, or the one Goldilocks chose, ‘just right’. Having consumed the porridge, sat in the ‘just right’ chair and slept in the ’just right’ bed, Goldilocks managed to escape the bears when they returned to their house. There are comparable terms in other languages and cultures. Thus, the term, ‘*lagom*’ is widely used in Sweden where it means ‘just enough’ or ‘just the right amount’ as well as ‘moderation’ and ‘in balance’ <https://en.wikipedia.org/wiki/Lagom>.

The Goldilocks principle has been applied to many phenomena; in economics, astronomy, physics, psychology, social sciences and in biology (e.g., Liu et al 2012; Drake et al 2014) with a few examples in reproductive biology and medicine. For example, Fowler and O’Shaughnessy (2013) highlighted the way in which fetal androgen production, especially testosterone, needs to be ‘just right’ to ensure the appropriate developmental trajectory of the fetus and offspring, and that inappropriate fetal androgen or androgen signalling – both too little and too much - is associated with disorders of male reproductive development as well as being implicated in Polycystic Ovarian Syndrome in women.

In a second example, Clancy (2013) considered what is ‘just right’ in balancing fetal needs vs maternal supply during pregnancy in great apes and humans in terms of the level of inflammation; essential during implantation but potentially predisposing to disorders such as gestational diabetes and choriodecidual inflammatory syndrome.

An overriding question relating to the Golidlocks Principle is: what determines ‘just right’ or “lagom”? In this paper, we address this question at the cellular level in the context of the development of the preimplantation embryo, where we propose that ‘just right’ is the capacity to develop successfully at the highest efficiency, i.e., to carry out the developmental programme in a faithful manner while expending the minimum amount of energy. Initially, we have done this by re-interpreting data by Guerif et al (2013) on energy homeostasis/pyruvate consumption in early cattle embryos. In addition, considerable use is made of the review by Johnson (2013) entitled ‘*Teaching the principle of biological optimization’* which provides a valuable guide to the need for energy efficiency, the uses to which energy is put and the factors which drive the optimization of energy use at all levels; from genes, proteins and physiological systems, to whole organisms and ecosystems. Before presenting these analyses, it is necessary to consider briefly, the energy metabolism of the early embryo.

NUTRITION AND METABOLISM OF THE EARLY MAMMALIAN EMBRYO

It has been known since the 1960s, that the nutritional needs of mammalian embryos throughout the preimplantation stage are remarkably simple. They may be cultured in simple physiological salts solutions supplemented with a few nutrients and serum albumin (reviewed by Biggers, 1998). Energy production throughout preimplantation development is largely aerobic (reviewed by Smith and Sturmey, 2012). Pyruvate is the preferred energy substrate for the first cleavage division (from 1 to 2 cells), and is obligatory for many species. As early development progresses a variety of nutrients can be utilised; notably, pyruvate, lactate, amino acids and endogenous fatty acids. The cleavage stages up to the morula are relatively quiescent in terms of oxygen consumption, widely accepted as the best overall marker of metabolic rate. As the blastocyst stage is reached, glucose consumption rises significantly; a large proportion of which is converted to lactic acid, at least in vitro. This is accompanied by a rise in oxygen consumption. The change in metabolic rate that coincides with blastocyst formation is due largely to the energy demands of the sodium pump required to form the blastocoel cavity, and of protein synthesis, associated with the first increase in the mass of the embryo which occurs at this stage.

THE EARLY EMBRYO AS A MODEL SYSTEM

It is worth emphasising that the early embryo, aside from its biological fascination, has a special advantage as a model system with which to consider energy homeostasis; namely, its availability as a discrete cellular entity. In other words, the molecular cell biology and biochemistry of early embryos may readily be studied on single cells (unfertilised or fertilised eggs) or small clusters of cells (cleavage stage preimplantation embryos) through to the blastocyst stage, which comprises about 100 cells. In marked contrast, most mammalian cells, apart from those in the extracellular compartments in the body, are rarely found individually, but present in highly organised multi-cellular tissues. Such cells are routinely studied in very large numbers (>106) which severely limits the scope to examine their biochemistry at the single cell level. Put another way, the early embryo provides an excellent system for studying intra- and inter-cellular differences.

At an applied level, understanding the basis of this variation is essential to resolving one of the major challenges facing In Vitro Fertilisation (IVF) and related technologies - to devise a robust, non-invasive test of cellular health with which to select single embryos for transfer into the uterus.

FACTORS INFLUENCING THE EFFICIENCY OF EARLY EMBRYOS AND CELLS

Competition for resources

Early embryos can exist with complete autonomy, as demonstrated by their capacity to develop in vitro, and their solitary existence obviates the need to compete for resources with other cells with their nutritional needs in vivo provided by the oviduct and uterus and their own endogenous reserves. However, the notion of autonomous preimplantation development needs to be questioned because of increasing awareness of embryo-maternal cell signalling interactions, whose role is only beginning to be clarified (reviewed in Leese and Brison, 2015). In marked contrast, one of the major constraints under which somatic cellular systems; cells, tissues and whole organisms, operate, is the need to compete for resources, as emphasised by Johnson (2013), such that the most efficient, those that are able to compete successfully, survive. A strong caveat is required in that cells in tissues, and tissues within the body, are constrained metabolically from becoming autonomous or ‘rogue’ cells or tissues by a variety of mechanisms; for example the presence of gap junctions between cells in tissues and of hormonal and neuronal mechanisms between tissues, which help maintain overall homeostasis (Brison et al 2014). Nevertheless, it can be said that one of the major drivers of cellular efficiency; the need to compete for external resources is largely absent in the early embryo.

Intrinsic factors

If the drive to compete is minimised, cells, tissues and organisms obviously still possess an intrinsic capacity for survival and those that make more efficient use of resources will be at an advantage (Johnson 2013). ‘Efficiency’ in an energetic sense implies carrying out a defined action with the minimum input of energy. In order to illustrate this, data from a system in which input and output are well-defined and can be measured quantitatively is required. Such criteria were fulfilled in a study by Guerif et al., (2013) on the relationship between the consumption of the essential nutrient pyruvate by 2-cell bovine embryos and their subsequent capacity to reach two different endpoints: (i) the subsequent stage of development; i.e., the 4-cell stage (ii) the blastocyst stage of development. Pyruvate is an appropriate nutrient to use in an energetic sense since it is largely oxidised to produce ATP and provides a measure of energy input. These data were also chosen because they are unusually detailed and include prospective as well as retrospective studies.

In the two types of experiment, bovine embryos were produced in an identical manner, via the in vitro fertilisation of in vitro-matured immature oocytes obtained from abattoir ovaries (Guerif et al., 2013).

Experiment (i)

The zygotes (fertilised eggs) were allowed to develop to the 2 cell stage before being incubated individually in 5 µl of culture medium 5%CO2/5%O2/90%N2 for 24 hours. The embryos were removed and allocated into two groups: (a) those that had developed to 4 cells (n=40) and (b) those that showed no development, i.e., remained at the 2-cell stage (n=30). The individual droplets in which the embryos had been incubated were then analysed retrospectively for their pyruvate content enabling the relationship between embryo development and metabolism (the consumption of pyruvate) to be determined. The data may be presented in a number of different ways. Traditionally, they might be shown as a table of values of pyruvate consumption (pmol per embryo per hour), as depicted in Table 1.

There was a significant difference in pyruvate consumption between the groups; those which exhibited development having higher values on average than those with no development (p=0.016). This is illustrated more strikingly in the second way of expressing the data – as a plot of mean values with confidence intervals (Figure 1)

However, in order to examine individual cellular efficiency and discover whether the Goldilocks Principle applies, it is more informative to visualise the data as distributions. Thus Figure 2 shows the spread of data for pyruvate consumption by 2-4 cell embryos.

A number of conclusions may be drawn from the data:

* There was a high attrition rate: only 40/70 (57%) of the 2-cell embryos developed to the 4 cell stage;
* There was considerable variation in pyruvate consumption, whether or not development occurred;
* There was considerable overlap between the data in the two categories and a Goldilocks Principle was not observed, in which case, the individual data points would have been distributed broadly or narrowly, with a considerable degree of overlap
* The differences in input in the developed group were very considerable: development occurred with values between 2 pmol pyruvate consumed/embryo/hour (very high efficiency) and 16 pmol pyruvate (low efficiency).
* Given these differences, it is possible to hypothesise that ‘low efficiency’ embryos which use a large amount of pyruvate to reach the next stage might struggle to maintain such a high consumption throughout development in contrast to the more efficient embryos, with a lower pyruvate consumption. Conversely, apparently highly efficient embryos (those that developed with very low pyruvate consumption), might struggle to continue to develop through subsequent cleavage divisions if continuing with such a low pyruvate consumption.

A caveat to these conclusions is the obvious capacity of the embryo to use other substrates and switch from one to another. Against this is the obligatory nature of pyruvate as a nutrient and the lack of data on a full balance sheet of the relative contribution of all other potential nutrients – ideally determined simultaneously; a technical challenge yet to be overcome. As indicated earlier, the best marker of metabolic capacity would be oxygen consumption (e.g. Lopes et al., 2007; Tejera et al., 2011), but as discussed by Leese (2012) this is difficult to measure on such a small amount of material, and comprises several components which have yet to be quantified at all the preimplantation stages. Given these constraints and the unique data set available, we consider pyruvate consumption presently provides the best option for considering energy efficiency throughout preimplantation embryo development.

In order to test the propositions in the final conclusion above, it was necessary to devise a prospective type of experiment in which, following metabolic profiling at the 2-4 cell stage, development could be monitored through to the blastocyst stage, which takes about 6 cleavage divisions over 6 days in the bovine. The difficulty with this type of experiment lies in the fact that bovine (as well as ovine and porcine) embryos are less viable if cultured singly, especially in extended culture; they prefer to be grown in groups (Gopichandran and Leese, 2006; Stokes et al., 2005). This problem was overcome as described in the paper by Guerif et al., (2013) as follows:

Experiment (ii)

The initial stages of the experiment were identical to experiment (i). Thirty bovine embryos were then incubated singly from day 2 to day 3 in small droplets of medium and pyruvate uptake was measured. On the basis of the results, the embryos were allocated into tertiles with 10 embryos per group, representing ‘high’ (>10pmol/embryo/h: T3 in Figure 3) ‘intermediate’ (4-10pmol/embryo/h:T2) and ‘low’ (<4pmol/embryo/h: T1) pyruvate uptakes respectively during the 24 hours of culture between days 2 and 3. The embryos were then cultured to the blastocyst stage (day 8) to provide a direct test of the Goldilocks Principle. Experiment (ii) was repeated six times.

The relationship between pyruvate uptake values and blastocyst formation can be seen in Table 2:

A better illustration of these data is obtained by plotting the full distributions of pyruvate uptake between 24-48 hours against blastocyst formation (Figure 3).

While not central to the proposition being addressed, Figure 3 includes the terms ‘optimum’ ‘pejus’ and ‘pessimism’ as in the original paper (Guerif et al 2013), illustrating hypothetically, the response of an embryo to stress. When the stress is mild, embryo metabolism shifts up or down from within the ‘optimum’ to the ‘pejus’ range in order to minimise or rectify the damage. However, since the damage is modest, metabolism can return to the ‘optimum range’ when it has been corrected. However, when the stress is severe, metabolism shifts irreversibly into the ‘pessimum’ range from which it cannot recover. For further discussion, see Guerif et al (2013).

The following conclusions may be drawn from this second data set:

* In line with the first experiment, there is considerable variability in the capacity of in vitro produced bovine 2-cell embryos to develop to the blastocyst stage. This is well-known, and the overall blastocyst rate (~35%) is consistent with the data of others
* Highest blastocyst rates were obtained with pyruvate consumption in the intermediate range; embryos in the higher and lower ranges were much less likely to form blastocysts. The data are therefore consistent with the Goldilocks Principle with an optimal “lagom” range of pyruvate uptakes, consistent with a high blastocyst rate
* Pyruvate uptake does not provide an all or nothing marker of bovine 2-cell embryo developmental capacity; the overlap between the categories was considerable, especially between the intermediate and higher ranges.
* The end point in these studies is blastocyst formation and it would be interesting to discover whether these embryos have the same potential for implantation and the capacity to give rise to live offspring.

The value of plotting such results as distributions in order to reveal optimal ranges, and, in this particular example, of a long time interval between metabolic assessment (day 2-3) and the measurement of development outcome (day 8), is illustrated by the study of Turner et al., (2004) on the pyruvate uptake of single human embryos generated via natural cycle IVF. Pyruvate consumption was measured over the first 24 hours following fertilisation prior to transfer on day 2 (40 – 50 hours post-insemination). The values for pyruvate were related retrospectively to the outcome; pregnant or not-pregnant (Figure 4).

These data indicate the existence of an optimal range of pyruvate uptake (between about 10 and 30 pmol/embryo/hour) within which a pregnancy can occur and that as with the data of Guerif et al considered above, embryos in the higher and lower ranges are less likely to lead to blastocyst development and the establishment of a pregnancy.

The quiet embryo hypothesis has been questioned by Gardner and Wale (2013) largely on the basis that blastocyst formation is associated with a dramatic increase in glucose consumption (i.e., a highly active as opposed to quiet, metabolism). In response, Leese (2012) proposed that what was required as a test of the quiet embryo hypothesis was not the ‘functional’ demand for high glucose but the overall metabolic cost of this process and that the challenge was to measure energetic efficiency alongside nutrient uptake and relate the data to developmental competence, as has been done in this paper. A further interpretation of the data of Gardner and Wale is to propose that the minimum threshold for glucose consumption required to make a blastocyst is set at a high level, but that within the range of values conducive to blastocyst formation there will be sub-ranges – of ‘too high’ and ‘just right’ consistent with a viable pregnancy in the long term.

KINETICS OF EARLY EMBRYO DEVELOPMENT

Another feature of the preimplantation embryo where the Goldilocks principle could be apparent is the speed of development. In the early days of IVF, when embryos were grown under what were likely to have been severely suboptimal culture conditions, a high speed of development was taken as an indicator of quality. However, as culture conditions and success rates improved, numerous studies were conducted, many of them large, correlating cleavage speed to implantation and live birth rates; more recently, with the introduction of the time-lapse technique in IVF laboratories, it has been possible for these associations to be investigated in a more precise manner. The data are now consistent with the proposition that the speed of development should be “just right” and that both too slow and too fast development results in poorer success rates, presumably indicating a non-optimal metabolic and/or genetic phenotype.

Early studies also showed that the sooner embryos underwent the first cleavage their prognosis for blastocyst development, pregnancy and live birth, was better than for embryos with a later first cleavage (Lundin et al., 2001; Salumets et al., 2003; Van Montfoort et al., 2004). The time used for cut-off was around 25-27 hours. However, with the implementation of time-lapse and the facility for observing embryo development continuously, it was found that the optimal time span was intermediate between fast and slow, and embryos that cleaved too rapidly (<24.3h) had a poor developmental potential (Meseguer et al 2011). Similar conclusions, showing a tighter time distribution for implanting than for non-implanting embryos, have been reached for a number of morphokinetic variables, such as number of cells and length of cell cycles. (Meseguer et al 2011, Cruz et al., 2012).

A specific illustration of the value of plotting the distribution of biomarkers of embryo health is provided in the retrospective analysis by Meseguer et al., (2011) who recorded the time taken for individual human IVF embryos to divide to 5 cells and related this to their subsequent capacity to implant following transfer (Figure 5) where NEG = negative implantation; POS = positive. The general pattern described above is replicated, with embryos more tightly distributed in the intermediate range (which in Goldilocks and Lagom terms is ‘just right’) more likely to give a positive outcome.

CONCLUSION: THE GOLDILOCKS ZONE

In light of the data appraised in this paper and the notion of a ‘quiet range’ of metabolic activity (Leese 2007), we propose a new term, a *‘Goldilocks zone’* within which embryos with maximum developmental potential will be located. The lower limits of the Goldilocks zone will be determined by the minimum, or threshold, value that nutrient /metabolic activity has to reach to ensure the fidelity of energy homeostatic mechanisms, and the upper limit by the physiological scope to increase cellular metabolism balanced against *the energy parsimony in almost everything they do* (Johnson, 2013). The existence of ‘ranges’ or ‘zones’ is obviously best revealed by plotting data as distributions and we believe that other areas of biology and medicine could benefit from this approach. The challenge is to discover where the limits lie for other cell types, tissues and whole organisms, in different situations, and their determinants.

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TABLE 1. Pyruvate consumption (pmol/embryo/hour) by 2 cell bovine embryos which developed or showed no development to the 4-cell stage

|  |  |  |
| --- | --- | --- |
|  | n | Mean (sd) |
| Development | 40 | 7.27 (3.98) |
| No Development | 30 | 5.19 (2.74) |

TABLE 2

Rate of blastocyst development according to the level of pyruvate consumption (pmol/embryo/hour) measured between day 2 and day 3. Values are mean +/-s.e.m.

|  |  |  |
| --- | --- | --- |
|  | Mean pyruvate consumption | Blastocyst rate (%) |
| Low pyruvate | 1.14+/-0.20 | 13 |
| Intermediate pyruvate | 6.14+/-0.27 | 68 |
| High pyruvate | 13.0+/-0.48 | 25 |

Figure legends

Figure 1 Pyruvate consumption (pmol/embryo/hour) by 2 cell bovine embryos which developed or showed no development to the 4-cell stage. Values are mean +/- 95% confidence intervals

Figure 2 Individual values for pyruvate consumption (pmol/embryo/hour) by 2 cell bovine embryos which developed or showed no development, to the 4-cell stage

Figure 3 Individual values for pyruvate consumption by bovine embryos assigned prospectively to one of 3 categories representing ‘low’,(T1) ‘intermediate’ (T2) and ‘high’ (T3) pyruvate uptakes respectively (<4pmol/embryo/h; 4-10pmol/embryo/h or; >10pmol/embryo/h) and cultured to the blastocyst stage. While not central to the proposition being addressed, Figure 3 includes the terms ‘optimum’ ‘pejus’ and ‘pessimism’ as in the original paper (Guerif et al 2013), illustrating hypothetically, the response of an embryo to stress. When the stress is mild, embryo metabolism shifts from within the ‘optimum’ to the ‘pejus’ range in order to provide resources to rectify the damage. However, since the damage is modest, metabolism can return to the ‘optimum range’. However, when the stress is severe, metabolism shifts irreversibly into the ‘pessimum’ range from which it cannot recover. For further details, see Guerif et al (2013).

Figure 4. Pyruvate uptake of single human embryos generated via natural cycle IVF. Pyruvate consumption was measured over the first 24 hour following fertilisation prior to transfer on day 2 (40 – 50 hours post-insemination. The values for pyruvate were related retrospectively to the outcome; pregnant or non-pregnant. From Turner et al (1994).

Figure 5 The time taken for individual human IVF embryos to divide to 5 cells in relation to their subsequent capacity to implant following transfer. Adapted from Meseguer et al (2011).