Use of Nonhuman Primates for the Development of Bioengineered Female Reproductive Organs

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Nonhuman primates (NHPs) have been widely used in reproductive biology, neuroscience, and drug development since a number of primate species are phylogenetically close to humans. In this review, we summarize the use of NHPs for nonclinical application in the reproductive system disorders including the loss or failure of an organ or tissue. Causes of infertility include congenital aplasia and acquired disorders of the reproductive organs. In addition, anti-cancer treatments can deplete ovarian follicles, leading to premature ovarian failure, infertility and long-term health risks. Along with a limited supply of human reproductive organs, anatomic/physiologic similarities to humans support the need for NHP models (New-World monkeys such as the common marmoset and Old-World monkeys such as cynomolgus and rhesus monkeys) to promote the advances in female infertility studies. For maintaining and executing animal studies using NHP, special protocols including animal care, anesthetic protocol, surgical technique, and immunosuppressive protocol are necessary. With a growing interest in the potential therapies such as endometrial tissue engineering, and ovary/follicle cryopreservation and grafting in Korea, this review can be useful in selecting appropriate animal models and can bridge between nonclinical studies and clinical applications by providing detailed information on the use of NHPs in the field of reproductive organ disorders.

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INTRODUCTION

Infertility affects about 15% of all couples during their reproductive lives, and its rate is on the increase [1]. The causes of infertility may vary from population to population. The main causes of infertility in women have been known to be tubal factor, ovulatory disorders, endometriosis, and no demonstrable cause [2]. Among them, unexplained causes account for approximately 15% to 30% of couples [3].

Many millions of women worldwide suffer from the diseases of the uterus and vagina. One important cause of infertility is congenital anomalies including Mayer-Rokitansky-Küster-

Hauser (MRKH) syndrome occurring in 1 out of 4500 female births. It is characterized by congenital aplasia of the uterus and of the upper part (2/3) of the vagina in women presenting with a normal female karyotype and secondary sexual characteristics, which have no option of having children and affects the woman's quality of life [4,5]. Another common cause of infertility is malignant uterine tumors [6] or benign diseases such as myoma or adenomyosis, which may distort the uterine cavity or interfere with embryo attachment in implantation. Leiomyomas may also cause endometrial cavity distortion, dysfunctional uterine contractility, and spontaneous abortion [7,8]. Acquired uterine severe damage such as intrauterine adhesions/scarring due to surgery or infections also can affect embryo implantation and uterine contractility, which leads to infertility and miscarriage [9].

The endometrium, inner lining of the uterus, is a unique tissue that undergoes dynamic, cyclical processes of growth, differentiation, shedding, and regeneration as part of the menstrual cycle. Severe damages of uterine endometrium lead to endometrial scar formation which prevents embryos from implantation

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and placentation, finally resulting in subfertility or pregnant complications [10,11].

The uterine cervix is a fibrous organ with connective tissue content and leads from the bottom of the uterus to the top of the vagina. During the pregnancy period, maintaining the anatomic shape of the cervix during a period of substantial fetal and uterine growth is crucial [12]. Abnormalities of the cervix, such as a short cervix, have been implicated in a high risk for preterm birth, which is a leading cause of mortality and morbidity in newborn infants. In the US, preterm birth occurs in more than 12% of all births, representing approximately 500,000 pregnancies per year [12,13].

Although the long-term survival rate for young cancer patients is steadily increasing every year because of the progress in chemotherapy and radiotherapy as cancer treatment, many cancer therapies have permanent detrimental effects on the quality of life for these survivors since these treatments are toxic to the gonads and subsequently deplete follicles in the ovary, which produce oocytes and secrete female hormones, leading to premature ovarian failure and infertility [14,15]. The incidence rate of premature ovarian failure depends mainly on the chemotherapeutic regimen and the patient's age. Among different anticancer chemotherapeutic agents, the most gonadotoxic alkylating agents cause cortical fibrosis and ovarian blood-vessel damage, leading to follicular depletion [16]. Also, female-specific mutagens platinum agents (i.e., carboplatin, platinol, paclitaxel and cisplatin) can cause chromosomal aberrations such as deletions, and rearrangements, resulting in embryo toxicity [16-18]. The patients who are facing treatment with gonadotoxic agents for non-malignant autoimmune disorders like systemic lupus erythematosus, those who need to undergo surgery for endometriosis, and those with genetic disorders like Turner syndrome also have a growing demand for the fertility preservation [19-21].

TREATMENTS OF INFERTILITY

Artificial uterine functional unit engraftment/transplantation

The tissue loss or organ failure is one of the most critical human health problems. Although hormonal therapies may currently be used for various disorders of the reproductive tract, they do not replace all the functions of a lost organ or tissue, including maintenance of normal physiological levels of sex hormones. Therefore, for women with a congenital aplasia of the uterus, such as in MRKH syndrome, or for those with acquired uterine defect caused by hysterectomy due to uterine cancer, bioengineered or donor uterine (tissue) transplantation is now a possible therapeutic option for women with absolute

uterine factor infertility (an absent or nonfunctional uterus) to bear healthy children [22].

One of the most important factors of uterine tissue engraftment/transplantation is maintenance of surrounding blood flow after transplantation and subsequent maintenance of pregnancy. Inadequate vascular supply to the uterus has been a major problem for transplantation [22,23]. Therefore, selection of blood vessels and evaluation of blood flow in the transplanted uterus can help improve success rate of uterus transplantation and subsequent pregnancy maintenance [24,25]. Mihara et al. [22] have reported that anastomosis of at least the three blood vessels, the bilateral uterine arteries and unilateral ovarian vein is necessary for recovery of perfusion and blood flow of a transplanted uterus or bioengineered functional tissue unit among all of the bilateral uterine arteries, ovarian arteries and veins, superficial uterine veins, deep uterine veins and ovarian veins.

In the first donor uterine transplantation trial conducted in Saudi Arabia in 2000, the transplanted uterus was finally removed due to necrosis [26]. In the recent report with a 23-year-old MRKH patient, the first menstruation was confirmed with the transplanted uterus at 1 month after uterus allotransplantation. However, pregnancy ultimately failed to progress although it was achieved from embryo transfer for more than 1 year, leading that a live birth after uterine transplantation remains challenging [27].

Tissue engineering

Tissue or organ transplantation is a conventional therapy for disorders of the reproductive organs, however, this treatment modality suffers from a limitation of supply [28]. This has led scientists in the field of tissue engineering as a great approach to treat the tissue loss or organ failure without the limitations of native materials available [29]. A wide range of biological materials, such as small intestine submucosa, or synthetic materials, such as polytetrafluoroethylene and polyetherurethane/poly-L-lactide, for tissue engineering have been investigated in order to create completely natural new tissues and replace the conventional therapies [30,31]. The biomaterials are designed to bring isolated cells together and also control the tissue structure until the newly formed tissues are stabilized with biomechanical properties [32,33]. They are then transplanted into a patient to survive and integrate structurally and functionally into the host [28]. Collagen, one of the basic structural elements of extracellular matrix, has been widely used as a framework for structural support of tissue and as a substance for regulating cell behavior including cell adhesion, migration and differentiation in wound repair and tissue regeneration [34,35].

Ovary/follicle cryopreservation

Ovary/follicle cryopreservation can be often the primary treatment option currently available for human female fertility preservation for the purpose of strain rescue in young prepubertal girls and female cancer patients at risk of losing their ovarian function [36,37]. Several freezing protocols for ovarian tissues including slow-freezing and vitrification are available. Slow-freezing requires a programmed cryo-machine for controlled-rate freezing of embryos, oocytes or ovarian tissues, and allows the formation and development of extracellular ice crystals that are harmful to the surrounding tissues [38-40]. In addition, Mathias et al. [36] have demonstrated that slow freezing renders the oocyte and granulosa cell DNA more vulnerable to fragmentation. Vitrification has been applied to ovarian tissues as an effective method in various species, including human [41], mouse [42], and monkey [43]. It is time saving and requires little special freezing equipment. And, it involves a fast cooling rate with a high concentration cryoprotectant, which prevents potential risk of cellular injury associated with intracellular ice crystal formation in the tissue during the cryopreservation process. However, the high concentrations needed for vitrification make cryoprotectants toxic to cells [44]. The recent advance of ultra-rapid vitrification procedures has been effective to increase the cooling rates. These approaches include open-pulled straw (OPS) vitrification [45] and solid-surface vitrification (SSV, a new freezing technique) [46].

If the patient is considered to be disease-free, the cryopreserved ovaries may be re-implanted to re-establish female fertility. For this, maintaining the developmental potential of primordial follicles in banked ovarian tissue and establishing oocyte development from the primordial follicle stage to the stage of developmental competence for pre-pubertal patients are necessary [47]. In general, autografting of the ovarian tissue can be grafted orthotopically (transplanting the ovarian tissue back to the ovarian pedicle) or heterotopically (transplanting to the nonphysiological region, like under the skin) [43]. In 2004, auto-transplantation of cryopreserved ovarian tissue has resulted in the delivery of a healthy baby [40]. However, to date, there are limited reports regarding live births, indicating a need for further improvements of the current technique associated with better survival, fertilization and pregnancy potential [48].

USE OF EXPERIMENTAL ANIMALS IN REPRODUCTIVE RESEARCH

Uterine tissue engineering

Animal models can be useful for identification of human disease state and pathogenic mechanisms which cannot be completely studied using simple *in vitro* models [49]. Accumula-

tion of data from basic uterine transplantation experiments conducted in many animals, including mice [50], rats [51], pigs [52], and sheep [53], has led to clinical application, the performance of uterine transplantation in humans in Saudi Arabia, Turkey, and Sweden [26,54-56].

Many studies have been conducted on the tissue engineering as a helpful approach to treat organ malfunction using biological/synthetic material and isolated cells. Li et al. [57] have tested collagen membranes incorporated with a collagen-binding domain-basic fibroblast growth factor (bFGF) in a rat model under the severe uterine damage, and found that this bFGF delivery system improved regeneration of uterine endometrium and muscular cells, vascularization, and pregnancy outcomes. Campbell et al. [58] have implanted the encapsulating myofibroblast-rich tissue as a uterine tissue autologous graft in the peritoneal cavities of rats or rabbits. This novel autologous uterine graft tissue was found to be thickened and developed the morphology of normal uterus [58]. For *in vivo* transplantation researches associated with absolute uterine factor infertility, Hellström et al. [59] have also developed a novel whole-uterus scaffold and improved decellularization protocol for uterus tissue engineering experiments in a rat model. Wang et al. [60] have investigated the possibility of engineering functional uterine tissue using autologous rabbit uterine smooth muscle and epithelial cells. Song et al. [61] have demonstrated that human embryonic stem cells-derived endometrium-like cells seeded onto collagen scaffolds recovered function of uterine horn in rats with severe uterine damage. Ding et al. [10] have sought to identify the effect of this collagen/bone marrow-derived mesenchymal stem cells (BM-MSCs) constructs in the regeneration of severely damaged uterus endometrium in a rat model. Accompanied with the promotion of angiogenesis by transplanted BM-MSCs for regeneration of uterine horns, the regenerated endometrium improved the pregnancy rates and embryos implantation at the wounded site [10].

Mostly, an impaired cervix is involved in impaired mechanical properties of cervical stroma. For investigating the dysfunctional uterine cervix, House et al. [62] have developed engineered three-dimensional cervical-like tissue constructs with cervical cells from premenopausal women undergoing hysterectomy with biochemical constituents and morphology similar to native tissue. Difficulty obtaining sufficient cervical tissue from pregnancy of sufficient size led the researchers to use many animal models (mouse [63], rat [64], primate [65], rabbit [66], and guinea pig [67]) for study on the cervix during pregnancy.

For patients requiring vaginal reconstruction, De Filippo et al. [68] have investigated the feasibility of engineering vaginal tissue *in vivo*. Biodegradable polymer scaffolds with vaginal epithelial and smooth muscle cells of female rabbits were implan-

ed into nude mice. Based on the results of immunocytochemical, histological, Western blot, and electrical field stimulation studies, this tissue-engineered constructs were found to show similar functional properties with native vaginal tissue [68].

Tissue engineering for ovary/follicle cryopreservation

Although much more work is needed in this area, limited is the availability of donated human ovarian tissue. This led that ovarian tissue cryopreservation and transplantation in animal models is needed [69]. Ovarian tissue cryopreservation followed by autologous transplantation of tissue pieces or whole ovaries has resulted in restoration of normal endocrine and reproductive function in mice [70,71], sheep [72], and rabbits [73]. Chen et al. [14] have reported that the *in vivo* function of frozen-thawed mouse ovaries treated by direct cover vitrification in allogenic orthotopic transplantation showed highly efficient in comparison to conventional vitrification and slow freezing regarding the follicular survival and pregnancy capability [14]. The rat model has also been used for studies of ovarian function after cryopreservation. Milenkovic et al. [74] have used the whole rat ovary to evaluate the post-thawing viability of the ovary following vitrification or slow freezing with different concentrations of DMSO, and found that a moderate concentration of the cryoprotectant DMSO for both vitrification and slow freezing showed higher follicular viability than a high DMSO concentration. Xing et al. [44] have used isolated rat follicles to identify the effects of the three freezing methods (SSV, OPS, and slow-rate freezing) on viability, ultrastructure

and developmental capacity of isolated follicles. On a basis of the results on viability, morphological alteration, and estradiol level, the SSV method is an appropriate, more convenient, and less expensive method for cryopreservation of isolated rat follicles in comparison to other freezing methods [44].

The sheep have also been used extensively in research on whole ovary cryopreservation [75]. After whole ovary cryopreservation and subsequent transplantation, live births have been reported in the sheep in spite of low live-birth rates during entire procedure [76]. Nicacio et al. [77] evaluated the effects of different cryopreservation protocols on development of bovine embryos that can easily be *in vitro* produced at a relatively low cost. It suggested that controlled freezing (1.2°C/min) can be an alternative approach for cryopreservation of bovine embryos. The guinea pig has also been known as a useful small animal model to investigate ovarian follicular development and oocyte maturation [78]. Xu et al. [78] have showed that whole guinea pig ovaries could be cryopreserved *in vitro* slow-freezing protocol.

USE OF NONHUMAN PRIMATES

NHPs and animal care

For human physiology, pathology, and toxicology work, 'New-World monkeys' such as *Callithrix jacchus* (the common marmoset) and the larger 'Old-World monkeys' such as *Macaca fascicularis* (cynomolgus monkeys) and *Macaca mulatta* (rhesus monkeys) are commonly used models [79-81] since a number

Figure 1. Different primate species and housing facility. (A) Macaques (cynomolgus and rhesus) and marmoset, (B) examples of cages and chair for nonhuman primates.

of primate species phylogenetically close to humans (Fig. 1A). The features of this species are described in Table 1.

The marmoset is a neotropical species residing in Brazilian forests, and adult marmosets range in weight from 150 to 600 g. The lifespan of the marmosets ranges around 20 years, and they attain sexual maturity at 15–24 months of age [82,83]. Along with small size and high fecundity in captivity, the marmosets have an approximately 28 days ovarian cycle, 8 days follicular phase, 20 days luteal phase, a relatively short gestation period (140–150 days), and a relatively large litter sizes (1–3 offspring per delivery), and a relatively large number of deliveries (twice a year) [82–84].

The body weights of adult cynomolgus monkeys range 2.5 to 5.7 kg for females and 4.7 to 8.3 kg for males. And, adult rhesus monkeys in weight range 4.4 to 10.9 kg for females and 5.5 to 12.0 kg for males [83]. The lifespan of cynomolgus and rhesus monkey ranges around 30 and 40 years, respectively [85,86]. Sexual maturity of cynomolgus monkey is reached at 46 months of age for the females and 42–60 months for the males. Rhesus monkey attains sexual maturity at 34–43 months of age for the female and 38 months of age for the males [83]. The entire duration of the ovarian cycle is around 28 days in cynomolgus and rhesus monkey. The follicular phase lasts 12 to 14 days, and the luteal phase lasts 14 to 16 days in this macaques [83,87]. And,

Table 1. Features of nonhuman primates

	Cynomolgus [81,83,85]	Rhesus [81,84,85]	Marmoset [80,81]
Common characteristics			
Birth weight	F: 260–310 g M: 340–400 g	F: 410 g M: 450 g	25–35 g
Adult weight	F: 2.5–5.7 kg M: 4.7–8.3 kg	F: 4.4–10.9 kg M: 5.5–12.0 kg	150–600 g
Life span	30 years	40 years	20 years
Reproduction characteristics			
Breeding (in captivity)	Non-seasonal	Seasonal (Sep to Feb)	Non-seasonal
Age at maturity	F: 46 months M: 42–60 months	F: 34–43 months M: 38 months	F: 20–24 months M: 15 months
Ovarian cycle	28 days	28 days	28 days
Follicular phase	12–14 days	12–14 days	8 days
Luteal phase	14–16 days	14–16 days	20 days
Gestation	160–170 days	164 days	140–150 days
Litter size	1	1	1–3
Interbirth interval	390 days	360 days	155–160 days

M: male, F: female

Figure 2. Sexual skin coloration and menstrual bleeding of cynomolgus monkeys. (A) A reddish color of normal monkey and the palest color of ovariectomized monkey, (B) cynomolgus monkey showing menstrual bleeding (open arrows).

the normal gestation of cynomolgus and rhesus monkey is approximately 160–170 days [83].

Females in a variety of nonhuman old-world primates possess pink-to-red sexual skin around the perineum to the base of the tail (Fig. 2). It has long been assumed that redness attracts greater sexual interest from opposite sex individuals. Changes in skin color are correlated with ovarian estrogen concentrations before ovulation, which acts to increase vascular blood flow. This sexual skin becomes palest at menstruation [88,89]. In addition, cynomolgus monkeys showed marked sexual skin edema and swelling associated with fluctuation of estrogen levels during the menstrual cycle [40]. In old-world monkeys, the sexual skin is thus considered to be an external indicator of normal estrogen cycle and reproductive state.

According to the United States National Research Council's "Guide for the Care and Use of Laboratory Animals" [90], the nonhuman primates (NHPs) are recommended to maintain in cages equipped with a wooden bar (Fig. 1B) with minimum space [Group 1 (up to 1.5 kg), a height of 76.2 cm and a floor area of 0.20 m²; Group 2 (up to 3 kg), a height of 76.2 cm and a floor area of 0.28 m²; Group 3 (up to 10 kg), a height of 76.2 cm and a floor area of 0.40 m²] in a temperature- and light-controlled room. All monkeys are also recommended to introduce fresh fruits, vegetables, bread, eggs, and nuts, and a commercial diet supplemented with stabilized vitamin C daily by trained staffs, and the marmosets should be provided a diet supplemented with vitamin D₃ since they are unable to synthesize vitamin D₃ without UVB radiation (e.g., "New World Primate Diet" for the marmoset; "2050 Teklad Global 20% Protein Primate Diet" for old-world primates) [81,83]. Primate facilities provide appropriate enrichment, including chew toys, foraging items, audio and visual stimulation, to improve the psychological wellbeing and welfare of captive animals, and prevent the occurrence of abnormal physiology [91]. Noteworthy, it is important to recognize that there are some NHPs presenting special hazards to handlers, particularly the danger of bites and zoonotic infections. Therefore, several procedures such as sitting in a primate chair (Fig. 1B) for conscious NHPs are recommended during procedures. And, the NHP cages are recommended to have a squeeze-back mechanism to restrain the animal at the front of the cage by having the trainer slowly move the cage forward. More importantly, appropriate anesthesia is safer for the handler and less stressful for the animal for many procedures [80].

Immunosuppression for allogenic transplantation in NHPs

The transplanted graft expresses antigens that are recognized as foreign by the recipient, leading to activation of allo-immune

response and subsequent transplant rejection [92]. Thus, organ transplant NHP models necessarily involve immunosuppressive medications to minimize graft rejection prior to allogenic transplantation although immunosuppressants can also induce complications and toxicities. Following allogenic uterine transplantation in two cynomolgus monkeys, one animal receiving three immunosuppressants (tacrolimus, mycophenolate mofetil, methylprednisolone) had temporary recovery of menstruation, whereas the other animal receiving two immunosuppressants (tacrolimus, methylprednisolone) showed uterine atrophy due to continuous ischemia [93]. Since the late 1970s to use azathioprine and cyclosporine A [94], various polyclonal and monoclonal antibodies are being utilized to prevent organ rejection. Commonly, polyclonal antibodies show activity against T cell activation/proliferation markers, including CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, human leukocyte antigen (HLA)-DR and HLA class I. And, monoclonal antibodies target CD3, CD25, and CD52 on the T or B cell surface [92]. This led us consider that proper immunosuppressive drugs and methods for allogenic transplantation should be selected for appropriate immunosuppression without unwanted toxicities depending on the type of allograft.

Anesthetic protocol and surgical technique for NHPs

Prior to surgery, menstruation can be checked every day to verify if normal ovulatory menstrual cycles occur at regular interval in old-world primates (Fig. 2B), not a non-menstruating species marmoset [95]. Since ultrasound (Fig. 3) can help for

Figure 3. Reproductive organs in cynomolgus monkey. Ultrasonography of monkey uterus and ovary.

the diagnosis of infertility by demonstration of follicular growth and uterine endometrial changes, NHPs can also be visualized by ultrasonography [96]. Pre-operative fasting is necessary to reduce the volume of stomach contents during surgery and prevent possible vomiting. In general, the duration of fasting is for at least 12 h for old-world monkeys, and for 4–6 h for the marmosets to avoid symptoms of hypoglycemia or hypotension [83].

Anticholinergic drugs (atropine or glycopyrrolate) are often necessary to counteract bradycardia and hypersalivation before beginning of surgery [83]. According to recommended volumes and sites of administration of substances to laboratory animals, well-established injectable anesthetics (ketamine, tiletamine-zolazepam, propofol, etomidate, barbiturates, opioids) and inhalation anesthetics (nitrous oxide, halothane, isoflurane, sevoflurane) can be used for the induction of sedation and anesthesia in NHPs [83,97–99]. Once anesthetized (Fig. 4A), the incision areas and electrode attachment sites of all animals are shaved thoroughly. Animals displaying abnormal electrocardiogram, such as tachycardia, arrhythmia, and ST segment deviation can be excluded from the study. To help avoid possible bladder injury during surgical procedure, a Foley catheter can

be placed for NHPs prior to surgery under general anesthesia. Physiologic parameters, including noninvasive blood pressure, heart rate, oxygen saturation, respiratory rate, end-tidal CO_2 , rectal temperature, and inspired O_2 (Fig. 4B) can be recorded in NHPs under the anesthesia or physically restrained for sample collection [79,97]. Hypothermia is common after the induction of deep sedation or general anesthesia. In particular, anesthesia with isoflurane has been associated with greater hypothermia than that with ketamine/midazolam or propofol [100], suggesting that body temperature should be maintained with a heating pad under anesthesia.

Following proper skin preparation using appropriate antiseptic agents, laparotomy is performed through the incision of the skin, abdominal musculature and peritoneum. Special care must be taken with monkeys to avoid the accidental perforation of the urinary bladder during the incision of the abdominal wall. The ovary and uterus are exposed and collected from anesthetized monkeys (Fig. 4C), and immediately transported to the laboratory on ice in appropriate media. Then, the peritoneum, abdominal musculature, subcutaneous tissue, and skin were sutured and closed. Anesthetic administration was dis-

Figure 4. Anesthesia and surgery. Anesthetized monkey (A) and anesthetic monitor, (B) for heart rate, SpO₂, respiratory rate, end-tidal CO₂, and rectal temperature, (C) collection of the ovary (open arrow) and uterus (closed arrow).

continued at the end of the surgical procedure, and every effort should be made to alleviate animal discomfort and pain by the administration of anti-inflammatory and analgesic agents. After surgery, wearing a jacket and a collar system for NHPs can protect the surgical area for possible scratching and subsequent infection. All monkeys are monitored daily for evidence of disease and significant changes in attitude, appetite, behavior, or vaginal bleeding by veterinarians.

Use of NHP in reproductive research

Autologous and allogenic uterus transplantation to NHPs

Gross morphological characteristics of adult uterus are species-specific. Laboratory rodents (mouse and rat) have a long duplex uterus with a dual cervix. Ruminants (sheep, goat and cow) have a bicornuate uterus with a small common corpus with a single cervix, whereas higher primates including humans have a single uterus with a single cervix (Fig. 5), indicating that NHP model is the most useful for reproductive researches to study features of human uterine development [101].

Several studies of autologous orthotopic uterine transplantation have been performed in 16 baboons [26] and 26 baboons [100,101] by the groups in Saudi Arabia and Sweden, respectively. Experiments conducted by Saudi Arabia showed a great success rate of anastomosis although detailed outcomes on recovery of menstruation and pregnancy were not described. In autologous uterine transplantation in Sweden, 30% of animals had menstruation, but pregnancy was unsuccessful owing to severe adhesion and tubal obstruction after surgery. A group in Japan has conducted autografts in 6 cynomolgus monkeys [22,25,104]. In these cases, anastomosis of vessels was particularly time consuming. 33% of 6 animals showed menstruation, and subsequently, pregnancy and delivery were achieved in 1 cynomolgus monkey model after autologous uterine transplantation [22,25,104].

Figure 5. Comparison of types of uterus in monkey (simplex) and rat (duplex).

Pregnancy by allogenic uterine transplantation has been shown in rats [51] and sheep [105]. However, more studies in NHPs are needed for important information on a pregnancy and delivery in human allogenic uterine transplantation. Allogenic uterine transplantation was conducted in 18 baboons with several immunosuppressants. Impaired blood flow, some degree of rejection, and necrosis were found, and no animals resumed menstruation. There have been no reports of pregnancy and delivery after allogenic uterine transplantation in humans [106]. A group in US has conducted allogenic uterine transplantation with cyclosporine in rhesus monkeys [107]. In two allograft cases using cynomolgus monkey with immunosuppression, temporary recovery of menstruation occurred in one animal along with long-term survival. Ischemia and atrophy in the uterus were found to be associated with rejection in these allografts [93].

Despite continuous uses of old-world monkeys including cynomolgus and rhesus monkeys on uterine transplantation, limited data has been gathered to attempt autologous or allogenic uterine transplantation using the marmosets. Therefore, a current important issue is to establish the marmoset as a useful model for uterine transplantation. The marmosets are comparable to rats in adult body size, and they are less costly to maintain than larger NHP such as macaques (rhesus, ~12 kg; cynomolgus, ~8.3 kg) or baboons (10–30 kg) [83]. With the benefits of its small size, more important reproductive characteristics of the marmosets, such as a relatively large litter sizes (2–3 offspring per delivery) and a relatively large number of deliveries (twice a year), provide many researchers valuable advantages that the marmoset can be a important model species for various aspects of human reproductive biology [47,82–84,108].

Ovary cryopreservation and transplantation using NHPs

Even though various animal models have been used in research on ovary cryopreservation and transplantation, information obtained by NHP study can be valuable for clinical translation of *in vivo* data to humans. Ting et al. [48] have indicated that vitrification maintained morphology of the stroma and secondary follicles of rhesus monkeys, suggesting that it can be a great approach for ovarian tissue cryopreservation with *in vitro* maturation of secondary follicles. Yeoman et al. [43] have demonstrated that co-culture of thawed ovarian tissue on mouse fetal fibroblasts and follicle-stimulating hormone (FSH) increases the viability of cynomolgus or rhesus follicles cryopreserved previously with slow-rate freezing or vitrification, providing possible evidence on improvement of graft survival and function.

The marmoset has been known as an excellent NHP model for studies on the ovarian tissue development after cryopreservation and/or xenografting [109]. von Schönfeldt et al. [108]

have analyzed the effect of FSH treatment on the resting follicles in cryopreserved marmoset ovarian tissues with a slow-freezing protocol, leading that FSH treatment alleviates depletion of the resting follicle and promotes the percentage of morphologically normal follicles in frozen-thawed marmoset ovarian tissues. Xenografting of cryopreserved marmoset ovarian tissue into ovariectomized immunodeficient mice has been described with good follicular survival rates and antral follicle formation [109]. von Schönfeldt et al. [47] have used the marmoset to established a NHP model for follicular development cryopreserved prepubertal ovarian tissue with a slow freezing protocol after xenografting into immunodeficient nude mice. Increased presence of good follicular morphology as an indicator of graft sustainment was found in frozen-thawed grafted ovarian tissues. Moreover, the shift from the primordial to primary follicle and the appearance of secondary and preantral follicular stages in ovariectomized nude mice can be used as indicatives for the grafting procedure and development potential of the tissues [47].

Autologous transplantation of cryopreserved ovarian tissue showing resumption of endocrine function has been performed in monkeys [108,109]. Schnorr et al. [111] have reported that cryopreserved autologous ovarian transplantation in cynomolgus monkeys induced the restoration of ovarian function accompanied with ovarian and menstrual cyclicity. Also, Lee et al. [110] have demonstrated pregnancy and live births from autologously transplanted rhesus monkey ovarian tissue. These successes of ovarian transplantation in these NHPs will provide useful data for women at risk for ovarian failure from chemotherapy, radiotherapy or other causes [110,111].

CONCLUSION

For the patients with severe reproductive organ disorders, there is a growing interest in the field of regenerative medicine, tissue engineering and transplantation as therapeutic options. However, the accessibility of human reproductive organs is highly limited to be provided for the experimental studies on these disorders. For this reason, the use of NHPs, which are genetically and physiologically the most similar animals to humans, has been strongly advocated to promote further advances between rodent studies and controlled clinical trials for female infertility despite a lack of resources and trained personnel for the monkey models. Currently, limited data are available on the use of NHPs for the development of bioengineered uterine tissue in comparison to other experimental animals. By providing an overview of female infertility and the use of NHPs, this review can help provide researchers with a selection of appropriate animal models depending on the specific objectives and

aims of their studies. Noteworthy, with the benefits of a relatively small size, a relatively high reproductive output, and a relatively short lifespan compared with old-world monkeys, the marmosets can be a useful model for human reproductive research such as bioengineered uterine and ovarian tissue transplantation.

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Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

The animal experimental procedures were performed after receiving approval of the Institutional Animal Care and Use Committee of the Biomedical Research Institute at the Seoul National University Hospital (SNUH-IACUC No. 15-0032).

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