

# “Extrauterine Fetal Growth- Current Achievements and Future Endeavors”

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## ABSTRACT

**Introduction:** Global population trends show varying dynamics across continents due to economic, social, and demographic factors. Africa faces rapid growth due to high fertility, while Europe struggles with declining populations and aging issues. By 2050, the world population is projected to reach 9.7 billion, with significant growth in Africa and Asia. Declining fertility rates are raising concerns about infertility and its social impact.

**Reproductive Advances:** Over the last six decades, advancements in Medically Assisted Reproduction (MAR) have revolutionized human reproduction. Techniques such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and embryo implantation have enabled conception, even in severe male infertility cases. More recent developments like uterine transplantation and *in vitro* gametogenesis (IVG) offer new reproductive possibilities, but unresolved fertility issues persist for some women, requiring alternatives like gestational carriers or artificial wombs.

**Methods:** A comprehensive literature review was conducted using PubMed and Google Scholar, focusing on studies related to ectogenesis, *in vitro* embryo culture, and artificial uterus design. Non-English studies were excluded. The review also explored current alternatives like uterine transplantation and gestational carrier programs.

**Ectogenesis and Ethical Considerations:** Ectogenesis, or gestation outside the human body, is emerging as a reproductive option for women with uterine dysfunction. Advances in artificial uterus technology may eventually support full-term pregnancies. However, the potential commodification of reproduction raises significant ethical concerns.

**Conclusions:** While reproductive technologies offer solutions for infertility, managing ethical considerations remains essential. Ongoing research in artificial womb technology, uterine transplantation, and reproductive innovations is critical for addressing complex fertility challenges.

**Keywords:** Gametogenesis; *In Vitro* Embryo Culture Differentiation; Artificial Uterus Design; *Ex Vivo* Implantation; Ectogenesis

**Abbreviations:** MAR: Medically Assisted Reproduction; IVF: In Vitro Fertilization; ICSI: Intracytoplasmic Sperm Injection; IVG: *In Vitro* Gametogenesis; HFEA: Human Fertilisation & Embryology Authority; AUI: Absolute Uterine Factor Infertility; MRKH: Mayer-Rokitansky-Küster-Hauser Syndrome; WHO: World Health Organization

Introduction

The global population trends are influenced by economic, social, and demographic factors. Africa is experiencing rapid growth due to high fertility, while Europe faces declining populations and aging challenges. Asia’s growth is slowing, though India is expected to surpass China’s population by 2024. Moderate growth is observed in North America, while Latin America and Oceania grow steadily. By 2050, the global population will reach 9.7 billion, with most growth in Africa and Asia. Issues like aging populations, fertility rates, and migration pose significant challenges [1-3]. The low fertility rate observed in some countries poses the problem of infertility as a further social problem in reverting the low fertility rate of the countries. The world population has been growing since 1960, with perspective growth World population trend From Statista, 2024 The regional demographic changes occurring worldwide are influenced by various factors, including economic conditions, cultural norms, quality of life, healthcare, and the level of individual freedom, whether under democratic or authoritarian regimes of secular or religious origin.

Reproductive Advances: Medically Assisted Reproduction (MAR)

The advancements in reproductive technologies over the past six decades have revolutionized human reproduction (Figure 1) [4-23], starting with contraception and progressing through *in vitro* fertilization (IVF) [5], intracytoplasmic sperm injection (ICSI) [6] and embryos implantation in the extracorporeally perfused human uterus [7-10]. These milestones enabled conception even in severe male infertility cases. They paved the way for uterus transplantation<sup>11</sup> and breakthroughs like *in vitro* gametogenesis (IVG) [12,13] as well as babies born with three genetic patrimonies<sup>14</sup> and the first attempt of partial ectogenesis<sup>10</sup>. MAR programs<sup>17</sup> have led to over 9 million births [20-27], but some women still face unsolvable fertility issues, requiring solutions like gestational carriers [18-20] or future artificial womb technologies <sup>10</sup>, [28-43]. This review addresses these developments, focusing on ectogenesis, MAR efficiency, and ethical challenges. Uterine transplantation and gestational carrier programs were also considered temporary solutions.

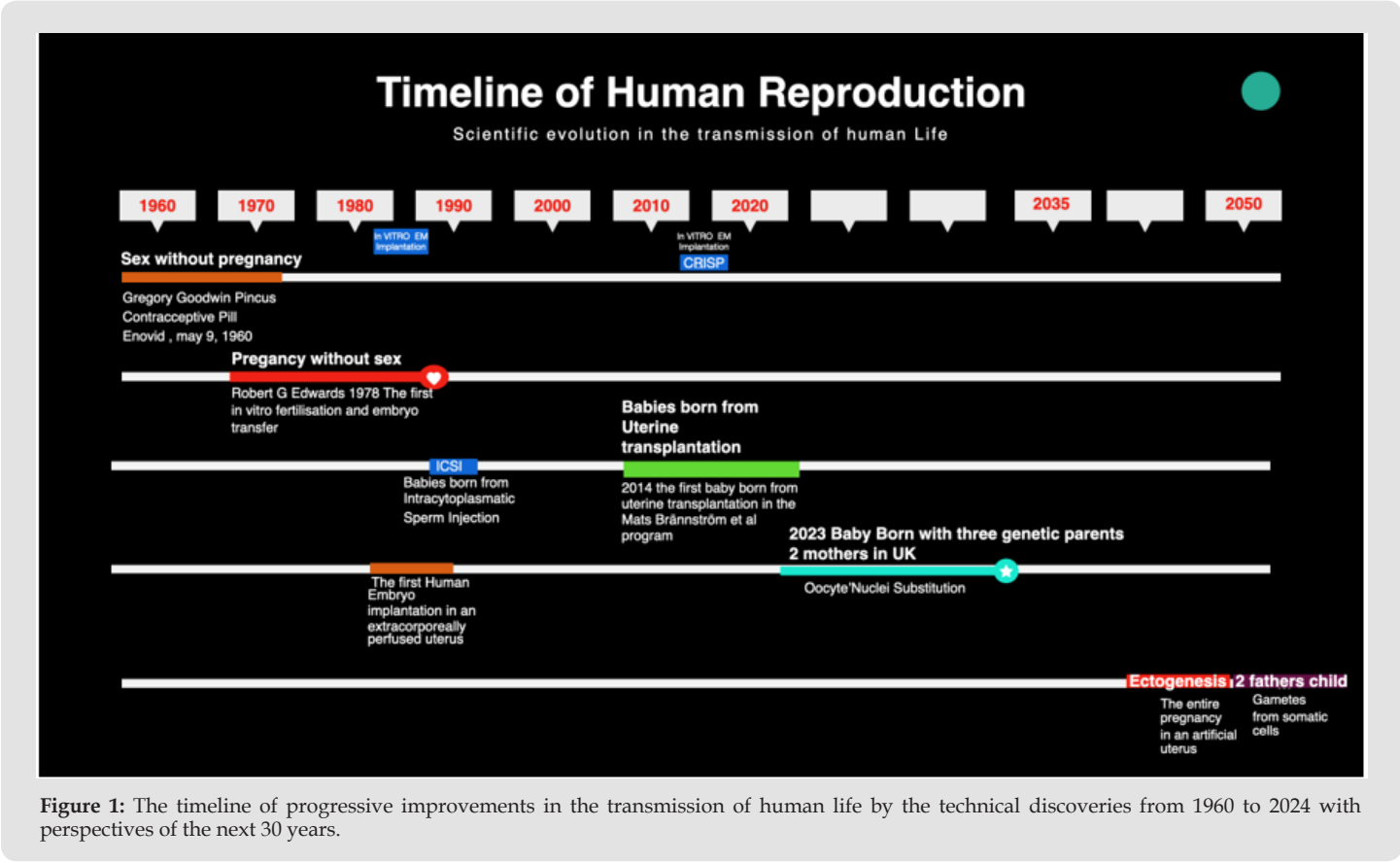


Figure 1: The timeline of progressive improvements in the transmission of human life by the technical discoveries from 1960 to 2024 with perspectives of the next 30 years.

The efficiency of medical treatment, mainly due to Robert Edwards and Patrick Steptoe's discovery, is constantly increasing, as here reported by HFEA. The graph describes the improvement of results obtained by MAR during the last ten years. From HFEA. It shows IVF pregnancy rates from 2012-2022, which comes from a report by the Human Fertilisation & Embryology Authority (HFEA) titled "Fertility Trends: Preliminary Trends in Fertility Treatment 2022." This data tracks the average success rates of IVF treatments across various age groups, noting significant improvements in pregnancy rates using fresh embryo transfers over the decade. This data is published in the HFEA's official reports, particularly the "Fertility Treatment 2021: Preliminary Trends and Figures" and subsequent updates for 2022 [21].

## Material and Methods

A comprehensive literature search was conducted using PubMed and Google Scholar to identify relevant studies on ectogenesis. The search focused on experimental studies in English related to gametogenesis, *in vitro* embryo culture differentiation, artificial uterus design, *ex vivo* implantation, ectogenesis, and related topics. Non-English studies or those lacking clear methods were excluded. The selected studies were critically analyzed to synthesize strategies for advancing ectogenesis technology. Additionally, current alternatives like uterine transplantation and gestational carrier programs were reviewed. The evaluation also considered reports on extracorporeal embryo or fetal development, incorporating both human and animal models. This review aimed to outline the design of artificial uteruses for full ectogenesis and explore potential future applications, requiring no specific statistical analysis for this descriptive synthesis.

## Ectogenesis and Ethical Considerations

Ectogenesis, or gestation outside the human body, is emerging as a solution for women unable to carry pregnancies due to uterine dysfunction [41-87]. Advances in artificial uterus technology could eventually support pregnancy from conception to birth. However, ectogenesis raises ethical issues concerning the potential commodification of reproduction, parenthood and the impact on societal and legal structures. Researchers must balance innovation with ethical scrutiny.

### Uterine Transplantation (UTx)

Uterine transplantation (UTx) [11] is a pioneering option for women with absolute uterine factor infertility (AUI), a condition affecting approximately 1 in 500 women of childbearing age, impacting around 200,000 women in Europe, 85,000 in the USA and up to 1.5 million globally, offering the chance to experience pregnancy through assisted reproductive technologies. Over 70 UTx procedures have been performed globally, leading to 23 live births as of 2021 [44]. UTx involves significant risks, including major surgeries and long-term immunosuppression, but offers a unique solution where surrogacy or adoption is not desired. Ongoing research aims to make UTx more acces-

sible, with advancements in surgical techniques and robot-assisted surgery [45,46]. Women with AUI have traditionally remained childless or pursued adoption or surrogacy. Women considering these alternatives can access information from various sources, such as government websites for adoption or organizations like the HFEA and fertility counseling services for surrogacy in other countries (ASRM Recommendations 2022) [44-48]. Despite the physical risks associated with UTx, including major surgeries and the need for immunosuppression, while there are no direct alternatives to UTx, adoption, and surrogacy should be thoroughly discussed during counseling to ensure informed consent, weighing the risks of UTx against the potential benefits [56-59]. Indications for uterine transplantation primarily focus on cases of absolute uterine factor infertility (AUI), which means a woman is unable to carry a pregnancy due to the absence or non-functioning of the uterus [11,44-50,59]. Common indications include

- **Congenital absence of the uterus (Mayer-Rokitansky-Küster-Hauser Syndrome - MRKH):** This is a condition where women are born without a uterus.
- **Uterine damage or loss due to surgery or medical conditions:** This may include hysterectomies due to severe hemorrhage, fibroids, or gynecological cancers.
- **Severe Uterine Malformations:** Conditions where the uterus is present but structurally incapable of supporting a pregnancy.
- **End-stage uterine disease:** Cases where severe endometriosis or fibroids have rendered the uterus non-functional for reproduction [11,44-50,59].

As of 2024, around 120 uterine transplants have been performed worldwide, with more than 60 live births resulting from these transplants. Globally, uterine transplantation is still considered a developing field, and most transplants have been performed using living donors, although some have also been from deceased donors [11,44-50]. Limitations currently include donor availability, recipient suitability, surgical challenges regarding success and complications, and recipient management post-UTx and during pregnancy [11,44-50,59].

### Use of Gestational Carriers

Gestational carriers offer an alternative to uterine transplantation [59] for women who cannot carry pregnancies due to uterine absence or malformations [50-54,75]. Conditions like Mayer-Rokitansky-Küster-Hauser Syndrome [50-54], uterine damage [57-68], Asherman's Syndrome [4,], and severe uterine abnormalities (e.g., Unicornuate [51], didelphys [75], bicornuate [52], septate, or T-shaped uteri) [60-63,72] often prevent pregnancy. Cervical incompetence, repeated IVF failures, and unsafe medical conditions like heart disease may also lead women to surrogacy. Around 22% of cases involve congenital absence of the uterus, while others stem from surgery, recurrent miscarriage, or repeated IVF failure. Surgical correction can sometimes

address these challenges, but alternatives like surrogacy or adoption are necessary in more severe cases [56].

- Surrogacy, both charitable and commercial, provides an option for biological parenthood but varies widely in legal and ethical standing across the globe [57,69,73-75]. Countries like the U.S. and Ukraine allow commercial surrogacy, whereas others, such as Canada and the UK, only permit altruistic surrogacy. Some regions, including France and Germany, ban all forms of surrogacy. Ethical debates focus on the surrogate's rights, financial compensation, and risks of exploitation, especially in cross-border cases<sup>56</sup>. Gestational surrogacy, where the surrogate has no genetic connection to the baby, is generally preferred due to its lower complexity and legal risks. The embryo is created using the intended mother's egg (or a donor egg) and the intended father's sperm (or donor sperm) through IVF. Traditional Surrogacy: Less common today, where the surrogate provides her egg and is inseminated with the sperm of the intended father or a donor. In this case, the surrogate is genetically related to the baby.

- Gestational surrogacy offers a valuable option for individuals or couples who cannot carry a pregnancy themselves, and it is often favored over uterine transplantation due to the complexity and risks involved with the latter [55-77].

### Artificial Womb Technology

The study of artificial womb technology has progressed steadily since the 1950s, with milestones including artificial placental systems for fetal sheep, IVF breakthroughs, and advancements in placental perfusion. In 2017, biobag systems allowed lamb fetuses to develop outside the womb for four weeks, mimicking natural conditions [85]. Researchers such as Bulletti [7-10,41-43], Watanabe T et al. [81], Partridge EA et al. [88], Zernicka-Goetz, M et al. [89], and Aguilera-Castrejon, A. et al. [90] with embryo implantation and embryo-fetal development as well as Sasaki H et al. [91], Takebe T et al. [92] and Rivron NC et al. [93] have furthered this field, focusing on stem-cell-based embryo development and placental exchange. These advancements aim to improve neonatal care and fertility treatments, laying the groundwork for supporting preterm human infants and addressing infertility issues.

The development of artificial womb technology has advanced through several critical milestones over the past decades:

- **1954 - Artificial Incubation of Chick Embryos:** Willier and colleagues demonstrated that chick embryos could be grown outside the womb, laying the groundwork for future artificial incubation across species [78].
- **1965 - Artificial Placenta for Sheep Fetuses:** Liley's experiments with lambs explored artificial placental systems in nutrient-rich fluid, attempting to mimic the placenta's function to support fetal development, though long-term viability remained a challenge [79].

- **1978 - Birth of Louise Brown (First IVF Baby):** Louise Brown's birth via *in vitro* fertilization (IVF) marked a turning point in reproductive technology, allowing human embryos to develop outside the body before implantation. This was a significant breakthrough for embryological research [5].
- **1984 - Artificial Placental Support for Fetal Sheep:** Bartlett and Callaghan advanced neonatal care by developing an artificial placenta that used ECMO (extracorporeal membrane oxygenation) to sustain fetal sheep, improving techniques for premature infant support [80].
- **1986 - Extracorporeal Perfusion of Human Uteri:** Human uteri from hysterectomies were kept in good condition through extracorporeal perfusion with warmed, oxygenated buffer solutions, advancing knowledge in uterine preservation [7].
- **1988 - Human Uterus Perfusion:** Bulletti and colleagues maintained hysterectomized uteri in good biological condition using prolonged extracorporeal perfusion, showing that embryo development might be sustained in an artificial environment [10].
- **1992 - Artificial Womb-Like Systems for Fetal Sheep:** Watanabe and his team at Tokyo Women's Medical University used ECMO and artificial amniotic fluid to sustain fetal sheep in a controlled, womb-like environment for about three weeks. This was a significant step forward in artificial womb technology [81].
- **1997 - Cloning of Dolly the Sheep:** Dolly's birth through somatic cell nuclear transfer was a major milestone in reproductive technology, showing that organisms could develop entirely outside their natural parent's body [82].
- **2016 - Mouse Embryo Development in Artificial Wombs:** In Japan, researchers successfully grew mouse embryos in a simulated uterine environment for several days, advancing extracorporeal embryo development [83].
- **2017 - Biobag for Preterm Lamb Fetuses:** Researchers at the Children's Hospital of Philadelphia developed a "biobag" that sustained preterm lambs for up to four weeks, marking a major leap in artificial womb technology and neonatal care [85].
- **2021 - Human-Mouse Hybrid Embryos:** Scientists created hybrid embryos with human and mouse cells to study early developmental processes outside the womb, shedding light on interspecies cellular interactions [86,87].
- **2022 - Mouse Embryos Created from Stem Cells:** Japanese researchers developed mouse embryos entirely from stem cells *in vitro*, demonstrating the potential for synthetic embryology in fertility treatments and regenerative medicine [88].

These developments have progressively built toward understanding and creating functional artificial wombs, with substantial implica-



tions for neonatal care and reproductive technologies. While the application of this technology to human gestation is still distant, these advancements could revolutionize the treatment of premature births.

### Artificial Placenta Development

Alongside artificial womb research, the development of artificial placentas began in the 1960s, aiming to replicate the natural placenta's function. Notable advances include:

- **1967 - Panigel's Ex Vivo Placental Perfusion System:** Enabled studying nutrient exchange between mother and fetus [89].
- **1980s - Advanced Placental Perfusion Models:** Improved understanding of drug transfer across the placenta [89,94].
- **1991 - Mellor's Sheep Placental Perfusion:** Contributed insights into fetal growth and nutrient transfer [92].
- **2006 - Kingdom's Placental Pathology Studies:** Investigated placental dysfunctions like pre-eclampsia using perfusion models [93].

In 2017, the biobag developed for preterm lambs simulated placental functions through oxygenation and circulation, representing an integration of placental perfusion and artificial womb systems [94].

### In Vitro Gametogenesis (IVG)

IVG research explores creating gametes from stem cells, providing fertility options for infertile individuals or same-sex couples. While significant progress has been made in animal models, human applications remain limited due to biological and ethical challenges. IVG could potentially revolutionize reproductive medicine by offering solutions to premature ovarian failure and reducing dependence on gamete donation [95-99].

### Embryo Development and Differentiation In Vitro

*In vitro*, the differentiation of embryos varies by species. For example, human embryos can be cultured for up to 14 days per the "14-day rule," while mouse embryos can survive up to 12 days, covering early organogenesis. Other species, like non-human primates, zebrafish, and pigs, show different survival percentages relative to their total gestation periods [100-105].

### Artificial Endometrium and Ectogenesis

Researchers have made progress in creating an artificial endometrium by co-culturing epithelial and stromal cells, which supports embryo implantation. Full ectogenesis, sustaining pregnancy entirely outside the body, remains a long-term goal. Research in the 1980s and 1990s by groups in Bologna and Mount Sinai demonstrated prolonged survival of uteri in extracorporeal oxygenated perfusion using hybrid methods [7-10].

### Artificial Womb Technology and Future Prospects

A significant breakthrough occurred in 2017 when scientists at the Children's Hospital of Philadelphia successfully sustained lamb fetuses in an artificial womb for up to 28 days. The bio-bag system replicated the womb environment, supplying essential nutrients and oxygen while the fetuses floated in a fluid-filled chamber (Supplementary Movies 1-5) [85]. The lambs showed normal development, suggesting future applications for human preterm infants, though human use is still distant [85]. In 2021, Israeli researchers grew mouse embryos in an artificial womb-like system for 11-12 days, around halfway through the typical mouse gestation period. While this experiment achieved a significant milestone in mammalian development outside the body, full-term development in an artificial environment remains a challenge [106].

### Ethical Considerations and Challenges

Current research shows promise for extending gestation *in vitro*, particularly in mice, but applying this to humans presents ethical and biological hurdles. The 14-day rule limits human embryo research, though advances in animal models pave the way for future breakthroughs [107,108]. In conclusion, while artificial womb technology and related developments in embryo and placental research are making rapid progress, significant challenges remain before they can be applied to humans. These technologies have the potential to transform neonatal care, reproductive medicine, and the treatment of premature births.

### The Drama of Premature Babies

**Key Global Statistics:** The World Health Organization (WHO) estimates that approximately 15 million babies are born prematurely each year, accounting for around 10% of all global births [109]. In the United States, the Centers for Disease Control and Prevention (CDC) reported a preterm birth rate of about 10.5% in 2021, a figure that has remained relatively stable in recent years [110]. In high-income countries such as the U.S. and parts of Europe, preterm birth rates typically range from 8-12%. In contrast, rates in low- and middle-income countries are often higher, partly due to limited access to prenatal and healthcare resources.

**Regional Breakdown:** Africa and South Asia have the highest rates of preterm births, with about 60% of global preterm births occurring in these regions. In countries like Malawi, the preterm birth rate can reach as high as 18%. Several factors can increase the likelihood of preterm births, including:

- Maternal age (both very young and older women are at higher risk)
- Multiple pregnancies (e.g., twins, triplets)
- Pre-existing medical conditions (e.g., diabetes, hypertension)
- Lifestyle factors (e.g., smoking, poor nutrition)
- Lack of access to prenatal care

These statistics highlight the global challenge posed by preterm births and emphasize the need for improved maternal healthcare to reduce the incidence of premature deliveries. The survival rate of preterm babies largely depends on the gestational age at birth and the quality of neonatal care. Advances in medical technology and neonatal care have significantly improved survival rates in recent decades.

### Mortality Rates by Gestational Age

**Extremely Preterm (Born before 28 Weeks):** These infants are at the highest risk of complications and mortality. In high-income countries, around 80-90% of babies born at 28 weeks survive. However, for those born at 24 weeks, survival rates drop to 40-60%, and for those born at 22-23 weeks, survival falls significantly to just 10-30%, depending on medical resources. Mortality rates are highest for babies born at 22-23 weeks, with 70-90% not surviving despite advanced care.

**Very Preterm (Born between 28-32 Weeks):** Survival rates improve dramatically for this group, with more than 95% surviving in high-income countries. The mortality rate for very preterm babies is about 5-10%, largely due to complications such as infections and respiratory issues, though these infants often require specialized care.

**Moderate to Late Preterm (Born between 32-37 Weeks):** Babies born after 32 weeks have excellent survival rates, often exceeding 98%, with most experiencing few long-term complications. Late preterm infants (34-36 weeks) have survival rates similar to full-term babies, with mortality rates under 1%.

**Global Differences in Survival Rates:** In high-income countries with advanced neonatal care, the survival rate for babies born at 28 weeks or later is over 90%. However, in low- and middle-income countries, survival rates for babies born before 28 weeks are much lower, often below 10-20%, due to limited access to specialized care. According to the World Health Organization (WHO), around 1 million preterm babies die each year due to complications related to prematurity, representing about 6-7% of the 15 million premature births annually [109]. Most of these deaths occur in low-resource settings where neonatal care is limited.

#### Key Factors Affecting Survival:

- **Gestational Age:** Earlier births carry lower survival rates.
- **Access to Neonatal Care:** Advanced care, such as ventilators and medications, dramatically improves outcomes.
- **Birth Weight:** Heavier premature infants tend to have better survival chances.
- **Infections and Complications:** Premature babies are vulnerable to life-threatening conditions like respiratory distress and infections [110].

Embryonic diapause is a reproductive strategy found in several mammals, including rodents, carnivores, marsupials, and bats, where

the development of the embryo is paused until conditions are more favorable for pregnancy. In rodents like mice and rats, environmental or physiological factors, such as lactation, trigger diapause by delaying the implantation of the blastocyst. Lactation-related hormonal changes prevent implantation until nursing ends, at which point implantation resumes, allowing the embryo's development to continue under optimal conditions [111]. Carnivores, such as bears, seals, and mink, experience diapause primarily to align birth with periods of resource abundance. For example, mink can undergo diapause for months, ensuring offspring are born in spring when food is plentiful, promoting higher survival rates [112]. In marsupials like kangaroos and wallabies, diapause provides a reproductive advantage by coordinating reproduction with environmental factors. Kangaroos can simultaneously manage an embryo in diapause, a young joey in the pouch, and an older joey feeding outside. The embryo's reactivation is controlled by cues such as food availability and optimizing reproductive success [113]. Bats also use diapause to synchronize birth with periods of abundant food, ensuring the young are born when conditions are most favorable for survival [114]. In humans, natural diapause remains speculative. Some suggest that variability in implantation timing (6-12 days after fertilization) could indicate a form of diapause, but this is generally seen as part of normal human reproduction, not evidence of a dormant state.

No direct evidence supports diapause in humans, as seen in animals like mice or kangaroos. However, assisted reproductive technologies (ART), such as *in vitro* fertilization (IVF), involve an artificial pause in embryonic development. During IVF, embryos can be cryopreserved at the blastocyst stage and later thawed for implantation. While this resembles diapause, it is a technology-mediated pause rather than a natural biological process. The ability to freeze and thaw embryos is a key advancement in reproductive medicine, but it differs fundamentally from natural diapause mechanisms. In conclusion, while embryonic diapause is well-documented in various animals, there is no definitive evidence of its natural occurrence in humans. Variation in implantation timing in humans likely represents developmental variability, and embryo freezing in ART, while a technological parallel, is not the same as natural diapause. As hypothesized, a cyto-static factor might promote diapause, opening possibilities for future innovations, but imagination precedes realization: "What does not yet exist, you must imagine before having it." [115]. Artificial Endometrium Since the birth of the first "test-tube" baby in 1978 (Steptoe and Edwards, 1978), the use of human embryos in research has sparked ethical controversy. Central to the debate is the "14-day rule," which prohibits culturing embryos beyond 14 days or the onset of the primitive streak. This rule, proposed over 40 years ago, is a widely accepted bioethical guideline [116].

However, recent scientific advances have challenged this rule, suggesting it may be possible to extend the culture period beyond 14 days [117], although specific ethical approval would be required [116]. The 14-day limit is based on our understanding of embryo development

during the post-implantation period, marking the end of full developmental potential and the onset of gastrulation, where embryonic cells differentiate and begin to form pre-neural cells. This stage is considered critical, as the embryo may start to perceive stimuli, transitioning into an early stage of human development [118,119]. Research by Morris and, more recently, by Hyun and colleagues has successfully cultured embryos close to the 14-day limit, suggesting this period could potentially be extended to 20 or 28 days, which would enable deeper studies of early fetal development [116]. An artificial endometrium, consisting of epithelial endometrial cells cultured on a 3D support matrix (e.g., Matrigel®), allows essential biological processes to occur in a structure that mimics the *in vivo* environment [120,121]. Similar cultures and their hormonal responses have been explored in prior studies [122-124]. Ectopic pregnancies, as seen in the Supplementary Artificial Uterus Design Proposal, demonstrate that embryos can implant and develop in non-standard locations, providing further insights for research. However, while pre-implantation embryos can be cultured *in vitro* [83], validated methods for post-implantation embryo culture still lack [125]. A recent study by Aguilera-Castrejon et al. [90] [87] provided the first successful illustration of post-implantation mouse embryo development outside the uterus, supporting growth until day 11 (Supplementary Video1-8) [87].

The system carefully controlled CO<sub>2</sub>, O<sub>2</sub>, and atmospheric pressure—key factors for effective oxygen delivery and cell growth [126,127]. Using this advanced culture system, the researchers tracked the stages of embryo development from early gastrulation (day 5.5) to hindlimb formation (day 11). To build an artificial endometrium, researchers used a co-culture of epithelial and stromal cells in a synthetic environment that mimics the natural uterine conditions. This process involves creating a three-dimensional structure using a scaffold or matrix, which supports the growth and interaction of these two types of cells, enabling them to mimic the natural endometrium (Supplementary: Artificial Uterus Design Proposal).

### Step-by-Step Process

**Matrix or Scaffold Selection:** A suitable matrix is selected to serve as the foundation for the artificial endometrium. In many studies, Matrigel, a biologically derived extracellular matrix, is used due to its ability to support the growth of epithelial and stromal cells. The matrix provides the necessary structural support for cellular attachment, growth, and differentiation [128].

**Co-Culture of Epithelial and Stromal Cells:** The epithelial cells, which line the uterine cavity, and stromal cells, which are found in the connective tissue of the uterus, are co-cultured on the matrix. This co-culture setup is crucial for mimicking the natural endometrial environment. Epithelial cells are responsible for providing a barrier and secretory function, while stromal cells offer structural support and help in the decidualization process, which is necessary for embryo implantation [120-122].

**Cell-Cell Interaction:** The interaction between the epithelial and stromal cells is key to replicating the natural endometrial environment. These cells communicate through signaling pathways, which regulate processes such as cell differentiation, proliferation, and hormonal response. In a well-developed co-culture system, these interactions mirror those found in the natural endometrium, allowing the artificial system to support embryo implantation and early pregnancy development [120-122].

**Hormonal Stimulation:** To replicate the natural menstrual cycle and pregnancy conditions, the co-culture system is treated with hormones such as 17 $\beta$ -estradiol and progesterone, which are crucial for endometrial preparation and embryo support. These hormones induce decidualization, a process by which the stromal cells change to become supportive of embryo implantation [129].

**Decidualization and Support of Embryo Implantation:** *In vitro*, decidualization is achieved by subjecting stromal cells to hormonal treatment replicating the conditions necessary for successful embryo implantation. This process has been extensively studied and well-documented in artificial systems, with research demonstrating that the co-culture of these cells can support embryo attachment and growth [130]. By combining epithelial and stromal cells in this controlled environment, it becomes possible to replicate the natural endometrium's functions [131-133]. This artificial endometrium can be used for various research purposes, including studying implantation, embryo development, and uterine disorders.

### Our Project to Build Up an Artificial Uterus Useful for Complete Ectogenesis

There are two approaches to creating artificial uteruses for ectogenesis: one focuses on the fetal maturation of immature fetuses (partial ectogenesis) (Supplementary Movies 1-5) [85] and another aimed at supporting life from conception to birth (total ectogenesis) for women unable to carry a pregnancy [134]. One project for complete ectogenesis (Supplementary Artificial Uterus Design Proposal) involves a titanium-based chassis with semi-permeable tubes (0.5-2 mm in diameter) that allow the exchange of gases and molecules (up to 70,000 Å), including dissolved oxygen, arranged in a one-square-centimeter grid [135]. On this structure, an organic matrix of Matrigel (an artificial matrix) is first prepared and seeded with cultured epithelial and stromal (or undifferentiated mesenchymal) cells [128]. This setup is enclosed in a 70 cm-diameter, thermally controlled glass dome with two airtight observation windows. The gas environment is electronically controlled, and perfusion through the system is managed by computer-regulated components that control temperature and liquid and gas circulation. Liquid perfusion is assisted by peristaltic pumps, handling oxygenation (acting as lungs) and purification (acting as kidneys), while gas is monitored for pO<sub>2</sub> and pCO<sub>2</sub>, with consumption tracked. The artificial endometrium is perfused with varying concentrations of 17-beta estradiol, progesterone, DHEA, and androstenedione, simulating physiological pregnancy levels. *In vitro*,

the decidualization of stromal and epithelial cells is well-documented [129,130]. After achieving the desired thickness and differentiation, a blastocyst is transferred into the system to begin implantation and development. The system includes sensors to monitor heartbeat and perfusion, ensuring the integrity of the decidua-trophoblast interface.

## Conclusion

In the past 70 years, there have been more scientific advancements in human reproduction than in all previous millennia. However, technical barriers still exist for women who lack functional uteruses or genitals due to birth defects, impending disease, surgery, or the absence of gametes due to age, illness, or medical treatment. Similarly, men can face infertility due to similar issues. Homosexual couples also face challenges, as their reproductive desires cannot be met without the use of gestational carriers or gamete donations. Cultural and religious prejudices, along with socio-economic challenges, further prevent some individuals from accessing options such as gamete donation, gestational carriers, and uterine transplants. Looking ahead, over the next 30 years, *in vitro* gametogenesis could become a solution for same-sex couples, eliminating the need for gamete donation, which is still not universally accepted for ethical and religious reasons [136]. Additionally, genetic interventions in embryos from families with hereditary diseases could become possible, as well as advancements in ectogenesis, leading to significant changes in social customs, marriage, and legal obligations [137-140]. This progress could address infertility challenges for many couples, reduce deaths and major complications for premature births, and eliminate the need for surrogate mothers or uterine transplants.

## Data Availability Statement (DAS)

The data that support the findings of this study are available from references included with the identifier. Data may also be available from the corresponding author upon reasonable request."

## Declarations, Ethics Approval and Consent to Participate, or Compliance with Ethical Standards

### Informed Consent/Patient Consent

"Not applicable. This article does not contain any studies with human participants or animals performed by the author."

### Consent for Publication

"Not applicable. This manuscript is a narrative review and does not include any identifiable personal data or case details that would require consent for publication."

### Ethics Approval

"Not applicable. As this study is a narrative review of existing literature, no ethical approval was required."

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## Authors Contribution

Francesco Maria Bulletti and Carlo Bulletti: Conceptualization. Romualdo Sciorio and Antonio Palagiano's research and screening literature coherent publications, Maria Elisabetta Coccia and Maurizio Guido, explored the research of gametogenesis and embryo differentiation *in vitro* functional to the ectogenesis.

## Conflict of Interest Statement

The authors declare no financial interests.

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