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ScienceDirect
Theriogenology 69 (2008) 2–9
Theriogenology
www.theriojournal.com

Are animal models as good as we think?

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Abstract

Models have been a tool of science at least since the 18th century and serve a variety of purposes from focusing abstract thoughts to representing scaled down version of things for study. Generally, animal models are needed when it is impractical or unethical to study the target animal. Biologists have taken modeling by analogy beyond most other disciplines, deriving the relationship between model and target through evolution. The ''unity in diversity'' concept suggests that homology between model and target foretells functional similarities. Animal model studies have been invaluable for elucidating general strategies, pathways, processes and guiding the development of hypotheses to test in target animals. The vast majority of animals used as models are used in biomedical preclinical trials. The predictive value of those animal studies is carefully monitored, thus providing an ideal dataset for evaluating the efficacy of animal models. On average, the extrapolated results from studies using tens of millions of animals fail to accurately predict human responses. Inadequacies in experimental designs may account for some of the failure. However, recent discoveries of unexpected variation in genome organization and regulation may reveal a heretofore unknown lack of homology between model animals and target animals that could account for a significant proportion of the weakness in predictive ability. A better understanding of the mechanisms of gene regulation may provide needed insight to improve the predictability of animal models. Published by Elsevier Inc.

Keywords: Animal; Model; Preclinical trials; Gene regulation

''Discovery consists of seeing what everybody has seen, and thinking what nobody has thought.'' Albert Szent-Gyorgyi (1957). Models help us see.

1. Model definitions

A model is a pattern, plan, representation, or description designed to show the structure or workings of an object, system, or concept (http://en.wikipedia.org/wiki/Animal_model). There are all kinds of models. Abstract models include molecular models, mental models, mathematical models, computer models, and conceptual models. Representational models include scale models, engineering models, fashion models, and role models. A detailed description of models for biomedical research is presented in two National Research Council reports, Models for Biomedical Research [1] and its 1998 update *Biomedical models and resources: current needs and future opportunities* [2].

2. Why do we need models?

We need models when we cannot put our hands on the object of our study (the cosmos, a molecule, string theory, etc.). An example of a conceptual model, proposed in the 50s, envisions epigenetic regulation as a ball rolling down hill between a series of mountain ranges [3,4]. This woodcut conceptual presentation

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0093-691X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.theriogenology.2007.09.030

clearly provides no insight into methylation of histones, etc. but it does serve to focus the mind. We need models when our study would benefit from simplification. We need models when it is considered ethically inappropriate to study the real thing. Animal models are useful when we do not have access to the target animal, it is impractical to use the target animal because of expense, or the logistics of acquiring or housing an adequate number of the target of study. However, as will be discussed below, the results of animal model studies have limitations. Thus, the questions that can be asked have constraints.

An animal model is a living organism in which the phenomenon of interest can be studied and in which the phenomenon resembles that in the target animal in some respect. The results of an animal model study can serve to characterize a system (e.g. spermatogenesis in ruminants), or predict the behavior of another particular animal (e.g. response of humans to influenza vaccine). One has a great deal more flexibility in choosing a model animal in the former case compared to the latter. The animal model, though often thought of as a one-to-one surrogate, is actually more like a representation, an analogy or in some cases the relationship between the model and the target is more abstract.

3. The model concept

The concept of models in science has been around for a long time. The idea of reasoning by analogy can be traced back to the 18th century when Kant in 1790 in ''The critique of judgment'' proposed that at least in a qualitative way, similarities among things could be used to predict cause and effect relationships, even if other differences exist [1].

This concept of modeling by analogy is pervasive in most fields of scientific investigations and was established long before the concept of hypothesis testing, which is a relatively new ''norm'' for experimentalists (Suggestions that experimental investigations should be based on hypothesis testing seems to have first appeared in 1959 – ''All experiments require a tentative theory to make it legitimately science, rather than random observations'' [5]. And somewhat surprisingly still appears to be an unsettled, evolving concept by some theorists [6]).

Representational models based in the natural sciences are common place. Ship builders can inform their designs by studying the performance of scaled down models. This is possible because hydrodynamic principles are scalable. Though the structure of biological science differs from that of physics, and does not have an equivalent scalable aspect (a goat is not a scaled down version of a cow), biologists also use the analogy concept. For example, the giant axon of squid has long been a model for studying nerve conduction [7], and the pigeon breast muscle has revealed much about intermediate metabolism, that by analogy predicts energy generation mechanisms in mammalian muscles [8].

But biologists have taken modeling by analogy beyond most other disciplines. The ''August Krogh Principle'' described by Krebs states ''For many problems there is an animal on which it can be most conveniently studied.'' [9]. This concept of ''unity within diversity'' is based on the relatedness of living organisms through evolution [1]. It has been argued that similarities in the way information is transmitted between generations (via nucleic acids), the way information is regulated (RNAi) and the way energy is generated (intermediary metabolism) support the notion that there are many other subsystems that are not only analogous, but homologous. Such homologies are clearly demonstrated in comparative genomics. The implications being that if two systems, components or entities are homologous then they are likely to function similarly. This concept appears to be an extension of the structure/function paradigm. As will be discussed later, in the context of gene regulation, the homology concept may turn out to be a somewhat superficial premise, or at least a concept that requires refinement when operating at molecular resolution.

The homology principle supports the notion that functions and structures appearing early in evolution are universally present in more evolved organisms. Thus, the study of metabolism in bacteria should reveal principles exhibited by mammals or the study of peptide hormones used by unicellular organisms for communication can serve as a good analog model for studying autocrine/paracrine communication in more complex multicellular organisms [10].

Furthermore, the homology concept supports Ernst Haeckel's theory of recapitulation (ontogeny recapitulates phylogeny) [11]. Though Haeckel's theory has fallen out of favor as not being precisely correct, the quest to disprove it has been informative [12,13]. Finally, the homology principle predicts that the closer the model organism and the target organism are to a common point of evolutionary divergence the more likely the model organism will serve as a good model.

However, homology is not a requirement for models to be good analogs. Organisms as evolutionarily distinct as plants and animals can be good analog models for one another. An example of such convergent evolution is

seen in the process of myoglobin/leghemoglobin-mediated oxygen diffusion exhibited by animals/plants. Clearly these oxygen carrying capacities evolved independently, but they are good analogs, if poor homologs [14]. In basic biological investigations, animal models are generally not intended as one-to-one models, but are intended to reveal an understanding of a process, function or structure rather than in directly establishing a connection between the process in the model species and the target species.

The vast majority of animals used for research each year are used as models by the biomedical community to address human health questions because of the obvious ethical concerns associated with human experimentation. A lot of animals are used for experimentation each year, but it is not easy to get a good count. In 1986 the U.S. Congress Office of Technology Assessment (OTA) reported estimates for animal model usage, by various special interest groups, ranged from 10 million to 100 million per year. OTA's own estimate was around 20 million annually (http://en.wikipedia.org/wiki/Animal_testing).

4. What makes a good animal model?

Many of us have an innate bias in choosing our model. We choose to use animals with which we have experience, animals for which we have housing and care readily available, and animals we can afford to support. But sometimes the model is chosen by happenstance, as was the case when ferrets were chosen for influenza studies because they just happened to be around for dog distemper studies or for the wrong reasons as was the case when pigeon breast muscle was chosen as a model for muscle energetics studies (because of poor analogy: their mitochondria are unique) [9]. The 1985 NRC report suggests we should choose animal models based on: appropriateness as an analog; transferability of information; genetic uniformity; background knowledge of biological properties; generalizability of the results; ease of experimental manipulation and ecological consequences and ethical implications [1]. These criteria seem to make sense from a theoretical point of view, but may not always be achieved when filtered through the practical constraints with which many investigators have to navigate in the real world.

5. Do animal models fulfill their promise?

The answer to that question is, it depends on the question being asked. There is no doubt the acquisition of scientific knowledge has been greatly facilitated by animal experimentation. The question we are asking here is more narrowly focused. Are animal models elucidating mechanisms and forecast outcomes of their target with adequate fidelity to validate this approach of scientific investigation? Or are such studies expanding our knowledge of the model animal without fully fulfilling their function as a tool for explaining systems or predicting behavior of the target animal?

We could present nearly an infinite number of examples. Here are a few examples related to reproduction that provide an answer to the above question: ''sometimes.''

Though sheep and goats are very good reproductive models for one another [15], standard procedures for superovualation of *Bos taurus* do not provide a good guidance for superovulating buffalo (*Bubalus bubalis*) [16]. This may suggest evolutionary relatedness may not always be a good criteria for model selection.

Embryo development and intrauterine stresses are often modeled. In one such case mouse, rat and bovine embryos have been employed to address the interesting observation that women with insulin-dependent diabetes mellitus tend to have an usually high proportion of female offspring [17,18]. Investigators found that high *in vitro* glucose environments favored female embryo survival thus leading to testable hypotheses to explain the mechanism [19–21]. Hasmster oocytes and embryo culture experiments have been invaluable in establishing concepts of for optimized culture conditions for other mammalian embryos, but the details have best been worked out when the target species material was available for study [22–24].

Due to the limited availability of human embryos and the ethical considerations for human experimentation, animals have been used extensively to address many aspects of reproductive issues such as fetal growth retardation or assisted reproductive technologies [25,26]. These studies demonstrated the value of animal models in better understanding the basic mechanisms involved but they recapitulate only some human phenotypes.

It is not uncommon for comparative animal experimental results to be informative in revealing biological differences that argue against using the species under investigation as models for one another. Oocyte maturation and embryo culture experiments are good examples of this concept. Maturation of bovine, human and mouse oocytes were shown to differ in their response to FSH/LH [27] and current optimized culture conditions, at least for the mouse, has diverged from the others [23,28].

The continued quest for embryonic stem cells has revealed species differences that continue to perplex.

What meaning is the scientific community to derive from the observations that Oct4 (POU) expression is restricted to the ICM of murine embryos but apparently not in bovine and porcine embryos [29]? What about the other morphological and differences in pattern of expression [30]? Those differences may lead to interesting evolutionary insights some day, but are not helpful for isolating embryonic stem cells from a variety of species of interest.

And then there are situations where our typical animal models are not very useful. It has been reported that in the USA more than 50% of human IVF treatments use intracytoplasmic sperm injection (ICSI). Unfortunately, rodents, rabbits and bovine oocytes do not behave as human oocytes. Only non-human primates seem to be an adequate animal model in this instance [26].

6. Animal models in biomedical research – testing the predictability of animal models

Though we realize the readership of this paper is primarily interested in livestock animal models, we have chosen to focus on the biomedical literature. It provides unique opportunities to evaluate animal models because a significant goal of that literature is devoted to validating the efficacy of the animal models to predict target response or behavior. There is no such animal model quality control literature for livestock per se, but maybe there should be. In biomedical research, animal models serve to study fundamental biological systems and diseases in a way that cannot be studied in humans. In these models, specific hypotheses and experimental therapies can be tested extensively within ethically accepted limits. Not only does it make good scientific sense to use animal models to understand human biology, it is required. The requirement for animal model studies as a prerequisite to any human clinical trials was codified as ‘‘The Nuremberg Code’’ after World War II [31].

The concept of the Nuremberg Code, that animal studies must precede human trials, is mandated by United State’s law when requesting approval for sale of a biomedical drug or device (Federal Food, Drug and Cosmetic Act, United States Code Title 21, Chapter 9). Animal models for genetic diseases have arisen spontaneously in a variety of species (e.g. mouse, cat, dog) or have been artificially created by transgenic/gene targeting. Numerous mouse mutant strains with mutations in genes related to human diseases are available. In some instances, such altered mice exhibit a phenotype similar to that seen in humans (chronic granulomatous disease [32], hemophilia A [33], spinocerebellar ataxia [34]) or displayed a somewhat more severe phenotype than in humans (ADA deficiency [35], Gaucher’s disease [36]).

Unfortunately, animal models often do not faithfully resemble the corresponding human disease. For example, one of the first surprises following the establishment of gene targeting was the lack of the typical Lesch-Nyhan phenotype in a mutant mouse deficient in hypoxanthine phosphoribosyl transferase (HPRT). This disconcordance turned out to be due to alternative and redundant metabolic pathways utilized by mice and man [37]. Similarly, mice deficient in the CFTR gene did not exhibit the pulmonary effects of cystic fibrosis but instead suffered from severe gastrointestinal obstruction [38]. This unpredicted observation is probably explained by a recent study which demonstrated even tissue-specific regulation has diverged significantly between human and mouse [39]. Discovering differences between the human disease and animal model phenotypes provides important insights into disease pathogenesis and the alternative schemes used by these species to deal with stress. But such differences, if not discovered, can interfere with developing viable therapies or testing the efficacy of novel treatments as a precursor to conducting clinical trials on human subjects. These undetected differences are likely one of the main reasons many human clinical trials fail. However, poorly designed and conducted animal experiments may also play a significant role [40].

The above examples demonstrate that even in simple scenarios of single gene disorders, transgenic/knock-out mice may not always provide a suitable model. What then are the chances of gaining an understanding of normal biological function or the pathogenesis of complex multi-gene disorders such as atherosclerosis, hypertension, diabetes, stroke, cancer, etc., with complex etiology that can further be confounded by including environmental components? Some researchers say the answer lay in systems biology [41].

Why are we seeing differences between so many rodent models and human disease? As indicated above, although biochemical pathways in mammals are generally well conserved. It is also important to consider the different life span between model animals and target species. Disorders that are manifested late in life may be difficult to accurately model in relatively short lived animals. Attempting to model bovine spongiform encephalopathy in mice has been thwarted by such latency issues [42]. The obvious differences in heart rate and metabolic rates of rodents and larger

mammals can influence drug clearance rates. Such ''mechanical'' differences are easily compensated for through well known mathematical relationships. But accounting for metabolic pathway differences is less straightforward. Furthermore, what kind of correction is appropriate to account for the direct correlation between mutation rate (nucleotide base substitution rate) and body size? Additionally, most large animal populations, including humans, are out bred whereas laboratory animals are usually inbred and are caged in environmentally controlled conditions.

FDA highlights the potential difficulties in interpreting animal model results by requiring preclinical trails to use two or more species (at least one rodent and one non-rodent). The result of preclinical animal model studies is often disappointing because in many cases even the use of multiple species of animal models fail to predict efficacy in human trials. Spectacular failures of animal models are not unprecedented. Recently, just minutes after injection of an anti-inflammatory drug, TGN1412, six volunteers suffered multiple organ failure. TGN1412 had been tested extensively in rabbits and monkeys with no serious side effects reported. A recent publication by the FDA indicates that the rate of success for a new medical compound entering Phase 1 clinical testing to reach the market is not greater than 8% [43]. Inadequate animal models are most likely one of the major hurdles in drug discovery and development. In a toxicology meta-analysis (combined statistical analysis of multiple studies that are based on similar hypotheses) of 150 compounds rodent experiments correctly predicted organ toxicity in humans in 43% of the studies and non-rodent animal models correctly predicted toxicity 63% of the time [44]. Critics of animal models have cited the TGN1412 drug trial as an example of why animal testing does not always have the desired predictive value. However, given the limited usefulness of computer models, in vitro models, and lower organism models, animal models remain the best alternative.

The failure of animal models to predict adverse outcomes in human clinical trials appears to be only one side of the argument that interpretation of some animal model studies can be misleading. It is interesting to speculate that animal models maybe just as likely to exhibit false positive results (compound or devise would be OK in humans but show adverse effects in animal studies) as they do false negatives results (OK in animal studies but have adverse outcomes in human trials). Given the ethical considerations associated with experimentation in humans, it is not likely we will ever know the false positive rate of animal models.

7. Are animal models losing their relevancy?

Once the sequencing of the human genome was proclaimed complete we were led to believe that the genomic era was in the past and the post-genomic era was our future. The goal of the post-genomic era is to decipher the sequence information in order to understand how structure and function of the genome in the context of cells, tissues, individuals and populations determine phenotype in normal, stressed and in disease states. Through high resolution genome sequencing it has become possible to identify sources of variability between individuals and make use of this information in an attempt to map genes affecting complex traits and multi-gene syndromes [45]. The genome was considered relatively stable except for occasional (once every thousand bases or so) single nucleotide polymorphisms (SNPs) or small scale deletions or duplications between individuals (for a recent review see [46]). Based on SNP comparisons, it was concluded that there is merely 1.23% difference between chimpanzee and human genomes [47] and nearly all (99%) of the sequence encoding proteins in the human genome align with homologous genes in mouse, and over 80% are clear 1:1 orthologs [48]. The discovery that mammalian genomes seem to be so similar strongly argues that the lack of precision in animal model predictability may have less to do with the inadequacy of the animal model concept and more to do with some external influences.

However, the high degree of genome conservation has recently been questioned by the discovery of variable numbers of duplications of large segments of DNA, ranging in size from thousands to millions of nucleotides, in normal populations. The surprising finding from the first map of copy number variation (CNV) in the human genome estimates that humans differ from one another by 12%, not a fraction of 1% as had previously been proposed [49]. As many as 2900 genes, or about 10% of known genes, are duplicated one or more times [50]. Similar CNVs have been identified between mouse inbred strains and will likely be a common characteristic of many other genomes (In some sense, plants such as ferns and wheat have taken this gene duplication strategy to an extreme, with their polyploidy genomes. For example, common durum wheat is a tetraploid and other wheat varieties are hexaploid.). It is, therefore, conceivable that this type of genetic variability may contribute to variability in gene expression among individuals and therefore phenotypic differences. It is probably too soon to speculate as to what, if any, influence copy number variation has on regulation or function of the genome, but at a minimum

it provides a new way of characterizing differences among individuals, breeds, species, etc. This unexpected variability may reveal reasons to question the usefulness of model organisms at some level. It remains to be seen if CNV exerts enough control over the genome to alter the predictive role of animal models, or if understanding the implications of CNV will help build algorithms that can be used to correct raw preclinical data for better predictability.

Intuitively a positive correlation between gene dosage (copy number) and mRNA abundance seems to make sense. It is less clear what influence the recently discovered transcription factor binding site infidelity may have on genetic control and ultimately phenotype [51]. One of the fundamental assumptions in comparative genomics is the factors (proteins) and elements that regulate the genomes of mice, cow and man are similar and can be identified by homology. That seems to hold true for genomic sequences which encode proteins, including transcription factors, those special proteins that control transcription initiation. Furthermore, it follows that the critically important components of the genome, that interact with transcription factors, and transcription factor binding sites (or *cis*-regulatory regions), are also conserved, so the current dogma goes. This obviously has some credence or investigators such as ourselves could not have utilized *cis*-regulatory regions from the sheep genome to appropriately control transgenes in mice and cows [52,53]. Although, in general, *cis*-regulatory regions are less tightly constrained (more permissive to mutation) then protein coding sequences, alignments of *cis*-regulatory regions of human and rodent genes revealed many blocks of highly conserved sequences [54]. Such strong sequence conservation suggested conserved function. Some new observations are starting to perturb the foundation of part of those concepts. The species differences in gene regulation beginning to emerge among species may be new reasons for caution in our interpretations of animal model results. Until recently the study of *cis*-regulatory regions has been conducted with laborious biochemical or functional assays, precluding a genome wide analysis. Since the development of the chromatin immunoprecipitation coupled to hybridization to DNA microarrays methodologies (known as ChIP-chip) high throughput scans are possible [55]. This technology allows for the, genome wide, isolation of DNA binding sites for given transcription factors. The first of these analyses are starting to appear. The new findings confirm that transcription factor proteins are evolutionarily conserved, but the location of their binding sites (*cis*-regulatory regions), and the number of binding sites relative to a controlled gene, and the sequence of the binding site are less well conserved in evolutionary terms. More surprisingly, this variation in *cis*-regulatory regions number, location, and configuration is observed among individuals within species [51].

If it can be logically argued that knowing the specific genetic makeup of a target animal will lead to more efficacious treatments an obvious corollary is a model will be a less precise predictor of the target animals response if the model animal's genome is regulated differently. The findings of the ENCODE project [54] suggest differences in gene regulation between species should be taken into account when considering the appropriateness of an animal model and the question it is being asked to address. Unfortunately, it is unlikely that the gene regulation of parameters of interest will be known for either the target or the potential model animals for many years.

8. Conclusions

The validity of the genetic discussion above is exemplified in dozens, and possibly hundreds of examples of genetic background differences within species influencing the outcome of studies [56,57]. If animal models are shown time and again not to precisely predict behavior of the target species, what good are they? We believe the literature supports the notion that animal models are an excellent basic science tool but are less useful for the purpose most are used for, as a tool for biomedical predictions. Unfortunately, there is currently no better alternative, at least if the target animal is human. The literature does seem to suggest if one is searching for a precise elucidation of a pathway, or wanting to discover the precise formulation for culturing tissues, or devising an optimized treatment regime to elicit a specific response, doing the study in the target animals is the only way to get there. On the other hand, models are useful for revealing generalized mechanisms. The earlier in evolution the process under investigation appeared (e.g. nucleic acid as an information storage device) the more likely that a model animal will serve as a good analog to study general principles, but not specific details. Obviously, the closer evolutionarily the model animal and the target animal, the more precision the model offers.

Using a rodent animal model to ask a question about livestock is risky unless the question being asked is conceptual. For example, much can be surmised about livestock embryo development by studying mouse embryos. The mouse embryo can reveal that transcription of the embryonic genome begins with a burst at a

particular stage of development, but the details of when it happens are only going to be revealed by studying the species of interest (e.g. bovine or porcine embryos [58,59]).

These concepts are probably intuitively obvious to most biologists. However, what was not obvious, at least to these authors, before reviewing the literature, is how seriously flawed the animal model paradigm is in biomedical research. That field, partly forced by law, is using animal models for a purpose, precise prediction, for which they are not well suited. The extraordinary high failure rate of animal models to predict human responses in the context of clinical trials argues for an alternative approach to assessing safety and efficacy. Possibly the information flowing from the ENCODE project and others will reveal enough about the genetic control of physiology to justify using particular animal models for very particular questions with the desired precision. In the meantime there are probably some warranted changes in standard experimental design parameters, such as selecting animal models based on pharmacological and pharmacokinetic similarities when testing drugs and testing therapies on animals that better simulate the disease state, including ancillary conditions. The problem of choosing the appropriate model animals and experimental designs going to be exacerbated when the era of personalized therapies, based on an individual's genome sequence, arrives.

Often animal models are justified, to granting agencies and others, as an analog of the target, a representation of the target animal. We often propose we will be able to extrapolate from our model studies. But in reality what we should do is use the results to build conceptual models that help generate testable hypotheses for the ultimate verification in the target species. We conclude that it is probably safer to use animal models to develop speculations, rather than using them to extrapolate.

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