Journal of Reproductive Immunology 132 (2019) 54–59

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Review article

When 3 Rs meet a forth R: Replacement, reduction and refinement of animals in research on reproduction

Petra Clara Arck*

Division of Experimental Feto-Maternal Medicine, Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

ARTICLE INFO

Keywords:
Reproduction
Mouse models
3R principle
Immunology

ABSTRACT

Research endeavors aiming to understand the maternal immune adaptation to pregnancy significantly rely on the use of animal models, such as mice and rats. These models have provided important insights into the pathophysiology of a number of pregnancy disorders in humans. However, the use of animal models in scientific research is a vividly debated and emotive topic. The 3R principles – replacement, reduction and refinement of research animals – have been propagated a few decades ago. The present review advocates a forward-thinking consciousness to address the 3R principles in research projects in the field of reproductive biology and immunology. Specific measures and alternative methods are being proposed to replace research animals by using e.g. tissue engineering approaches, biobank-derived tissue, 'placenta-on-a-chip' devices or *in silico* methods. The latter may involve data queries from repositories now available to provide single cell sequencing information on reproductive tissues. Reduction of research animals by gestational imaging and a wealth of suggestions for refinement are proposed. Taken together, the measures and guidelines introduced in this review are expected to spark a reconsideration of experimental designs in the area of reproductive biology and immunology in order to implement 3R principle where applicable.

1. Introduction

Successful reproduction requires the intricate and tailored adaptation of the maternal immune and endocrine system throughout the entire period of gestation. If such adaptation is not adequately mounted, pregnancy complications such as spontaneous miscarriage, preterm birth and preeclampsia can occur (Arck & Hecher, 2013). Thus, research endeavors aiming to understand such adaptational processes have continuously increased over the last decades in order to apply insights arising from normally progressing pregnancies towards unraveling the pathogenesis of pregnancy complications. These endeavors significantly relied on the use of animal models, such as mice and rats (Fig. 1). Animals and humans share an ancestral history, although the last common ancestor of mice and humans dates back approx. 75 million years (Winter et al., 2004). As outlined in a seminal review by the Markert group, animal models may be of limited value to understand the anatomy and physiology of placentation, due to the evolutionary tissue diversity (Schmidt et al., 2015). However, animal models allow understanding the pathophysiology of a number of disorders in humans, as well as developing treatments that are safe and effective. Here, the inclusion of mouse and – although to a lesser extend – rat models is particularly relevant when immunological aspects are being addressed, since a large number of genes coding for the immune response orthologue to humans are still present in mice, along with similar immune response patterns.

Random, but prominent examples mirroring the pivotal relevance of findings from research animals include the identification of human epidermal growth factor receptor 2 as a proto-oncogene in rodents, which has improved the understanding of pathogenesis of breast cancer and opened therapeutic avenues that now save lifes (Pegram and Ngo, 2006; Saad et al., 2019). Similarly, immune checkpoint inhibitors to treat cancer targeting the molecules cytotoxic T-lymphocyte-associated protein 4, programmed cell death protein (PD-1) and the PD-1 ligand have also evolved from insights in mice (Ai and Curran, 2015). Also, findings from animal models have significantly sparked the development of neutralizing antibody immunotherapy for HIV-1 (Nishimura and Martin, 2017). Moreover, although from a more historic perspective, the successful demonstration of bactericidal action on pathogens in mice has led to the breakthrough for penicillin (Kardos and Demain, 2013). Similarly, vaccine quality control tests date back to animal experiments pursued by 19th century scientists, including Louis Pasteur, Robert Koch, Ernst von Behring or Paul Ehrlich, to name only a few.

* Corresponding author at: Division of Experimental Feto-Maternal Medicine, Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Campus Research (N27), Martinistr. 52, 20246, Hamburg, Germany.
E-mail address: p.arck@uke.de.

https://doi.org/10.1016/j.jri.2019.03.004
Received 15 February 2019; Accepted 25 March 2019
0165-0378/

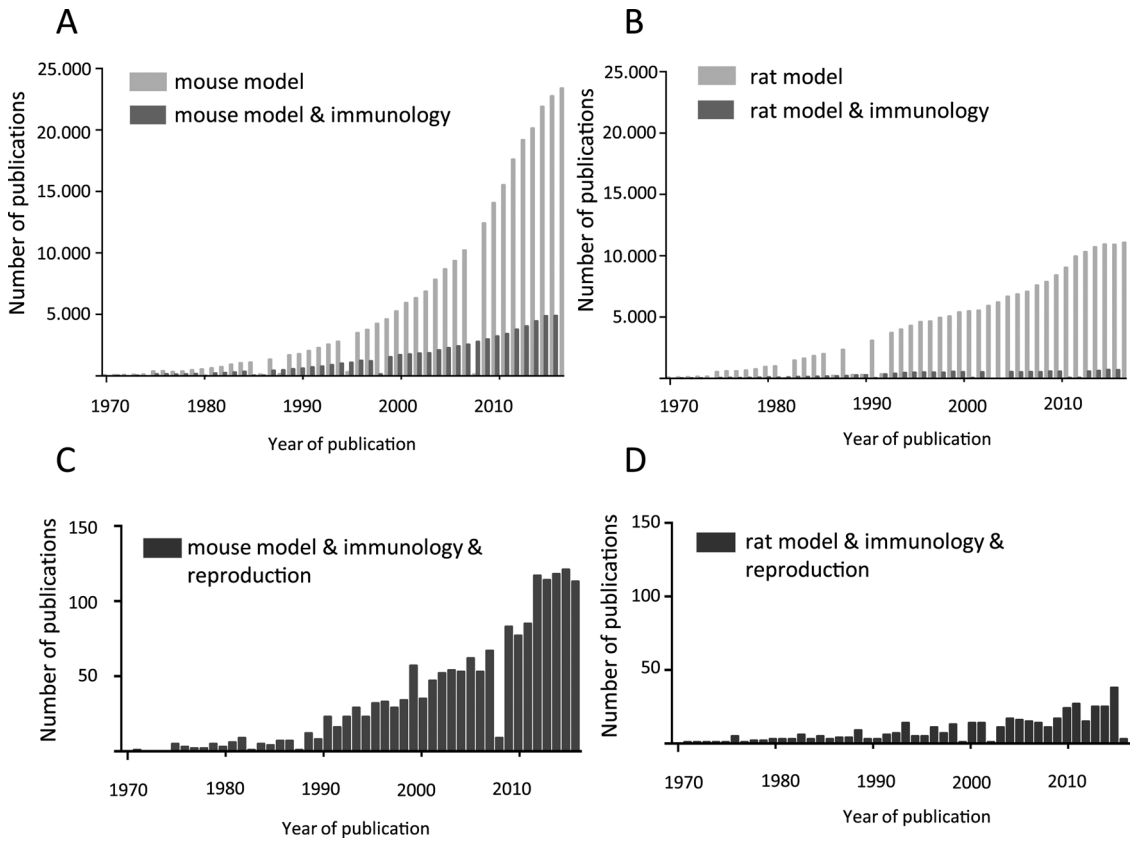

Fig. 1. PubMed-based queries using the search items 'mouse model' and 'mouse model & immunology' in order to obtain the related number of publication per year since the year 1970 (A). The search items 'rat model' and 'rat model & immunology' was used to provide the related number of publication per year since the year 1970 (B). (C) The search described in (A) has been refined by adding the search item 'reproduction', which yielded to the number of publications per year since 1970. (D) Same approach as in (C) for rat models .

Remarkable illustrations of the critical importance of finding from mouse models for human reproduction and their translational relevance are the identification of pregnancy-protective role of $CD4^+$ regulatory T cells in mice (Aluvihare et al., 2004), which coincide with the recognition of their functional role in mitigating pregnancy success in humans (Sasaki et al., 2004). Similarly, the tolerogenic phenotype of dendritic cells and related promotion of reproductive success has been identified using mouse models (Blois et al., 2005), and this unique phenotype of dendritic cells could be confirmed in reproductive tissues in humans (Kammerer et al., 2003; Hsu et al., 2012). Also, the pregnancy-protective role of galectin-1 was described in mice (Blois et al., 2007), which facilitated the interpretation of findings in galectin-1 in human pregnancies (Ramhorst et al., 2012; Tirado-Gonzalez et al., 2013). Lastly, blocking the action of tumor necrosis factor (TNF) in during threatened pregnancies in mice was shown to reduce the number of fetal losses (Arck et al., 1997; Kang et al., 2016) and also ameliorated pregnancy outcome in humans (Winger and Reed, 2008). Research endeavor using mouse models currently provide significantly insights into the impact of the endocrine-immune cross talk during pregnancy, as fetuses in pregnant mice lacking the progesterone-receptors specifically on dendritic cells show signs of intrauterine growth restriction (Thiele et al., 2019), an observation that can be expected to sparks such targeted analyses using human cells in *in vitro* approaches.

Despite these prominent examples, the use of animal models in scientific research is generally a vividly debated and emotive topic. This is not a recent development, and statements from prominent researchers date back more than a century. For example, Charles Darwin declared in a correspondence with a Swedish researcher in 1881: "On the other hand I know that physiology cannot possibly progress except by means of experiments on living animals, and I feel the deepest conviction that he who retards the progress of physiology commits a crime against mankind. Anyone who remembers, as I can, the state of this science half a century ago must admit that it has made immense progress, and it is now progressing at an ever-increasing rate." (Darwin-online, see Web reference list).

1.1. The principles of the 3Rs: replace, reduce and refine

Moreover, the use of animal models also causes concern for researchers and lay individual alike, due to the experimentally induced pain and suffering in the research animals. Hence, the so-called principles of the 3Rs have been propagated in the later Nineteen Fifties and reinforced in Europe (Directives of the European Parliament, see Web reference list), aiming to Replace, Reduce and Refine the use of animals in scientific research. A number of major funding agencies nowadays require that applicants address how the 3R principle has been implemented in planned research endeavors. Such requirements often cause weariness and concerns among researchers, fearing that the competitiveness of their experimental designs is jeopardized if the animal models that have often been tediously developed and are preferably included in projects are forced to undergo a 3R make over.

On the other hand, a forward-thinking consciousness to address the 3R principles in research projects also holds a great potential. It forces researchers to think outside the mind frame of personal preferences and consider alternative approaches that can be used to understand the mechanisms involved in e.g. successful reproduction, which may in fact even further increase the biological significance of findings and hence, the competitiveness of insights in terms of publication and recognition.

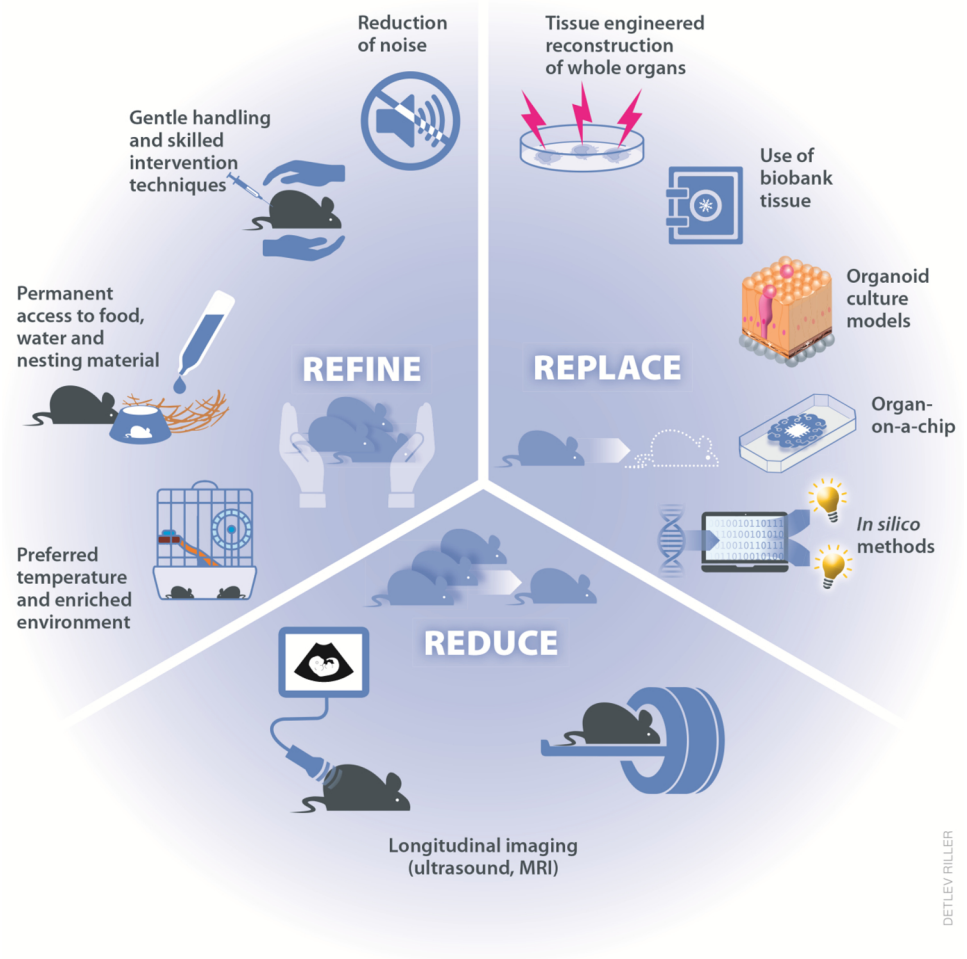

Fig. 2. Scenario depicting measures to implement the 3R principle in experimental research designs.

1.2. Methods to replace the use of research animals and their potential relevance for research in reproduction (Fig. 2)

A wealth of examples in life sciences now highlights that outstanding science as well as the incorporation of the 3R principles in the experimental design are not mutually exclusive. Robust alternative methods that completely replace the use of research animals include tissue engineering approaches in order to reconstruct whole organs. Here, culture systems have been developed that e.g. enable stem cells to grow and expand into fully functioning three-dimensional organ-specific tissue (Huch et al., 2013), for example the development of a human embryonic brain model in 3D, using human induced pluripotent stem cells (Lancaster et al., 2013). Also, *in vitro* culturing of multi-cellular spheroids has significantly advanced by combining the hanging drop method and microfluidics, hereby amending experimental options for culturing spheroids (Frey et al., 2014). Additionally, biobanks generated from biopsy tissue have become instrumental to grow organoids that mirror molecular fingerprints of diseased patients (van de Wetering et al., 2015). In fact, an organoid culture model of trophoblasts has recently been introduced. These organoids show long-term genetic stability and can differentiate into syncytiotrophoblast and human leukocyte antigen-G-positive extravillous trophoblast cells. These trophoblasts organoids show typical *in vivo* features of first trimester placentas, ranging from the secretion of placental-specific peptides and hormones as well as epigenetic fingerprints (Turco et al., 2018). Hence, the trophoblast organoid model holds the potential to improve our understanding of human placental development and the cross talk between trophoblast cells and the maternal immune response to fetal antigens.

Similarly, 'placenta-on-a-chip' microdevices have been introduced. For example, microfluidic channels separated by a thin extracellular matrix membrane can be seeded with human trophoblasts (JEG-3 cells) or human umbilical vein endothelial cells onto the opposing sides. The resulting microarchitecture of the confluent cellular monolayers on the membranes can then be considered as a micro-engineered 'placental barrier', which shows metabolic features such as glucose transport and metabolism similar to observations in vivo (Lee et al., 2016). Similar approaches use human term cytotrophoblasts for coating matrices (Huang et al., 2016). The proposed use of immortalized cell lines from placental malignancies, such as choriocarcinoma-derived JEG-3 cells, or cells isolated from healthy term placental cells may create a limitation of this model, which may be overcome by the targeted use of selected placental cells. One example might be the isolation of cells from diseased placental tissue, e.g. gestational diabetes, in order to study placental metabolism.

However, limited or missing access to high-quality biological tissue and related clinical metadata may create a major obstacle for the inclusion of the proposed approaches to replace research animals in experimental designs. In order to address this limitation, an improved biobanking infrastructure is urgently needed, aiming to benchmark tissue quality via systematic collection, transport and storage of human tissue and cells, whilst obtaining consent and developing procedures to provide clinical metadata.. In fact, a pilot project named PLACENTA (PathLink Acquired gEstatioNal Tissue bAnk) assessed the feasibility to create a biobank of fresh gestational tissue (Linder et al., 2018). Albeit the number of samples in this trial is very low, pivotal standard to

ensure maintenance of high tissue quality have been proposed to allow for molecular, cellular and proteomic analyses of the tissue. Similarly, a population–based, prospective cohort study called 'Alliance for Maternal and Newborn Health Improvement (AMANHI)' – funded by Bill & Melinda Gates Foundation through a grant to the World Health Organization – has been initiated in order to provide gestational biological tissue and clinical metadata from pregnant women and their children in sub–Saharan Africa and south Asia for the exploration of future hypotheses (Amanhi Maternal Morbidity Study Group, 2016). Also, via the Biobank Graz, a publicly-owned, non-profit central research facility of Medical University of Graz, a wealth of tissues including placental specimens can be made available for research purposes (Sargsyan et al., 2015).

In silico methods, in which biological experiments are carried out entirely in using computer models, as well as computational biomechanics have also become powerful and predictive tools to replace research animals. These approaches allow understanding cellular characteristics (Li et al., 2009) as well as the pathogenesis of diseases, such as cardiovascular ailments, diabetes or preterm birth (Britton et al., 2013; Westervelt and Myers, 2017). Although some of these efforts may fall short to fully predict cellular functions due to the inability to incorporate cellular and molecular dynamics, or simply lacking sufficient computer processing power, the great potential of *in silico* approaches may result from the increasing access to repositories of genetic sequences obtained from single cells sequencing using human samples, for example derived from placenta, decidua or peripheral blood of pregnant women. In fact, single cells analyses of first-trimester placentas and matched maternal blood and decidual cells have recently been published, which allowed for the identification of regulatory interactions that prevent harmful innate or adaptive immune responses at the feto-maternal interface (Vento-Tormo et al., 2018). Similarly, a comprehensive map of cell types in human first-trimester placenta and decidua has been charted (Suryawanshi et al., 2018). Single cells analysis of human specimen obtained from early preeclamptic placentas provided insights into cellular heterogeneity and enabled reconstruction of the trophoblast differentiation trajectory in pathological pregnancy conditions (Tsang et al., 2017). Besides these observations, the data generated within these single cell sequencing studies have been deposited and these publicly accessible repositories now allow for additional analyses using *in silico* approaches. Hence, further interpretation of findings, as well as integration of the various data sources into heterologous data sets for a differential analyses has become within reach.

1.3. Methods to reduce the use of research animals and their potential relevance for research in reproduction

Approaches to reduce the number of research animals in experimental designs are also emerging. Here, imaging techniques can now be applied longitudinally in e.g. mice, hereby avoiding that higher numbers of mice need to be euthanized on sequential gestational days in order to perform kinetic organ assessments. Imaging approaches include methods that are either non-invasive or show a low degree of invasiveness, such as ultrasound (Zhang and Croy, 2009). More recently, three-dimensional high-frequency ultrasonography has been proven to successfully detect, visualize, and characterize embryo-implantation sites during early murine pregnancy (Peavey et al., 2017). It further allows for the detection of fetal, placental or decidual changes, including small volume fluctuations, and monitoring of a potential progression of a pathology. Also, testing the efficacy of therapeutic intervention can be accommodated by ultrasonography. Compared to ultrasonography, magnetic resonance imaging allows for the longitudinal evaluation of fetal, placental or decidual adaptations at even higher resolution (Yadav et al., 2016). Here, dynamic contrast-enhanced MRI datasets have led to the development of computationally simple methods to quantify and automate differentiation in different placental compartments in mice (Remus et al., 2013; Kording et al., 2015). Clearly, the imaging approaches significantly advance experimental designs relying on the discontinuation of pregnancies by sacrificing mice for gross tissue and histopathologic characterization on distinct gestational days. Moreover, they reduce inter-animal variability which may be a confounder when analyzing tissue obtained on sequential gestational days from different animals. However, whilst the widely used description of imaging approaches as 'non-invasive' may be overtly appeasing, as sedation and immobilisation of mice using inhalation anesthetic medication is needed, which may interfere with the experimental outcome and cause distress for the mice.

Noteworthy, since detection of vaginal plugs as a confirmation of pregnancy in mice may be unreliable, a model to predict pregnancy early in mice has been proposed by using the weight gain in dams. This predictive model facilitates pregnancy detection in mice before mid-gestation, hereby reducing the number of mice that are sacrificed on distinct gestational days due to false positive pregnancy assumptions (Seaborn et al., 2011).

1.4. Methods to refine the use of research animals and their potential relevance for research in reproduction

Refinement approaches may be the easiest to adopt in experimental designs, as they may be implemented by simply measures. These include picking mice up in cupped hands or by using a home cage tunnel, instead of lifting them out of the cage by the tail. These gentler handling methods have been shown to reduce anxiety in mice (Hurst and West, 2010). Also, procedures of substance administrations that may cause pain and discomfort should be frequently supervised and re-evaluated with regard to options for refinement. A number of video tutorials and freely accessible reading material is now available to allow conscious decisions on how to improve animal handling and avoid unnecessary pain and discomfort (Online tutorials on animal refinement, see Web reference list). These include – if possible and applicable in experimental settings – the use of minimally irritant formulation of substances and vehicles for injections and other routes of application, the avoidance of injection sites that are highly painful, such as in the footpath, reduction of injection-related pain by using new, sharp needles with the narrowest possible gauge for every injection and using the lowest possible injection volume.

Also, housing conditions can significantly improve the well-being of research animals such as rodents, including provision of refuges and nesting material and – although a matter of course - free access to water and food (Gaskill et al., 2013). Group housing of female mice meets their social needs and aids thermoregulation. Also, all stressors should be reduced to a minimum level, such as noise from ventilation systems or music listened to by animal caretakers. Ambient temperature should be appropriate to rodent physiology and heating blankets or devices should be used after interventions. If such behavioral needs are not met, the animals can suffer mental and physical stress, which may jeopardize the experimental outcome. Thus, refinement strategies not only improve the well-being of research animals, but also improve quality and reproducibility of science.

1.5. Outlook

It is unlikely that research involving animals can be completely replaced in the near future. Noteworthy, whilst encouraging the implementation of the 3R principle, funding bodies and research associations even propose to reduce the regulatory burden associated with obtaining an ethical approval to conduct experiments involving research animals (Recommendations to reduce regulatory burdens, see Web reference list), as a great deal of inequality currently exists with regard to such regulations between countries. These inequalities render some scientists at disadvantage in terms of competitiveness, as they are facing lengthy and time-consuming national approval procedures.

However, regardless of these obstacles, efforts should be made to replace and reduce the number of research animals and to refine experimental and environmental conditions, even though confirmation of proof-of-concept and validity of the measures requires investments in infrastructure and training. The measures introduced in this article may spark reconsideration of experimental designs in the area of reproductive biology and immunology in order to implement the 3R principle.

Conflict of interest

The author declares that writing of this article was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Literature Research, conceptualization and writing: P.C.A..

Acknowledgments

Own work cited herein was partly supported by research grants provided by the German Research Foundation to P.C.A. (KFO296, AR232/26-2 and AR232/27-1).

References

Ai, M., Curran, M.A., 2015. Immune checkpoint combinations from mouse to man. Cancer Immunol. Immunother. 64, 885–892.
Aluvihare, V.R., Kallikourdis, M., Betz, A.G., 2004. Regulatory T cells mediate maternal tolerance to the fetus. Nat. Immunol. 5, 266–271.
Amanhi Maternal Morbidity Study Group, 2016. Burden of severe maternal morbidity and association with adverse birth outcomes in sub-Saharan Africa and south Asia: protocol for a prospective cohort study. J. Glob. Health 6, 020601.
Arck, P.C., Hecher, K., 2013. Fetomaternal immune cross talk and its consequences for maternal and offspring's health. Nat. Med. 19, 548–556.
Arck, P.C., Troutt, A.B., Clark, D.A., 1997. Soluble receptors neutralizing TNF-alpha and IL-1 block stress-triggered murine abortion. Am. J. Reprod. Immunol. 37, 262–266.
Blois, S., Tometten, M., Kandil, J., Hagen, E., Klapp, B.F., Margni, R.A., Arck, P.C., 2005. Intercellular adhesion molecule-1/LFA-1 cross talk is a proximate mediator capable of disrupting immune integration and tolerance mechanism at the feto-maternal interface in murine pregnancies. J. Immunol. 174, 1820–1829.
Blois, S.M., Ilarregui, J.M., Tometten, M., Garcia, M., Orsal, A.S., Cordo-Russo, R., Toscano, M.A., Bianco, G.A., Kobelt, P., Handjiski, B., Tirado, I., Markert, U.R., Klapp, B.F., Poirier, F., Szekeres-Bartho, J., Rabinovich, G.A., Arck, P.C., 2007. A pivotal role for galectin-1 in fetomaternal tolerance. Nat. Med. 13, 1450–1457.
Britton, O.J., Bueno-Orovio, A., Van Ammel, K., Lu, H.R., Towart, R., Gallacher, D.J., Rodriguez, B., 2013. Experimentally calibrated population of models predicts and explains intersubject variability in cardiac cellular electrophysiology. Proc. Natl. Acad. Sci. U. S. A. 110, E2098–105.
Frey, O., Misun, P.M., Fluri, D.A., Hengstler, J.G., Hierlemann, A., 2014. Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis. Nat. Commun. 5.
Gaskill, B.N., Karas, A.Z., Garner, J.P., Pritchett-Corning, K.R., 2013. Nest building as an indicator of health and welfare in laboratory mice. J. Vis. Exp. 51012.
Hsu, P., Santner-Nanan, B., Dahlstrom, J.E., Fadia, M., Chandra, A., Peek, M., Nanan, R., 2012. Altered decidual DC-SIGN(+) antigen-presenting cells and impaired regulatory T-cell induction in preeclampsia. Am. J. Pathol. 181, 2149–2160.
Huang, X., Luthi, M., Ontsouka, E.C., Kallol, S., Baumann, M.U., Surbek, D.V., Albrecht, C., 2016. Establishment of a confluent monolayer model with human primary trophoblast cells: novel insights into placental glucose transport. Mol. Hum. Reprod. 22, 442–456.
Huch, M., Dorrell, C., Boj, S.F., Van Es, J.H., Li, V.S.W., Van De Wetering, M., Sato, T., Hamer, K., Sasaki, N., Finegold, M.J., Haft, A., Vries, R.G., Grompe, M., Clevers, H., 2013. In vitro expansion of single Lgr5(+) liver stem cells induced by Wnt-driven regeneration. Nature 494, 247–250.
Hurst, J.L., West, R.S., 2010. Taming anxiety in laboratory mice. Nat. Methods 7, 825–U1516.
Kammerer, U., Eggert, A.O., Kapp, M., Mclellan, A.D., Geijtenbeek, T.B.H., Dietl, J., Van Kooyk, Y., Kampgen, E., 2003. Unique appearance of proliferating antigen-presenting cells expressing DC-SIGN (CD209) in the decidua of early human pregnancy. Am. J. Pathol. 162, 887–896.
Kang, X.M., Zhang, X.X., Zhao, A.M., 2016. Macrophage depletion and TNF-alpha inhibition prevent resorption in CBA/J x DBA/2 model of CpG-induced abortion. Biochem. Biophys. Res. Commun. 469, 704–710.
Kardos, N., Demain, A.L., 2013. Ernst Chain: a great man of science. Appl. Microbiol. Biotechnol. 97, 6613–6622.
Kording, F., Forkert, N.D., Sedlacik, J., Adam, G., Hecher, K., Arck, P., Remus, C.C., 2015. Automatic differentiation of placental perfusion compartments by time-to-peak analysis in mice. Placenta 36, 255–261.
Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P., Knoblich, J.A., 2013. Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379.
Lee, J.S., Romero, R., Han, Y.M., Kim, H.C., Kim, C.J., Hong, J.S., Huh, D., 2016. Placenta-on-a-chip: a novel platform to study the biology of the human placenta. J. Matern. Fetal Neonatal Med. 29, 1046–1054.
Li, S., Brazhnik, P., Sobral, B., Tyson, J.J., 2009. Temporal controls of the asymmetric cell division cycle in Caulobacter crescentus. PLoS Comput. Biol. 5, e1000463.
Linder, J.E., Batey, K., Johnston, R., Cohen, E.M., Wang, Y., Wang, X., Zaleski, N.M., Rogers, L.M., Mcdonald, W.H., Reyzer, M.L., Judd, A., Goldstein, J., Correa, H., Pulley, J., Aronoff, D.M., 2018. The PathLink acquired gestational tissue bank: feasibility of project PLACENTA. J Reprod Biotechnol Fertil 7, 14–27.
Nishimura, Y., Martin, M.A., 2017. Of mice, macaques, and men: broadly neutralizing antibody immunotherapy for HIV-1. Cell Host Microbe 22, 207–216.
Peavey, M.C., Reynolds, C.L., Szwarc, M.M., Gibbons, W.E., Valdes, C.T., Demayo, F.J., Lydon, J.P., 2017. A novel use of three-dimensional high-frequency ultrasonography for early pregnancy characterization in the mouse. Jove-J. Visual. Exp. 128, e56207.
Pegram, M., Ngo, D., 2006. Application and potential limitations of animal models utilized in the development of trastuzumab (Herceptin): a case study. Adv. Drug Deliv. Rev. 58, 723–734.
Ramhorst, R.E., Giribaldi, L., Fraccaroli, L., Toscano, M.A., Stupirski, J.C., Romero, M.D., Durand, E.S., Rubinstein, N., Blaschitz, A., Sedlmayr, P., Genti-Raimondi, S., Fainboim, L., Rabinovich, G.A., 2012. Galectin-1 confers immune privilege to human trophoblast: implications in recurrent fetal loss. Glycobiology 22, 1374–1386.
Remus, C.C., Sedlacik, J., Wedegaertner, U., Arck, P., Hecher, K., Adam, G., Forkert, N.D., 2013. Application of the steepest slope model reveals different perfusion territories within the mouse placenta. Placenta 34, 899–906.
Saad, E.D., Squifflet, P., Burzykowski, T., Quinaux, E., Delaloge, S., Mavroudis, D., Perez, E., Piccart-Gebhart, M., Schneider, B.P., Slamon, D., Wolmark, N., Buyse, M., 2019. Disease-free survival as a surrogate for overall survival in patients with HER2-positive, early breast cancer in trials of adjuvant trastuzumab for up to 1 year: a systematic review and meta-analysis. Lancet Oncol. (20), 361–370.
Sargsyan, K., Macheiner, T., Story, P., Strahlhofer-Augsten, M., Plattner, K., Riegler, S., Granitz, G., Bayer, M., Huppertz, B., 2015. Sustainability in biobanking: model of biobank graz. Biopreserv. Biobank. 13, 410–420.
Sasaki, Y., Sakai, M., Miyazaki, S., Higuma, S., Shiozaki, A., Saito, S., 2004. Decidual and peripheral blood CD4(+)CD25(+) regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol. Hum. Reprod. 10, 347–353.
Schmidt, A., Morales-Prieto, D.M., Pastuschek, J., Frohlich, K., Markert, U.R., 2015. Only humans have human placentas: molecular differences between mice and humans. J. Reprod. Immunol. 108, 65–71.
Seaborn, T., Moulin, J.A., Cote, M., Tremblay, Y., 2011. Promoting the "3Rs" principle in developmental biology with early and convenient diagnosis of pregnancy in mice. J. Reprod. Dev. 57, 655–659.
Suryawanshi, H., Morozov, P., Straus, A., Sahasrabudhe, N., Max, K.E.A., Garzia, A., Kustagi, M., Tuschl, T., Williams, Z., 2018. A single-cell survey of the human first-trimester placenta and decidua. Sci. Adv. 4.
Thiele, K., Hierweger, A.M., Riquelme, J.I.A., Solano, M.E., Lydon, J.P.A., Arck, P.C., 2019. Impaired progesterone-responsiveness of CD11c+ dendritic cells affects the generation of CD4+ regulatory T cells and is associated with intrauterine growth restriction in mice. in press. Front. Endocrinol. (10), 96.
Tirado-Gonzalez, I., Freitag, N., Barrientos, G., Shaikly, V., Nagaeva, O., Strand, M., Kjellberg, L., Klapp, B.F., Mincheva-Nilsson, L., Cohen, M., Blois, S.M., 2013. Galectin-1 influences trophoblast immune evasion and emerges as a predictive factor for the outcome of pregnancy. Mol. Hum. Reprod. 19, 43–53.
Tsang, J.C.H., Vong, J.S.L., Ji, L., Poon, L.C.Y., Jiang, P.Y., Lui, K.O., Ni, Y.B., To, K.F., Cheng, Y.K.Y., Chiu, R.W.K., Lo, Y.M.D., 2017. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. Proc. Natl. Acad. Sci. U. S. A. 114, E7786–E7795.
Turco, M.Y., Gardner, L., Kay, R.G., Hamilton, R.S., Prater, M., Hollinshead, M.S., Mcwhinnie, A., Esposito, L., Fernando, R., Skelton, H., Reimann, F., Gribble, F.M., Sharkey, A., Marsh, S.G.E., O'rahilly, S., Hemberger, M., Burton, G.J., Moffett, A., 2018. Trophoblast organoids as a model for maternal-fetal interactions during human placentation. Nature 564, 263–267.
Van De Wetering, M., Francies, H.E., Francis, J.M., Bounova, G., Iorio, F., Pronk, A., Van Houdt, W., Van Gorp, J., Taylor-Weiner, A., Kester, L., Mclaren-Douglas, A., Blokker, J., Jaksani, S., Bartfeld, S., Volckman, R., Van Sluis, P., Li, V.S.W., Seepo, S., Pedamallu, C.S., Cibulskis, K., Carter, S.L., Mckenna, A., Lawrence, M.S., Lichtenstein, L., Stewart, C., Koster, J., Versteeg, R., Van Oudenaarden, A., Saez-Rodriguez, J., Vries, R.G.J., Getz, G., Wessels, L., Stratton, M.R., Mcdermott, U., Meyerson, M., Garnett, M.J., Clevers, H., 2015. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161, 933–945.
Vento-Tormo, R., Efremova, M., Botting, R.A., Turco, M.Y., Vento-Tormo, M., Meyer, K.B., Park, J.E., Stephenson, E., Polanski, K., Goncalves, A., Gardner, L., Holmqvist, S., Henriksson, J., Zou, A., Sharkey, A.M., Millar, B., Innes, B., Wood, L., Wilbrey-Clark, A., Payne, R.P., Ivarsson, M.A., Lisgo, S., Filby, A., Rowitch, D.H., Bulmer, J.N., Wright, G.J., Stubbington, M.J.T., Haniffa, M., Moffett, A., Teichmann, S.A., 2018. Single-cell reconstruction of the early maternal-fetal interface in humans. Nature 563, 347–353.
Westervelt, A.R., Myers, K.M., 2017. Computer modeling tools to understand the causes of preterm birth. Semin. Perinatol. 41, 485–492.
Winger, E.E., Reed, J.L., 2008. Treatment with tumor necrosis factor inhibitors and intravenous immunoglobulin improves live birth rates in women with recurrent

spontaneous abortion. Am. J. Reprod. Immunol. 60, 8–16.
Winter, E.E., Goodstadt, L., Ponting, C.P., 2004. Elevated rates of protein secretion, evolution, and disease among tissue-specific genes. Genome Res. 14, 54–61.
Yadav, B.K., Neelavalli, J., Krishnamurthy, U., Szalai, G., Shen, Y.M., Nayak, N.R., Chaiworapongsa, T., Hernandez-Andrade, E., Than, N.G., Haacke, E.M., Romero, R., 2016. A longitudinal study of placental perfusion using dynamic contrast enhanced magnetic resonance imaging in murine pregnancy. Placenta 43, 90–97.
Zhang, J.H., Croy, B.A., 2009. Using ultrasonography to define fetal-maternal relationships: moving from humans to mice. Comp. Med. 59, 527–533.

Web references (last accessed February 15, 2019)

Darwin-online.
http://darwin-online.org.uk/content/frameset?itemID=F1353&viewtype=text&pageseq=1.
Directives of the European Parliament.
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063.
Online tutorials on animal refinement.
http://www.procedureswithcare.org.uk.
https://research.unc.edu/files/2017/03/Mouse-Handling-and-Techniques.pdf.
https://www.nc3rs.org.uk/mouse-handling-video-tutorial.
https://www.aalaslearninglibrary.org.
Recommendations to reduce regulatory burdens.
http://www.faseb.org/Portals/2/PDFs/opa/2017/FASEB-Animal-Regulatory-Report-October2017.pdf.
https://www.dfg.de/download/pdf/dfg_im_profil/reden_stellungnahmen/2018/genehmigungsverfahren_tierversuche.pdf.