Advanced Drug Delivery Reviews 176 (2021) 113865

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/adr

Women-specific routes of administration for drugs: A critical overview

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ARTICLE INFO

Article history:
Received 13 May 2021
Revised 7 July 2021
Accepted 9 July 2021
Available online 16 July 2021

Keywords:
Drug delivery
Drug therapy
Gynecology
Microbicides
Nanomedicine
Obstetrics
Women's health

ABSTRACT

The woman's body presents a number of unique anatomical features that can constitute valuable routes for the administration of drugs, either for local or systemic action. These are associated with genitalia (vaginal, endocervical, intrauterine, intrafallopian and intraovarian routes), changes occurring during pregnancy (extra-amniotic, intra-amniotic and intraplacental routes) and the female breast (breast intraductal route). While the vaginal administration of drug products is common, other routes have limited clinical application and are fairly unknown even for scientists involved in drug delivery science. Understanding the possibilities and limitations of women-specific routes is of key importance for the development of new preventative, diagnostic and therapeutic strategies that will ultimately contribute to the advancement of women's health. This article provides an overview on women-specific routes for the administration of drugs, focusing on aspects such as biological features pertaining to drug delivery, relevance in current clinical practice, available drug dosage forms/delivery systems and administration techniques, as well as recent trends in the field.

Contents

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https://doi.org/10.1016/j.addr.2021.113865
0169-409X/

1. Introduction

Women and men are naturally different in terms of their biology. These dissimilarities are most evident in the distinctive sexual characters and reproductive physiology typical of each sex, but also occur in more elusive ways, from anatomical features down to the basic cell and molecular levels. Biological differences can have a tremendous impact in health and disease [1], and justify distinct responses to drug therapy [2]. For example, women are recognized for being more prone to experience side effects when using antithrombotic drugs [3], diuretics [4], serotonin reuptake inhibitors [5], or antipsychotics [6]. These observations are related with variations in pharmacokinetics and/or pharmacodynamics. Moreover, women undergo lifetime changes related with hormonal status and reproductive physiology, and these have been associated with modified response to multiple drugs, thus requiring specific attention [7]. During pregnancy, understanding the effects of drugs is not only vital to the mother, but also to the fetus. Despite these concerns, biomedical and medical research has focused heavily on males, which alone justifies why many deleterious effects of drugs in women were not identified until several years after market approval [8]. This paradigm, however, has been slowly changing, largely fueled by a general demand from various scientific, clinical and regulatory authorities for the mandatory inclusion of sex as a biological variable that needs to be addressed in pre-clinical and clinical drug testing [9–11].

Apart from problems created by the misrepresentation of females/women in pre-clinical and clinical research, biological sex differences open unique possibilities for the development of specific clinical approaches, including the way drugs are presented to the body. Much alike the field of pharmacological sciences as a whole, the relevance of women-specific routes of drug administration has often been overlooked, despite (in some instances) their long-standing usage and well-established place in medicine. The present article provides an overview and discussion on the routes of administration for drugs that are exclusively used for women. These stem from anatomical differences between women and men regarding primary and secondary sexual characteristics. Routes are divided into genital (vaginal, endocervical, intrauterine, intrafallopian and intraovarian), pregnancy-related (extra-amniotic, intra-amniotic and intraplacental) and breast intraductal. Although debatable, this classification provides a convenient systematization for the purpose of this work and its rationale is justified along the text. As much as possible, the standardized terms preferred by the US Food and Drug Administration [12] and the European Medicines Agency [13] are used. Exceptions include the intrafallopian and intraplacental routes, which are not considered in guidance documents issued by these last regulatory agencies. We will overview pertinent biological features of the anatomical sites in question, current medical use, and technological aspects and biopharmaceutics of drug products considered for each route, as well as recent advances and trends. The discussion will further incorporate brief historical insights, and basic description of administration techniques and procedures.

2. Genital routes

Virtually all compartments of the female genital tract have been explored and used as routes of administration for drugs. These include the vagina, the cervix, the uterus, the fallopian tubes and the ovaries (Fig. 1). Each route will be considered individually over the following sub-sections.

2.1. Vaginal

2.1.1. Background

The administration of drugs into the vagina is one of the oldest forms of pharmacotherapy. The vagina was among the five routes of drug administration considered in ancient Egypt, with first known written records about this practice dating back to circa 1850 BCE [14]. Although vaginal administration of drugs continued throughout the following ages across multiple cultures and until present times, its documentation has been often erratic and limited to contraception, abortion and pregnancy practices that were in large part developed during classical antiquity. Oral tradition and wisdom related with midwives further played an essential part in the continued use of the vaginal route [15]. No major changes occurred until roughly 150 years ago, when considerable advances started being built upon chemical, biological, anatomo-physiological and medical knowledge accumulated since the Renaissance [16]. For example, the first commercial contraceptive pessary (W. J. Rendell's Wife's Friend) based on quinine was introduced in 1885 [17], thrusting a whole new era of spermicide products. The studies by Macht [18] and Robinson [19] in the 1910s-1920s on the ability of different compounds to undergo vaginal

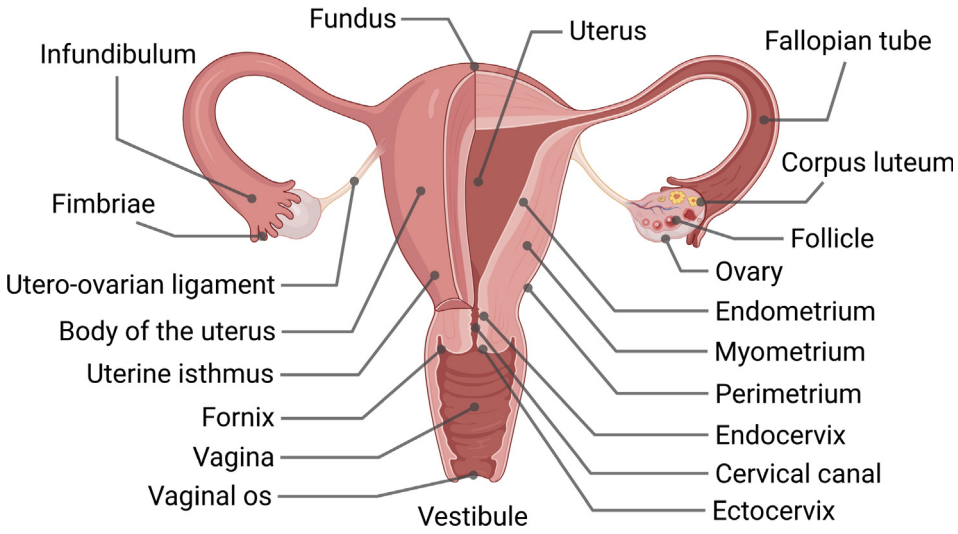

Fig. 1. Anatomy of the female genital tract (front view).

absorption were of particular importance, opening the door for new medical applications. Modern contraception would not be the same without the pioneer studies in the 1920s on the extraction and purification of estrogens [20] and progesterone [21] from the ovary, which propelled research in biochemistry and physiology and culminated in the 1960 s with the commercialization of oral hormonal contraceptives and, later on, led to the development of vaginal contraceptive rings. The interesting story of another key drug in modern obstetrics, dinoprostone (prostaglandin E2), also had several highlights throughout the twentieth century. All started with the intriguing report by Kurzrok and Lieb in 1930 on the effects of semen in the contractility of uterine tissue as related to the presence of the then unknown prostaglandins [22]. Isolation and chemical structure elucidation of dinoprostone was not possible until the 1960s [23], but such discovery quickly led to the clinical development and regulatory approval in the late 1970s of vaginal dinoprostone for inducing labor.

Modern drug products intended for vaginal administration have a well-established role in the therapeutic armamentarium, and benefited from the achievements made by the pharmaceutical industry regarding manufacturing technologies, quality control and clinical assessment. Over the last three decades, extensive advances regarding the vaginal route have been prompted in particular by the development of topical microbicides intended to prevent sexual transmission of pathogens, particularly HIV-1 [24]. The administration of drugs in the vagina presents several commonly advocated advantages that have sustained, at least partially, the use of this route until our days [25–27]. For instance, it allows easy and comfortable self-administration, most of the times without the need for intervention of a healthcare provider. Although some dosage forms are intended to be administer by hand, insertion often requires the use of a suitable applicator, either mandatorily (*e.g.*, liquids, semi-solids and foams) or optionally (*e.g.*, ovules, tablets, capsules). Vaginal products do not need to be sterile, and are usually cheap and relatively easy to manufacture. When drugs are used for managing local conditions, intravaginal administration means that lower doses are required (namely as compared to the oral route), which leads to reduced systemic exposure and lower onset of adverse effects. Also, from a psychological perspective, it makes sense for women to administer a drug product at the site where healing or protection is required [28]. Even in cases where systemic drug delivery is intended, the extensive vaginal absorption of relevant drugs such as sex hormones may allow achieving pharmacologically active blood levels with low doses. Disadvantages of the vaginal route are mostly related with non-medical issues such as gender specificity (which may demote drug developers from "targeting" only roughly half of the population), cultural and intimacy issues related with the manipulation of genitalia, or long-standing misconceptions regarding the safety of medical products inserted into the vagina [29]. Education is therefore paramount in expanding the use of the vaginal route for the administration of drugs. Some vaginal products may also cause discomfort during usage – which are typically related with messiness and leakage of liquid or liquefying systems – or even interfere with sexual intercourse and pleasure [30]. Physiological changes undergone by women throughout their life (including those occurring during menstrual cycle and pregnancy) may have considerable impact in drug and dosage form performance, and are important challenges in product design.

2.1.2. Features of the vagina and impact on drug performance

The vagina is a tubular mucosal site connecting the cervix to the vestibule (Fig. 1). Although comprising a descendent plane that drives expulsion of any intravaginally inserted objects, its sagittal S-like shape allows the upper part of the vagina to be on an almost horizontal plane when individuals are in the standing position [31]. This favors retention of products that are inserted near the cervix. This area is also largely deprived of free nerve endings and allows objects placed therein to be typically unperceived by users. The vaginal mucosa comprises an outer layer of non-keratinized stratified squamous epithelium lying on top of a vascularized lamina propria. The epithelial layer is responsive to hormonal stimulation, being thicker or thinner under estrogen or progestogen influence, respectively [32]. It also constitutes the major barrier to permeability and absorption. Still, multiple compounds (including several with high molecular weight) have been shown able to undergo transport across human vaginal mucosa *ex vivo*, presenting in many cases higher flux values than those observed in intestinal tissues [33–36]. Different studies using animal vaginal mucosa have also confirmed the potential of the vagina to serve as an interesting access site for systemic drug delivery [37–43]. Still, extensive permeability appears to be restricted to low molecular weight hydrophobic compounds. Drug transport occurs primarily by intercellular or transcellular passive diffusion depending on specific compound solubility [44], although receptor-mediated translocation is also possible [45–48]. Moreover, permeation enhancers have been shown useful in enhancing drug transport across animal vaginal mucosa, but the utility of these compounds needs to be balanced with their potential to induce deleterious effects on the epithelium, particularly if long-term use is intended [49–51]. Enzymatic degradation of drugs (namely therapeutic peptides and other labile compounds) at vaginal tissues and fluids is possible, but considerably lower than, for example, at the gastrointestinal tract [52,53].

The mucosal surface is covered by a scanty acidic mucous fluid that comprises for the larger part a mixture of cervical mucus (source of mucin) and tissue transudate, but also contains minor contributions from vestibular glands secretions, host cells and urine residues. The relatively low pH of this fluid (3.5–4.5) is related to the presence of lactic acid resulting from the degradation of host-produced glycogen, as mediated by lactobacilli present in natural microbiota [54]. This is representative of reproductive years and stems from high estrogen levels that lead to intense glycogen synthesis by epithelial cells. Drops in estrogen (*e.g.*, during pregnancy, and in pre- or post-menopause) or depletion of lactobacilli (*e.g.*, upon bacterial infection) results in an increase in pH to near neutral values [55]. The presence of menses and semen is also able to transiently augment pH and, possibly, impact drug kinetics by direct interference of components of these fluids [56]. Work conducted over the last two decades also contributed to the definitive recognition of cervicovaginal mucus as a chief barrier to the transport of drugs, pathogens and other materials [57–59]. Importantly, altered vaginal microbiota can induce important changes to the mucin fiber mesh that defines mucous fluids, and alter the barrier properties to particulates and important pathogens such as HIV-1 [60–62]. Microbiota can also determine the performance of drugs administered in the vagina by mediating their degradation and modulating interactions with epithelial cells, with recognized impact on efficacy, as shown in recent studies involving microbicides [63,64]. Additionally, dysbiosis can contribute to changes at the vaginal epithelium and, thus, potentially alter drug permeability [61,65]. These data highlight the importance of the highly dynamic cervicovaginal microbial community in the performance of vaginal drug products beyond their influence on basic aspects related with pH-dependent solubility and ionization (that can impact, for instance, permeability), and provide valuable hints for future development of strategies for vaginal drug delivery.

Access of drugs to the blood circulation following mucosal permeation is achieved through the vaginal veins of the utero-vaginal plexus, which ends up draining into the inferior vena cava via the pudendal vein. This enables bypassing early hepatic metabolism.

At the same time, the vaginal route provides a vein-to-artery countercurrent transfer of absorbed drugs that favors access to the uterus. This is due to the extensive vascular interconnections between the vagina and the uterus, and originates a regional accumulation mechanism that was coined in the 1990s as the uterine first-pass effect [66]. Such preferential transport seems to occur only at the upper third of the vagina [67], and may be beneficial for compounds intended to have a pharmacological action at the uterus, such as sex hormones and analogues [68].

2.1.3. Role in current clinical practice

The vaginal route has been primarily used for the administration of drugs intended to manage local conditions. One particularly important application is the treatment of infection. Topical administration of drugs such as metronidazole and clindamycin for bacterial vaginosis (BV), or various imidazole compounds (*e.g.*, clotrimazole and miconazole), nystatin and boric acid for vulvovaginal candidiasis (VVC) are often recommended [69,70]. Different commercial products are available in the form of ovules (also known as vaginal suppositories), tablets, creams and gels, several of which are sold as over-the-counter. Topical treatment of bacterial and *Candida* spp. infection appears to be as effective as oral regimens and causing limited systemic adverse effects. Alternative topical treatments have been studied and even routinely used in clinical practice (*e.g.*, probiotics for BV or plant extracts for VVC), but efficacy remains unclear [71,72]. Still, research on new agents and treatment approaches is deemed urgent because of the emergence of drug resistance across pathogens causing vaginal infections. The human papilloma virus (HPV) is another important pathogen causing infection in the lower genital tract. Vulvar warts associated with the infection can be treated by topical self-application of imiquimod or podofilox available as creams or solutions [70]. Highly concentrated trichloroacetic acid solutions (80–90% in volume) can be used for the treatment of both vulvar and cervicovaginal warts, but application needs to be performed by a physician. Particular care should be taken in order to avoid spreading beyond the lesion due to the caustic nature of the solution. The use of a cotton-tipped applicator is recommended. Intralesional injection of interferon may further be a useful alternative for the management of cervicovaginal warts [73].

Contraception can be attained by the vaginal administration of spermicides such as nonoxynol-9, octoxynol or benzalkonium chloride, although with only mild efficacy [74]. This is a longstanding practice, and several commercial products are available in a variety of dosage forms including gels, films, tablets, ovules, foams or sponges. For example, the Today® sponge (Mayer Labs) is a quite popular over-the-counter contraceptive in the United States and is available since 1983 (although being discontinued between 1995 and 2005 due to manufacturing issues) [75]. The sponge comprises nonoxynol-9 dispersed in a disk-shaped deformable polyurethane sponge (approximately 7.6 × 3.8 cm) that allows fitting the cervix with its concave side (Fig. 2) [76]. Once in place, the sponge creates an additional barrier to sperm migration. The system further includes a fabric loop band on the side intended to face the vagina, which allows easy removal after usage. Several spermicides have further been shown to possess antimicrobial activity *in vitro*, and were thus suggested as potential microbicides for preventing sexual transmission of pathogens. However, different clinical trials failed to demonstrate this possibility and, in some cases, actually showed that spermicides can increase the likelihood of women to be infected by HIV-1 [77]. This apparent paradoxical outcome was correlated with the deleterious effects that spermicides have on the epithelial barrier and the onset of pro-inflammatory events facilitating viral transmission [78].

Fig. 2. The Today® contraceptive sponge. Reprinted from [76] by permission of Springer Nature, Copyright (2020).

Hormonal contraception is also possible using the vaginal route. The excellent absorption of different progestogens and estrogens through the mucosa, as well as the prevention of their rapid premature elimination due to extensive hepatic metabolism, allows obtaining adequate systemic hormone levels for inhibiting ovulation. The challenge here is to devise strategies that allow sustaining drug release in the vagina for extended periods, thus avoiding the need for frequent dosing. The vaginal ring was initially developed in the 1970s in order to address this issue [79], but its clinical use was not widely successful until the introduction of the NuvaRing® (Merck) in the early 2000s. Vaginal rings are flexible, toroid-shaped polymeric systems that not only allow controlled drug release, but also enable excellent retention due to the slight pressure exerted on the vaginal wall. This dosage form is intended to be self-inserted deeply inside the vagina without the need of an applicator, where it can reside for weeks or even months without causing discomfort to users. Materials such as polysiloxanes, poly (ethylene-*co*-vinyl acetate) (EVA) or polyurethanes, among others, and different manufacturing designs have been used for producing rings and controlling the release of drugs [80]. For example, the NuvaRing® is an EVA reservoir-type ring – *i.e.*, the core comprises a blend of active ingredients and polymer, which is then covered by a non-medicated sheath of plain polymer (Fig. 3**A**) – that is able to release etonogestrel and ethinyl estradiol at constant rates of roughly 120 μg/day and 15 μg/day, respectively, for at least 21 days [81,82]. Combination rings allow maintaining stable and low blood levels of delivered hormones (Fig. 3**B**), despite providing similar efficacy to hormonal contraceptives delivered by other routes [83]. This can potentially lead to lower onset of adverse effects. Sex hormones delivered by the vaginal route have also been used for other purposes such as hormone replacement therapy. For instance, intravaginal low dose estrogens, with or without progestogens, are highly effective in the management of symptoms associated with decaying estrogen levels during menopause [84]. Despite controversy dealing with increased incidence of breast cancer and coronary heart disease in women undergoing replacement treatment with estrogens, the benefits appear to exceed risks, and different vaginal products are currently available in the form of creams, tablets or rings. Luteal phase support with vaginal progesterone during assisted reproduction is another well-established clinical practice [85]. Here, the uterine first-pass effect may be particularly beneficial since direct action in the uterus is intended. Vaginal progesterone is also used for preventing pre-term birth [86], but its efficacy has recently been challenged [87]. A new segmented silicone-based ring combining levonorgestrel with anastrozole (an aromatase inhibitor) is currently under clinical testing for the long-term treatment of endometriosis [88].

Cervical ripening and induction of labor can be attained by the vaginal administration of prostaglandins such as dinoprostone or

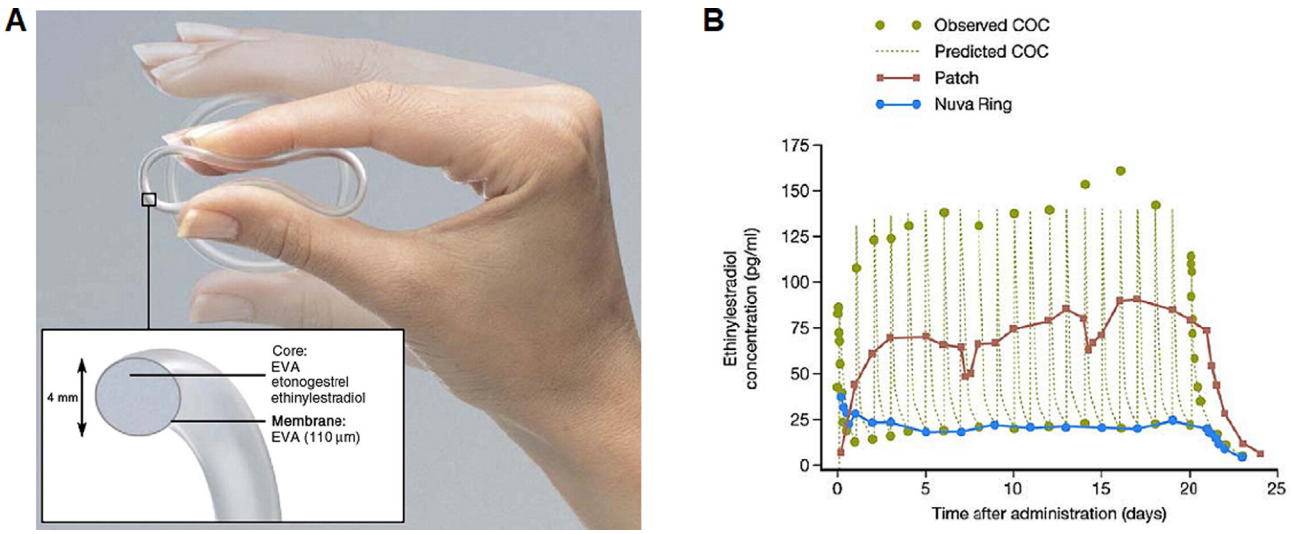

Fig. 3. **(A)** The combined contraceptive vaginal ring NuvaRing® (54 mm in diameter). **(B)** Mean serum ethinyl estradiol concentration–time curves for women treated with NuvaRing® (n = 8), a transdermal combination contraceptive patch (Evra™, Ortho-McNeil – releases 20 μg of ethinyl estradiol/day; n = 6) or a daily combination oral contraceptive (COC, Microgynon®, Schering – contains 30 μg of ethinyl estradiol; n = 8). *Panel A* modified from [81], Copyright (2007), and *Panel B* modified from [83], Copyright (2005), with permission from Elsevier.

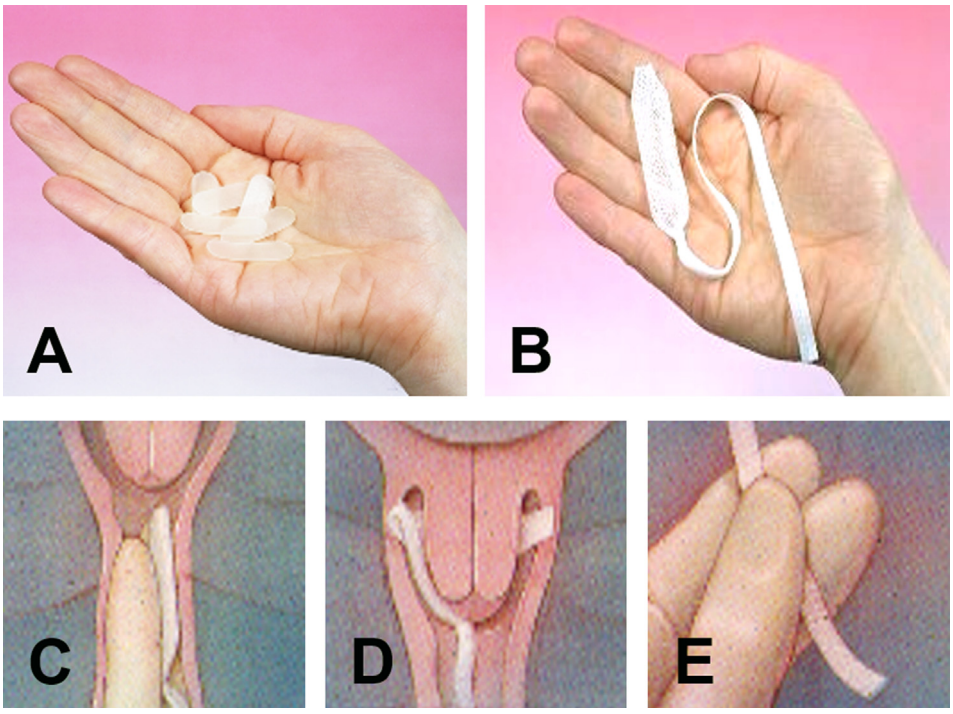

Fig. 4. The Cervidil®/Propess® vaginal delivery system. **(A)** Individual inserts containing dinoprostone and **(B)** the complete system. **(C-E)** The system is intended to be inserted near the cervix, where it stays until retrieval at the onset of labor or in the case of adverse effects to mother and/or fetus. Image kindly provided by Dr. Janet Halliday.

misoprostol [89]. Although gels, ovules or tablets are also available, both drugs have been formulated as controlled release inserts based on non-biodegradable crosslinked polyethylene glycol hydrogels that were specifically designed for vaginal administration. Inserts are encased in a knitted medical grade polyester net that ends in a tail intended for retrieval of the system (Fig. 4). For example, Cervidil® (available as Propess® in some countries; Ferring) is a commercially available dinoprostone insert that releases its drug payload at an approximate rate of 0.3 mg/h over a period of 24 h, after being placed near the cervix by a healthcare provider [90,91]. A similar misoprostol insert (Misodel™/Mysodel le™, Ferring) was also available [90], but was recently withdrawn from the market due to lack of commercial success. Vaginal prostaglandins, in particular misoprostol, have further been used for pregnancy termination, alone or preceded by oral administration of the antiprogestogen synthetic steroid mifepristone [92]. Misoprostol in the form of tablet can be self-administered following medical initiation of the treatment. These should be placed deeply in the vagina, and women should remain in a recumbent position for approximately 30 min in order to assure retention. Vaginal administration of misoprostol is considered more effective than the oral route for both first and second trimester pregnancy

[93,94], which could be justified by the extensive absorption of the drug across the mucosa [95].

Although local treatment is not part of the standard of care for cancer, various clinical case reports indicate that this could be a viable strategy. Dosage forms such as vaginal creams, gels and suppositories have been recently used for the vaginal administration of 5-fluorouracil or imiquimod with relative success in the treatment of lesions associated with vulvar, vaginal and cervical intraepithelial neoplasia [96,97]. The use of photosensitizers (*e.g.*, 5-aminolevulinic acid) for subsequent photodynamic therapy has also been shown promising, but definitive clinical evidence on efficacy and safety are missing [98]. One particular issue preventing the widespread clinical use of these products concerns damage induced to adjacent healthy tissues [99].

Beyond safety and efficacy issues, one key aspect when considering the vaginal route relates with acceptability and preferences of women. This is no trivial matter and can ultimately determine the success (or failure) of a product or a clinical approach. Multiple studies have been conducted in order to determine which dosage forms, specific attributes of products, applicators (when applicable), and type of usage are preferred by current and prospective users under different clinical settings [100–111]. Results, however, have been heterogeneous and idiosyncratic, but it appears that women are generally receptive to the vaginal administration of drug products. Factors such as age, sexual activity, partner attitude, socio-economic status, cultural issues, clinical problem being addressed, and previous experiences seem to play relevant roles in determining product preferences, while counseling and proper training appear to be effective in optimizing acceptability and enhance adherence. Overall, accumulated knowledge advises that multiple products and strategies should be available for women to choose from, and in order to provide individuals with the best fitting solutions to their lifestyle and clinical needs.

2.1.4. Recent advances

Progress in vaginal drug delivery over the last decades has been chiefly driven by research and development efforts in the field of anti-HIV vaginal microbicides. These products are intended to be present in the vagina around the time of sexual intercourse in order to prevent male-to-female viral transmission events occurring at the mucosa [24]. Epidemiologist Zena Stein at Columbia University is often cited as being responsible for establishing the foundations of modern microbicides in 1990 with her seminal commentary article on HIV prevention [112]. In particular, she called for the development of strategies and products that could empower women with new options for protection against sexual transmission of the virus. The following years were marked by multiple clinical trials testing compounds and products that were already available in the market (namely containing the spermicide nonoxynol-9) or specifically developed as microbicides (*e.g.*, formulations containing cellulose sulfate, naphthalene 2-sulfonate polymer, C31G, or carrageenan) [113]. However, results from these studies were disappointing [114–119]. These so-called first generation microbicides were mostly formulated as gels and had non-specific antimicrobial activity – which actually led to their proposal for the prevention of multiple sexually transmitted infections –, and impacted the vaginal environment in a deleterious way. Toxicity was, in some cases, even responsible for increased viral transmission [114,115]. These harsh outcomes highlighted the need for vaginal products that interfered minimally with natural local defense mechanisms. Changes induced by products to the vaginal environment are often subtle and cannot be readily identified using standard clinical evaluation tools such as colposcopy examination [120]. Toxic insult can translate into modifications of natural microbiota [121,122], and/or the onset of pro-inflammatory response at both the lower and upper genital tract, which can be monitored by changes in levels of soluble immune mediators or immunohistochemistry [123–126]. Importantly, different excipients considered safe for vaginal administration due to their previous extensive use in commercial products were also identified as being able to trigger pro-inflammatory responses and cause mucosal damage, thus potentially increasing the susceptibility to HIV acquisition [127]. Deleterious effects can result from inherent toxicity of the materials used or be related with changes induced to the whole formulation. The implications of these advances have now transcended the microbicides field and constitute important guidance for the development of safe vaginal products, namely those most likely to be used during sex [128–130]. For instance, the World Health Organization has recently issued draft recommendations concerning pH (5.0 to 7.0) and osmolality (below 1200 mOsm/kg) of lubricants used with condoms [131].

Notwithstanding the disappointment of early clinical results, research in the field of vaginal microbicides turned its attention towards potent antiretroviral drugs and different formulation approaches. Such efforts soon paid off, with the first report of a successful clinical trial arriving in 2010. The CAPRISA 004 phase IIb study was able to show that a 1% tenofovir gel could reduce male-to-female HIV-1 transmission by 39% [132]. The gel also decreased the transmission of type 2 herpes simplex virus (HSV-2) [133]. Even if only partial protection was observed, this study established the much needed and long sought proof-of-concept for microbicides. However, other 1% tenofovir gel formulations used according to different administration schemes were unable to replicate protection against HIV-1 in subsequent phase III clinical trials [134,135]. One important lesson emerging from these and other trials was related with adherence: microbicides were not used consistently, in part due to their coitus-dependent status. Indeed, parallel efforts led by the nonprofit organization International Partnership from Microbicides focused on the development of a vaginal ring for the delivery of dapivirine, an investigational non-nucleoside reverse transcriptase inhibitor (NNRTI). Similarly to contraceptive rings, the system allows maintaining tentatively protective levels of the drug in the vagina for one month, or even longer, without the need for frequent administration as in the case of tenofovir gels [136]. It comprises a matrix silicone-made ring containing 25 mg of dapivirine that was initially developed by researchers at Queen's University Belfast [137]. Results from two independent phase III clinical trials released in 2016 showed that the ring was acceptable, safe and could decrease male-to-female transmission of HIV-1 by 27% to 31% [138,139]. Again, adherence to ring use was key in determining vaginal drug levels and the degree of protection. Efficacy results were further confirmed in two subsequent open-label extension trials [140,141]. The ring has recently received favorable recommendation from the European Medicines Agency [142] and the World Health Organization [143], and is now on the final stages of the evaluation process for regulatory approval in several sub-Saharan African countries. Additionally, different versions of the dapivirine ring are under development in order to co-deliver other drugs [144,145]. Of particular interest is the ongoing clinical testing of a combination ring of dapivirine and levonorgestrel, which constitutes a multipurpose prevention technology (MPT) product for simultaneously prevention of HIV-1 infection and unplanned pregnancy [146]. A similarly intended ring containing tenofovir and levonorgestrel is also undergoing clinical testing [147,148]. Overall, tenofovir and dapivirine studies reinforced the need to focus on the safety of vaginal products, and also highlighted the positive and nearly linear correlation between local antiretroviral drug levels and efficacy against HIV-1 transmission.

The microbicides field further contributed to the engineering of novel and improved delivery systems and dosage forms that could be useful for the vaginal administration of drugs. For instance,

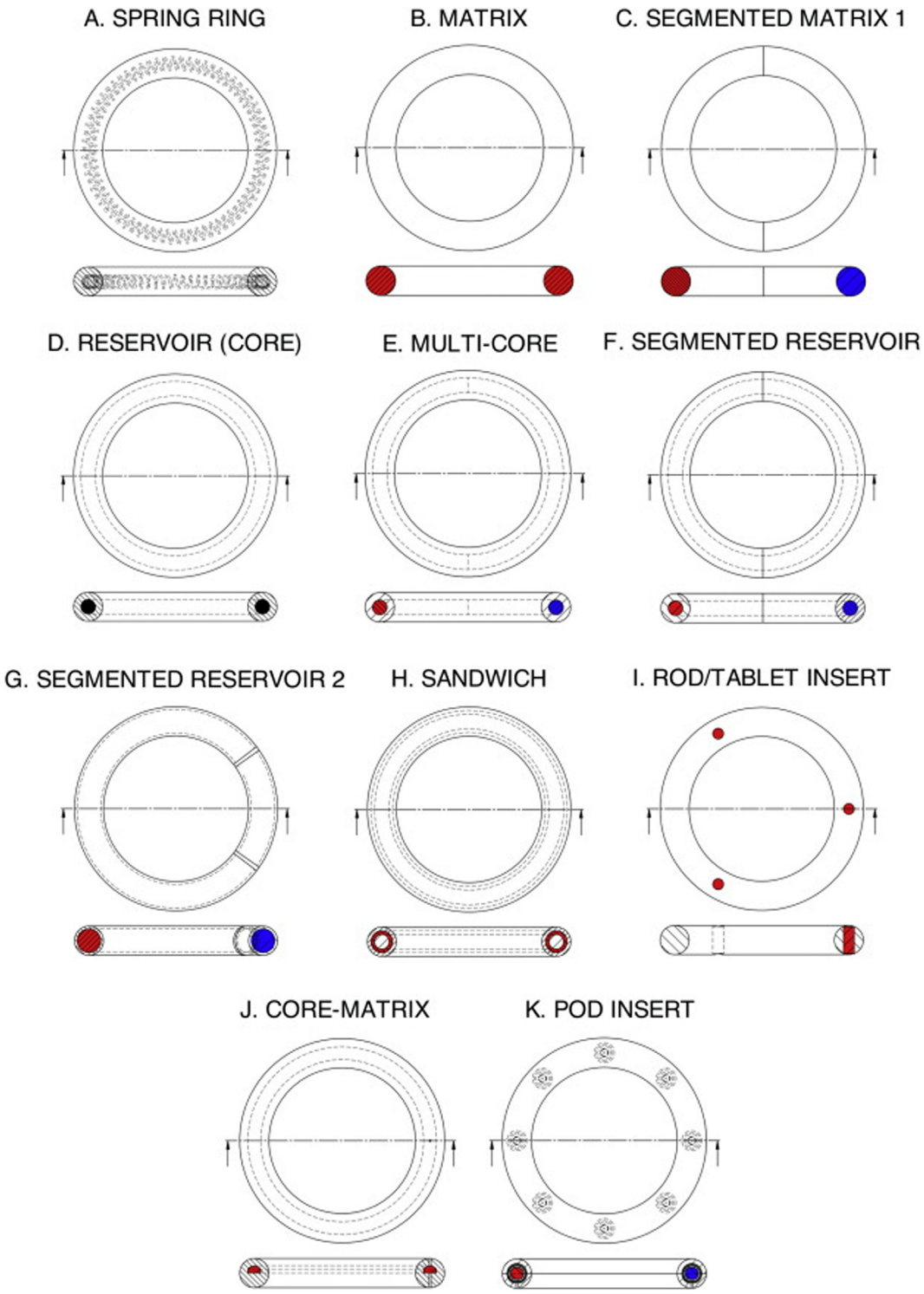

Fig. 5. Full ring (upper) and cross-sectional (lower) views of the various vaginal ring designs reported in the scientific literature for the delivery of HIV microbicides. Darker shading represents the location of the active agent(s). Reprinted from [149], Copyright (2016), with permission from Elsevier.

vaginal ring technology evolved from previously established basic designs (namely matrix and reservoir types currently available in the market), to innovative approaches that will undoubtedly allow diversifying clinical applications (Fig. 5) [149]. Of particular interest are rod/tablet insert or pod insert rings. In these last, the ring serves mostly as a supporting frame allowing administration and retention in the vaginal cavity, while the drug(s) is(are) actually incorporated into small rods/tablets or pods that are inserted into discrete gaps along the toroid-shaped system. An additional permeable or semi-permeable membrane is also included at the interface in the case of pod insert rings in order to lock pods and control drug release. These designs offer potential advantages over more traditional designs such as the combination of multiple drugs irrespective of their physicochemical compatibility, the custom selection of drug doses and release profiles, and the effective release of biopharmaceuticals, as well as the re-utilization of ring frames in

the case of rod/tablet insert rings [150–154]. These advanced rings are particularly interesting for developing MPT products. As an example, Smith, Moss *et al.* [155] developed a pod insert silicone ring for the vaginal delivery of tenofovir alafenamide hemifumarate, acyclovir, etonogestrel and ethinyl estradiol in order to prevent HIV-1 and HSV infections, as well as unplanned pregnancy. Suitably animal-sized rings were tested in a macaque model and were shown able to sustain the release of all four compounds and yield potentially clinically relevant drug levels for over 30 days.

Collectively referred to as inserts, solid dosage forms such as vaginal tablets, ovules and soft capsules have also benefited considerably from advances in the field of microbicides [156]. Modulation of drug release from inserts has been extensively explored for providing either fast or slow release profiles, depending on specific needs. Strategies to do so include proper ingredient selection (*e.g.*, use of disintegrants [157] or binding/mucoadhesive agents [158] for accelerating or slowing drug release, respectively), choice of manufacturing techniques (*e.g.*, freeze-drying [159–161] or granulation [162,163] for accelerating or slowing drug release, respectively), or the development of more complex systems such as osmotic pumps [164]. The use of inserts has also been considered interesting for allowing the vaginal administration of biological agents such as lactobacilli that are capable of restoring the local microbiota and even producing active compounds *in situ* [165,166]. Information extracted from acceptability studies involving the evaluation of putative microbicide inserts were further important in providing hints regarding which organoleptic properties are important for boosting adherence by users [167–169].

The vaginal film is another long-standing dosage form being explored for microbicide research. Previous market experience and relative popularity of a few spermicide film products sustain that these systems are acceptable and versatile for vaginal drug delivery [170]. Recent years saw important developments regarding the use of new materials and film design (*e.g.*, multilayered systems), the co-formulation of multiple drugs, the incorporation of drug-loaded nanocarriers, and the development of stimuli-sensitive formulations (*e.g.*, responsive to pH changes observed upon ejaculation), which were mainly intended to allow sustained/modified drug release or to improve mucosal tissue penetration of active compounds [171–179]. Films have also been recently proposed for the delivery of antibodies with spermicide activity that could be useful as non-hormonal contraceptives [180]. Electrospun fiber mats are related to films in terms of macroscopic appearance (with the notable exception of transparency or translucence) and utilization, and have been receiving increasing interest for application in vaginal drug delivery, namely for the development of MPTs. Polymeric microfibers or nanofibers are feasible to manufacture at the industrial scale, able to yield high drug payloads (including of drug combinations), versatile in terms of design and drug release tunability, and capable of providing extensive mucosal coverage [181–185]. Fiber mats were recently shown to be well accepted by sub-Saharan African women in need for MPT products during focus group discussions, thus supporting further development of this type of drug delivery systems [186].

Another interesting but seldom explored approach to the topical administration of drugs is the modification of commonly used

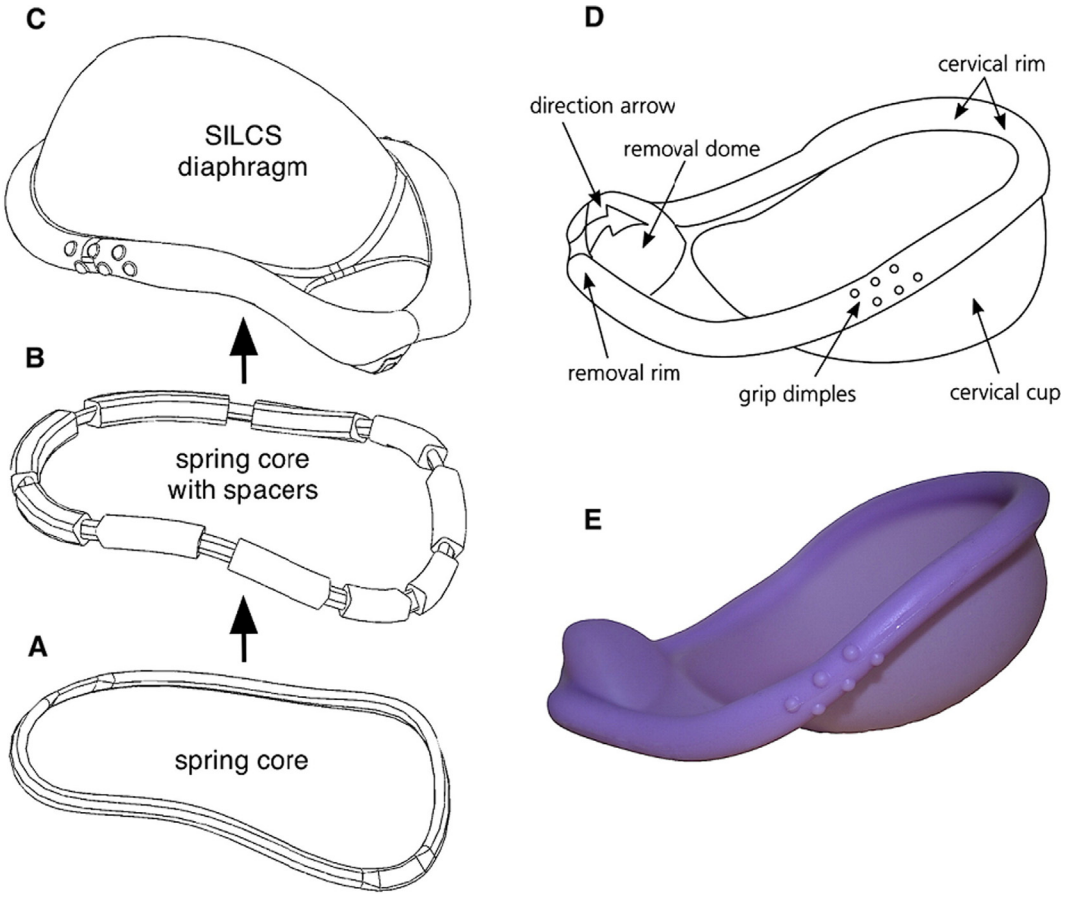

Fig. 6. The SILCS diaphragm device. **(A-C)** Three-step injection molding manufacturing process, **(D)** device features and **(E)** photograph of the device. Reprinted from [187], Copyright (2013), with permission from Elsevier.

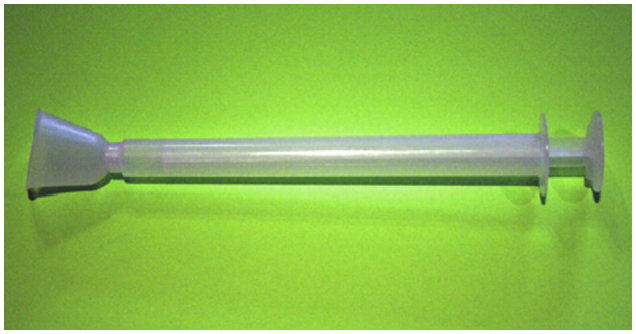

Fig. 7. CerviPrep™ device for localized therapeutics to the cervix. This device consists of a cylindrical applicator with a cervical cap attached to one end (on the left). As the cap is automatically positioned over the cervix, deployment of the device provides localized drug delivery to the target tissue while limiting exposure to and absorption by the surrounding vaginal tissue. Reprinted from [190], Copyright (2012), with permission from Elsevier.

vaginal devices. These last include tampons, support pessaries, female condoms, cervical caps or diaphragms. In a rare but interesting study, Major *et al.* [187] modified a SILCS diaphragm – which was originally developed by the nonprofit organization PATH as a barrier contraceptive – for the delivery of dapivirine. The drug was incorporated in the polyoxymethylene spring core of the diaphragm during manufacturing (Fig. 6), and the system was shown able to provide zero-order release kinetics *in vitro*. In another example, Kramzer *et al.* [188] demonstrated the feasibility of modifying a female condom with the NNRTI UC781 by incorporating the drug into the polymeric capsule used for insertion of the device.

The vaginal route may be further used for focal drug delivery targeting well-defined areas, most notably the cervix. Such approach seems to be particularly interesting for treatment of cervical cancer, as it presents the potential to reduce exposure of healthy mucosal tissue to cytotoxic drugs [189]. For example, Hodge *et al.* [190] reported on the ability of a new type of applicator termed CerviPrep™ (WVU Research Corporation; Fig. 7) to adapt to the ectocervix and deliver a gemcitabine gel into the cervical canal. The applicator allowed maximizing drug concentrations in the cervical mucosa, while reducing leakage to surrounding tissues and yielding minimal systemic exposure. Recently, Varan *et al.* [191,192] proposed the use of bioadhesive hydroxypropylcellulose films incorporating paclitaxel:cyclodextrin complexes and cidofovir-loaded polymeric nanoparticles (NPs) for localized treatment of cervical cancer associated with HPV. The incorporation of active molecules onto pre-prepared films was achieved by using inkjet printing, thus allowing for flexible dose selection. These films have the potential to be easily placed over or near the opening of the ectocervix, and remain in place due to their mucoadhesiveness. Polymeric fiber mats could similarly be useful for cervical cancer therapy [193,194].

2.1.5. Nanotechnology-based drug delivery

Considerable interest and recognized achievements in the field of nanomedicine over recent years have fueled extensive research in nanotechnology-based systems for vaginal administration. Apart from nanostructures featuring inherent activity against different pathogens (*e.g.*, dendrimers or metal-based NPs), research has focused on understanding mucosa-nanomaterial interactions and the development of drug nanocarriers that could be helpful in disease prevention and therapeutics [195,196].

In general, NPs are thought to be able to distribute throughout the mucosa and oppose natural cleansing mechanisms of the vagina [197]. However, the behavior of NPs is largely influenced by colloidal properties, particularly by surface chemistry that determines the interactions that are established with the mucin fiber network composing mucus. One important paradigm shift seen over roughly the last 15 years relates to the usefulness of establishing adhesive interactions with mucins when considering vaginal drug delivery [198]. Contrary to the long-standing thinking that favored mucoadhesive NPs for mucosal drug delivery, mucus-penetrating (or mucus-diffusive) nanosystems started being considered not only for improving mucosal distribution, but actually for enhancing vaginal retention. While the effect on distribution is easily understandable – *i.e.*, enhanced transport in mucus favors extensive coverage of the vaginal mucosa –, the impact on retention may be at first counter-intuitive. It can be explained, however, by the ability of mucus-penetrating nanosystems to rapidly penetrate mucus and reach deeper layers that are largely unstirred and undergoing minimal shedding [199]. The contributions of Hanes and collaborators have been critical in developing mucus-penetrating NPs and understanding their behavior in mucus. In particular, these researchers found that dense PEG-modification of the surface of otherwise adhesive NPs (100–500 nm) was effective in allowing almost unhindered transport in cervicovaginal mucus [200–202]. Surface modification can be achieved either by covalent bonding of PEG or by adsorption of PEG-poly(propylene oxide)-PEG co-polymers (poloxamers). Importantly, mucus-penetrating NPs cause minimal disturbance to the native structure of mucus (as opposed to adhesive counterparts) [203], an important feature due to the key role that this fluid plays in maintaining vaginal homeostasis. Different *in vivo* studies demonstrated that indeed mucus-penetrating NPs can better distribute (Fig. 8) [204–206] and retain for prolonged time frames (particularly associated with mucosal tissue) [207] in the vagina of mice. Transport of mucus-penetrating NPs (and drugs) can further be enhanced by using hypotonic vehicles that trigger fluid absorption and advective movement towards the mucosal surface [208]. Interestingly, the development of anti-PEG antibodies can reverse the mobility of densely PEG-coated NPs [209], which could be a downside for repeated administration of this type of nanocarriers. Alternative mucus-inert coating materials such as poly(2-ethyl-2-oxazoline) [210] or certain grades of partially hydrolyzed poly(vinyl alcohol) [211] have been proposed and could help circumvent this problem. Additionally, the safety of using intravaginal PEG-modified poly(lactic-*co*-glycolic acid) (PLGA) NPs has been recently challenged, namely in the presence of *C. albicans* infection [212,213]. Thus, additional work is recommend in order to clarify whether toxicity issues could jeopardize future applications of such nanosystems.

In general, NPs (75–200 nm) administered in the vagina of mice are largely restricted to the lower genital tract, although some studies indicate that such systems can migrate up to the uterus and, possibly, the ovaries [214–218]. Limited observations in humans following the vaginal administration of technetium 99 m-sulfur NPs (100 nm) appear to corroborate these findings [219]. Leakage is also typically extensive following vaginal instillation in mice [207,216]. Furthermore, NPs are able to penetrate deep into murine mucosal tissues and undergo cell uptake [214,216,218], and even reach distant anatomical sites such as proximal lymph nodes [220,221]. These last observations, in particular, suggest that the vaginal administration of nanocarriers could be an interesting approach for the delivery of antigens in order to elicit an immune response. One important consequence of the distribution of drug-loaded NPs is the modification of pharmacokinetics. For example, dapivirine-loaded polycaprolactone NPs were able to enhance drug levels in vaginal fluids (4.2-fold increase in the area-under-the-curve) and vaginal tissues (5.1-

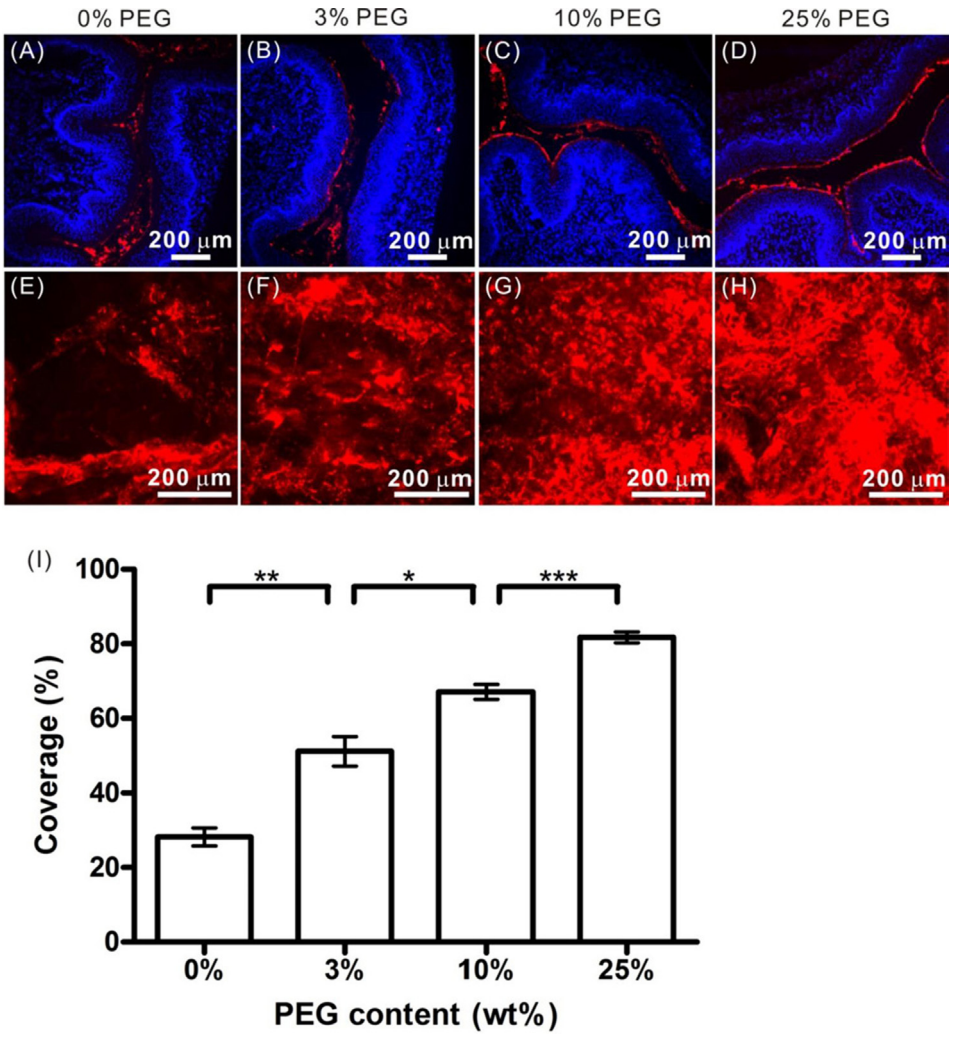

Fig. 8. Distribution of NPs bearing different degrees of PEG-modification in the mouse vagina *in vivo*. The distribution of red fluorescent **(A, E)** PLGA (0% PEG; mucoadhesive), **(B, F)** PLGA-$PEG_{3\%}$ (3% PEG), **(C, G)** PLGA-$PEG_{10\%}$ (10% PEG), and **(D, H)** PLGA-$PEG_{25\%}$ (25% PEG; mucus-penetrating) in transverse cryosections of estrus phase mouse vaginal tissue (upper row) and on flattened estrus phase mouse vaginal tissue (bottom row). **(I)** The percent surface coverage by NPs on the flattened vaginal tissue surface was quantified as percent coverage ± standard error of the mean. Reprinted with permission from [205]. Copyright (2015) American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fold increase) up to 24 h after administration to mice, as compared to the free active molecule [216]. In another study using pregnant mice, Hoang *et al.* showed that a mucus-penetrating nanosuspension of progesterone (260 nm drug particles coated with poloxamer 407) allowed obtaining higher drug levels at the cervix (2.3-fold increase) and blood plasma (4.9-fold increase) up to 6 h post-administration when compared to a commercially available gel [222]. A trend towards higher uterine drug levels was also noted in the case of animals treated with the nanosuspension. In general, pharmacokinetics studies indicate that systemic drug exposure is relatively low when drug-loaded nanocarriers are used [216,222]. This does not necessarily mean that drugs are unable to be absorbed and reach blood circulation, but rather reflects the minute doses typically needed to obtain pharmacologically relevant genital concentrations upon intravaginal administration.

Despite the considerable advances in understanding the behavior of nanomaterials upon vaginal administration, generated data are almost exclusively derived from studies using small rodents. How much of it actually applies to the human scenario is unknown as translation from animal models to humans appears to be tricky due to the stringent interspecies anatomical and physiological differences. A central aspect of the use of nanocarriers relates to their safety, not only to women, but also to fetuses in case of pregnancy. However, available data are meager, particularly regarding chronic use. Another important issue of the use of drug-loaded nanocarriers pertains to their administration. Although animal studies typically rely on the administration of nanosystems in the form of aqueous suspensions, liquid systems may not be adequate for clinical translation. Different platforms have been proposed for the incorporation of nanocarriers in order to obtain putative dosage forms that could be acceptable by women, and even enhance the performance of incorporated drugs [223]. Some of the most promising include polymeric films [175,177], electrospun fiber mats [224,225], gels [226,227], thermosensitive gels [228–230], foams [231], and vaginal rings [232,233]. Microarray patches have also been recently proposed as an interesting platform for the intravaginal administration of a nanosuspension of the NNRTI rilpivirine [234]. In this case, the nanosuspension was injected directly into the vaginal epithelium of rats by means of dissolving microneedle tips and allowed prolonging local drug residence.

The range of potential clinical uses for which intravaginally administered drug nanosystems have been proposed is wide, as denoted in Table 1. Microbicides are the dominant application, but important contributions have also been made for the purpose

Table 1
Examples of potential applications of different nanosystems intended for vaginal administration of drugs.

Intended application	Nanosystem (average diameter)	Drug payload	Key findings	Ref.
Prevention of HIV-1 transmission	Eudragit® S-100/PLGA NPs (250 nm)	Tenofovir	*In vitro* drug release rate from NPs was approximately 4-times higher when pH was increased to near neutral values	[235]
	PLGA NPs functionalized with an anti-CD4 antibody (280 nm)	Saquinavir	NPs were able to target CD4 + T Sup-T1 cells *in vitro*, resulting in higher intracellular drug accumulation, as compared to non-functionalized NPs	[236]
	Polycaprolactone NPs (199 nm)	Dapivirine	NPs were safe and were able to enhance vaginal dapivirine levels up to 24 h post-administration, as compared to the free drug in suspension	[216]
	Anti-HLA-DR antibody-functionalized PEG-PLGA NPs (232 nm)	Anti-SNAP-23 siRNA	NPs incorporated into a polymeric film crossed an epithelial cell monolayer, targeted HLA-DR + KG-1 cells and knocked down SNAP-23 expression	[172]
	PLGA NPs (66 nm)	Rilpivirine	NPs incorporated into a thermosensitive gel prevented vaginal HIV-1 transmission in humanized BLT mice; degree of protection was dependent on the time mediating administration and viral challenge	[237]
	PLGA NPs (275 nm)	Efavirenz	NPs incorporated into a polymeric film were safe and improved local residence of efavirenz, namely when compared to films containing free drug	[174]
	Cell-adhesive HPG-PLA NPs (131 nm)	Elvitegravir	Cell-adhesive properties were achieved by converting vicinal diols on HPG to aldehydes; these NPs exhibited higher vaginal retention in mice and enhanced local drug levels, as compared to mucus-penetrating HPG-PLA NPs	[238]
	Poloxamer 288/HPMC-stabilized drug nanocrystals (243 nm)	CSIC	Nanocrystals increased *in vivo* murine genital tissue penetration of the NNRTI by roughly one order of magnitude, as compared to the drug in suspension	[239]
	Antibody-conjugated PEI/PEG-PLGA NPs (251 nm)	Anti-alpha4beta7 integrin antibody	NPs incorporated into a gel preferentially bounded to mucosal T cells expressing high levels of alpha4beta7 integrin within 72 h after a single administration to rhesus macaques	[240]
	Lactoferrin NPs (74 nm)	Tenofovir and curcumin	NPs combining both drugs were safe and provided higher vaginal drug levels in mice, as compared to free compounds	[241]
Prevention of HSV-2 infection	Poloxamer 407-stabilized drug:zinc nanosuspension (65 nm)	Acyclovir monophosphate	Mucus-penetrating NPs protected 53% of mice challenged intravaginally with HSV-2 *vs.* 16% for animals treated with soluble drug	[204]
	PLGA NPs (161 nm)	Anti-nectin-1 siRNA	Efficient, dose-dependent *in vivo* knockdown of murine nectin-1 by NPs allowed protecting mice from HSV-2 infection	[242]
Treatment of cervicovaginal cancer	PLGA NPs (158 nm)	Camptothecin	NPs were safe to use and prevented the development of vaginal tumors in a mouse model	[243]
	Poloxamer 407-coated PLGA NPs (239 nm)	Paclitaxel	NPs were more effective than mucoadhesive counterparts (*i.e.*, without poloxamer 407) and the free drug (topical Taxol®) in suppressing tumor growth and prolonging survival in a mouse model	[244]
	Poly(acrylic acid)/beta-cyclodextrin-based nanogel (71 nm)	Paclitaxel	The mucoadhesive nanogel was safe, increased drug residence and improved tumor growth suppression in an orthotopic cervical cancer mouse model, as compared to free paclitaxel	[245]
Treatment of HPV infection	PEG-modified liposomes (181 nm)	Interferon alpha-2b	Liposomes improved *ex vivo* penetration of interferon alpha-2b across sheep vaginal tissue	[246]
Treatment of VVC	Lecithin-stabilized drug nanosuspension (247 nm)	Amphotericin B	Nanosuspension incorporated into a thermosensitive gel was more efficient than a commercial effervescent tablet containing the drug in reducing inflammation associated with *C. albicans* vaginitis in mice	[247]
	Chitosan NPs (207 nm)	Miconazole	Therapeutic efficacy of NPs was similar to that of a commercial miconazole cream when tested in a mouse model of *C. albicans* vaginitis	[248]
	Chitosan NPs (294 nm)	Miconazole and farnesol	Fungal burden reduction using NPs was comparable to that of a commercial miconazole cream (albeit with less inflammatory response) in a mouse model of *C. albicans* vaginitis	[249]
Gene therapy	Poly(beta-amino ester) NPs (240 nm)	CRISPR/Cas9 single guide RNAs targeting porcine endogenous retroviruses	Efficient local genome editing was achieved when NPs incorporated into a montmorillonite-based gel was tested in a pig model	[227]
HIV-1 mucosal vaccine	TAT-coated APS/rAd nanocomplexes (96 nm)	HIV-1 gag p24 encoding vector	PEG corona provided by APS rendered mucus-penetrating properties, while TAT enhanced uptake by epithelial and immune cells; nanocomplexes elicited robust mucosal immune response in mice	[250]
Prevention of pre-term birth	Poloxamer 407-stabilized nanosuspension (261 nm)	Progesterone	The mucus-penetrating nanosuspension dispersed in a hypotonic vehicle was safer and more effective than a commercial micronized progesterone gel in preventing pre-term birth in a mouse model	[222]
	Poloxamer 407-stabilized nanosuspensions (216 nm or 306 nm)	Trichostatin A or progesterone	Combination of intravaginal trichostatin A and progesterone nanosuspensions was effective in preventing inflammation-induced pre-term birth in mice, rendering live neurotypical offspring (intraperitoneal administration of the combination was ineffective)	[251]
Treatment of polycystic ovary syndrome	Transethosomes (133 nm)	Progesterone	Daily use of transethosomes incorporated into a mucoadhesive gel was superior to commercial micronized progesterone capsules in improving endometrial thickness, echogenicity, serum drug levels and pregnancy rate when tested in women	[252]

Abbreviations – APS: Amino-(ethylene oxide)$_n$-(allyl glycidyl ether-succinate)$_m$; CSIC: 5-Chloro-3-phenylsulfonylindole-2-carboxamide; HLA-DR: Human Leukocyte Antigen-DR isotype; HPMC: hydroxypropyl methylcellulose; HPG: hyper-branched polyglycerols; PLA: poly(lactic acid); rAd: recombinant adenovirus; TAT: transactivating transcriptional activator peptide.

of cancer treatment, management of vaginal infections, gene therapy, mucosal vaccination, and prevention of pre-term birth.

2.2. Endocervical

The cervix provides a passageway between the vagina and the uterus (Fig. 1). Although minute in size (2–3 cm in length and width), the cervical canal undergoes considerable dimensional changes along the menstrual cycle and, in particular, during pregnancy and labor [253]. Despite its direct contact with the vagina, the cervix has distinctive features. For example, it is coated by a locally produced mucus layer presenting near neutral pH [254]. The production and viscoelastic properties of this fluid are considerably influenced by hormonal changes observed throughout the menstrual cycle, which determines its barrier properties [255]. The cervix also marks the epithelial transition between the vaginal and the endometrial mucosa. The ectocervix is lined by stratified squamous nonkeratinized epithelium, while the endocervix comprises simple columnar epithelium. Data from studies using cervical explants indicate the ability of different microbicide compounds to undergo extensive permeation across the mucosa [256–259].

Endocervical (or intracervical) administration of drugs into this restricted anatomical site has been utilized in clinical practice for a few specific purposes. For instance, administration of silver nitrate or ferric subsulfate (Monsel's solution) can be useful in treating bleeding following routine cervical polyp removal in the gynecologist office [260]. The endocervical route can also be used for local instillation of prostaglandins in order to induce labor, although its advantages over vaginal administration are questionable [261]. This can be achieved by using a dinoprostone-containing colloidal silicon dioxide/triacetin gel such as Prepidil® (Pfizer), which is available in various countries for this specific purpose. Different experimental gel formulations have further been found useful for endocervical administration of dinoprostone in clinical trials [262,263], while other labor inducing drugs have been tested using this route [264,265]. Endocervical instillation needs to be performed by a physician using sterile technique, and involves the use of a syringe coupled to a catheter or an otherwise adequate applicator that is inserted across the vagina. Poor retention in the cervical canal is a common issue for fluid and even semi-solid formulations, and the patient is usually recommended to remain in the supine position for a few minutes after administration.

Solid systems have been proposed in order to provide better endocervical residence of topically applied drugs, although with little clinical translation. The simplest solution comprises the insertion of tablets or suppositories that are available for other routes of administration [266]. However, this practice is not recommended. Cervical tents (or cervical dilators) have also been adapted for the endocervical administration of drugs. Tents are typically rod-shaped, hygroscopic solid devices that swell upon the absorption of local fluids, and have been in use at least since the mid-nineteenth century in order to force the dilation of the cervix and induce labor [267]. One example of a medicated tent is Lamicel™ (Medtronic Xomed), a polyvinyl alcohol-based endocervical insert (67 mm in length and 3–5 mm in diameter) delivering magnesium sulfate as a collagenolytic and osmotic agent (Fig. 9) [268–270]. The product was commercially available in the United States from the 1980s to 2008 for inducing labor or for cervical preparation prior to surgical abortion, but ended up being withdrawn due to futility. Other rod-shaped, non-degradable systems have also been developed during the 1970s envisioning long-term contraceptive use, but their inability to stay in place limited their utility [271]. Alternative systems featuring more complex designs were further developed in order to enhance residence for prolonged periods of time (months to years). For example, T- or Y-shaped, silicone-based devices impregnated with sex hormones were devised and were shown able to control the release of various compounds and prevent pregnancy in animal tests and even in clinical trials during the 1980s [272–274]. Curiously, and despite its hormonal content and release, such devices appear to act solely at the cervical level by altering mucus and impairing the ability of spermatozoids to migrate into the uterus. The development of other more acceptable and equally effective contraceptives, however, led to the discontinuation of the development of these products. Even so, similar systems could find alternative applications today, namely those requiring prolonged and localized drug delivery to the cervix (*e.g.*, cervical cancer therapy). For instance, Sherwood *et al.* [275] recently developed different cervical inserts composed of polycaprolactone and poloxamer blends for the localized delivery of withaferin A, a steroidal lactone with potential application in cancer treatment. *In vivo* testing in a goat model showed that inserts were able to provide measurable tissue levels after 90 days of use, but could not prevent considerable leaching of the drug towards the vagina. Also, onset of tissue damage, inflammation and infection were identified depending on the design of inserts, which was optimized in order to allow retention [275].

Another use for the endocervical route involves the termination of cervical ectopic pregnancy, which can be achieved by direct injection of methotrexate into the gestational sac [276,277]. Less toxic agents such as potassium chloride [278], vasopressin [279] or ethanol [280] may be useful alternatives, and are particularly interesting in cases of heterotopic pregnancy cases. Administration is performed under ultrasonographic guidance and typically involves transvaginal injection inside the gestational sac or, eventually, in its vicinities (*e.g.*, in the case of vasopressin) using a needle guide device. Paracervical block (*i.e.*, injection of a local anesthetic such as lidocaine in the submucosa at the ectocervix and/or at the cervical side of the fornices) is also usually performed as a complementary measure for pain management during cervical ectopic pregnancy termination [281].

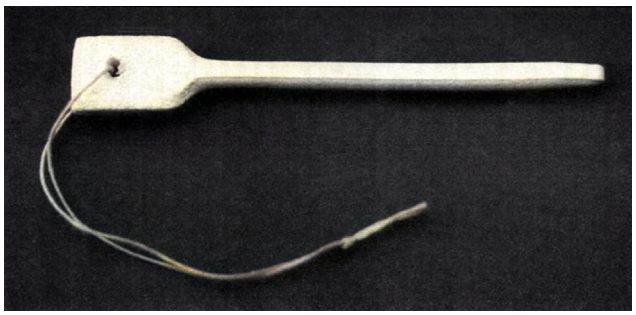

Fig. 9. Lamicel™ osmotic cervical dilator (67 mm × 3 mm tent is depicted). The larger flat section stays on the vaginal side, and the string attached to the bottom is intended for retrieving the medicated tent after usage. Adapted from [268], Copyright (1991), with permission from AWHONN, the Association of Women's Health, Obstetric and Neonatal Nurses and Elsevier.

2.3. Intrauterine

The uterus is an important route for the administration of drugs, being particularly useful for long-term contraception. Other clinical applications encompass local anesthesia in preparation of biopsy [282] or surgery [283], and pregnancy termination (including viable or cornual and cesarean scar ectopic cases) [284], which will be discussed latter on under the sub-sections dealing with the intra-amniotic and the extra-amniotic routes.

The uterus consists of a pear-shaped hollow organ (Fig. 1) that is enlarged during reproductive years and even more pronouncedly in pregnancy. Is natural inner chamber provides a relatively large

space in which differently shaped devices can be easily fitted and retained for prolonged periods of time without being perceived by users. The uterine wall, in particular, undergoes considerable changes throughout life in response to ovarian hormones. The luminal surface is covered by a mucous membrane – the endometrium – that is thicker around ovulation, but ends up shedding in the absence of fertilization, thus leading to menses. The endometrium comprises a simple columnar epithelial layer associated to the lamina propria resting upon the myometrium. The uterine wall is highly vascularized and provides an excellent gateway for blood and lymphatic drainage, while avoiding the hepatic first-pass effect. A combined analysis of permeability data using uterine tissue from rodents indicates that the endometrium is highly permeable to negatively- and non-charged compounds and even high molecular weight molecules, without apparent influence of hormonal status [285]. In particular, when administered into the uterus in the form of solution or incorporated into different sustained release systems (*e.g.*, osmotic mini-pumps, polyurethane matrices and pellets), the bioavailability and pharmacological effects of biopharmaceuticals such as calcitonin (3.4 kDa), insulin (5.8 kDa) and erythropoietin (30.4 kDa) were close to that of sub-cutaneous injection in rats [285,286]. Despite its potential to provide access to systemic circulation, nuisance around administration determines that the utility of the intrauterine route is restricted to very potent drugs that allow sustaining pharmacological effects over prolonged periods (months to years). One particular drug class has been studied: progestogens. The human uterus appears to provide a preferential target and accumulation site for these sex hormones, as demonstrated in an *ex vivo* human perfusion model [66]. This effect is justified, at least partially, by the local high expression of progesterone receptors, which appears to be relatively stable throughout the menstrual cycle [287,288]. Moreover, clinical pharmacokinetics of intrauterine levonorgestrel has been extensively studied and supports the preferential local action of the drug, in striking contrast with its low systemic exposure [289,290].

Intrauterine administration of drugs is a mildly invasive procedure that needs to be performed by specifically trained physicians, and involves risks such as mechanical injury and triggering of pelvic inflammatory disease. Access to the uterus is achieved transvaginally, and aided by a catheter for liquids and semi-solids, or by a specific insertion device in the case of solid systems. Direct administration of drugs can also be performed during surgical procedures involving the uterus [291].Removal of drug delivery systems may further be necessary in planned cases after intended usage (*e.g.*, non-degradable solid products), or due to the onset of medical complications. Topical administration is usually intended, but mucosal injections may also be useful in some particular cases, such as in the administration of local anesthetics [283].

The most successful and well-established systems designed for the administration of drugs are intrauterine devices (IUDs) or intrauterine systems. These are typically intended for long-term (up to several years) prevention of unplanned pregnancy [292], and its origins can be traced back to the beginning of the twentieth century [293]. According to numbers from 2019, IUDs are the most widely used reversible female contraceptive method, with an estimated 159 million users worldwide [294]. Their usefulness has also been demonstrated for emergency contraception [295]. Although inert IUDs can have a contraceptive effect, devices based on copper (Cu-IUDs) or releasing progestogens are preferred due to their lower failure rates [296]. Different designs have been proposed throughout the years, namely in order to enhance intrauterine retention (Fig. 10). However, T-shape IUDs comprising one central shaft attached to two flexible arms that allow the device to stay in place are currently the most popular ones (Fig. 11) [297]. These IUDs are placed by a physician deeply into the uterus with the aid of an insertion tube or a specific insertion system (Fig. 12) that maintains both arms retracted during the procedure [298]. Insertion is relatively simple and safe to perform, although expulsion, uterine perforation and infection may occur. A monofilament thread attached to the bottom of the T is left within the cervix and extending towards the vagina in order to allow easy inspection of the correct placement of the device throughout its use, as well as to aid removal by a physician.

Cu-IUDs were the first largely successful IUDs and are still in use. For example, ParaGard® T 380A (CooperSurgical) is a commercial Cu-IUD available in the United States since 1984. The device comprises a T-shaped, polyethylene-based frame that is partially covered by copper wire. Other Cu-IUDs featuring minor variations in design are available worldwide, as well as frameless Cu-IUDs (*i.e.*, without the plastic T-shaped frame) or the intrauterine ball (copper beads included in a nickel titanium alloy frame; Fig. 13) [299,300]. These systems are able to release copper ions that impair sperm mobility and induce an endometrial inflammatory response opposing embryo implantation [301]. Main adverse effects of these systems include increased menstrual pain and bleeding, particularly in T-shaped Cu-IUDs. The modification of Cu-IUDs with indomethacin has been proposed to mitigate these problems. Drug incorporation within a matrix elastomeric reservoir [302] or by applying polyelectrolyte film coatings [303] allows sustaining the release of indomethacin. For example, the gamma Cu380 IUD is a T-shaped stainless steel/copper-wired system modified with two indomethacin-loaded silicone elastomer beads at the tip of each arm that is available in China [304].

Progestogen-releasing IUDs were initially developed and shown clinically effective in the early 1970s, leading to the approval of the first commercial product Progestasert® (Alza) in 1976 [305,306]. This progesterone-releasing IUD was available until 2001 and was effective for up to one year. Progestogens (mainly levonorgestrel) act locally by causing endometrial atrophy and inhibiting

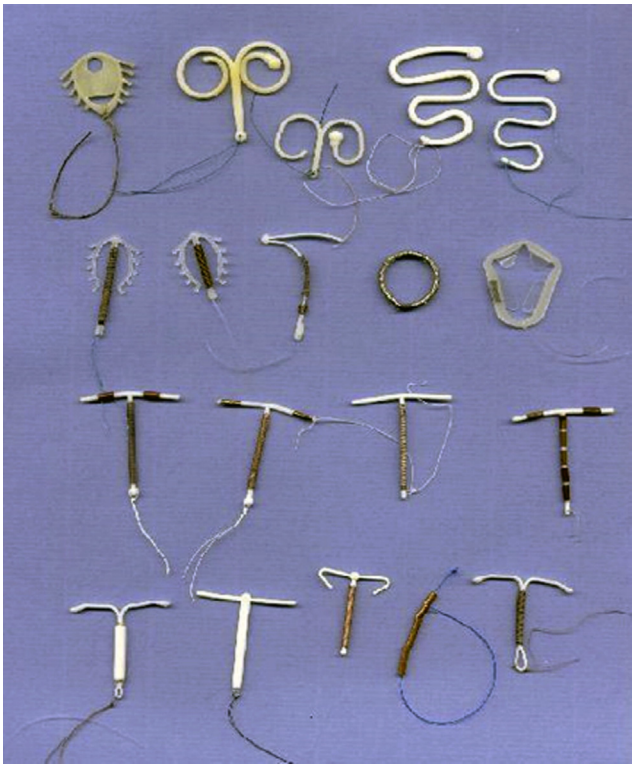

Fig. 10. Various current and historical intrauterine devices/systems. Reprinted from [296], Copyright (2014), with permission from Elsevier.

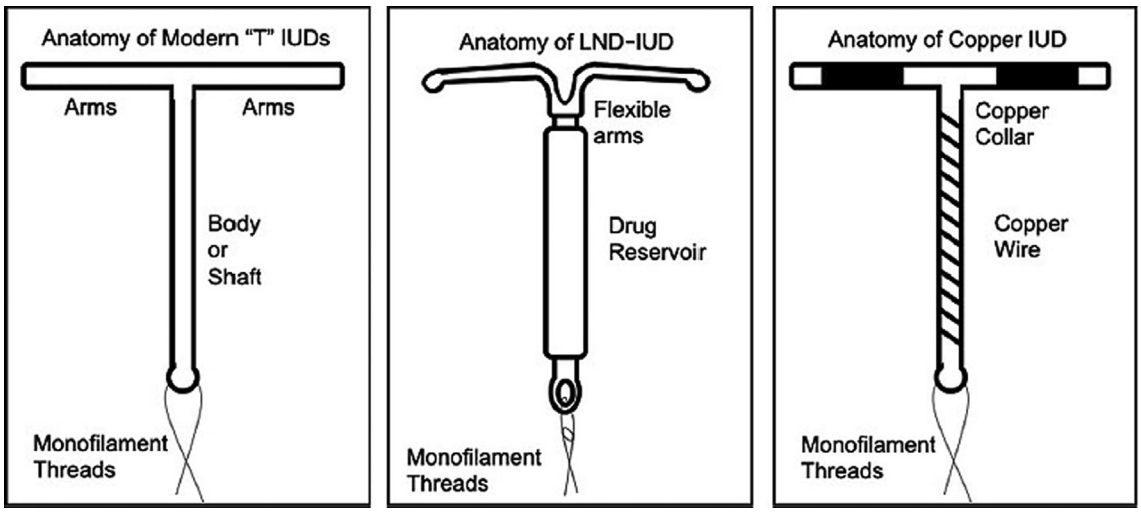

Fig. 11. Anatomy of modern T-shaped IUDs, levonorgestrel (LND)-releasing IUD, and non-hormonal copper-based IUD. Reprinted from [297], Copyright (2019), with permission from Elsevier.

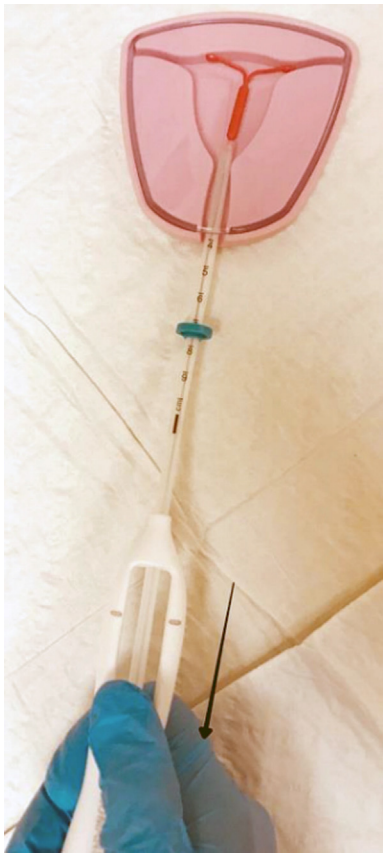

Fig. 12. Simulated insertion of a T-shaped IUD (in red). Release of the device in the uterine cavity is achieved by pushing the slider toward the inserter (indicated by the black arrow). Adapted from [298] by permission of Springer Nature, Copyright (2020). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

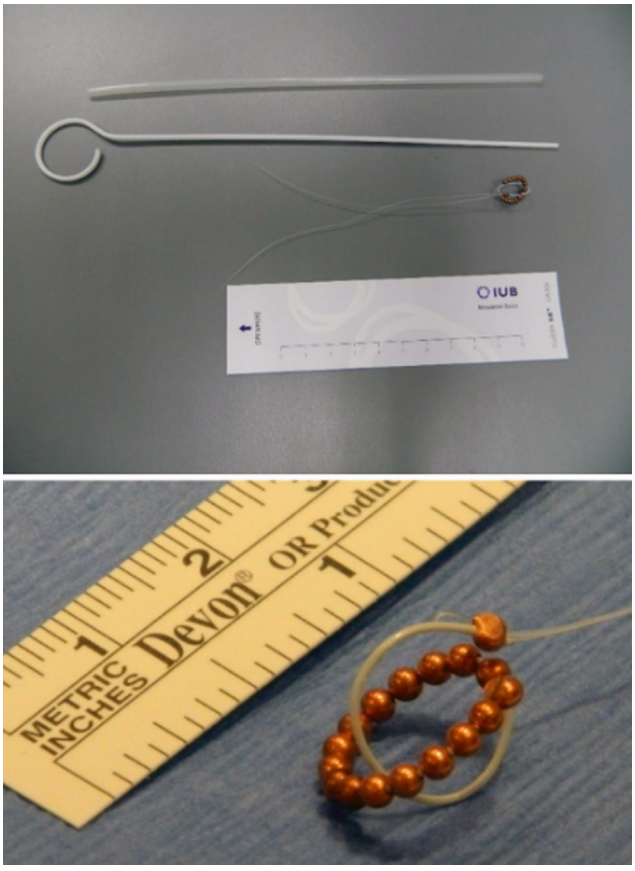

Fig. 13. The IUB™ (OCON Healthcare) intrauterine ball device with insertion kit (top) and released (bottom). Reprinted from [299] by permission of Springer Nature & Federation of Obstetric and Gynecological Societies of India, Copyright (2014).

embryo implantation, as well as by thickening cervical mucus and limiting the ability of spermatozoids to migrate into the uterus [301]. Anovulation can occur with IUDs featuring high release rates of levonorgestrel [289]. Reduction in menstrual bleeding is also observed for this type of IUDs, thus justifying their use for the treatment of menorrhagia [307]. Different levonorgestrel-releasing IUDs featuring similar T-shaped design are available in the market today. One example is the Mirena® intrauterine system (Bayer), the first of its kind to be approved by the FDA in 2000 (please refer to the review by Rose and colleagues [308], published previously in *Advanced Drug Delivery Reviews*, for an excellent overview on the development of this product). The device contains 52 mg of levonorgestrel in a hollow cylindrical polydimethylsilox-

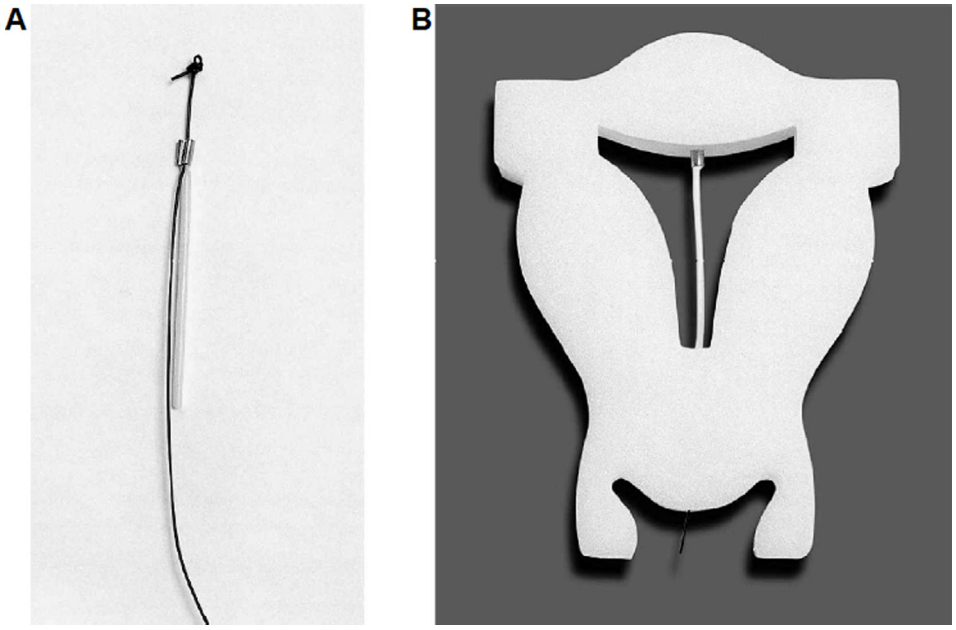

Fig. 14. The FibroPlant® levonorgestrel intrauterine system: **(A)** close-up of the system, and **(B)** after insertion in a uterine model. Reprinted from [310] with permission of Taylor & Francis, Copyright (2001).

ane reservoir coated by a release rate controlling silicone membrane and mounted on the body of a T-shaped polyethylene frame (32 mm × 32 mm). Mirena® also contains barium sulfate that makes it radiopaque and readily traceable by X-ray examining. The drug is initially released at a rate of approximately 20 μg/day but decreases to around 10 μg/day and 9 μg/day after the fifth and sixth years, respectively [296]. Even with such low amounts of drug being released, Mirena® has been demonstrated effective, and the FDA has recently approved an extension of its maximum period of use from five to six years [309]. Contraceptive efficacy at lower release rates also justified the development of other similarly sized/shaped levonorgestrel-releasing IUDs such as Kylena® (Bayer; 17.5 to 7.4 μg/day over 5 years) or Jaydess®/Skyla® (Bayer; 14 to 5 μg/day over 3 years). Frameless progestogen-releasing IUDs have further been designed and are available in the market. One example is FibroPlant® (Contrel Research), a small IUD (30 mm × 0.12 mm) that releases 14 μg/day of levonorgestrel from an ethylene–vinyl acetate copolymer rod covered by a non-medicated membrane of the same material (Fig. 14) [310,311]. The reservoir is fixed to an anchoring filament by a metal clip, and the whole system is intended to be implanted within the fundal myometrium.

Despite extensive and successful clinical use of levonorgestrel-releasing IUDs, accessible information regarding manufacturing processes and availability of relevant *in vitro* characterization methods of these systems is limited. Recent work by the group of Diane Burgess at the University of Connecticut in collaboration with the FDA attempted to mitigate these issues, and provided important insights into how manufacturing parameters influence the quality and release performance of drug reservoirs [312]. They also contributed to the establishment of accelerated conditions for *in vitro* testing and understanding of the mechanisms involved in the release of levonorgestrel from IUDs [313]. Another exciting and emerging topic in the field is the possibility for using additive manufacturing in order to prepare drug-releasing IUDs. Technical versatility of this technology allows fast development of personalized systems (*e.g.*, featuring different sizes, drug content, reservoir geometry) that can be used to administer multiple compounds, alone or in combination, thus opening a vast range of possibilities for intrauterine administration of drugs [314–316].

Apart from IUDs, other approaches have been proposed for intrauterine administration of drugs, particularly in order to enhance the residence and distribution of drugs intended for the management of discrete medical conditions. For example, thermosensitive gels have been proposed for the intrauterine administration of beta-estradiol for the treatment and prevention of intrauterine adhesions – a common consequence of surgical or otherwise invasive procedures involving the uterus [317]. The ability of these pharmaceutical formulations to undergo sol–gel transition upon heating to body temperature allows optimizing initial distribution in the uterine cavity and further prevent leakage. Different poloxamer-based formulations were shown effective in regenerating the endometrium and preventing uterine adhesions in rats, namely by providing enhanced uterine residence and sustained release of beta-estradiol for several days at the site of action [318,319]. Similar thermosensitive gels containing keratinocyte growth factor, alone or in combination with epsilon-polylysine (used for increasing mucoadhesion), have also been shown effective *in vivo* using animal models [320,321]. In another approach to the same clinical problem, Cai *et al.* [322] proposed a basic fibroblast growth factor-loaded gelatin methacryloyl/alginate composite porous scaffold fabricated using a microfluidics device, which featured anti-adhesive properties and promoted endometrial healing in rats. Bovine serum albumin was incorporated into the scaffold as a proxy of biopharmaceutical compounds, and was shown able to be released *in vitro* in a sustained fashion up to roughly 100 h.

2.4. Intrafallopian

Intrafallopian (or intratubal) administration of drugs is uncommon, but may be of value in particular circumstances. For instance, this route has been shown feasible for the relief of pain with topical anesthetics upon surgery involving the fallopian tubes (*e.g.* salpingectomy and tubal sterilization) [323,324], or the treatment of unruptured ectopic pregnancy with different agents such as

methotrexate, potassium chloride and hyperosmolar glucose, among others [325,326]. Administration of liquid formulations originally intended to be used by parenteral routes can be conducted either transvaginally with sonographic guidance or via laparoscopy under general anesthesia, using catheter assisted injection. Reports vary regarding the volume of formulations being administered, but are usually within 5–10 mL [323,324,327]. In any case, sterility must be assured, and administration procedures find parallel in well-established assisted reproduction techniques such as transvaginal intratubal insemination (ITI) and laparoscopic gamete/zygote intrafallopian transfer (GIFT/ZIFT) [328].

Due to its restricted use, characterization of this mucosal route is nearly non-existent, namely concerning local and systemic drug exposure. Limited clinical data on the utilization of methotrexate in women undergoing ectopic pregnancy management indicated that serum drug levels obtained after transvaginal intrafallopian administration, namely directly into the gestational sac, were overall similar to those obtained for intramuscular administration [329,330]. These results appear to support the high permeability of the oviducts, which can also be inferred from the histological and anatomical properties of this area. Indeed, the mucosa is covered by leaky simple columnar epithelium and possesses a highly vascularized lamina propria. The presence of fimbria (fingerlike projections) at the infundibulum additionally increase the area available for drug permeation/absorption [32]. Moreover, the vascular permeability of the fallopian tubes and oviductal fluid production is dependent on the menstrual cycle and appears to be regulated, at least partially, by vascular endothelial growth factor [331,332]. This could result in considerable variability in drug disposition depending on hormonal status, although further studies are necessary in order to confirm such possibility.

2.5. Intraovarian

Clinical reports on the use of the intraovarian route are scarce and usually involve experimental medical interventions. Despite its relative small size (similar to that of an almond), the ovary can accommodate relatively large volumes of liquid (up to about 5 mL) upon slow injection into the stroma, as inferred from cell therapy procedures [333,334]. Access to the ovaries can be achieved transvaginally [335] or through laparoscopy [334]. Transvaginal ultrasound imaging is used for guiding an adequate administration device (*e.g.*, an aspiration needle coupled to a biopsy line) up to the ovary in the case of former, while forceps may be used in the case of the laparoscopic approach in order to immobilize the ovary and facilitate needle injection. Administration is always performed following aseptic technique and typically under anesthesia, although associated discomfort/pain seems to be tolerable to allow an anesthesia-free transvaginal intervention [336]. Additionally, free access to the ovary is possible for the administration of drugs during laparotomy [337].

Pre-clinical and clinical research in new applications for intraovarian administration of drugs has been limited. Recent studies using animal models showed the potential of local administration of neosaxitoxin [338] or a miRNA-expressing lentiviral vector [339] for the management/diagnosis of polycystic ovaries. Clinical observations from a limited number of case studies published in the 1980s appear to indicate the potential efficacy of intraovarian injections of carboprost for the termination of ovarian or tubal ectopic pregnancies [340,341], but these have not been further validated by randomized clinical trials. Direct administration of tracers (*e.g.*, activated charcoal solution [342], or ^{99m}Tc colloid albumin and patent blue V [343]) into the ovaries has been suggested as a possible approach for the detection of sentinel nodes in cancer patients [344]. However, the procedure is not recommended due to the risk of capsule rupture and tumor cell extravasation into the abdominal cavity. The injection of tracers in the uteroovarian ligament may be a useful alternative [345,346].

3. Pregnancy-related routes

The genital tract, in particular the uterus, undergoes dramatic changes in size and organization during pregnancy in order to accommodate the growing fetus (Fig. 15). The development of the placenta and the amniotic sac that divide the uterine space between maternal and fetal sides creates the opportunity for the establishment of new routes of administration for drugs – the extra-amniotic, intra-amniotic and intraplacental routes. These

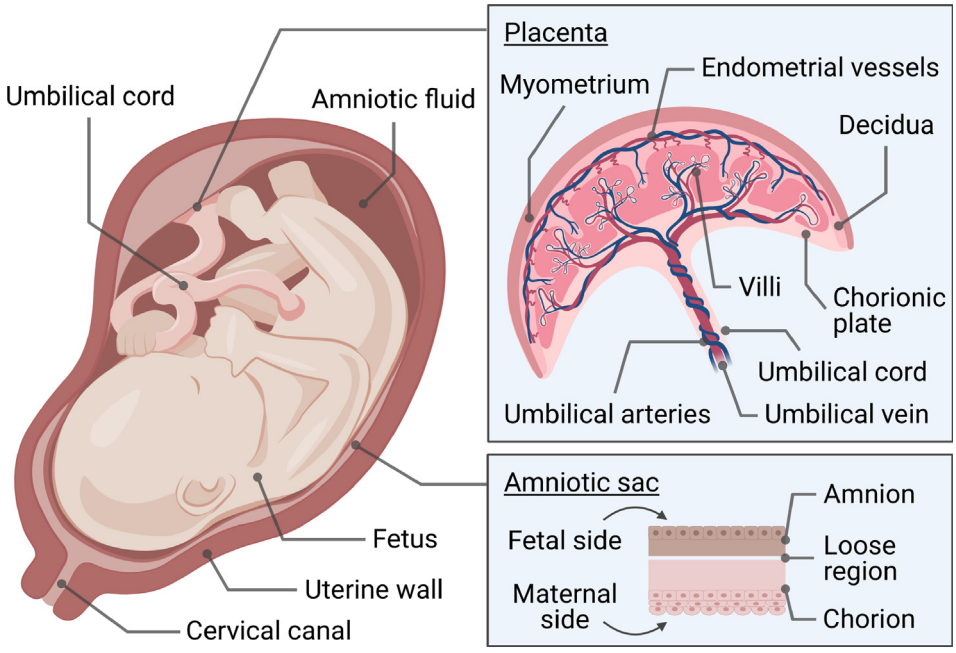

Fig. 15. The uterus accommodating the fetus and associated structures during late pregnancy.

can be considered as particular cases of the intrauterine route, but are considered separately due to their specificities, namely related to the maternal-fetal coexistence.

3.1. Extra-amniotic

The extra-amniotic route comprises the uterine space defined by the modified wall of the uterus (decidua) and the amniotic sac. Its dimensions vary considerably depending on the stage of pregnancy. Special care should be taken during access and administration of drugs in order to avoid unintentional damage of the amniotic sac, which comprises two loosely attached fetal membranes – the chorion and the amnion. The former faces the extra-amniotic space and decidua, while the previous surrounds the amniotic fluid and the fetus. The extra-amniotic administration of drugs has been traditionally considered for the termination of pregnancy, and may involve the use of hypertonic, prostaglandin or ethacridine lactate solutions/gels administered via a transvaginal catheter [347]. However, the availability of safer and less invasive methods led to the near discontinuation of this approach in clinical practice. The extra-amniotic route has also been shown adequate for inducing labor using prostaglandins, but apparently without any advantage over other less invasive methods [348]. Depending on the volume of fluid used, induction can also occur as a result of mechanical action on the amniotic sac. This effect can be obtained by simple extra-amniotic saline infusion (EASI) at a rate of 30–60 mL/h using a Foley catheter, typically in combination with other methods [349].

Permeability of drugs across fetal membranes should be considered upon extra-amniotic administration of drugs, particularly if fetal exposure is of concern. Both the chorion and the amnion contribute to the selective semi-permeable nature of the amniotic sac to water, electrolytes and small molecules, which can vary throughout the course of pregnancy [350,351]. Such variations appear to be at least partially related with hormonal stimuli [352,353]. Definitive studies are missing, but there is a fair amount of evidence that different drugs can extensively cross the amniotic sac. For instance, Menjoge *et al.* [354] conducted *ex vivo* experiments and found that fluorescein isothiocyanate (FITC; 0.39 kDa) could permeate human fetal membranes, while a G4-PAMAM dendrimer (16 kDa) had low transport rate. Both chorion and amnion appeared to have contributed similarly to the barrier effect. Additional information on the permeability properties of the amnion alone comes from the use of this membrane in ophthalmic reconstructive transplantation [355]. *In vitro* experiments indicate that drugs such as ofloxacin (0.36 kDa) [356], moxifloxacin (0.40 kDa) [357] and, surprisingly, bevacizumab (149 kDa) [358] may not only penetrate the amniotic membrane, but can also then be released back in a sustained fashion over several hours/days. These data suggest that the amniotic sac may be able to act as a natural reservoir site for drugs. The apparent permeability of macromolecules (77 kDa FITC-dextran used as model) across human chorioamniotic membranes may be increased by the use of penetration enhancers (*e.g.*, sodium lauryl sulfate, cetyltrimethylammonium bromide, *N*-methyl-2-pyrrolidone, bupivacaine, lidocaine), as shown in *ex vivo* experiments [359]. This effect was further enhanced when combined with low frequency ultrasounds [360] and/or upon FITC-dextran encapsulation in PLGA NPs (≈135 nm) [361]. Even if safety issues cannot be ignored, permeability enhancing approaches could be useful for devising strategies for the delivery of drugs to the fetus in a minimally invasive way. Another issue that needs to be considered when considering extra-amniotic administration of drugs is the presence of important molecular transporters at chorioamniotic membranes. ATP-binding cassette transporters such as MRP1, MRP2, MRP5, BCRP and CERP are present in the amnion and the chorion, but with variable distribution [362–364]. The role of these and possibly other transporters on the transfer of drugs across the amniotic sac is not understood and further studies are required.

3.2. Intra-amniotic

The intra-amniotic route encompasses the space inside the amniotic sac that is partially occupied by the fetus and filled with amniotic fluid. Composition, osmolality and volume of this natural fluid vary throughout the course of pregnancy. In the second trimester, when the intra-amniotic route is most frequently considered, the amniotic fluid features lower osmolality than that of blood plasma and high concentrations of urea, creatinine and uric acid resulting from its content in fetal urine [365]. Average volume steadily increases from around 20 mL to 770 mL between 10 and 28 weeks of pregnancy, peaking at around 900 mL around week 34; the amount of fluid remains relatively unaltered until week 38, but then starts reducing dramatically [365,366]. Such changes need to be considered when calculating adequate doses in order to achieve effective drug concentrations in the amniotic sac. Importantly, the amount of amniotic fluid that is swallowed daily by the fetus is remarkably high (50–140 mL/day/kg of fetal weight) [367], and may provide a gateway for fetal enteral drug delivery.

Clinical use of the intra-amniotic route has been typically restricted to the administration of prostaglandins or hypertonic solutions for second trimester pregnancy termination, usually in combination with other methods [347]. It can also be helpful for the management of local pathological conditions such as infection, or for the prevention of pre-term labor [368,369]. The procedures involved in intra-amniotic administration of drugs are similar to those of amniocentesis [370]. Access to the inside of the amniotic sac is achieved using a needle inserted via abdominal puncture and ultrasound imaging for guidance. Local anesthesia may be used in order to minimize pain during insertion and removal of the needle. Fetal membranes are thought to limit exposure of the mother to molecules administered in the amniotic fluid, but, as discussed in the previous sub-section, drug transport towards the maternal side can occur even if delayed. This may explain why the intra-amniotic administration of prostaglandins can stimulate the uterine wall and induce contractility, but its onset usually takes more time as compared to the extra-amniotic route [347].

Fetal exposure to drugs administered in the amniotic space is variable and may even be low [371]. Still, the continuous oral intake of amniotic fluid by the fetus opens a window of opportunity for achieving relevant fetal systemic drug levels, as shown in different animal studies and in a few clinical studies. For example, Mann *et al.* [372] demonstrated the ability of intra-amniotic desmopressin to sustain marked fetal antidiuresis in ewes, presumably due to drug ingestion and intestinal absorption. Likewise, Burd *et al.* [373] showed that G4-PAMAM dendrimers (3–5 nm) could reach several fetal organs after intra-amniotic administration to pregnant mice. The amount of dendrimers recovered from pups was, however, only around 1–2% of the total administered dose. In a follow-up study in rabbits, dendrimers were shown to have differential biodistribution depending on surface functionalization [374]. Around 20–30% and 10% of hydroxyl-terminated and carboxyl-terminated dendrimers, respectively, were retained in the fetal compartment at 24 h after administration. The remaining fractions were found in maternal tissue and urine, presumably due to transport via the placenta. Interestingly, dendrimers were able to reach the fetal brain in quantities that were considered by the authors of the study as still sufficient to allow their use as nanocarriers for *in utero* therapy of neuro-inflammation and brain injury [374]. In another study using a mouse model, Hermes *et al.* [375] observed that an experimental therapeutic protein, EDI200, could achieve sufficient fetal blood plasma levels for *in utero* treat-

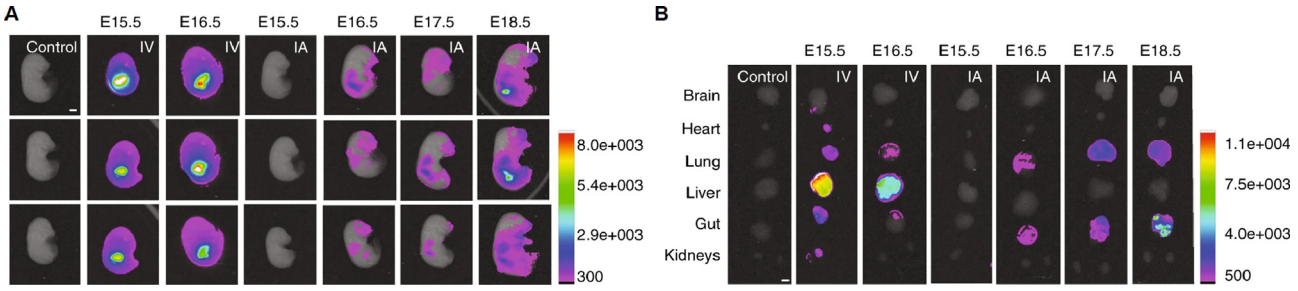

Fig. 16. Biodistribution of DiD-loaded PLGA NPs 3 h after either vitelline vein (IV) or intra-amniotic (IA) injection in **(A)** individual fetuses, and **(B)** fetal organs (control $n = 9$ fetuses, E15.5 IV $n = 10$ fetuses, E16.5 IV $n = 7$ fetuses, E15.5 IA $n = 7$ fetuses, E16.5 IA $n = 8$ fetuses, E17.5 IA $n = 9$ fetuses, E18.5 IA $n = 10$ fetuses). Scale bars = 2 mm. "E" indicates embryonic age in days. Fluorescence and x-ray imaging was performed on a Bruker Carestream In-Vivo MS FX PRO (Billerica, MA). Modified with permission from [394], under the terms of the Creative Commons Attribution 4.0 International License (Copyright 2018, Ricciardi *et al.*, https://doi.org/10.1038/s41467-018–04894-2).

ment of X-linked hypohidrotic ectodermal dysplasia. This was presumably related with the ability of the protein to retain in the amniotic sac due to poor transport across fetal membranes, and to be extensively absorbed at the intestine of fetuses. Indeed, EDI200 possesses an Fc fragment in its structure that can promote neonatal Fc receptor-mediated absorption [375]. The therapeutic merits and safety of the approach have been further observed in dogs, non-human primates and, most significantly, in humans [376–378]. Furthermore, the intra-amniotic administration of levothyroxine was able to yield high fetal blood plasma levels and was shown useful in the management of fetal hypothyroidism in some clinical case reports [379,380].

The possibility of using the intra-amniotic route for *in utero* gene therapy has been a particularly exciting topic of research over the last couple of decades [381,382]. There are several potential advantages for this approach, namely the avoidance of immune response and disease onset, as well as high vector-to-cell ratio, facilitated access to stem cells, and gene expression in daughter cells as a result of early transduction of progenitors [381]. However, injection of gene delivery vectors into the amniotic fluid leads to considerable dilution and may not allow achieving feasible concentrations for therapy. In fact, most animal studies typically consider direct fetal administration (*e.g.*, intravenous, intramuscular, intraperitoneal, intracardiac or intratracheal) [383–389]. Although technically challenging, data from studies involving sheep and non-human primates sustain that direct fetal administration could be feasible to implement in humans. Still, a few animal studies have also demonstrated the feasibility of gene injection into the amniotic fluid surrounding the fetus, mostly by using viral vectors [390–392]. In one particularly interesting study, Alapati *et al.* [393] tested an adenoviral vector for CRISPR-Cas9-mediated gene editing in fetal lungs after intra-amniotic administration to female mice bearing fetuses with severe diffuse parenchymal lung disease. Lung targeting was enhanced by promoting fetal breathing movements with the co-administration of intra-amniotic theophylline and by maternal inhalation of carbon dioxide, thus leading to higher efficacy of the treatment. In another recent work, Ricciardi *et al.* [394] demonstrated the possibility of using PLGA NPs as carriers for triplex-forming peptide nucleic acids (PNAs) and single-stranded donor DNAs for intra-amniotic gene therapy. They observed preferential accumulation of 200 nm NPs in fetal lungs and gut (Fig. 16), and amelioration of anemia in a thalassemic mouse model. Overall, these studies support the tremendous potential of the intra-amniotic route for fetal gene therapy and clinical translation may be expectable in the near future.

Apart from pre-clinical studies in gene therapy, direct access to the fetus through the intra-amniotic route can also be useful in very particular clinical situations. One such case is the *in utero* management of fetal tachycardia along with abnormal buildup of fluids (hydrops fetalis). The onset of edema is associated with the failure of the transplacental route (*i.e.*, passage through or across the placenta of oral or intravenous drugs administered to the mother) and an alternative access to the fetus is required. Drugs such as amiodarone [395–397], digoxin [398], adenosine [399] or flecainide [400] have been described has effective in different clinical cases of fetal tachycardia. Initial access to the amniotic sac is conducted as described above. Drugs can then be injected through the umbilical vein (similarly to what happens with cordocentesis) or directly to the fetus. Intraumbilical and intracardiac injections provide rapid access to fetal blood circulation, but are riskier and more traumatic than intraperitoneal or intramuscular administration [401,402]. Naturally, direct administration of drugs to the fetus comprises considerable risk and needs to be conducted by highly-specialized personnel.

3.3. Intraplacental

The placenta constitutes a remarkable exchange hub between mother and fetus, but also an important barrier to the transport of several drugs [403,404]. In such cases, the direct administration of drugs into the placenta may be of value. Animal data support the feasibility of this approach for the management of extrauterine pregnancy [405], treatment of hematopoietic disorders [406] and gene therapy [407,408]. Direct injection of drugs in the placenta during pregnancy is rare in clinical practice, but may be useful in singular cases such as the *in utero* treatment of large chorioangiomas with ethanol (intratumoral injection) [409,410]. Access to the placenta is typically achieved by ultrasound-guided transabdominal puncture (paralleling chorionic villus sampling used for prenatal genetic testing), although transvaginal access to the placenta may also be feasible. Another relevant application of this route involves the management of post-partum retained placenta. Expulsion of the placenta can be promoted by administering uterotonic drugs such as oxytocin (10–100 IU) or misoprostol (800 μg) through the umbilical vein [411,412]. Efficacy of the procedure is dependent on proper administration. Uterotonics are usually injected in 30 mL of saline using the technique introduced by Pipingas and colleagues [413], which involves deep insertion of an infant nasogastric feeding tube into the umbilical vein (Fig. 17) [414].

4. Breast intraductal route

Contrary to the previous routes that find support on anatomical structures exclusively present in women, the breast is ubiquitous to all humans. However, anatomical and functional particularities – as well as the high incidence of associated pathologies (namely cancer) – make the breast a potentially relevant target for focal

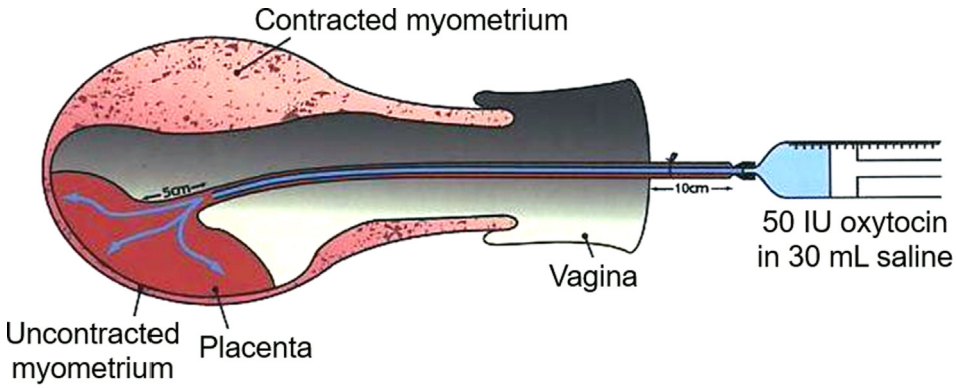

Fig. 17. The Pipingas technique for umbilical vein injection. A nasogastric tube is threaded down the umbilical vein to the placental bed, and then withdrawn by 5 cm and tied. Next, 30 mL of solution are injected down the catheter. This technique achieved optimal filling of the placental bed capillaries in the clinical study conducted by Pipingas *et al.* [413]. Adapted from [414], Copyright (2008), with permission from Elsevier.

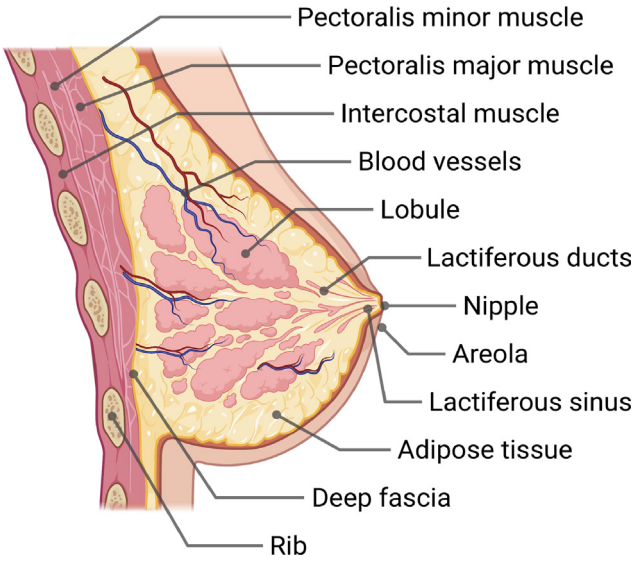

Fig. 18. The anatomy of the breast and associated structures in women (sagittal section).

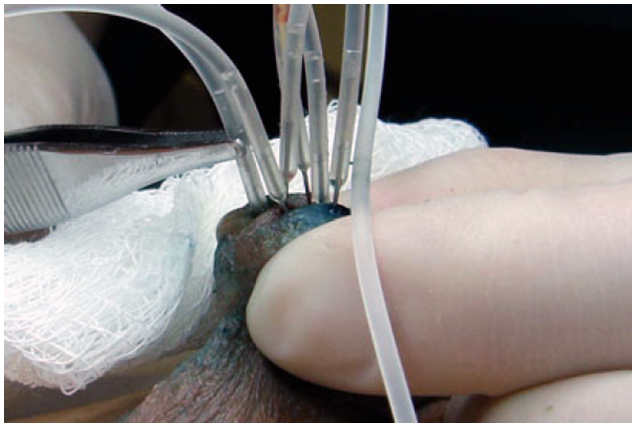

Fig. 19. Insertion of cannulas into multiple ducts. Adapted from [418], by permission of Springer Nature, Copyright (2013).

drug delivery in women (Fig. 18). The administration of drugs directly into the lactiferous ducts via the nipple opening is a relatively new approach for the treatment of ductal carcinoma *in situ* (DCIS) [415,416]. This form of non-invasive breast cancer affects the epithelial cell lining of the lactiferous ducts and can precede invasive forms of the disease. Breast intraductal administration of antineoplastic drugs seems particularly interesting for low-grade forms of DCIS and could provide a minimally invasive alternative to standard treatment, which often includes breast-conserving surgery or even mastectomy [417]. One readily identifiable advantage relates to its localized effects and need for lower drug amounts, which helps avoiding systemic adverse events. Moreover, therapy can be precisely directed to the affected region of the breast and reach terminal duct lobular units [415,416]. Drugs are administered through a sialography catheter inserted into one or more pre-selected ducts (Fig. 19) after local anesthesia at the base of the nipple [418–420]. The correct cannulation can be assessed by pre-administering a contrast agent and performing a ductogram (Fig. 20) [421,422]. The drug of interest is typically dissolved or dispersed in normal saline or 5% dextrose and injected slowly over 30–90 min at volumes that vary between 0.5 and 10 mL/duct. Discomfort and mild pain are associated with the procedure, but are usually well tolerated and fade within 48–72 h [419–422].

Seminal work in the mid-2000s by Okugawa *et al.* [423] and Murata *et al.* [424] demonstrated the efficacy of breast intraductal administration of anti-cancer agents such as paclitaxel, 4-hydroxytamoxifen or PEGylated liposomal doxorubicin (Doxil®) in the prevention and treatment of breast cancer in rodent models. Additional animal studies demonstrated the potential of using other clinically approved drugs such as 5-fluorouracil or carboplatin, but not albumin-bound paclitaxel NPs or methotrexate [421]. Multiple preliminary reports from early clinical studies have soon followed and confirmed the feasibility of breast intraductal injection for the treatment of DCIS [425–429]. Although generally safe, breast intraductal injection of Doxil® or carboplatin can still induce local inflammatory response on a dose-dependent manner [419,420]. Higher doses of carboplatin were also shown able to determine the onset of mild systemic adverse effects, namely vomiting and nausea, which were correlated with the extensive and rapid transfer of the drug into the blood circulation. Systemic distribution of doxorubicin was delayed when liposomes were administered intraductally, and overall lower than what would be expectable for the intravenous administration of Doxil® [419–421]. Despite these promising results, future complementary efficacy and long-term safety studies are needed in order to clinically validate breast intraductal administration of anti-cancer drugs for the management of DCIS. Of particular concern is the report by Chun *et al.* [430] on the long-term development of malignant

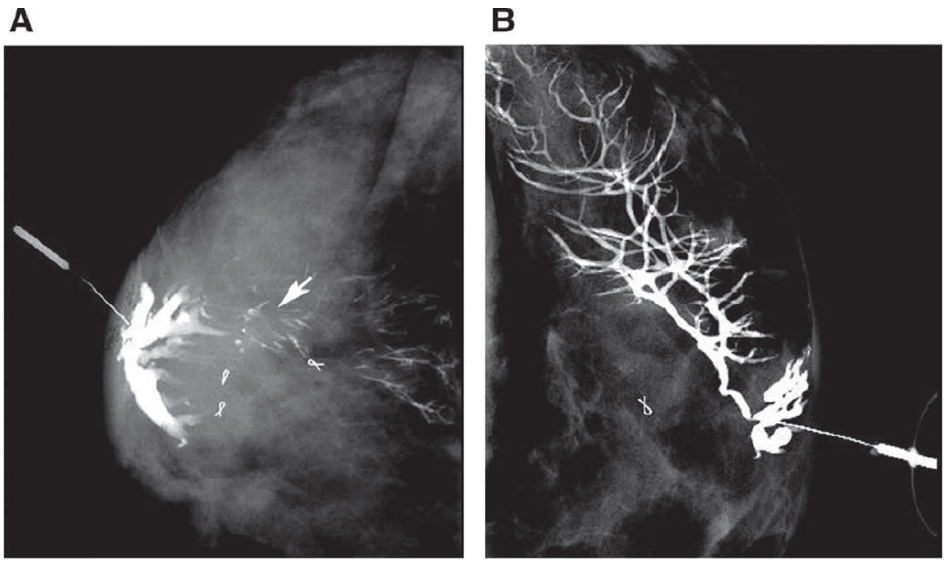

Fig. 20. Representative ductograms from two women participating in a clinical trial. **(A)** A ductogram from a 29 year-old participant, which outlines dilated ducts surrounding the area of the tumor, located in the upper aspect of the right breast (arrow). **(B)** A ductogram from a 37 year-old participant. The ductogram outlines a normal branching duct coursing superior to the site of malignant calcifications in the tail of the left breast. Adapted from [421] with permission from AAAS, Copyright (2011).

mammary tumors in mice treated intraductally with PEGylated liposomal doxorubicin.

Interest originated by early promising clinical results triggered additional pre-clinical work for advancing breast intraductal administration of drugs. Animal studies recently conducted by Wang *et al.* [431] were able to show the potential of clinically approved fulvestrant, an estrogen receptor antagonist, for intraductal treatment of DCIS. In another interesting study, Yoshida *et al.* [432] proposed alpha-particle emitting ^{225}Ac linked-trastuzumab for targeted delivery to HER-2-expressing tumor cells. Breast intraductal administration of the immunoconjugate not only allowed prolonged retention of the radioactive isotope in the mammary gland (thus ameliorating systemic toxicity), but also appeared to reduce the onset of tumors in a DCIS mouse model, as compared to the intravenous route. The potential of nanomedicine has also been explored for breast intraductal administration of different anti-cancer compounds. For example, curcumin-loaded NPs were shown useful for chemoprevention of breast cancer in a rat model [433]. The association of breast intraductal administration and the use of NPs based on an *N*-isopropylacrylamide/vinylpyrrolidone/acrylic acid co-polymer not only allowed using lower doses of curcumin than what would be required by the oral route, but also resulted in a larger reduction of the size of tumors as compared to the intraductal administration of the free drug. The benefit of using drug-loaded NPs for breast intraductal chemoprevention was also recently demonstrated by Al-Zubaydi *et al.* [434,435] for ciclopirox-zinc complexes and ciclopirox prodrugs using a DCIS rat model. Lastly, Brock *et al.* [436] identified the *HoxA1* gene as a potential driver of DCIS progression and were able to successfully silence it in transgenic C3(1)-SV40TAg mice using siRNA. The genetic material was associated to lipid-like amino alcohols-based NPs that were administered bi-weekly for nine weeks by intraductal instillation. The nanosystem was shown able to reduce tumor incidence by 75% as compared to untreated mice. Collectively, these positive results seem to be largely justified with the prolonged intraductal retention of nanocarriers and drugs for up to several days, as evidenced by various studies in rats [437–440]. The association of cytotoxic drugs to nanocarriers may additionally be helpful in improving safety and reducing the onset of local adverse effects [441,442].

5. Concluding remarks

Distinctive anatomical features of women provide unique opportunities for the establishment of specific routes for drug delivery that have been used in clinical practice. Vaginal administration of drugs is particularly prominent due to its long-standing use and current applicability, namely for contraception and treatment of local infection, as well as for the management of other women's health issues. Considerable advances made over recent years regarding the vaginal route were intrinsically related to the development of topical microbicides intended to prevent the sexual transmission of pathogens, namely HIV-1. The dapivirine ring, in particular, is on the verge of being the first vaginal product licensed for topical pre-exposure prophylaxis. General but valuable lessons have also been learned during the development process of microbicides, namely regarding the onset of sub-clinical toxicity by drugs and products administered intravaginally, as well as issues concerning women's acceptability and preferences that have been previously overlooked by those working with this route. At the same time, research efforts for developing nanotechnology-based carriers for vaginal drug delivery have been considerable, and nanomedicine is standing out as a promising approach for innovation in the field.

The worldwide utilization of IUDs for preventing unplanned pregnancy justifies alone the importance of the intrauterine route, but the accumulated experience with these systems could also sustain other clinical applications, namely by using innovative materials and engineering strategies. Even so, practical utility of IUDs is limited by the need for highly potent drugs that are compatible with long-term intrauterine use. Major safety concerns regarding IUDs seem to be long gone, although their nature (large amounts of potent drugs incorporated into a reservoir and extended uterine residence) justifies continuous research on potential toxicity issues and clinical monitoring. Other genital routes also have properly justified, but more restricted applications in drug therapeutics. Pregnancy offers challenges for local drug delivery (*e.g.*, toxicity to the fetus), but also exciting possibilities. Extra-amniotic and intra-amniotic administration of drugs has gone a long way over the last few decades, from being a last resort clinical option, to standing as a consolidated medical practice in obstetrics and

maternal-fetal medicine. Recent and expectable advances in prenatal diagnostics may justify the need for increasing pharmacological interventions targeting the fetus in the years to come, which could thrust the development of new approaches involving localized drug delivery. Finally, the breast intraductal route is the newest addition to women-specific routes for the administration of (anticancer) drugs, but still requires further clinical validation in order to stand as a firm option for pharmacotherapeutics.

Despite the considerable advances in the development of women-specific drug delivery systems – in many cases with successful translation into commercial products – different challenges remain, particularly for disruptive technologies. Progress along the development pipeline may be impaired by technical and scientific issues such as insufficient regulatory guidance and standards, lack of suitable animal models for pre-clinical testing, or difficulties in manufacturing scale-up that are often associated with the need for specialized equipment. Ethical issues related with pre-clinical testing (*e.g.*, use of non-human primate models for microbicide research) and clinical trials during sensitive phases of women's lives – particularly in pregnancy – may further constitute major barriers for successful development. These hurdles seem to, at least partially, justify the poor interest of most pharmaceutical companies in investing in the field.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financed by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project "Institute for Research and Innovation in Health Sciences" UID/BIM/04293/2019. The Graphical Abstract, and Figs. 1, 15 and 18 were created with BioRender.com.

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