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EMBRYO QUALITY AND SELECTION IN NON-HUMAN SPECIES

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ABSTRACT

Embryo evaluation, culture, and developmental biology are integral components of assisted reproductive technologies (ART) and hold profound implications for human fertility and reproductive health. This review explores the utilization of non-human primates (NHPs) as pivotal models in advancing our understanding of embryonic development and refining ART procedures. Tracing the evolutionary proximity of NHPs to humans, it underscores their significance in elucidating fundamental principles of embryo biology. The review highlights the importance of standardized embryo evaluation criteria and the integration of innovative technologies, such as time-lapse imaging, in enhancing embryo selection and improving IVF outcomes. Furthermore, it discusses the challenges and opportunities in current embryo culture methodologies, emphasizing the need for continued refinement to support long-term embryo viability. Additionally, it explores the diverse applications of NHPs in disease modelling, contraceptive development, and embryonic stem cell research, underscoring their invaluable contributions to biomedical research. By comparing key developmental milestones among humans, NHPs, and mice, this review provides insights into species-specific differences in embryonic development. In summary, this comprehensive examination elucidates the pivotal role of NHPs in advancing our understanding of embryo biology and enhancing the success of ART procedures.

INTRODUCTION

The progress from blastocyst to early organogenesis is crucial in human embryonic development, marking the differentiation of germ layers and the formation of early tissues and organs (Peng & Tam, 2022; Haniffa et al., 2021). This stage requires the coordination of morphogenesis and cellular differentiation, a key focus in developmental biology and regenerative medicine. Due to ethical constraints and the delicate nature of human embryos, much research has shifted to surrogate animal models, including rodents, sheep, rabbits, pigs, bovines, and non-human primates (NHPs), as well as human pluripotent stem-based embryo models (Lindenberg et al., 1989; Nakamura et al., 2016; Bao et al., 2022).

Recent advancements have allowed the culture of human embryos in vitro up to 14 days post-fertilization, revealing significant developmental milestones but also encountering technological and ethical limits (Shahbazi et al., 2016; Deglincerti et al., 2016; Xiang et al., 2020). Although the ISSCR has relaxed the 14-day rule, no new reports have emerged. As close relatives to humans, NHPs provide an excellent model for studying human embryo

development. Since the first successful in vitro fertilization (IVF) in non-human primates in 1984, there has been significant progress in Assisted Reproductive Technologies (ART) (Bavister et al., 1984; Clayton,1984). Initially focused on optimizing culture conditions and developing embryo transfer techniques, most studies have used rhesus macaques due to their availability and similarity to humans. However, the rise of AIDS-related research has limited their availability for reproductive studies.

NHP ARTs were initially aimed at supporting human ARTs, offering critical insights and new technologies, as direct research on humans is often impractical or unethical. Despite advancements in human ART, significant challenges remain, necessitating the use of NHPs to achieve further breakthroughs. This includes developing better culture media and methods for selecting viable embryos, as well as contributing to embryonic stem cell biology and technology (Bavister, 2004; Yao et al., 2023).

Until recently, ART research with nonhuman primates primarily aimed to support human ARTs, as direct experimentation on humans is often impractical or unethical. Despite this, nonhuman primate ARTs have lagged behind due to the earlier establishment and rapid advancement of human ARTs since the first IVF [Edwards *et al*., 1969], and the birth of Louise Brown in 1978. This head start has made nonhuman primate ARTs appear to be catching up. Moreover, nonhuman primate ART advancements heavily rely on human ART technologies, like recombinant gonadotropins, making them costly. Consequently, there has been limited technology transfer from primate research to human clinical practice. Nonetheless, certain challenges in human ART require breakthroughs that can only be achieved through nonhuman primate models.

Improvements could be made, by devising even better culture media for IVP or through objective methods for selecting the most viable embryos for transfer. Moreover, there is great potential for nonhuman primate ART to make a major contribution to embryonic stem cell biology and technology, neglecting difficult ethical and logistical problems inherent in human ES cell studies and applications [Bavister, 2004; Yao et al., 2023]. Mouse embryos are well-studied, providing insights into maternal-to-zygotic transition, EGA, and lineage segregation.

Human pre-implantation development, however, is less understood due to ethical constraints. Single-cell genomics has recently advanced our knowledge of molecular changes during human pre-implantation, such as transcriptional dynamics, DNA methylation, and X inactivation. Given the differences between human and mouse development, nonhuman primates (NHPs) may serve as better models for human pre-implantation studies.

Rodents, especially mice, are prominent in mammalian biology due to ease of breeding, short generation times, and available genome engineering technologies. However, significant differences between rodent and human biology have highlighted the need for models closer to humans. NHPs, sharing more genetic and physiological similarities with humans, are increasingly recognized as crucial for bridging basic research and clinical applications. Animal models are essential for understanding disease mechanisms and developing treatments. Traditional models include spontaneous and induced animal models, and embryo-engineered models. While rodent models are useful, they often fall short in replicating human disease processes.

NHP models, due to their genetic and physiological closeness to humans, provide more accurate representations of human diseases. Embryo-engineered NHP models are particularly valuable for studying gene functions and disease mechanisms. NHP models are pivotal in exploring complex human diseases and developing treatments. They offer significant insights into diseases like diabetes, where deriving pancreatic beta cells from monkey ES cells could advance research.

The cytogenetics of ES cells and immune rejection issues need thorough investigation in NHPs before human applications. Parthenogenetic ES cells present another research avenue, though controversial in humans, reinforcing the need for NHP models. The aim of evaluating embryo quality is to identify markers predicting pregnancy rates more accurately than current methods, improving single embryo transfer protocols. Both invasive

(PGS) and non-invasive (morphological assessment, spent culture media analysis) approaches are used. Research in NHPs can address challenges in embryo normality and immune rejection, guiding better practices in human IVF.

In brief, the construction of NHP disease models offers a valuable research platform for human diseases, particularly for those complicated diseases that cannot be successfully modelled in other animals. The use of NHP models to authenticate disease treatment strategies before the clinic can forecast treatment effects more accurately and supply a powerful reference for clinical treatment.

NHP disease models have endorsed the development of translational medicine and brought new hopes to realize the underlying disease mechanisms and explore disease treatment methods. Thus, NHPs have a requisite status in the field of life sciences. A huge amount of factors like embryo quality and selection have a say in the likelihood that a single attempt at IVF will result in live birth. It is decisive that rigorous research investigates new technologies prior to their being accepted into routine clinical practice, and that clinicians incessantly educate themselves about the progress in this swiftly sprouting field.

In summary, studies on surrogate animal embryos, human pluripotent stem-based embryo models, and in vitro culturing of human embryos have collectively enriched our knowledge of human embryonic development by providing complementary approaches to investigate the intricacies of early embryogenesis and unravel the mysteries of human life's genesis.

OBJECTIVE

1. Assess progress and limitations of ARTs in NHPs, focusing on enhancing implantation rates after embryo transfer.
2. Investigate developmental milestones in human embryonic development, emphasizing NHPs' role as surrogate models for early embryonic studies.

STATEMENT OF PROBLEM

The field of assisted reproductive technologies (ARTs) in nonhuman primates (NHPs) faces significant challenges hindering progress and application. Despite advancements, including improved success rates, achieving optimal implantation rates after embryo transfer remains elusive, akin to human ARTs. Challenges include difficulties in the successful assembly of identical twins in rhesus monkeys, limited protocols for in vitro maturation of primate oocytes, and the absence of reliable non-invasive markers for assessing oocyte and embryo quality. Furthermore, the development of chemically defined embryo culture media and the lack of genetic and physiological data on in vivo-produced embryos impede advancements in ARTs. Inadequate funding allocation for NHP ART research exacerbates these challenges, highlighting the need for increased emphasis and investment to realize the full potential of NHP models in advancing ARTs for research and clinical applications.

HYPOTHESIS

Hypothesis 1: Improved implantation techniques in NHPs will enhance ART success rates and outcomes, advancing biomedical research and addressing human health challenges.

Hypothesis 2: Protocols for in vitro maturation of primate oocytes will boost ART efficiency in NHPs, potentially improving reproductive outcomes for humans.

Hypothesis 3: Non-invasive markers for assessing oocyte and embryo quality in NHPs will refine embryo selection, enhancing ART success rates and reducing risks.

RESEARCH METHODOLOGY

1. Literature Search Strategy:

Conducted systematic searches using academic databases such as PubMed, Scopus, Science Direct and Web of Science. Utilized relevant keywords and search terms related to assisted reproductive technologies (ARTs), nonhuman primates (NHPs), and specific challenges and advancements in the field.

2. Inclusion and Exclusion Criteria:

Established clear criteria for selecting literature based on relevance to the topic, publication date, and source credibility. Excluded studies that did not focus on ARTs in NHPs or were not published in peer-reviewed journals.

3. Literature Selection Process:

Screened titles and abstracts to identify potentially relevant studies and reviewed full texts of selected articles to determine their suitability for inclusion in the literature review. Ensured consistency and transparency in the selection process to minimize bias.

4. Data Extraction and Synthesis:

Extracted key information from selected studies, including study objectives, methodologies, findings, and conclusions. Organized extracted data into thematic categories to facilitate analysis and synthesis. Identified common themes, trends, and gaps in the literature related to NHP ARTs.

5. Critical Appraisal:

Evaluated the quality and rigour of included studies, considering factors such as study design, sample size, and potential biases. Acknowledged limitations and strengths of individual studies to provide a balanced interpretation of the literature.

6. Data Analysis and Interpretation:

Analysed synthesized data to identify overarching patterns, discrepancies, and areas of consensus within the literature. Interpreted findings in light of research objectives and hypotheses, drawing connections between different studies and themes. Generated insights and conclusions based on the collective evidence presented in the literature.

ANALYSIS

Several key findings and implications in the field of early embryonic development and the use of non-human primate (NHP) models for research purposes:

Advancements in Developmental Biology: The review highlights the progress made in understanding the morphological and molecular features of early human embryonic development, particularly focusing on the period from blastocyst formation to early organogenesis. The use of NHP embryos as surrogate models for studying human embryonic development shows promise in providing valuable insights into embryogenesis.

Technological Innovations: The establishment of 3D culture systems for studying cynomolgus monkey embryos up to 25 days post-fertilization demonstrates the potential for mimicking the uterine microenvironment and enhancing embryonic development in vitro. These advancements offer new opportunities for studying cellular compositions, developmental trajectories, and epigenetic features during early organogenesis.

Challenges and Ethical Considerations: The review addresses the limitations and ethical considerations surrounding in vitro culture of human embryos beyond 14 days post fertilization, emphasizing the need for careful oversight and adherence to regulations. The discussion on human-monkey chimeric studies raises important ethical concerns regarding neural and reproductive system chimerism, highlighting the necessity for cautious and step-by-step advancements in research.

Implications for Disease Research: The potential for creating identical macaque monkeys for disease research and vaccine production could significantly impact comparative studies and reduce the number of animals required for research purposes. This approach holds promise for advancing disease research and improving treatment outcomes.

Future Directions: The literature review underscores the need for further optimization of culture systems to enhance embryo development, as well as the importance of community involvement and ethical oversight in human-non-human primate interspecific chimaeras research. The review sets the stage for future studies focusing on refining culture techniques, addressing ethical concerns, and advancing knowledge in developmental biology and regenerative medicine.

CONCLUSION

Despite substantial improvements in assisted reproductive technology (ART) for non-human primates (NHPs), implantation success rates remain around 20%, paralleling human ART success rates (Wright et al., 2005). This highlights the potential for significant improvements in primate ART efficiency. Achieving the successful creation of identical twins in rhesus monkeys remains a challenge, yet it holds promise for advancing biomedical research. addressing the critical research needs outlined, such as advancing protocols for in vitro maturation of primate oocytes, identifying non-invasive markers for assessing oocyte and embryo quality, and developing chemically defined embryo culture media, is essential for the advancement of assisted reproductive technologies (ARTs) in both monkeys and humans.

Furthermore, obtaining genetic and physiological data on in vivo-produced embryos and establishing additional non-human primate (NHP) models for ARTs are urgent priorities. Educating the public and government about the invaluable role of NHPs in disease modeling is crucial to garnering increased support for this vital research area [Bavister, 2004]. By addressing these research needs and fostering greater awareness, we can pave the way for significant advancements in ARTs and biomedical research.

While fundamental developmental processes are conserved between rodents and primates, there are significant species differences among primates themselves, such as in implantation patterns. For instance, human and chimpanzee embryos fully implant into the endometrium, whereas cynomolgus and rhesus monkey embryos only

partially do so [Nakamura et al., 2016; O'rahilly & Müller, 2010]. Therefore, careful consideration of these species differences is crucial in primate research aimed at deducing aspects of human biology.

In light of advancements in human ESCs/iPSCs, various induction methods for differentiation, including organoid formation, have been developed. These methods have successfully replicated gene expression oscillations during mitogenesis. With their versatility and ease of application, in vitro human pluripotent stem cell (PSC) differentiation models are considered alternatives to post-implantation human tissues. Additionally, extended culture systems for preimplantation human embryos, known as ex vivo culture models, have emerged. Although ex vivo culture requires human embryos, it offers a model closer to in vivo systems than in vitro models, suggesting that both in vitro and ex vivo systems are valuable for studying human post-implantation development [Deglincerti et al., 2016; Lv & Zhang, 2019].

Moreover, in vitro and ex vivo models offer valuable insights into human development, they may introduce experimental artifacts, necessitating validation against in vivo systems. Ethical concerns surrounding human embryo destruction, culture duration limitations, and gene-editing experiments persist. Conversely, in vivo experiments with NHPs, including ex vivo culture of cynomolgus monkeys, offer opportunities to address species differences and ethical challenges. Additionally, the availability of iPSCs from Great apes presents a promising avenue for bridging species gaps. Utilizing cross-platform and cross-species analyses will be crucial for overcoming these challenges and advancing our understanding of human development. Ultimately, NHPs will continue to play pivotal roles in human embryology, remaining indispensable for future research endeavours [Warnock, 1985; Niu et al., 2019].

RECOMMENDATION

Based on your findings, recommend future research directions or actions.

For instance:

1. Invest in developing improved ART protocols and non-invasive diagnostic tools.
2. Foster collaborative research efforts and resource sharing to enhance cost-effectiveness and research output.
3. Increase public awareness and governmental support for NHP research to address human health challenges.

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