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Contraceptive Special Issue

Preclinical contraceptive development for men and women

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Abstract

This manuscript endeavors to present research considerations for the preclinical development of non-hormonal contraceptives. Topics include (1) how advances in genomics and bioinformatics impact the identification of novel targets for non-hormonal contraception, (2) the importance of target validation prior to investment in a contraceptive development campaign, (3) considerations on targeting gametogenesis vs gamete maturation/function, (4) how targets from the male reproductive system are expanding women's options for 'on demand' contraception, and (5) some emerging non-hormonal methods that are not based on a specific molecular target. Also presented are ideas for developing a pipeline of non-hypothalamic-pituitary-gonadal-acting contraceptives for men and women while balancing risk and innovation, and our perspective on the pros and cons of industry and academic environments on contraceptive development. Three product development programs are highlighted that are biologically interesting, innovative, and likely to influence the field of contraceptive development in years to come.

Summary sentence

Considerations for preclinical contraceptive development.

Key words: contraception.

Introduction

The field of contraceptive development is in an exciting time, as highlighted at the Eunice Kennedy Shriver National Institute of Child Health and Human Development Contraceptive Development Meeting hosted by the Baylor College of Medicine, 03-06NOV2019. The meeting focused on the development of non-hormonal contraceptives for men and women, including multipurpose prevention technologies (MPTs) that include both contraceptive and anti-infective technologies. While the authors are aware that several promising hormonal approaches for men and women are under development [1], and that these have rightfully garnered keen public interest, they are beyond the scope of this review.

Here we will focus on the literature that presents a spectrum of approaches under development for contraceptive practice and covers critical considerations for moving forward in preclinical development. Specifically, we present herein: (1) how advances in genomics and bioinformatics have impacted the identification of novel targets for non-hormonal contraception, (2) the importance of target validation prior to investment in a contraceptive development campaign, (3) how targets from the male reproductive system are expanding women's options for "on demand" contraception, and (4) some emerging non-hormonal methods that are not based on a specific molecular target. Also presented are ideas for developing a pipeline of non-hypothalamic-pituitary-gonadal (non-HPG) contraceptives for men and women that provides the optimal balance of

risk and innovation; the pros and cons of contraceptive development in industry and academic environments; and a few emerging product development programs that are biologically interesting and—in our opinion—likely to enhance the field of contraceptive development in years to come.

In the last 20 years, the effort to develop additional contraceptive technologies for men and women that do not act via the HPG axis has gained momentum. For the sake of organization, we classified these into two categories: targeted (specific) and non-targeted (non-specific).

Targeted non-hormonal contraception

Targeted methods for contraception are generally focused on specific modulation (typically inhibition) of a defined molecular target (i.e., enzyme, ion channel, and transporter) required for the formation of a functional sperm or a developmentally competent egg. Inherent to these strategies is specific modulation of a defined molecular target required for a biological process critical to fertility, such as meiosis, osmotic regulation, sperm motility, energy production, or sperm-zona pellucida binding [2–5].

A common approach employed to date involves evaluating and targeting gene products that are expressed specifically—or at least preferentially—in the male and/or female reproductive tract [6–8]. The expectation is that having a target expression profile limited to the reproductive tract will correlate to restricted on-target activity. By extension, this characteristic will increase the likelihood of obtaining a safety profile necessary for a product that will be used by a generally healthy individual for a long period of time (potentially decades).

During the first years of this century, several large pharmaceutical companies (e.g., Wyeth, Organon, Schering AG) identified and validated contraceptive targets [9–14] and had development programs for targeted non-hormonal contraceptives, but most of these efforts were abandoned by 2008 and, unsurprisingly, few significant details of these programs were published. Interest in contraceptive development from large- or mid-sized pharmaceutical companies has been nearly non-existent since. However, government and philanthropic organizations, including the National Institutes of Health (https://www.nih.gov), the Bill and Melinda Gates Foundation (https://www.gatesfoundation.org), and the Male Contraceptive Initiative (https://www.malecontraceptive.org) have all increased funding in the development of novel contraceptive methods during that time.

Most targeted contraceptives are directed toward the male reproductive system

For many years, the molecules targeted for modulation to achieve a contraceptive effect were identified from studies of basic reproductive biology. As more genes and proteins were discovered via investigation of reproductive function, the characterization and expression profile often demonstrated a reproductive tissue-specific localization [15–18]. The tissue-specific localization was more frequently described for genes/proteins of the male reproductive tract than the female reproductive tract. These observations were confirmed by the study of whole organs, micro-dissected tissue and isolated cell types of the reproductive system and non-reproductive tissues in conjunction with large scale "-omics" platforms, in association with increasing bioinformatics capabilities in the male [9, 11, 19–22] and from mural granulosa cells, cumulus granulosa cells, and purified oocytes from the ovaries of mice 48 h postpriming with pregnant mares serum gonadotropin (PMSG) or 12–14 h after standard PMSG + human chorionic gonadotropin priming (unpublished—Johnston). The 2003 publication by Shultz et al. [23] combined Affymetrix (Santa Clara, CA) microarrays and bioinformatics to study testicular gene expression and estimated that ≈4% of the mouse genome is dedicated to haploid male germ cell-specific expression. This is an enormous contribution of the mammalian genome to the process of male gamete development and these data were the first to elucidate why, if reproductive tract specificity is a desired criteria for a contraceptive target, most would be found solely or primarily in the male reproductive system [1, 24, 25]. The use of more sensitive expression profiling techniques [11, 21] demonstrated that many of the identified reproductive tract-specific genes do not have absolute testis specificity, but typically reveal an enormous fold difference in expression between reproductive and non-reproductive tissues. The trend of identifying fewer reproductive tissue or reproductive tract-specific genes as assay sensitivity increases is not surprising and will continue as more sensitive quantitative measures of gene transcription (e.g., RNAseq) are used for such studies. Rather than a characterization as "testis-specific" or "reproductive tract-specific," target selection will use a measure of the degree of tissue "selectivity" (e.g., fold-difference in expression levels), in combination with consideration of other identified sites of expression, target validation, and "druggability" (discussed below).

The expectation that a greater number of contraceptive targets would be found in the male reproductive system as compared to the female reproductive system was further confirmed by the ReproGenomics Program at The Jackson Laboratory. The group performed random, whole genome mutagenesis studies using N-Ethyl-N-nitrosourea (ENU) mutagenesis coupled with a phenotype screening for breeding failure as the only phenotype. Treatment with ENU generally results in random single base pair mutations via alkylation of nucleic acids [26, 27]. Approximately 75% infertile mutant lines showed infertility in the male only, 10% showed infertility in the female only, and about 15% exhibited infertility in both species [28].

While decades of observation and the more recent "omics" studies outlined above demonstrate that there are a far greater number of contraceptive targets in the male reproductive tract as compared to the female reproductive tract, there have been several targeted non-hormonal female contraceptive development programs, including wee 1-like protein kinase 2 [29], phosphodiesterase 3A [30, 31], follicle stimulating hormone receptor [32], zona pellucida glycoprotein 3 [33], and zona pellucida glycoprotein 2 [3]. In addition, there are also non-hormonal product development programs targeting male targets for use as female-controlled contraceptives (see section below about human contraceptive antibody (HCA)).

Target validation

By itself, tissue specificity or a restricted tissue distribution of genes or proteins is insufficient to warrant the investment of resources toward undertaking a contraception development campaign. Target validation is also required. For contraception, target validation can be defined as the demonstration (typically in one or more mammalian species) that modulation of the proposed target results in infertility or an effect on the fertilization process consistent with infertility. The most common validation approaches utilize genomic-based methods. Validation by non-genomic methods typically relies on treatment of gametes with candidate contraceptive compounds,

which are often toxic or inhibitory substances. None of the validation methods are ideal, and potential shortcomings of various approaches and methods are noted below.

An example of validation for a non-targeted method designed to inhibit sperm function is the demonstration that an agent (e.g., a pH buffer intended for vaginal administration or other cytotoxic agent) can disrupt sperm viability or motility in vitro. Defined concentrations of sperm can be exposed to known concentrations of a specific test agent followed by monitoring of sperm motility and/or viability. The direct sperm monitoring method is often criticized due to the experimental need to liquefy and wash sperm thereby eliminating seminal plasma, ejaculatory components, and female reproductive tract components, such as cervical mucus. While liquification and washing is necessary to generate a suspension with a defined sperm concentration, it compromises the physiologically relevant milieu to which sperm are normally exposed. If the test agents are cytotoxic to sperm, appropriate experimental approaches are required to (1) monitor systemic exposure and (2) evaluate toxicity with respect to exposed areas of the female and male reproductive tract. If significant systemic exposure is identified, additional toxicology studies would be required.

Targeted gene deletion has provided a powerful strategy to validate the requirement for the presence/activity of specific gene products in the reproductive process and is currently the most commonly used method for validating specific contraceptive targets. Targeted gene deletion can be conducted at relatively low cost ($15K), performed quickly (time to determine infertility is about 9 months) and with great precision. Techniques are widely accessible to basic researchers as well as industry scientists. Large-scale coordinated efforts are underway to systematically delete each known gene within specific model organisms. For example, the Knockout Mouse Project (KOMP; https://www.komp.org/) helps validate targets by establishing knockouts of every gene in the mouse genome and performing basic characterization of each knockout, including fertility assessment.

There are inherent risks in interpreting knockout data from mice as being relevant to human reproduction. The expression profile of the target should be carefully evaluated in both species. Genes thought to be orthologous may in fact have different functions, thus highlighting the need for continued work on the basic biology of human reproduction as well as in model systems. In addition, while targeted deletion is an accepted method of validation, it falls short of being a fully accurate representation. The targeted deletion strategies generally result in the loss of not only the complete activity/function of the protein of interest, but also the presence of that protein. If the protein target is not produced, then the biological effects resulting from the normal physical interactions of the target protein (e.g., with individual proteins, protein complexes, or with cellular factors) will be affected and likely lead to greater dysfunction than observed through specific pharmacologic inhibition of target function (i.e., the effect will be over represented). In addition, it is highly unlikely that pharmacologic inhibition of a target will reach 100% as represented by the complete loss of the target protein activity, complicating interpretation of the data. The development of genetically modified animals with baseline target expression and reduced target function (e.g., mutation of the target sequence leading to reduced but not complete loss of function) would be a more realistic indicator of pharmacologic inhibition in the physiological system, but it is acknowledged that the development of those models with current technology can only be achieved, if at all, through the expenditure of far greater resources than are required for standard gene knockout studies. A long-term strategy may include characterization of infertile models generated from the targeted deletion approach followed by mutagenesis approaches that result in loss of function without elimination of target expression.

Alternatively, infertility in heterozygous null animals may also be interpreted as strong validation. This method may better represent the effect of pharmacologic inhibition, and it is more cost effective.

Another concern in evaluating fertility in knockout animals is that the product of the deleted gene is often absent throughout development, potentially allowing compensatory mechanisms to offset the loss. Genes important for fertility may be replaced during development by redundant mechanisms or adaptive responses that are not predictable. Such an occurrence would not be analogous to the expected clinical situation in which a healthy adult is exposed to a contraceptive agent. An alternative strategy is conditional gene disruption, albeit with the concerns noted above for targeted deletion.

Genome wide association studies (GWAS) of infertility patients represent a novel approach to conduct both target identification and provide a degree of validation [34–37]. An exciting aspect of GWAS studies is that they inform the clinical population of interest; humans. However, pitfalls of GWAS-identified targets are: (1) suitable preclinical models for product efficacy testing may not exist, (2) the same concerns as noted above for genetically modified animals are also relevant for understanding the level of target dysfunction and the degree of pharmacologic inhibition needed to accurately mimic genetic dysfunction, and (3) the data must be carefully interpreted; in GWAS studies the infertility effect may be the result of genetic variation in multiple genes or from multiple single nucleotide polymorphisms.

Target "druggability"

In addition to tissue specificity and validation, a third characteristic that must be carefully considered prior to initiating a drug development program is "druggability." Historically, druggability meant that the target involved a mechanism of action that was amenable to functional modulation by a small molecule. Common classes of targets that are thought of as "druggable" included kinases, ion channels, ion transporters, dehydrogenases, deubiquitinases, phosphatases, and G Protein Coupled Receptors. With respect to contraceptive development, problems with targeting these groups of molecules arise when common substrate binding sites are used. For example, targeting a kinase ATP binding site will inhibit the activity of the kinase of interest, but ATP binding sites are common to many molecules and the importance of specificity/selectively discussed above is highly compromised, if not forfeited.

Targeting structural proteins and protein:protein and protein:nucleic acid interactions were once considered too difficult for any therapeutic intervention. However, the past decade has seen strong scientific advances in this type of approach [38–40], not to mention greater acceptance of using these interactions to develop therapeutics. These important research and development efforts improve our understanding how to best target these interactions. To date, however, protein:protein and protein:nucleic acid inhibitors have not been developed with a side effect profiles suitable for the development of a contraceptive.

Novel screening techniques and mechanisms to target protein degradation may play an important role in expanding acceptance of what is considered "druggable." DNA-encoded chemical library screening can be used to rapidly interrogate millions of DNA-tagged chemicals per library by measuring binding (affinity) rather than

inhibition of target function. On the surface, identifying molecules that bind may appear inferior to molecules that inhibit function, but there are two important points to consider. First, a two-step process of identifying molecules that bind followed by a screen for inhibition may be worthwhile given the enormous number of compounds that can be rapidly screened for binding using DNA-tagged chemicals. Second, identification of molecules with specific and high affinity binding to the target are of interest regardless of whether they cause target inhibition. They could be integrated with emerging technologies such as Proteolysis Targeting Chimaeras (PROTACs), which direct the targeted protein into cell protein degradation machinery, abolishing the entire target molecule and thus target activity. The synergistic use of these two technologies are expanding the classification of molecules that are considered "druggable."

The characteristics of tissue specificity, validation, and "druggability" are all important. However, satisfying all three of these characteristics does not ensure program success. Prime examples are the testis-/sperm-specific glycolytic pathway of enzymes glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase (PGK2) and lactate dehydrogenase (LDHC). All play key roles in sperm energy metabolism and are (1) testis specific (2) validated by targeted deletion [5, 41, 42], and (3) enzymes, an accepted "druggable" class of molecules. None of them have been successfully targeted as contraceptives. A key impediment to the development of these targets is their similarity to isoforms that are expressed outside the reproductive tract, especially within the catalytic domains. Similarity to non-reproductive tract-specific molecules is a frequent impediment to the development of validated, reproductive-specific targets. This problem helps explain why the emerging technology of PROTACs described above is intriguing, as PROTACs require target-specific binding (not target-specific functional inhibition), thereby allowing targeting outside of conserved catalytic/functional domains. There is general agreement that it is easier to identify target-specific binding as opposed to target-specific inhibition because the non-catalytic regions of isoforms are less similar than the catalytic domains of isoforms.

Site of action of contraceptives

There is a frequent discussion in the field of contraception—particularly male contraception, about the ideal site of contraceptive action (e.g., early in spermatogenesis leading to azoospermia or postspermatogenesis to perturb sperm maturation/motility). This topic has not been addressed at length in the literature and we have chosen to mention it briefly. Although discussion of this topic could include considerations for both male and female contraceptives, here we focus on the male.

As described above, there are many male reproductive specific/selective targets involved in the processes of spermatogenesis and sperm maturation and their sites of target action exist throughout the male reproductive tract. Some researchers argue that it is most beneficial if a method results in oligospermia or azoospermia. Other researchers suggest that it is preferable to have a method that acts against fully developed sperm. A third position is that the best method results in the production of morphologically abnormal sperm, which are incapable of fertilization. We will address each and provide comment.

Methods that result in oligospermia or azoospermia are advantageous because (1) total sperm number is a reliable indicator of infertility, (2) the measurement of total sperm output from ejaculates is an excellent (indirect) biomarker of pharmacodynamics, (3) samples of ejaculate are generally easy to obtain from clinical trial participants, (4) both the quantity and concentration of sperm in those samples can be determined in a non-subjective manner and, perhaps most importantly, (5) although male hormonal contraceptive methods have yet to complete successful clinical development, their long history has been extremely important in establishing the regulatory pathway for male contraceptive methods that result in azoospermia. The primary disadvantage of these methods is the long duration required to establish and reverse a contraceptive effect. The most highly studied methods that result in azoospermia/oligospermia are hormonal in nature where spermatogenic failure occurs pre-meiotically. These methods are associated with a median time to contraceptive effect of 2.73 months [43], and the time needed for reversal is generally accepted to be similar. The significant reduction in testicular size is often raised as a potential disadvantage, although clinical studies suggest that it may not be an impediment to patient compliance (Johnston, personal communications).

Systemically acting methods of male contraception directed toward mature sperm are also under development [44–46]. Their primary advantage is quick onset of contraceptive efficacy (potentially within hours) and rapid reversibility [44]. As spermatogenic output is not affected testicular size does not change. The primary concern with this approach is whether these methods render nearly all sperm—hundreds of millions of sperm—incapable of fertilization for the duration of sperm viability. The regulatory pathway (e.g., required Investigational New Drug Application (IND) enabling studies, clinical trial design) has not been established for such products, which some view as an impediment. Clearly, new regulatory pathways will need to be established, as is generally the case with innovation.

The two scenarios above related to male contraception represent the physiological extremes of spermatogenic output (complete ablation, full production). Other methods under development may result in sperm that are morphologically abnormal [7]. As might be expected, the pros and cons of these methods fall "in the middle" of the pros and cons of the other target scenarios discussed. Time to contraceptive effect is reduced compared to oligospermia/azoospermia methods but is not as rapid as intervention at the level of mature sperm. Testicular volume may be affected, but if the methods allow meiosis prior to apoptosis or if morphologically abnormal sperm are produced, the reduction in volume will not be to the extent of methods targeting oligospermia/azoospermia. If the degree of morphological abnormality is variable in the sperm population (as is often seen via targeted deletion), a heterogeneous population of sperm may result, leading to questions about whether some sperm may retain functionality and fertilizing capacity.

Ultimately, the requirement for any approved method of contraception is that it be safe and effective. Contraceptives that fulfill these requirements, regardless of site of activity, are worthy of consideration for development.

Lessons from research on the development of targeted non-hormonal methods of contraception

We have chosen to highlight three programs, Na, K-ATPase α4, HCA and Eppin. Each program is reviewed in greater detail in this special issue of Biology of Reproduction (BOR). We chose to highlight these three programs because the research associated with their development has provided important insights into approaches for contraceptive product development.

Na,K-ATPase

Na K-ATPase is the primary plasma membrane ion transport system that maintains low-intracellular sodium and high-extracellular potassium levels in the cell, thus regulating plasma membrane potential and intracellular pH. This transporter protein is formed by the association of two subunits, designated alpha (α) and beta (β). In mammals there are four Na^+/K^+-ATPase alpha subunits ($\alpha1$, $\alpha2$, $\alpha3$, $\alpha4$) and three beta subunits ($\beta1$, $\beta2$, $\beta3$). The predominant subunits in mammalian sperm are $\alpha1$, $\alpha4$, $\beta1$ and $\beta3$; the $\alpha4$ subunit is testis-specific and expressed only in spermatocytes and round spermatids. Male mice lacking the Na^+/K^+-ATPase $\alpha4$ subunit are sterile, validating it as a target for male contraception.

The development of Na^+/K^+-ATPase $\alpha4$ selective ouabain analog inhibitors has been described [47]. Incubation of isolated rat caudal epididymal sperm with 10 nM of a compound designated Compound 25 for 1 h significantly decreased all sperm motility parameters and inhibited capacitation by approximately 70%, suggesting a potential use as a female controlled, vaginally-administered on-demand method. In addition, the effects of Compound 25 were investigated following oral administration in rats. When orally dosed at 5 mg/kg for 3, 6, 9, or 12 days, isolated caudal epididymal sperm demonstrated a nearly 40% reduction in total motility and 50% reduction in progressive motility compared to untreated control animals. Taken together, these studies suggest that inhibitors of this target (and potentially the same inhibitor) could potentially be developed as both a systemically acting male-based contraceptive or as a female-controlled on-demand contraceptive.

Human contraceptive antibody (HCA)

The strategy of administering exogenous monoclonal antibodies that agglutinate and trap sperm in the lower female reproductive tract was postulated years ago [48–50]. Several proposed monoclonal antibodies have been directed against a male reproductive tract-specific carbohydrate on CD52, designated CD52g. CD52 is also known as CAMPATH-1 antigen, a glycosylphosphatidylinositol (GPI) anchored protein found in multiple locations in the body, including lymphocytes [51]. The CD52g variant contains a unique male reproductive tract N-linked carbohydrate antigen that distinguishes it from CD52. CD52g is expressed and secreted by the epididymal epithelium, vas deferens, and seminal vesicles [52], is found in seminal plasma and is tightly bound to sperm via the GPI anchor [53]. The unique N-linked glycan epitope(s) has been shown to be a target for the two best characterized antisperm antibodies against CD52g, the human H6-3C4 monoclonal, and the murine S19 mAb [54]. The mechanism(s) by which CD52g transitions from the epithelium of the epididymis, vas deferens, and seminal vesicles to sperm has not been elucidated. The molecule is also detectable in semen exosomes and in a soluble form in seminal plasma.

A human IgM antibody that interacts with CD52g was identified from immortalized B cells produced from an infertile woman with a high titer of sperm-immobilizing antibodies [55]. The antibody was designated H6-3C4. HCA was derived from the published variable sequence of H6-3C4 and engineered with an IgG1 Fc region rather than the natural IgM Fc region found on H6-3C4, allowing opportunities to modify the valency and structure through protein engineering. Initial studies have demonstrated that HCA has both contraceptive and anti-infective properties (Trichomonas vaginalis and HIV-1). The anti-fertility effects result from rapid agglutination and immobilization of sperm. The anti-infective properties arise from agglutination of the infective agents following incubation with seminal fluid which contains CD52g. The goal of the HCA development program is to produce an on demand topical contraceptive using vaginal films or suppositories as delivery systems, or a longer-term method using constant delivery of antibody via a device such as a vaginal ring. This development program demonstrates how a male reproductive tract-specific target can be used as a female-controlled method.

Eppin

Eppin is a sperm surface protein found in primates and human. Although it has been detected on sperm as they enter the human ductuli efferentes, the highest levels of Eppin expression are epididymal. During ejaculation, Eppin becomes saturated with Seminogelin-1 (SEMG1). Following ejaculation, SEMG1 bound to Eppin on sperm is hydrolyzed by activated protein-specific antigen (PSA) [56] secreted from the prostate gland. In a normal fertilization scenario, ejaculated sperm coated with SEMG1 are incapable of forward motility. The ability for forward motility is gained following SEMG1 cleavage by PSA. Failure to remove SEMG1 due to a lack of PSA activity results in seminal hyperviscosity and infertility [57–60]. Antibodies against Eppin that block the Semenogelin binding site on Eppin have been used to map the interaction site and small molecule inhibitors have been developed to inhibit the binding of Semenogelin to Eppin. Recently a lead molecule, EP055, was described and tested. Four macaques were infused initially with a low dose of EP055 (75–80 mg/kg) and approximately >70% inhibition of ejaculated sperm motility was observed at 30 h and 78 h postinfusion. In a second experiment, 2 weeks later a dose of 125–130 mg/kg of EP055 was administered [44] and 6 h postinfusion sperm motility fell to approximately 20% of pretreatment levels. At the 30 h postinfusion time point the sperm were immotile. Partial recovery of sperm motility was observed at 78 h postinfusion and full recovery was observed in all animals at 18 days postinfusion. No signs of toxicity were observed. Although the plasma half-life for EP055 was short (10.6 min) significant concentrations of EP055 were identified in semen samples at 6-h (0.539±0.186 mM), 30-h (0.376±0.168 mM), and 78-h (.101±0.030 mM) postinfusion.

Further testing will determine whether EP055 has the properties to serve as a novel male contraceptive. In the meantime, there are two important findings noted from this study: first, Eppin appears to be a suitable target for pharmacologic male contraception. Second, Eppin is a target for the development of an "on demand" male contraceptive.

Non-targeted methods for contraception

Across therapeutic areas within industry, most drugs under development are "targeted" compounds (e.g., small molecule and antibody) designed to modulate a specific molecule (biological process) of interest. However, within the field of contraceptive development, a significant percentage of development programs act via mechanisms that are non-specific. These programs are intriguing as they do not rely on the identification of a specific modulator, and so generally have reduced development time, less cost, and lower risk. Several of these programs are currently in, or approaching, clinical development.

One product, Amphora, is in late stage clinical development (http://www.evofem.com/). Amphora is a non-cytotoxic spermicide composed of citric acid, L-lactic, acid and potassium bitartrate that functions by maintaining the acidity of the vagina following coitus and was approved by the FDA as a vaginal lubricant in 2004. In

addition, data suggest that Amphora may be effective as a topical anti-infective [61]. For a recent review of Amphora, see Nelson [61].

In addition to Amphora, two other potential non-specific, non-hormonal products are Polyphenylene Carboxymethylene (PPCM) by Yaso Therapeutics (https://www.yasotherapeutics.com/) and Ovaprene® by Daré Biosciences. PPCM is a topically applied polymer under development for contraception and inhibition of sexually transmitted diseases. Ovaprene® is an intravaginal ring (IVR) that releases ascorbic acid and ferrous gluconate and contains a knitted polymer barrier to prevent sperm from progressing into the cervical canal. Daré Biosciences recently completed a postcoital test clinical study (NCT03598088) and reported positive results in a press release.

Advances in delivery devices will also drive contraception development

Dermal patches, microneedles, intrauterine systems, intravaginal rings, and vaginal films are important drug delivery systems in the field of contraception. The development of these and other medical devices, such as condoms, sponges, and diaphragms, requires less time and cost than small molecule therapeutics developed via medicinal chemistry and have a more rapid and reduced development risk profile, thereby providing a product developmental strategy more amenable to incremental changes over time.

In the current arena of contraceptive development, the large capital investment is absent to drive a portfolio of high-risk medicinal chemistry-based development. The coupling of novel or improved delivery devices with clinically approved active pharmaceutical ingredients (API) greatly reduces risk while allowing continued innovation. Two examples of medical device technologies that have improved significantly over the last decade are vaginal films and intravaginal rings.

Vaginal films are thin strips of polymeric water-soluble compounds formulated with an API that dissolves and is released locally when placed into the vaginal cavity [62]. Depending on the therapeutic indication and the desired characteristics of the API(s) to be delivered, the active ingredient may or may not be absorbed systemically. The films are engineered to dissolve at a specific rate following contact with vaginal fluids to form a viscous gel, with user preferred characteristics (e.g., color, odor, taste, softness, and flexibility) [63]. Significant advances in the field of vaginal film development have been realized primarily by research efforts in the HIV/anti-infective field and have included the introduction of new in vitro model systems to quantitatively evaluate film disintegration times [64] and the incorporation of nanoparticles to facilitate multiple drug doses [64, 65]. The development of vaginal films capable of antibody delivery has not been described, although a phase I clinical trial of MB66, a combined anti-HIV and anti-HSV monoclonal antibody film was recently concluded (clinicaltrials.gov; Identifier: NCT02579083). In addition, while vaginal films have historically been viewed as an "on demand" delivery method, the development of vaginal films capable of providing contraceptive protection over an extended duration (e.g., weeks) for multiple coital events may be feasible.

Intravaginal rings have a history spanning over 40 years [66, 67]. Specific reviews have been published on the use of IVRs for contraception [68, 69] and anti-infective therapeutics [70]. A highly significant recent advance in the field of IVR development involves the incorporation of technology engineered into the ring design to allow determination of user compliance during clinical trials and thus ensure validity of the data obtained. In 2015, Boyd et al. [71] demonstrated the successful incorporation of temperature sensors into an IVR that could accurately monitor ring insertion or removal in cynomolgus macaques. The technology was extended in 2017 by Moss et al. [72] who developed and tested a simplified, lower cost temperature monitoring system in sheep. Thirty-three simulated insertion and removal cycles were performed. These important product developments could be crucial for accurately measuring participant compliance during clinical trials and are likely the first of several data recording/monitoring systems that could be incorporated into vaginal rings to strengthen understanding of patient adherence. This type of measurement system could also allow for better understanding the vaginal environment to which an IVR is exposed during a clinical trial (e.g., pH sensors to measure coital events, etc.).

Considerations for pipeline development

A product development pipeline is the set of programs under development that an organization (e.g., drug companies, philanthropic organizations) is supporting at a point in time. An important consideration for any product development pipeline is how to maximize the available resources (dollars, personnel, etc.) for maximal return on investment. It is imperative to balance risk and innovation. Highly innovative work typically carries a significant risk that there will be no return on investment—an unsustainable condition for any organization. Conversely, if a pipeline is developed where success is highly probable and minimally risky, the products in the pipeline often lack innovation and fail to distinguish themselves from existing products, and consequently do not earn enough market share to justify the investment. An important consideration for any product development pipeline is to balance risk/innovation.

The mechanism of action of current combined female hormonal contraceptives is effectively the same as those developed almost 60 years ago. That statement could be interpreted to imply that there has been a lack of innovation with respect to this contraceptive method, yet quite the opposite is true. The history of hormonal female contraceptives demonstrates a succession of constant but modest innovation in nearly all areas aside from the modulation of the HPG axis, including delivery methods (e.g., transdermal patches, subcutaneous implants, and vaginal rings), new molecules (e.g., new generations of steroids and steroid receptor modulators) and perhaps most importantly dose modifications. The result has been greater choices for women and improved safety. Although the development time required for new drug products is significant, companies have minimized risk not by changing the basic therapeutic paradigm, but rather by embracing low/modest risk innovation associated with product improvement. This has been an acceptable business strategy because robust consumer demand made the financial risk/benefit ratio acceptable. Many companies employed this strategy with great success.

Around the turn of the millennium, several leading pharmaceutical companies with products in the contraceptive market established in-house research and development groups to develop innovative non-hormonal based contraceptives. Although the reason for initiating these programs was not publicly disclosed, one might guess that these companies questioned whether significant innovation was left to exploit in the female hormonal contraceptive product portfolio and wanted to lead a paradigm shift toward non-hormonal contraception. Surprisingly, given their past successes with female hormonal contraception, there is no evidence that any large

pharmaceutical company pursued male hormonal contraception as part of an R&D strategy during that time. Companies did invest in the high risk, high innovation medicinal chemistry-based programs to develop non-hormonal systemic contraceptives, as did other investors in the contraception space, including the United States National Institutes of Health. The degree of financial commitment of large pharma to develop novel non-hormonal contraceptives is difficult to determine, as private companies closely guard information relative to their research investments. Yet by 2010, large pharma R&D investment in medicinal chemistry-based non-hormonal contraceptive product development was undetectable. Fortunately, government and private funding agencies continued to invest but also without a successful targeted non-hormonal program entering the clinic [1, 24].

So, does this mean that targeted medicinal chemistry-based projects should not be pursued for contraception? Not at all. Compelling results in medicinal chemistry-based programs include the recent findings from the Na K-ATPase $\alpha 4$ and Eppin programs described above. Medicinal chemistry-based programs, which are long in duration, high risk and high investment—yet innovative—are an important component of a product development pipeline. Additional resources could be directed toward product development programs that are less complex, and faster to clinical development, even if potentially less innovative compared to programs focused on the development of systemic non-hormonal contraceptives. Such programs are likely to include a focus on the development of delivery devices, the development of polymer-based products such as PPCM, barrier methods such as Ovaprene, or natural antibodies or bioengineered derivatives thereof (e.g., HCA described above). Data from these programs demonstrate the value of projects with a shorter and less expensive product development that may ultimately reach market and serve the goal of allowing for greater choice of non-hormonal contraceptive methods.

Product development in industry vs academic settings

The two authors of this review have over 80 years of experience in the field of reproductive biology, including many years that focused on developing novel contraceptive methods. During this time, they also gained insight into the administrative and cultural workings of industry, academic and philanthropic settings. During the preparation of this manuscript, the benefits and limitations of each setting on contraceptive development were frequently discussed. The authors' observations are briefly summarized here as a comparison of large industry (large pharma) vs academia; the two places deemed to provide the greatest contrast. The authors have great respect for (and experience in) the small company environment, but feel this area lies between the two extremes discussed. It is also worth noting that the goal of this section is not to criticize either setting discussed in the comparison. Rather, it is to highlight the strengths of both settings and to tentatively suggest that cooperation between the different groups could prove synergistic by leveraging the strengths of each. We view this as an important and viable consideration for the future.

Large pharma's product development armamentarium is extraordinary and importantly, not limited to their technological and financial resources. It also includes comprehensive drug development expertise and experience, effective establishment, and management of cross-functional drug development teams. These teams utilize outstanding cross-discipline communication, responsive project and portfolio management, regulatory expertise, a culture that promotes short-term deliverables to meet established guidelines, and an unambiguous delineation of responsibilities and intellectual property assessment, development, and prosecution.

Arguably, these characteristics are not the typical hallmarks of academic culture. However, aside from financial resources, many of these characteristics can be adopted, and some universities are actively pursuing progress on these fronts. For example, some academic centers are implementing well-equipped product/drug development centers, hiring highly qualified personnel (frequently from industry), and developing teams with cross functional capabilities.

Attributes that will be more difficult to change within academic product development sites are those associated with fast-paced communication and production of short-term deliverables. In the industrial culture, the success and survival of a product development team are predicated on meeting timelines and advancing the project. An employee's reputation, performance assessment, career trajectory, and even their continued employment can be tied to the outcome of a project. Group survival, program survival and individual indispensability are primary motives of every member. In academia, the unit of survival is the research laboratory, which relies on the lead investigator to obtain and retain funding, and to publish papers which are the academic currency for promotion and tenure. Ceding control of the laboratory's priorities, deliverables and timing of those deliverables for the benefit of a team effort is not standard practice within the academic culture.

Furthermore, authority structures differ between industry and academia. In industry, if timelines for the team effort are not met, job performance metrics, and career trajectories may be affected. In academia, collaborative bonds may be strong, but ultimately the degree of influence one lead investigator has over another's laboratory function is insignificant. Comparatively, academia has a long-standing culture of autocratic laboratory management.

If those are pharmaceutical industry strengths, what are the strengths of the academic environment? The four most obvious are: (1) a deeper understanding of the biological system being modulated (especially in smaller therapeutic areas), (2) passion, (3) the amount of time to reach milestones, (4) the ability to rekindle a project, and (5) ability to obtain financial support from multiple sources.

For the very large therapeutic areas within industry, such as oncology or neurology, staff often have their formal training in the specific discipline, followed by many years of focused employment in those therapeutic areas, and so the knowledge base is very deep. However, when smaller therapeutic area programs are established (e.g., contraception), frequently industry personnel will be repurposed from another therapeutic area where the focus has been downgraded. The logic behind such shifts is that the important principles of drug development will not need to be learned because they are already known, and that any newly required therapeutic area-specific scientific knowledge can be obtained with further study. However, it is extremely difficult to make up for years of training in a specific therapeutic area. Significant pitfalls may be encountered if a conceptual understanding of the biology of the therapeutic area (e.g., the blood testis barrier, capacitation, and spermiogenesis) and (often overlooked) the experimental knowledge associated with the area (e.g., gamete collection and handling, in vitro fertilization) is lacking or absent. Moreover, where a knowledge gap exists because a person has transitioned from the area of his or her education and training, another gap can insidiously arise- one involving passion (or lack thereof) for the work.

In academic research, the grant cycle is typically 5 years. For product development grant awards, this allows generous time to

work through difficulties such as those related to assay development or compound synthesis. Programs that may have experienced limited productivity during a grant cycle can still compete for additional funding. In industry, however, timelines for project advancement are short. Within industry, preclinical drug development projects have major milestones every 18–24 months. If milestones are not met, the project terminates, the product development teams disband, and resources are reassigned.

Within academic settings, an unsuccessful preclinical program can gain new life—a tremendous benefit to the academic culture. In academia, programs are often dropped due to insufficient funding, not a management structure that terminates support for the project with definitive finality. If a program stalls in academia, the investigator can reapply to the original sponsor or appeal to alternative funding sources in hopes of more favorable reviews. Modified research strategies or new information regarding validation of the target may convince a review panel to resurrect a project that had been suspended for years. One such example is Lactate Dehydrogenase C (LDHC), which was not pursued as a target for lack of funding. LDHC was among the first identified testis-specific gene products, demonstrated to be required for male fertility in mice [5] and confirmed in human [73, 74]. One of the major difficulties in the LDHC program was the high degree of similarity in the subunits of LDH tetramers (approximately 75%). However, recently obtained preliminary data demonstrates that the innovative DNA-encoded chemical libraries provide a new and promising screening strategy for LDHC to identify inhibitors able to selectively target LDHC from the other forms of LDH. After a gap in funding of several years, it has recently been announced by the Male Contraceptive Initiative that funded research on LDHC has begun again. This scenario is not uncommon within academia but is different from industry where when a program based on a specific target fails, the program is unlikely to be reinitiated later.

Summary

The 2019 NICHD Contraceptive Development Meeting demonstrated that the research and development area is innovative, active, and vibrant. As noted above, funding from government and philanthropic sources such as the NICHD, Bill and Melinda Gates Foundation and the Male Contraceptive Initiative are now the major drivers of these initiatives. Given typical attrition rates in product development, at most a small number of the programs presented will progress to the clinic. Even so, priority should be placed on supporting novel and innovative ideas forward to the extent practicable, so the discoveries are maximized. Inevitably, clinical development and regulatory approval of the best candidate products would benefit greatly from increased industry involvement. This is an obstacle that many leaders in the field, working together, must seek to mitigate. As emerging products spur greater interest, future support could be tapped from small biotech, midsize specialty pharma, large pharma or even financial investment institutions (e.g., venture capital). The best course of action for the field is to stay focused on innovative contraception product development to provide the impetus for investment.

Conclusions

Our experience leads us to the following conclusions. First, several product development programs exist today that demonstrate exciting possibilities for the future of contraceptive development, such as on-demand male contraception and MPTs. Second, product development pipelines should be balanced in terms of risk vs innovation, marketing strategy, and customer expectations. Third, the development of novel delivery devices and innovative biological technologies will continue to help drive contraceptive development. Fourth, non-targeted contraceptive development programs offer advantages of shorter product optimization timelines, which equate to reduced preclinical development costs and time to market. The environments for product development in industry and academia are inherently different, and important lessons can be learned from understanding the strengths of each setting and the opportunities for meaningful collaboration.

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