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# Virtues and limitations of the preimplantation mouse embryo as a model system

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## Abstract

The mouse is the most widely used model of preimplantation embryo development, but is it a good model? Its small size, prolificacy and ease of handling make the mouse a relatively low cost, readily available and attractive alternative when embryos from other species are difficult or expensive to obtain. However, the real power of the mouse as a model lies in mouse genetics. The development of inbred mouse strains facilitated gene discovery as well as our understanding of gene function and regulation while the development of tools to introduce precise genetic modifications uniquely positioned the mouse as a powerful model system for uncovering gene function. However, all models have limitations; the small size of the mouse limits tissue availability and manipulations that can be performed and differences in physiology among species may make it inappropriate to extrapolate from the mouse to other species. Thus, rather than extrapolating directly from the mouse to other species, it may be more useful to use the mouse as a model system for developing and refining hypotheses to be tested directly in species of interest. In this brief review, the value of the preimplantation mouse embryo as a model is considered, both as a model for other species and as a model for the mouse, as understanding the virtues and limitations of the mouse as a model system is essential to its appropriate use.

### Keywords

Mouse; Embryo; Preimplantation; Comparative; Model

## 1. Introduction

Claude Bernard (1885) developed the foundation for the modern use of models in biomedical research when he began using dogs to model aspects of human physiology. Animal models have been widely ever since and it has been suggested by the American Medical Association that many medical advances in the 20$^{th}$ century involved the use of animal models in some way (as cited in Lafollete and Shanks [1]). Bernard reasoned that humans and animals were similar enough that results from animal experimentation could be extended by analogy to humans [2]. This perspective requires that the model and target have common, causally connected properties and that no differences between the model and target invalidate the use of the model [1]. Since the days of Bernard, it has become clear that significant differences among species often make it inappropriate to extend results by analogy from one species to another, leading some to conclude that animal models are generally not suitable for testing hypotheses about the causal mechanisms underlying human physiological and disease

processes [1]. This does not diminish the value of models as tools for discovery. In this context, the use of models leads to the development of new hypotheses to be tested in target species [3]. This distinction between what have been referred to as causal analog models (CAMs) and hypothetical analog models (HAMs) is important as it explains much of the controversy surrounding the use of models [1]. Often, models used to generating insight about how a system may work in another species (HAMs) are not useful as CAMs because functional similarities do not always imply causal similarities. Thus, the answer to the question "is the mouse embryo a good model" will depend on how the mouse embryo is being used. As a CAM, the mouse embryo will often not be a good model for other species, such as the human or the bovine, but functional similarities between mouse, human and bovine embryos make the mouse embryo an invaluable HAM by overcoming many of the logistical, ethical and financial challenges of working with embryos from other species. Understanding of the virtues and limitation of the mouse as model for preimplantation embryo development will aid those considering working with this model system. In this brief review the value of the preimplantation mouse embryo as a model is considered, both as a model for other mammalian species and as a model for the mouse.

## 2. Why the Mouse?

The small size, short generation interval, high fecundity, and ease with which mice can be bred make them relatively inexpensive to work with and overcomes some of the challenges of working with embryos from humans and other mammals. Yet the mouse shares characteristics of interest with other species, especially the human, that make it a useful model. Ninety nine percent of mouse genes have human homologs, gene order is conserved between mice and humans, and mutations in mouse genes and their human homologs often have similar consequences [4]. Thus, genome organization and genetic regulation in humans and mice are similar enough that the mouse is often used as model system for humans.

Relative to other model systems, the mouse has several unique advantages. Enabled by the creation of inbred strains, a vast body of knowledge of mouse genetics has developed over the years and the tools available for introducing genetic modifications in the mouse are unmatched in any other mammalian species. Modern mouse genetics started with the creation of inbred strains. Mice from an inbred strain are nearly identical genetically and can be considered almost clonal, reducing phenotypic variability and making it possible to use fewer animals to complete an experiment [5,6]. Today, a diverse array of inbred strains exists, serving as a rich source of models for studying gene function as well as gene-gene and gene-environment interactions that underlie physiological and disease processes.

Inbred mice became a more powerful model once tools and strategies for introducing precise genetic modifications were developed, allowing the effects of genetic modifications to be examined on a uniform genetic background [7]. The number of different lines of mice created using these approaches and others is not known; however as of September 1, 2007, 10,914 unique mouse strains and 36,232 embryonic stem cell lines were listed in the International Mouse Strain Resource (www.informatics.jax.org/imsr/), as available from repositories, such as the Jackson Laboratory. Major initiatives underway around the world aim to create mouse or embryonic stem cell lines containing null mutations for every gene in the mouse genome within the next 5 years [8]. The availability of such resources and the availability of tools to create genetically modified mice provide an opportunity to probe gene function that is unmatched in any other mammalian species at this time, giving the mouse a unique advantage as a model.

### 2.1. Mouse embryos

Widespread use of mouse embryos as a model of preimplantation embryo development is likely due more to the advantages of the mouse as a model, as described above, than to the characteristics of the mouse embryo. Indeed, some authors have concluded that embryos from other species, such as the bovine, are a better model system in some situations [9]. Yet the mouse embryo remains in widespread use as a tool for understanding embryo biology, for developing Assisted Reproductive Technologies (ART), and as a component of quality systems for human IVF programs. The complete scope of work using mouse embryos is far beyond what can be covered in this paper; instead an effort has been made to compare mouse embryos to bovine and human embryos and explore how the mouse has been used as a model for preimplantation embryo development.

## 3. Comparative Aspects of Embryo Biology

Mammalian embryos appear similar. They are approximately the same size and develop similarly, starting as a one cell zygote, progressing through the blastocyst stage before hatching and implanting. During this series of changes, the metabolic needs of the embryo change, they become transcriptionally active at zygotic genome activation, and differentiation begins as the cells that compose the embryo change from totipotent to pluripotent to multipotent. Viewed abstractly, it seems reasonable to extrapolate from one species to another, but generalizations can be misleading. For example, while embryos from different species go through similar developmental stages, the timing varies by species as illustrated in Table 1. These differences and many others make it inappropriate to directly extrapolate from one species to another.

### 3.1. Biochemical/biophysical

Mouse embryos were among the first embryos to be cultured. This early work helped lay the foundation for the development of culture media and in vitro culture practices in use today [10]. Over time, differences among species became apparent, bringing into question the value of the mouse embryo as a model for improving media formulations, leading some to conclude that bovine embryos are a better model for the human [11]. Interestingly, strain specific differences in development are observed during mouse embryo culture [12,13]. For example, in some cases the optimum media for one strain may be detrimental to the development of embryos from another.

Some of the key differences among embryos from different species relate to metabolism. Lactate, pyruvate and glucose are common components of media that can be used as an energy source by embryos. However, the ability to utilize glucose varies among species. Human embryos do not utilize glucose due to limited availability of hexokinase [14]. The situation is more complicated for the mouse, as embryos from some strains can metabolize glucose while others cannot [15,16]. Cattle embryos are able to metabolize glucose, although this is affected by culture conditions [17]. A nitrogen source is also needed for embryos to develop. This can be supplied by serum, albumin or amino acids.

Amino acid uptake and utilization also differ among species with mouse embryos not requiring amino acids to develop to the blastocyst stage, in contrast to bovine and human embryos. Differences among species in the use of amino acids, may be due to their multiple functions, such as acting as an osmolyte in bovine embryos [18] or possibly forming ammonia. Culture conditions affect more than metabolism as the ability of embryos to regulate intracellular pH is also affected by culture conditions. Mouse embryos are less sensitive to and recover more easily from changes in pH than either human or bovine embryos [11,19]. Thus, although the mouse played an important role in the pioneering work on embryo culture, the apparent

differences among species and even among strains mean that caution should be exercised when extrapolating from one strain to another or from mice to other species.

### 3.2. Implantation

During implantation the embryo comes into close contact with the endometrium to allow the exchange of nutrients and waste products. In mice, implantation follows the loss of the zona pellucida as the lumen of the uterus narrows, bringing the embryo and endometrium into close contact where the embryo invaginates into the endometrium eventually forming a discoid placenta. Implantation in mice is eccentric occurring on the anti-mesometrial side and unlike many other species decidualization can occur in response to an artificial stimulus. Implantation can be delayed in mice, during which time the embryos are dormant and the uterus is quiescent [20]. Implantation is centric in cattle and the embryo fuses to the endometrium at spots throughout the endometrium via cotyledons. In contrast, the embryo trophoblast penetrates the epithelium and invades the stroma as embryos implant in humans forming a discoid placenta. Given the differences in implantation strategies among these species, the mouse has limited utility as a model for studying the physiology of implantation in cattle or humans. However, there is some commonality across these species, as implantation is controlled by ovarian steroids and many of the same growth factors and cytokines, making the mouse a useful model for examining the control of implantation [21].

## 4. The Mouse Embryo as a Model System

Mouse embryos have been used as a model system for many years for a variety of purposes including furthering our understanding of embryo biology, improving ART and assuring quality of embryo culture and manipulation systems.

### 4.1. Use of the mouse to understand gene function and regulation in embryos

Mouse embryos have been used extensively as a model system for studying gene regulation during mammalian embryogenesis. The variety of tools available for studying mouse genetics make the mouse well suited as a model system and more is likely known about gene regulation in the mouse than any other species. Consequently, the depth and breadth of information in this area are outside the scope of this brief review and the reader is referred to several recent reports [22–26]

An area particularly relevant to the study of preimplantation embryo development is imprinting. Animals produced using ART may differ phenotypically from animals produced by natural mating. Some of these changes include long-term negative effects on offspring lasting into adult hood [27]. In cattle and sheep, in vitro handling of embryos is associated with large offspring syndrome (LOS) [28] and in humans the use of ART has been associated with an increase in the prevalence of certain genetic disorders including Angelman Syndrome and Beckwith-Weidemann Syndrome [29,30]. These disorders share a common cause- aberrant genomic imprinting. There is some commonality in imprinted genes among mice, cattle, and humans, and mice have been used as a model to study aberrant imprinting [31]. Li et al 2005 [32] used inbred mice to create a model in which allele specific changes in DNA and histone methylation could be followed. Crossing two species of inbred mice to create an F1 hybrid with polymorphisms in imprinted alleles allowed parental alleles to be distinguished, making it possible to observe allele specific changes in DNA and histone methylation, and for examining the mechanisms by which ART may affect phenotype. While the phenotypic changes resulting from aberrant imprinting vary by species [27], the mechanisms underlying these changes appear similar, making the mouse a good model for furthering our understanding of the mechanisms controlling imprinting.

### 4.2. Assisted reproductive technologies

The scarcity of human embryos and the ethical and logistical challenges associated their use make it necessary to use models for developing new technologies. Mouse embryos are frequently used in the development of ART that will ultimately be applied to human embryos. Notably, the mouse has played an important role in the development of IVF [33], the development of culture systems [10] and embryo cryopreservation [34], and the reader is referred to these reviews for more detailed discussion of these areas. Mouse embryos continue to play a role in the development of new assisted reproductive techniques; once demonstrated to be safe and effective in the mouse, these techniques can be applied to embryos from other species, a strategy that is recommended but not always followed [35].

Examples of situation where the mouse has been used as a model for developing and testing techniques to be used in human ART programs include strategies for overcoming oocyte specific deficits and assisted hatching. In some individuals, oocyte specific defects in the ooplasm are thought to adversely affect fertility. It may be possible to overcome these defects by transferring ooplasm from a healthy oocyte to the defective one, allowing embryo development to proceed normally. While difficult to test with human embryos, the mouse embryo has been used as a model for evaluating the safety and efficacy of ooplasm transplantation. Examples include germinal vesicle transplantation and cytoplasmic transfer [36]. While effective, work with mice has also demonstrated that this approach may result in epigenetic changes [35].

Advanced age, hormonal treatment, in vitro culture and cryopreservation have all been implicated in zona hardening or thickening, changes that make embryo hatching more difficult [37–39]. Yet, successful hatching of the embryo is essential if implantation is to occur. Assisted hatching has been proposed as a possible solution, but results have been conflicting and recent recommendations suggest assisted hatching be used for embryos with poor prognosis [40]. The lack of benefit reported in some studies, may have resulted from the strategies used, as some can result in entrapment of the embryo [41]. Lyu et al. 2005 [42] attempted to use mouse embryos to develop a technique that overcomes this problem. A strategy was developed to partially dissect the zona pellucida mechanically, creating a longer slit than could be created using a laser, reducing the risk of embryo entrapment. This approach was subsequently tested using human embryos and found to be successful. However, better hatching does not necessarily correlate with implantation and clinical data are now needed to further evaluate this technique [42].

### 4.3. The use of mouse embryos in quality control systems

Robust quality systems are an essential part of IVF programs, preventing the exposure of embryos to harmful substances or conditions. Quality systems relying on mouse embryos as a model for human embryos have long played a role in bioassays for screening media and materials that may come in contact with embryos and the need for and value of such bioassays was recognized soon after the development of IVF [43,44]. Although criticized as not sensitive enough, too variable, and not predictive of the outcome of IVF [4,45,46], this approach has been fruitful as part of quality improvement efforts. Using a mouse embryo assay, the toxic effect of some gloves [47], contaminants from commonly used labware [48], affects of exposure to light [49] and endotoxins [50] were identified as were the detrimental effects of a transient drop in pH, changes in water quality, and temperature fluctuations [51]. Thus, the insight gained from the use of the mouse embryo assay has led to further definition of best practices and improvements in human IVF success.

Some of the controversy regarding the perceived value of the mouse embryo assay is likely the result of the conditions under which the assay was conducted. The strain of mouse used,

stage of embryo used and the culture conditions employed will affect the outcome [51,52]. This fits with our observations (unpublished data), compiled while working with hundreds of mouse strains, indicate considerable variability in the in vitro developmental capacity of embryos. Interestingly, this variability has not been exploited to create a more sensitive assay. Most mouse embryo assays are conducted with CD-1 or hybrid animals. Embryos from inbred strains could be a better model, but this has not been tested. The stage of embryo also impacts the outcome, with one-cell embryos being more sensitive than two-cell, four-cell or eight-cell embryos [51,53]. Embryos can be made even more sensitive to culture conditions when the zona pellucida is removed [50,54]. The type of media also influences the outcome of the assay [53,55]. In particular, the absence of protein from the media improves the sensitivity of the assay [48]. This may be due the ability of bovine serum albumin as well as other components of serum to bind contaminants, or could be due to the increased fragility of the embryos when cultured in protein-free media.

Taking into account the sources of variability described above, it should be possible to construct a mouse embryo assay which is more predictive and may be of use for the routine quality control of media used in IVF. Ideally, however, endpoints other than blastocyst formation should be used, such as the number of cells in the embryo or the rate of development based on observation at multiple time points. These endpoints reveal more subtle changes in embryo development that are likely more predictive than the more gross observation based on the formation of blastocysts [9,53].

## 5.0. The mouse as a model for the mouse

The basic aspects of reproduction remain the same across mouse strains- all are polyovular, exhibit spontaneous estrous cycles of short duration, corpus luteum function is induced by mating, the kinetics of embryo development are approximately the same, etc.[56]. However, among inbred strains there is variability in many traits including response to superovulation, developmental capacity of embryos in vivo and in vitro and in the efficiency with which embryos can be used to produce transgenic mice [57–60]. In fact, differences among strains could likely be found for almost any trait imaginable. This is one of the characteristics of mice that make them a powerful model system. However, it also means that the mouse is not always a good model for the mouse. Results obtained in one strain cannot always be extended by analogy to another strain as can be seen from the examples previously cited in this article. Those considering using the mouse as a model for the first time may not be cognizant of these differences, leading to frustration and possibly misinterpretation of data. Given the diverse number of mouse strains that exist and the differences among them, the mouse can also be considered a model for the mouse.

## 6.0. Summary and Conclusions

The use of the mouse as a model of preimplantation embryo development has advanced our understanding of mammalian embryos and aided the development of ART by overcoming some of the financial, ethical and logistical challenges of other model systems. The mouse has proven to be an excellent model in many regards, but it is important to not over generalize. While insight can often be gained by using one species as a model for another, results often cannot and should not be extrapolated directly from one species to another. With the mouse, the same issue arises at times when comparing mouse strains. Developing an understanding of mouse biology, the characteristics of the mouse strains to be used and the biology of the target species will aid in the proper use of the mouse as a model.

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**Table 1**

Comparison of embryo development in humans, cattle and mice.

| | Human | Cow | Mouse |
|---|---|---|---|
| Oocyte diameter (μm) | 150–180 | 150–180 | 90–100 |
| Stage at zygotic genome activation | 4-cell | 8-cell | 2-cell |
| Time to reach | | | |
| 2-cell stage (hours) | 30 | 36 | 12 |
| Blastocyst (hours) | 120 | 150 | 70 |
| Hatching (hours) | 150 | 200 | 100 |
| Implantation (days) | 9 | 30 | 4 |

Comparison of the timing of significant events in embryo development in humans, cattle and mice [21] and adapted from Menezo and Herubel 2002 [11].