R E V I E W

Animal models of contraception: utility and limitations

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Abstract: Appropriate animal modeling is vital for the successful development of novel contraceptive devices. Advances in reproductive biology have identified novel pathways for contraceptive intervention. Here we review species-specific anatomic and physiologic considerations impacting preclinical contraceptive testing, including efficacy testing, mechanistic studies, device design, and modeling off-target effects. Emphasis is placed on the use of nonhuman primate models in contraceptive device development.

Keywords: nonhuman primate, preclinical, in vivo, contraceptive devices

Introduction

There is an urgent need for investment in preclinical development of novel contraceptive methods. Despite an increase in the variety of available contraceptive products, nearly half of pregnancies in the USA are unplanned.[1] Unintended pregnancy is associated with significant public health consequences, including increased risk of low infant birth weight, preterm delivery, and maternal depression.[2] Contraceptive choice and effective use is associated with a wide range of variables, including but not limited to, socioeconomic characteristics,[3] childbearing goals,[4] and method-specific experiences or attitudes.[5] This variability suggests that increased contraceptive choice may improve usage and compliance; however, newly marketed contraceptive products are often variations in the dose and delivery method of well-established steroidogenic products.[6]

Barriers to development of novel contraceptives have been multidimensional, including regulatory and corporate limitations.[7] One difficulty originates from the unique character of newer contraceptives, which combines aspects of pharmaceuticals, biologics, and devices in a single medical product. Therefore, development and optimization of these products includes not only evaluation of pharmacokinetics, toxicology, efficacy, and mechanisms of action, but must also consider device design and user acceptability. These aspects can be challenging to interrogate, but their successful preclinical modeling increases the likelihood of success in human clinical trials.

In this review, we draw on examples of contraceptive modeling in multiple species to illustrate how challenges to preclinical development have recently been addressed. We begin with a brief discussion of the utility of rodent and nonhuman primate species for discovery of novel contraceptive targets, as well as means by which contraceptive efficacy is evaluated in preclinical models. We move on to discuss species-specific anatomic and physiologic considerations impacting evaluation of contraceptive

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device design and mechanism of action. Finally, we review models used for characterization of extra-contraceptive effects. Emphasis is placed on nonhuman primate models for contraceptive device development. Normative reproductive values for species used in contraceptive modeling are summarized in Table 1.

Contraceptive discovery

Characterization of processes underlying folliculogenesis, ovulation, luteal maintenance, and implantation in animal models has facilitated identification of potential pathways for contraceptive intervention and assisted reproductive technologies.[8] In order to achieve a contraceptive effect with minimal side effects, the expression and function of these targets should be unique to the reproductive tract or pathway. Ideally, an event occurring near fertilization is targeted in order to avoid affecting early gametogenesis.[7]

Rodent models are well suited for in vivo contraceptive target discovery. The availability of advanced genetic engineering technologies, such as CRISPR/Cas systems,[9] as well as resources for comprehensive phenotypic analysis[10] of genetically engineered mice facilitate discovery of fertility and contraceptive targets.[11] These targets fall across the hypothalamic–pituitary–gonadal axis and include a number of reproductive modulatory strategies, including vaccines, receptor antagonists, and hormonal analogs. Immunocontraceptive targets include zona pellucida glycoproteins,[12] spermatic antigens,[13] or hormones such as gonadotropin-releasing hormone or human chorionic gonadotropin.[14] Receptor-level modulation has been attempted at endometrial targets necessary for receptivity and decidualization, such as tissue-specific serine proteases,[15,16] follicle-stimulating hormone,[17,18] prostaglandin E2,[19] and progesterone receptors.[20]

Due to similarities with human reproductive anatomy and physiology, contraceptive target discovery also occurs in nonhuman primate species. For example, mRNA expression of eight proteases was upregulated 12 hours post-ovulation in rhesus macaques subjected to an ovulatory stimulation protocol. Moreover, direct follicular injection of a metalloproteinase inhibitor prior to ovulation was associated with inhibition of follicular rupture.[21] Similarly, pre-ovulatory gonadotropin surges potentiate phosphodiesterase 3A-mediated oocyte maturation.[22] Treatment of female macaques with a phosphodiesterase inhibitor was associated with a reduced rate of pregnancy in one preclinical trial.[23] Antagonism of endometrial targets necessary for implantation, such as leukemia inhibitory factor[24] or vascular endothelial growth factor,[25] has also undergone proof of concept testing. Development of compounds with antagonistic or modulatory activity at the progesterone receptor (antiprogestins, progesterone receptor modulators) has been evaluated in nonhuman primates as well as rodents. Parenteral administration of an antiprogestin is associated with reversible suppression of menstruation in artificially cycled rhesus macaques.[26] Similarly, intrauterine delivery of two antiprogestins, ulipristal acetate[27] and Schering ZK 230 211,[28] has been evaluated in ovariectomized, artificially cycled rhesus and stump-tailed macaques, respectively. In both cases, endometrial atrophy and amenorrhea were associated with intrauterine administration of a range of doses.

Contraceptive efficacy

Preclinical evaluation of contraceptive efficacy presents many challenges to the investigator. When selecting a model species, differences in reproductive physiology should be taken into consideration. Rodent ovarian follicular dynamics differ from those of monovulatory nonhuman primates in several important ways, including follicular size, number of follicles released, and hormonal control of ovulation and luteal maintenance.[29] A post-copulatory prolactin surge is

Table 1 Comparison of normative reproductive values in species used for contraceptive modeling

Species	Common name	Adult female size (kg)	Median ovarian cycle length (days)	Breeding seasonality	Menstruation	Gestation length (days)
Macaca fascicularis	Cynomolgus macaque	2.5–5.7	29.4	Year-round breeder	Yes	164
Macaca nemestrina	Pig-tailed macaque	4.7–10.9	32	Year-round breeder	Yes	170
Macaca mulatta	Rhesus macaque	5.3	28	Seasonal breeder	Yes	165
Papio anubis	Olive baboon	14.7–17	30	Year-round breeder	Yes; difficult to visualize	170
Mus musculus	Mouse	0.018–0.035	4–5	Year-round laboratory	No	19–21
Rattus norvegicus	Rat	0.25–0.35	4–5	Year-round laboratory	No	21–23
Oryctolagus cuniculus	Rabbit	2–5	Post-copulatory ovulation with sexual receptivity every 4–17 days	Year-round laboratory	No	30–33

required for luteal development in rodents, while a mid-cycle luteinizing hormone (LH) surge is sufficient amongst primates.[30] Old World monkey species, such as macaques and baboons, exhibit pituitary release of LH and gonadal LH receptor morphology that closely resembles that observed in humans. Conversely, New World monkey species, such as marmosets and squirrel monkeys, release chorionic gonadotropin from the pituitary and express a modified form of the gonadal LH receptor.[31]

Prospective evaluation of contraceptive efficacy is difficult in women. In Phase I and Phase II clinical trials, markers of reduced fertility, such as ovulatory or spermatogenesis rates, are utilized in place of pregnancy.[7] The use of pregnancy as an endpoint is a significant advantage in animal modeling of contraceptives. For example, Peluffo et al evaluated a prostaglandin E2 receptor 2 antagonist in a contraceptive trial with cynomolgus macaques.[19] Following pretrial treatment, group-housed females were introduced to a single male. Pregnancy was detected via serum progesterone and macaque chorionic gonadotropin levels and viability was confirmed with abdominal ultrasound. Pregnancies were terminated prior to 32 days' gestation with parenteral mifepristone or intrafetal methotrexate.[32] Thirty-eight percent of macaques receiving the prostaglandin E2 receptor 2 antagonist became pregnant in comparison with 80% in the vehicle-treated control group. In rodents, quantitative evaluation of contraceptive efficacy is easily determined by comparison of implantation rates in control and experimental uterine horns at necropsy. However, when evaluating preclinical contraceptive efficacy in rodents, investigators and regulatory bodies must define acceptable levels of reduction in gestational rates in these polyovulatory species.

In addition to use of pregnancy as an endpoint, animal modeling permits standardization of ovarian cyclicity. Ovarian cycle length can be synchronized through provision of exogenous hormone in ovariectomized macaques. This is typically performed in seasonally anovulatory species, such as rhesus macaques. In this protocol, animals are implanted with a subcutaneous capsule of estradiol for 14 days. A capsule containing progesterone is added from days 14–28, and then removed to stimulate menstruation. This protocol has been successfully used to synchronize and control ovarian cycle variability in a cohort of macaques prior to a contraceptive trial.[28] Disadvantages include potential impacts of exogenous progesterone, such as vaginal epithelial thinning and differences in background hormonal milieu, between ovariectomized and intact animals, as Old World primate species have a large circulating pool of adrenal-origin androgen.[29]

Where collection of preovulatory follicles for in vitro analysis is of interest, a controlled ovarian stimulation protocol has been described for the rhesus and cynomolgus macaque.[19,33] In these protocols, injection of human chorionic gonadotropin is followed by a course of recombinant human follicle-stimulating hormone, a gonadotropin-releasing hormone antagonist for prevention of endogenous gonadotropic surge, and finally a single preoperative dose of recombinant LH to promote follicular maturation without rupture. This protocol facilitates progressive development of a dominant follicle, but replaces endogenous LH with exogenous human chorionic gonadotropin in order to control the timing of ovulation.[30]

Where exogenous control of ovarian cycle is not desired, cycle monitoring is often still critical to the study of contraceptive efficacy. In rodents, the ovarian cycle is noninvasively monitored through cyclical changes in vaginal cytology.[34] In Old World monkey species with menses, swabs of the vaginal anterior fornix can be examined for the presence of blood or sperm when animals are being bred. Alternatively, the external genitalia can be visually examined. In baboons, menses may be difficult to visualize. Rather, perineal skin tumescence is reliably used as a visual proxy for the ovarian cycle.[35] External observations may be complemented by fecal, urine, or serum hormonal assays in most nonhuman primate species.[36–38]

Contraceptive device design

Due to their similarity to human reproductive anatomy and physiology, nonhuman primates are advantageous for late-stage preclinical evaluation of both contraceptive design and efficacy. Preclinical safety and feasibility is generally evaluated in rodents and rabbits. These species are advantageous due to the presence of dual uterine horns, a short ovarian cycle, and reduced cost relative to nonhuman primates, although challenges with device scale and differences in reproductive physiology may limit the translational value. Model characteristics are summarized for each contraceptive device in Table 2. For the purposes of this discussion, contraceptive devices will refer to both hormonal and nonhormonal intrauterine devices (IUDs), intravaginal rings, transdermal patches, and subcutaneous implants. Nonhormonal barrier methods such as condoms or diaphragms will not be discussed.

Intrauterine devices

Intrauterine contraception currently available to consumers in the USA includes two levonorgestrel intrauterine systems (LNG-IUS) and a copper IUD, the T380A (Paragard®; Teva

Table 2 Comparison of animal models of contraceptive devices

Device	Species	Advantages	Disadvantages
IUD	Baboon	Size permits human-use IUD Transcervical deployment possible Year-round breeder	Expensive
	Macaque	Well characterized	Expensive Hysterotomy placement of IUD Some species are seasonal breeders
	Rodent	Less expensive Well characterized	Filamentous or rod IUD only Hysterotomy placement of IUD
IVR	Macaque	Validated ring for NHP use Well characterized	Ring is smaller than human model More basic vaginal pH than human
	Sheep	Similar body mass to human Less expensive than NHP	Seasonal breeders IUD, NHP Not well characterized
Transdermal delivery systems	Pig	Well characterized in in vitro and in vivo assays Resembles human skin	Expensive
	Macaque	Resembles human skin and reproductive physiology	Expensive
	Rabbit	Less expensive	More heterogeneous drug absorption

Abbreviations: IUD, intrauterine; IVR, intravaginal ring; NHP, nonhuman primate.

Pharmaceuticals, Petah Tivka, Israel). Available LNG-IUS systems include Mirena® (32×32 mm; 20 µg of LNG released per 24 hours; Bayer Healthcare Pharmaceuticals, Wayne, NJ, USA) and Skyla® (28×28 mm; 14 µg per 24 hours; Bayer Healthcare Pharmaceuticals).[39] Intrauterine contraception is associated with an excellent contraceptive efficacy rate, comparable with that of tubal ligation, and requires less reliance on user compliance than other contraceptive methods.[40] In some women, the LNG-IUS also confers noncontraceptive benefits such as amenorrhea.[41]

Larger species of nonhuman primate such as the baboon have a uterine lumen size that can accommodate human-use IUDs. An LNG-IUS (Mirena®) can be transcervically deployed in the baboon uterus. In ex vivo and in vivo evaluation of LNG-IUS placement in three baboons, magnetic resonance imaging and transabdominal ultrasound indicated successful uterine fundic deployment of the IUS.[42] Successful implementation of a human-use copper IUD has been described in pigtail macaques. The GyneFix-200 mini® (Contrel Research, Ghent, Belgium) is a copper IUD consisting of four 5×2.2 mm cylinders on a suture. Devices were placed via hysterotomy and maintained for 3 months.[43]

As suggested by the aforementioned studies, species differences in cervical anatomy affect whether devices can be deployed transcervically or require hysterotomy for placement. Unlike the baboon, which has a relatively straight cervical canal,[42] the cervix of most macaque species is sigmoidal, making transcervical deployment of IUDs impractical.[44] The necessity for IUD placement via hysterotomy and use of retention sutures may incite a local inflammatory response that can impact interpretation of data

on toxicity and contraceptive efficacy. Interestingly, a recent study reports successful transcervical delivery of polidocanol foam in the rhesus macaque.[45] Atraumatic cervical cannulation was achieved with ultrasonographic or laparoscopic-guided placement of a series of cannulas used for gradual cervical dilation. This technique was not successful with all animals, however, and requires significant technical expertise. Similarly, nonsurgical transcervical cannulation in the rabbit has been reported,[46] although not in the context of intrauterine system placement. The ability to atraumatically cannulate the rabbit cervix may increase its utility as an animal model for IUD development.

Modification of the IUD shape and size is typically required for smaller species. A straight silicone elastomer ("silastic") rod or a single silk, nylon, or copper filament[47] has been utilized as an experimental alternative in smaller nonhuman primates and nonprimate animal models of intrauterine contraception. This type of device can be successfully implemented in many species, including rodents, with modifications easily made for individual variations in uterine size. Tubes can also be loaded with different pharmaceutical agents. For example, in one study that sought to evaluate the impact of an antiprogestin-releasing IUD in stump-tailed macaques, a 12 mm silastic tube was used to deliver one of three compounds with antiprogestin activity.[28] Tubes were placed in the uterine body lumen via hysterotomy and migration prevented with a myometrial stay suture. Successful deployment of similar rod-shaped devices of lengths ranging from 10 mm to 15 mm are reported in other nonhuman primate studies.[27] A filamentous IUD model has been well established in mice, rats, and guinea pigs.[48–50] In these models,

a single length of silk or other monofilament suture is placed transcervically within a single uterine horn. The contralateral horn is used as a control. Other devices successfully deployed via hysterotomy in rodents include copper or silicone rods[51–53] and metal or silicone coils.[54] Successful placement of IUDs from 5 mm to 35 mm in length are described in these studies. While the rod or filament IUDs offer flexibility with regard to scale and compound delivery, they do not necessarily mimic the biomechanical properties of larger IUDs.

This variability in size and type of device tested in preclinical studies has impacted our understanding of the contraceptive mechanism of action of the IUD. In both humans and animals, the mechanism of action is likely multimodal, occurring through alterations in the cervical mucous plug, endometrial receptivity, and germ cell or embryonic viability.[55] It is important to understand the contraceptive mechanism of action of the IUD, as it may impact user acceptability in light of patient preference for avoidance of post-fertilization effects.[56] In mice and rats, embryonic destruction within the uterine lumen is correlated with the presence of luminal polymorphonuclear leukocytes in the presence of a filamentous or copper IUD.[57,58] In women, evidence suggests that pre-fertilization destruction of oocytes or sperm may be the more common contraceptive mechanism, particularly with the copper IUD.[55,59,60] However, study of pre-fertilization events is difficult to perform noninvasively in women, and conclusions have been drawn from relatively small sample sizes. Therefore, it would be advantageous to prospectively evaluate the mechanism of action of the IUD in an animal model. However differences in IUD size and placement, as well as species-specific differences in reproductive physiology, have been difficult to overcome.

Intravaginal rings

An intravaginal ring (IVR) releasing etonogestrel and ethinyl estradiol (NuvaRing®; Merck and Co, Whitehouse Station, NJ, USA) is currently approved for contraceptive use in the USA. IVRs offer an appealing means of drug delivery. Transmucosal vaginal drug delivery results in good bioavailability and comparable pharmacodynamic effects, without the side effects associated with oral administration.[61] Moreover, IVRs offer a coitally independent means of drug delivery that is associated with high levels of user acceptability.[61] Most preclinical studies of IVRs have been performed in macaque species and sheep.[62–66]

Recently, significant efforts towards development of combined contraceptive and microbicide delivery via IVR have resulted in a renewed interest in preclinical testing of vaginal ring models.[62] Scaling of IVR size, stiffness, and surface area can present translational challenges. Promadej-Lanier et al tested three different IVR sizes in pigtail and rhesus macaques, and in both species, a 25 mm circumferential and 5 mm cross-sectional diameter silicone ring was associated with minimal induction of proinflammatory cytokines in the lower reproductive tract.[63] Additionally, no visual evidence of irritation or inflammation was noted on colposcopic evaluation, although the smaller ring was significantly stiffer than commercially available models. This model of ring has since been used in several studies evaluating IVR delivery of antiretroviral agents in both pigtailed and cynomolgus macaques.[64–66] These studies report acceptable levels of ring retention, although one noted that on necropsy 28 days post-placement, 25×5 mm rings were located mid-vagina in the smaller cynomolgus macaque.[66] Subsequent trials with a 20×4.5 mm ring were associated with successful pericervical retention.

Differences in scale and vaginal pH also present challenges to interpretation of pharmacokinetic data. For example, in vivo IVR delivery of the microbicide dapirivine was evaluated in the cynomolgus macaque.[66] Vaginal fluid concentrations of dapirivine were significantly lower than those reported from previous human clinical trials. The macaque vagina is more basic (pH 7) than that of the human (pH 4). The more basic vaginal environment may result in differences in drug protonation and consequent pharmacodynamic properties. Furthermore, the larger human IVR has a correspondingly greater surface area for drug delivery than the smaller macaque ring. It should also be noted that differences in vaginal epithelial morphology exist even between the different macaque species. For example, the vaginal epithelium of the rhesus macaque is more keratinized than that of humans or pigtail macaques.[67] Care should therefore be taken when extrapolating the pharmacokinetics of vaginally delivered topical drugs between species.

The sheep is a potential preclinical alternative to the nonhuman primate for evaluation of IVRs. Advantages of the sheep model include a body mass and cervicovaginal canal size similar to women. Visual and histologic evaluation of the reproductive tract may be performed with ease. The sheep vagina is lined by stratified squamous epithelium, and although the vaginal submucosa is thinner than in humans,[68] the vaginal epithelium of the sheep does not undergo pronounced histologic changes coincident with the estrous cycle, as is observed in the rodent.[69] Disadvantages of the sheep model include a seasonal estrous cycle and a paucity of data on reproductive tract mucosal immunology. As a result of the sheep's larger size, human-sized IVRs can

be tested without the scaling issues previously discussed. For example, the pharmacokinetic and toxicologic properties of a hydrophilic polyurethane IVR loaded with tenofovir were evaluated in sheep.[70] A 55×5.5 mm ring was used, which is comparable to the size of the NuvaRing (54×4 mm). These rings were maintained for 90 days, with only one case of expulsion.

Transdermal and subdermal delivery systems

Transdermal delivery systems include patches, gels, and sprays. A single transdermal patch is currently on the US market for sustained delivery of ethinyl estradiol and norelgestromin (Ortho Evra®; Janssen Pharmaceuticals, Inc., Beerse, Belgium). A 68 mg etonogestrel subdermal implant (Nexplanon®; Merck and Co) is also approved for use.

Transdermal drug absorption is affected by numerous variables, including species and drug characteristics. Amongst laboratory animal species, the pig, macaque, and rabbit are the most frequently utilized for study of topical drug applications.[71] Porcine ear skin is frequently used in vitro for early preclinical validation of percutaneous drug permeation.[72] Percutaneous absorption rates for testosterone and hydrocortisone as well nitroaromatic compounds is similar in vivo in rhesus macaques and humans.[71] In a study examining transdermal patch formulations of levonorgestrel and 17 beta-estradiol, good correlation was found between in vivo pharmacokinetic and pharmacodynamic data in rabbits and the results of Phase I clinical trials in women.[73] However, in percutaneous absorption studies of other hormonal compounds such as testosterone, data from rabbits is not predictive of human values.[71] Study of subdermal drug delivery is less impacted by heterogeneity of skin morphology than transdermal formulations; however, as with oral drug delivery, it is subject to species differences in drug distribution and metabolism. A comprehensive discussion of preclinical evaluation of injectable or oral contraceptives is beyond the scope of this review, so we direct the reader to an excellent review by Andersson et al for a more detailed analysis of assessment of circulating female reproductive hormones in animal models.[29]

Extra-contraceptive effects

Drug or device extra-contraceptive effects may impact user health and safety as well as method acceptability. Animal models are useful for characterization of contraceptive off-target effects where human studies are unethical or otherwise not feasible. For example, it is difficult to evaluate the interaction between sexually transmitted infection (STI) rates and hormonal contraception in human populations due to significant confounds, such as condom use. Condoms prevent STIs but are used at significantly lower rates by individuals employing other contraceptive methods.[74] In particular, equivocal evidence on a causal relationship between human immunodeficiency virus acquisition and depot medroxyprogesterone acetate use in women[75] has increased interest in modeling this interaction using macaques infected with unmodified or recombinant simian immunodeficiency virus (SIV or SHIV, respectively). These models were recently reviewed in a publication by McNicholl et al.[76] The same group has also developed a model of SHIV infection in copper IUD-implanted pigtail macaques.[43] In addition to SHIV, well characterized nonhuman primate models of *Chlamydia trachomatis*,[77] *Mycoplasma genitalium*,[78] *Trichomonas vaginalis*,[79] and genital papillomavirus[80] have been developed, and could be utilized for pathogen challenge studies of combined contraceptives.

Additionally, the vaginal microbiota of many species of nonhuman primate have been described both with[81] and without a contraceptive device.[82] As vaginal microbiota may contribute to host mucosal immune function, characterization of alterations in these bacterial communities is of particular importance for development of dual-action vaginal rings or IUDs that provide simultaneous contraception and microbicide-mediated STI protection. Several broad-spectrum microbicides have failed in clinical trials due to induction of mucosal damage with repeated use and increased risk of transmission of human immunodeficiency virus.[62] Use of more stringent preclinical product selection criteria, including description of the impact of a product on vaginal microbiota, may enhance understanding of microbicide epithelial toxicity and STI acquisition risk with contraceptive or dual-action products in a well characterized pathogen challenge model.

Associations between contraceptive use and obesity can also be explored in animal models. Although systematic review suggests no causal relationship between weight gain and combined oral contraception (COC), weight gain is a common complaint in COC users.[83] The impact of COC (ethinyl estradiol/LNG) on body weight, physical activity, caloric intake, percent body fat, and basal metabolic rate was compared in cohorts of healthy and obese female rhesus macaques over an 8-month treatment period. A 9% decrease in body weight was observed in the obese animals, with no significant difference observed in the normal animals. A significant increase from baseline in basal metabolic rate

was observed with COC treatment in both groups.[84] A study using the same COC dosing protocol observed increases in both serum adiponectin and resistin from baseline in both obese and healthy weight macaques.[85]

Conclusion

Validated animal models are crucial for preclinical prediction of drug safety and efficacy that cannot be determined in vitro. Due to similarities with humans in reproductive anatomy and physiology, nonhuman primates are attractive candidates for modeling of contraceptive devices, understanding of their contraceptive mechanism of action, and evaluation of contraceptive efficacy. Other species, such as rodents or rabbits, also serve important roles in preclinical dosing optimization, safety pharmacology, and contraceptive target discovery. Leverage of extensive genetic and phenotypic tools in murine models has enabled discovery of pathways in reproductive biology suitable for contraceptive intervention. However, interspecies differences in follicular, luteal, and endometrial dynamics may reduce the translatability of these discoveries. Nevertheless, with careful thought given to selection of animal models, preclinical modeling enhances contraceptive development and ultimately improves patient safety and contraceptive choice worldwide.

Disclosure

The authors report no conflicts of interest in this work.

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