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Review

Potential seminal transport of pharmaceuticals to the conceptus

Anthony R. Scialli[a,*], Graham Bailey[b], Bruce K. Beyer[c], Ingrid Brück Bøgh[d], William J. Breslin[e], Connie L. Chen[f], Anthony M. DeLise[g], Julia Y. Hui[h], Graeme J. Moffat[i], Jane Stewart[j], Kary E. Thompson[k]

[a] *Reproductive Toxicology Center and Scialli Consulting LLC, Washington DC, USA*
[b] *Janssen R&D, Beerse, Belgium*
[c] *Sanofi U. S. Inc., Bridgewater, NJ, USA*
[d] *Novo Nordisk A/S, Måløv, Denmark*
[e] *Lilly Research Laboratories, Indianapolis, IN, USA*
[f] *ILSI–Health and Environmental Sciences Institute, Washington DC, USA*
[g] *Novartis Pharmaceutical Corporation, East Hanover, NJ, USA*
[h] *Celgene Corporation, Summit, NJ, USA*
[i] *Amgen Inc., Thousand Oaks, CA, USA*
[j] *ApconiX Ltd., Macclesfield, UK*
[k] *Bristol-Meyers Squibb, New Brunswick, NJ, USA*

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ABSTRACT

Small molecule pharmaceutical products are assumed to reach concentrations in semen similar to those in blood plasma. Exposure modeling for these small-molecule products in humans assumes a daily dose of 5 mL of semen and 100% absorption from the vagina with distribution to the conceptus through the maternal systemic circulation. Monoclonal antibody drugs are present in semen at concentrations about 2% or less of those in blood, and the modeling used for small molecules will over-estimate the possibility of conceptus exposure to immunoglobulins. It is not known whether peptide products reach semen, but in general peptide medications are destroyed by vaginal peptidases, and conceptus exposure is predicted to be minimal. Theoretical exposure routes to pharmaceuticals that might result in exposure of the conceptus greater than that of maternal systemic exposures include direct access through the cervical canal, adsorption to sperm for carriage into the oocyte, and direct delivery from the vaginal veins or lymphatics to the uterine artery. There is some evidence for direct access to the uterus for progesterone, terbutaline, and danazol, but the evidence does not involve exposures during pregnancy in most instances. Studies in mice, rats, rabbits, and monkeys do not suggest that exposure to small molecule pharmaceuticals in semen imposes risks to the conceptus beyond those that can be predicted using modeling of systemic maternal exposure. Monoclonal antibody and peptide exposure in semen does not pose a significant risk to the conceptus.

Contents

* Correspondence to: Scialli Consulting LLC, 2737 Devonshire Pl NW #120, Washington DC 20008-3459 301-514-3081, USA.
E-mail address: ascialli@scialliconsulting.com (A.R. Scialli).

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1. Introduction

Semen is a mixture of spermatozoa produced in the testicular seminiferous epithelium and seminal fluid originating from the epididymis, seminal vesicles, prostate, and to a lesser extent, other male accessory glands. Concern about the presence of pharmaceuticals (both small and large molecules) in semen and their transport to sexual partners has been reflected in recommendations or requirements that men receiving certain pharmaceutical products use condoms during sexual activity [1–3]. These recommendations have come from industry authors [1], the European Union Heads of Medicines Agency [2], and the U.S. Food and Drug Administration, which also provided guidance on quantitative assessment of risk [3]. Although there are concerns about potential drug toxicity in sexual partners of treated individuals, the possible transfer of pharmaceuticals to the developing conceptus is the predominant consideration when condom use is recommended, particularly during clinical trials with new pharmaceutical products when potential effects on gametes or the conceptus are not yet fully investigated.

Possible mechanisms of seminal transfer of pharmaceutical products and other chemicals have been reviewed, most recently in 2005 [4]. Since that publication, additional research has become available to inform risk assessment when considering possible conceptus exposure to pharmaceuticals or other chemicals in semen. Some of the additional research has been performed by a consortium of members of the Health and Environmental Sciences Institute (HESI) Developmental and Reproductive Toxicology Technical Committee [5]. The present review paper was prepared by an expert group coordinated by HESI and summarizes the current understanding of the potential for seminal transfer of pharmaceuticals to female gametes, the developing embryo, and fetus. The possible role of male exposures on transmissible genetic or epigenetic alterations of sperm chromatin and possible effects of pharmaceutical exposure on sperm function will not be considered here, although in the absence of specific transport mechanisms large peptides and proteins are not expected to enter the cell and interact with cellular DNA to pose a genotoxic risk [6,7].

2. Components of semen

The role of semen is to deliver spermatozoa to the female genital tract. Semen ejaculated into the vagina coagulates immediately and liquefies again after approximately 20 min, allowing spermatozoa to enter the cervix from which they are released to the uterine cavity and fallopian tubes [8]. By contrast, semen gains direct access to the uterine cavity in some other species including rat, pig, and horse [9,10]. Mammalian spermatozoa develop in the seminiferous tubules of the testis and undergo additional maturation during transit through the epididymis, which also stores spermatozoa. The epididymis gives rise to the vas deferens, which transmits spermatozoa. Behind the bladder, the vas deferens and seminal vesicles join to form the ejaculatory duct, which opens into the urethra at the base of the prostate gland the fluid of which is mixed with spermatozoa and seminal vesicle fluid to form semen.

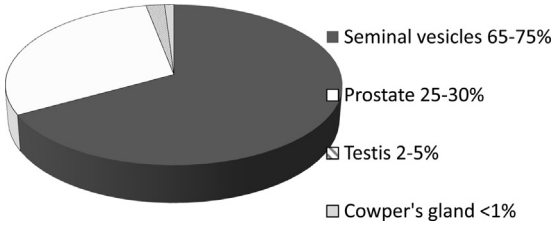

Fig. 1. Components of human semen.

The volume of human ejaculate ranges from 2.3 to 5.0 mL, with an average of 3.4 mL (review of 30 studies by Owen and Katz [11]). Spermatozoa make up only a small portion of whole human semen, *i.e.*, 1–5% of the total volume [12]. The testicular contribution to semen volume is minimal (Fig. 1). The so-called blood–testis barrier consists of tight junctions between Sertoli cells proximal to the spermatocytes, limiting access of blood-borne chemicals to postmitotic germ cells and to the lumen of the seminiferous tubules. Because most of the seminal volume originates from the seminal vesicles and prostate, partition of pharmaceuticals to these glands is more important than is testicular access in influencing semen concentration.

Seminal plasma components have nutritive, buffering, and protective functions for the spermatozoa. The contributions from the different accessory glands and the composition of the seminal plasma differ to some degree between different species. In the human, 65–75% of semen originates from the seminal vesicles (Fig. 1), and constituents include acid phosphatase, citric acid, inositol, calcium, chloride, magnesium, zinc, potassium, sodium, fructose, glucose, ascorbic acid, and prostaglandins. As the semen passes through the prostate, alkaline prostatic fluid is added, comprising about 25–30% of semen. The buffering capacity of this fluid protects sperm in the acidic vaginal environment until the sperm gain access to the cervical mucus. Proteins and amino acids are also present in seminal plasma, and albumin constitutes approximately one-third of the total protein concentration in semen [11].

The pH of human semen is a matter of some debate, and there is considerable variation in the pH measurements reported by different researchers. Nevertheless, the pH of semen lies generally slightly above neutral with a reported range of 7.4–8.4 (reviewed by Owen and Katz [11]). Semen has buffering capacity much higher than that of most other body fluids and maintains its pH near neutral even in the acidic vaginal environment, providing the spermatozoa with the opportunity to enter the neutral pH environment of the cervical mucus.

3. Vaginal and cervical enzymes

Enzymes in the vagina and in cervical mucus have been described, some of which are involved in protein degradation, especially aminopeptidases (*i.e.*, exopeptidases). While no *in vivo* data

on the approximate rate of protein degradation in the vagina could be identified, studies on vaginal homogenates show that the enzymatic activity of aminopeptidases is similar in rats, rabbits, and humans, with *N*-aminopeptidases exhibiting the highest activity [13]. Enzyme activity also varies with the menstrual cycle [14].

Human tissue kallikrein-related peptidases are expressed throughout the female reproductive system, including in cervicovaginal fluid. Kallikrein-related peptidases are localized in the glandular epithelium of the fallopian tubes and endometrium, the cervical mucus-secreting epithelium, and vaginal stratified squamous epithelium, and their concentrations peak in cervico-vaginal fluid after ovulation [15]. Kallikrein-related peptidases are also secreted in the male prostate gland and cleave C-terminals of proteins to several amino acid residues to enhance the liquefaction of ejaculated semen in the female reproductive tract [16].

Insulin can be considered as an example peptide drug. Endopeptidases might play an important role in insulin degradation, because *in vitro* degradation of insulin in small intestinal mucosal homogenates is rapid, and the presence of protease inhibitors reduces degradation considerably. The *in vivo* hypoglycemic response and pharmacological availability of insulin administered directly into the small intestine is insignificant [17]. Insulin is thought to be hydrolyzed by endopeptidases [18], and the proteolytic degradation of insulin in the vagina is comparable to that in the ileum [19,20], supporting the assumption that bioavailability of insulin, and most likely also other peptides, *via* vaginal absorption is negligible.

4. Risk assessment based on systemic distribution

It is standard practice to assume that any pharmaceutical present in the circulation of a man will gain access to his semen. When seminal concentrations of the pharmaceutical are available, this default assumption can be modified. In the absence of data on seminal concentration of a pharmaceutical, it can be assumed that at most, seminal concentrations of small molecular weight pharmaceutical compounds will be an order of magnitude higher than blood, plasma, or serum concentrations. This assumption is based on the empirical observation that semen:blood concentration ratios were 11 or less for most reported chemicals [4]. An exception was chromium, which was measured in an even higher concentration in semen from some metalworkers; however, the authors of that report could not exclude environmental contamination of the specimens after collection [21]. For large molecular weight drugs such as monoclonal antibodies, the concentrations found in semen are much lower than plasma concentrations [22].

To estimate a worst case exposure of a woman to low molecular weight pharmaceuticals in semen, the amount in a 5-mL ejaculate can be assumed to be 100% absorbed every day. The volume of distribution can be used to calculate the concentration of the chemical in the woman's blood, although the FDA draft guidance recommends considering distribution in a 5000-mL blood volume. Even for molecules that are concentrated in semen, the resulting concentration in the woman's blood is typically three or more orders of magnitude less than the concentration of the chemical in the blood of the man who provided the semen [4]. The concentration calculated for the woman's blood can be assumed to be available to a developing conceptus, and a comparison can be made to concentrations that have been identified as potentially harmful in clinical or non-clinical studies.

As an example of such a calculation for thalidomide, the maximum plasma concentration in a man after an 800-mg dose (twice as high as the recommended maximum clinical dose) is 4420 ng/mL [23]. Consistent with draft FDA guidance, this concentration can be assumed to be present in semen [3]. A 5-mL "dose" of semen would contain $4420 \times 5 = 22{,}100$ ng of thalidomide. Vaginal absorption of the entire amount and distribution in a 5000-mL maternal blood volume would produce a maximum concentration of 4.42 ng/mL. The maximum concentration of thalidomide in rabbit plasma at the no observed adverse effect level is 824 ng/mL [24], giving a predicted human exposure multiple of 186 for thalidomide exposure in semen. According to the FDA draft guidance, no further evaluation would be required. However, this exposure multiple is much lower than the >4000-fold obtained by using actual human seminal concentration of 1000 ng/g after a 400-mg thalidomide dose [25], semen weight of 4 g, a volume of distribution of 16,000 mL, and 100% absorption as detailed in the publication by Hui et al. [26], so the recommended procedure for exposure estimation in this case would be highly protective.

For large-molecule drugs including monoclonal antibodies, this modeling will markedly overestimate possible conceptus exposure, given the low concentration in semen, ≤2% of serum [22,27] and/or the limited vaginal absorption, 3.5–4 orders of magnitude below intravenous absorption [22,28]. For immunoglobulins, access to the embryo has been demonstrated to be very low during the first trimester [29,30]. These considerations are discussed below.

5. Other exposure routes to the conceptus

Besides absorption of chemicals from semen across the vaginal epithelium into the maternal systemic circulation, access to the conceptus might in theory be possible by three other mechanisms: migration of the chemical through the cervical canal, carriage by the fertilizing spermatozoon into the oocyte, and transport from vaginal venous or lymphatic drainage to the uterine artery through a so-called counter-current mechanism. There are data to address each of these potential mechanisms as discussed below.

5.1. Access through the cervical canal

5.1.1. The non-pregnant cervical canal

The cervical canal contains mucus produced by endocervical glands (reviewed by Becher et al. [31]). Properties of the mucus change during the menstrual cycle and are affected by estrogen and progesterone. The estrogen-dominated mucus at mid-cycle can be penetrated by sperm for several days prior to ovulation when the mucus is well hydrated, but during the luteal phase dominated by progesterone, the mucus is less hydrated and constitutes a barrier to the passage of sperm [32]. Carbohydrate accounts for about 80% of the mucus by weight and consists of oligosaccharides that protect a protein core (reviewed by Carlstedt and Sheehan [33]). The basic subunit of the mucus consists of 3–5 oligosaccharide-rich regions flanking a 400-amino acid naked protein region, with about 10 subunits in a linear array making up a filament of mucus [34]. These glycoprotein filaments form a meshwork that makes up the structural elements of the mucus plug.

Although it is possible that large molecules are blocked by restricted space between the meshwork of glycoproteins that make up the nonpregnant and pregnant mucus plug, the mesh spacing of human mucus from the cervix and other locations has been reported to be 90–3000 nm, which is large enough to permit the passage of immunoglobin G (IgG) and many globular proteins (reviewed by Olmsted et al. [35]). The use of fluorescent probes in samples of human preovulatory cervical mucus showed *in vitro* the diffusion of virus-like particles (38 and 55 nm) and proteins from 50 to 160 kDa (lysozyme, myoglobin, pepsin, amylase, lactoferrin, IgGs, IgAs) through mucus occurred as readily as diffusion through saline. Diffusion of IgM (950 kDa) and polystyrene microspheres (59–1000 nm) was impeded [35]. Because this preovulatory cervical mucus was more hydrated than mucus at other times during

Table 1
Vital dyes administered vaginally to rats prior to coitus (from Thompson et al. [38]).

Dye	Molecular weight (Da)	Log *P*
Toluidine blue	374	0.9
Acid blue 40	473	−0.46
Acid blue 29	616	−5.65
Brilliant green	483	2.02
Sudan black B	457	8.81

the menstrual cycle and during pregnancy, these findings might not be informative about the permeability of mucus during pregnancy, which is not hydrated.

Another study used cervical-vaginal secretions pooled from various times during the menstrual cycle excluding the periovulatory period and identified mucus pore sizes of 50–1800 nm using non-mucoadhesive nanoparticles [36]. The authors proposed that interference with virus transmission through cervical mucus was more likely based on adhesive interactions than steric hindrance. These coated nanoparticles have been evaluated primarily as potential carriers for the delivery of pharmaceuticals through mucus-coated tissues. It is not clear to what extent contamination with vaginal secretions altered the properties of cervical mucus during the collection process, which used a self-applied menstrual collection device.

Techniques for the measurement of drug transfer through mucus have been reviewed [37]. Most studies have used experimental animal gastric mucus or human ocular mucus. We are not aware of studies involving the penetration of chemicals through human cervical mucus.

5.1.2. The cervical canal during estrus and pregnancy

Additional investigation focused on whether chemicals applied to the rat vagina prior to coitus gained access to the upper genital tract [38]. Females were treated with intravaginal toluidine blue on the afternoon of proestrus and cohabited with males overnight. No dye was identified in the reproductive tract the following morning. In a modification of this experiment, female rats were treated with one of several vital dyes (Table 1) and cohabited with males for 135 min with the room lights off. The males were removed and the females necropsied 30–60 min later. Dye was found on the fur of the male genital region and in the copulatory plug but not in the female genital tract above the cervix. The vagina was stained by the dyes with the exception of acid blue 29 (a protein stain) and sudan black (a fat stain). This study suggested a lack of passage of small molecules through the cervix during estrus, when the cervical canal should have been at its most patent.

Studies have been performed in pregnant laboratory animals to address the possibility of chemical transfer through the cervix. Toluidine blue (molecular weight 270 Da; 0.5 mL) was administered intravaginally to gestation day (GD) 12 rats [38]. The animals were restrained in a head down position for 15 min or monitored for leakage and licking behavior for 10 min after dosing and were killed 15, 120, or 240 min after dosing. The reproductive tract was removed, visually inspected, and examined histopathologically. There was no evidence of dye penetration past the cervix (Fig. 2). Similar results were obtained using pregnant mice.

If a pharmaceutical product in the vagina were to pass through the cervical canal into the uterine cavity during pregnancy, it is not a foregone conclusion that the conceptus would be exposed. The human blastocyst enters the uterus 3–4 days post-conception and implants from 6 to 7 days post-conception into the decidua of the uterus, where it becomes entirely buried beneath the uterine epithelium by 9 days after conception. As the conceptus grows, the overlying decidua bulges into the uterine cavity and is called the decidua capsularis. By the end of the first trimester, the decidua capsularis meets and fuses with the decidua on the opposing uterine wall, called the decidua parietalis, thereby obliterating the uterine cavity. During the first trimester the uterine cavity is a slit-like space (Fig. 3). A pharmaceutical within the uterine cavity would not have direct access to the conceptus but would need to diffuse through the decidua and trophoblast to gain access to the amniotic space and the embryo.

5.2. Transmission of chemicals to the upper female genital tract and oocyte during coitus

In humans, semen deposited in the vagina in the days before ovulation results in the rapid uptake of sperm by the cervical mucus, from which the sperm are released over days into the upper genital tract. Of the hundreds of millions of spermatozoa deposited in the vagina, typically ten to a few hundred sperm and on occasion, up to one thousand sperm reach the fallopian tube [39,40]. Human seminal fluid is believed to be excluded from the uterus based on the observation that semen in the uterus causes cramping and inflammation, attributed to seminal prostaglandins (reviewed by Boomsma et al. [41]). By contrast, seminal proteins have been identified in the uterus of the rat after coitus, although entry of these proteins into the oviducts has not been documented [9]. The access of seminal proteins and perhaps other seminal chemicals to the uterine cavity appears to be an important difference between rats and humans.

The entry of a chemical into the human uterus or fallopian tube after vaginal application or exposure during coitus has not been described. In a human subject, vaginal application of a gadolinium-labeled spermicide in the mid-follicular phase of the cycle (about 6 days prior to ovulation) resulted in identification by magnetic resonance imaging (MRI) of the label in the vagina and the endocervical canal [42]. Studies using the same imaging technique reported the vaginal distribution of gel after simulated coitus using an artificial penis or after real intercourse [43–48]. The images presented in these papers do not show label above the cervix, but the authors did not comment on whether they evaluated the upper genital tract for access of the gadolinium. Interpretation of the images is limited by the bright signal from normal endometrium with the MRI protocols used in these studies, but in no case was there continuity of the signal with contrast in the vagina or endocervix.

It is clear, however, that human sperm are transported at mid-cycle through the uterus to the fallopian tube within minutes after being deposited in the vagina [49]. This transport is independent of sperm motility and has been attributed to uterine peristalsis, as demonstrated by the transport of inert albumin spheres 5–40 μm in diameter [50]. Microorganisms also can ascend from the cervix to the fallopian tubes as indicated by instances of salpingitis as a consequence of endocervical infection with gonorrhea or chlamydia. Spread to the fallopian tubes of at least some of these infections has been attributed to breakdown of the cervical mucus barrier at the time of menses [51].

Some pharmaceuticals and other chemicals have been found to bind to spermatozoa, raising the possibility of transfer to the oocyte during fertilization. Rabbit, boar, and human spermatozoa showed ultraviolet fluorescence after incubation with tetracycline, suggesting that the tetracycline in this *in vitro* exposure system had bound to the spermatozoa cell [52]. The amount of binding was not quantified, and the fertilizing ability of the bound spermatozoa was not evaluated. In a male rabbit given radiolabeled thalidomide by mouth, the concentration of label in a washed ejaculated spermatozoa sample was 0.05 μg/mL when the blood concentration was 6.81 μg/mL and the seminal fluid concentration was 9.60 μg/mL, all expressed as thalidomide equivalents [53]. In a second rabbit, spermatozoa-associated thalidomide was 0.02 μg/mL. Fertilizing ability was not assessed but in another publication, mating and fer-

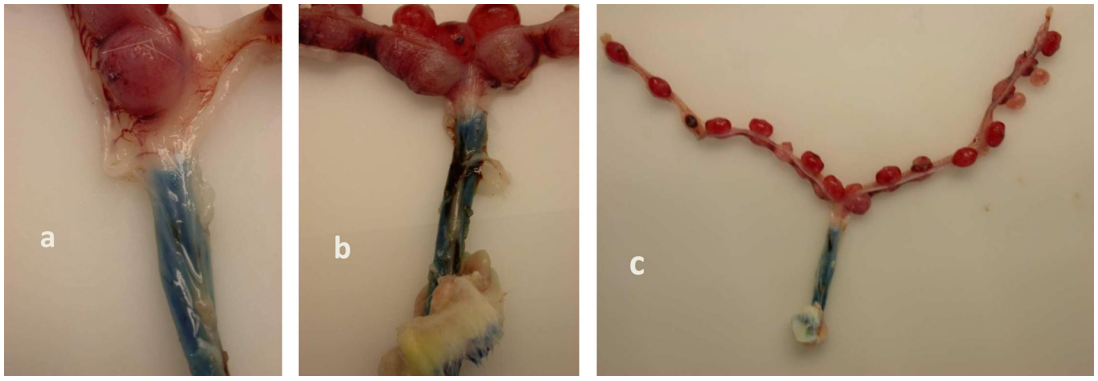

Fig. 2. Reproductive tract of pregnant rats (a) 15, (b) 120, and (c) 240 min after intravaginal administration of toluidine blue (Thompson et al. [38]).

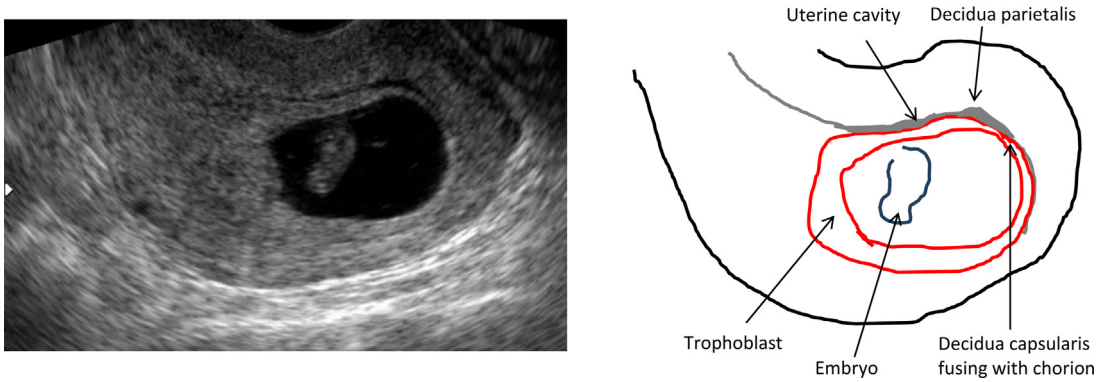

Fig. 3. Sonogram (left) of human pregnancy about 5 weeks after conception and line tracing of relevant structures.

tility (number of pregnancies per number of rabbits that mated) of male rabbits were unaffected by oral treatment with thalidomide up to 500 mg/kg/day [23]. Sperm motility, count, and concentration were also unaffected by thalidomide treatment. Cocaine added to washed human spermatozoa was adsorbed with an estimated 3600 binding sites per sperm cell [54]. Spermatozoa motility and viability was not affected by incubation with cocaine at up to 670 μM. Assuming a human oocyte volume of 3.59 nL and a fertilizing spermatozoon carrying 3600 cocaine molecules, the resulting concentration of cocaine in the oocyte would be 5×10^{-7} mg/L, compared to a recreational adult blood cocaine concentration of ~1 mg/L.

Spermatozoa concentrations of metals were measured in samples from men working in a refinery or polyolefin factory in Finland [55]. Mean spermatozoa metal concentrations in mg/kg of ashed specimens were cadmium 0.003, lead 0.03, and aluminum 0.93. These measurements were lower than in a group of healthy semen donors, which the authors attributed to sound industrial hygiene measures and the rural residence of the workers compared to the urban residence of the semen donors. In the combined workers and semen donors, there was a negative association between sperm or semen aluminum concentration and sperm motility. No other measures of spermatozoa quality were associated with metal concentrations in spermatozoa or semen, and there were no estimates of possible adverse effects of metal exposure on a fertilized oocyte.

In summary, where detailed analyses have been undertaken, it is possible to detect some small molecule pharmaceuticals or chemicals on or within spermatozoa. However, where comparable data exist, these concentrations are several log orders lower than plasma concentrations.

5.3. Countercurrent transfer

Based on the demonstration in menopausal women that vaginally administered progesterone has greater effects on the endometrium than would be predicted based on systemic progesterone concentration, it has been suggested that there is a countercurrent transfer mechanism whereby a chemical in the vaginal venous or lymphatic effluent can diffuse into the neighboring uterine arterial circulation [56]. This mechanism might result in higher concentrations of a chemical in the uterine circulation than in the systemic circulation. It has been shown in menopausal women that vaginal progesterone administration results in nearly two-fold higher progesterone concentrations in the uterine artery than in the radial artery [57].

Although data from menopausal women treated with progesterone might not apply to pregnant women or to other pharmaceutical products, the transfer of progesterone and terbutaline from the vagina to the uterus was demonstrated in perfused hysterectomy specimens obtained from premenopausal women [58,59]. The preferential transfer of vaginally administered danazol, which has a modified steroid hormone structure, to the uterus and ovary of premenopausal women was inferred based on similar tissue concentrations after hysterectomy in women given 100 mg vaginally and women given 400 mg orally in the face of much lower serum concentrations after vaginal administration [60]. Terbutaline given during pregnancy also might reach the uterus directly from the vagina rather than systemically when used for the treatment of preterm labor [61]. It is not known if this property would apply to first-trimester exposures. Thus, the possibility of preferential transfer of a chemical from the vagina to the uterus exists although it would require absorption through the vaginal mucosa

Table 2
Human evidence for the preferential access of vaginal small molecules to the uterus.

Medication	Model	Findings	Reference
Progesterone	Functionally agonadal women preparing for embryo donation	Vaginal progesterone produced higher endometrial tissue concentrations of progesterone than did intramuscular progesterone in spite of higher serum progesterone concentrations after the intramuscular administration	Miles et al. [74]
	In vitro perfused human uterus with vaginal cuff	^{3}H-progesterone placed on the vaginal cuff resulted in uptake of label in the endometrium and myometrium of the uterus beginning at 1 h of perfusion and peaking at 5 h. Effects were greater in uteri removed during the luteal compared to the follicular phase of the cycle	Bulletti et al. [59]
	Menopausal women undergoing hysterectomy	Uterine artery progesterone concentrations were higher than radial artery progesterone concentrations	Cicinelli et al. [57]
Terbutaline	Pregnant women	Vaginal administration inhibited the contractions of premature labor without systemic effects on blood pressure or heart rate and at very low systemic venous concentrations	Kullander and Svanberg [61]
	In vitro perfused human uterus with vaginal cuff	^{3}H-terbutaline placed on the vaginal cuff resulted in uptake of label in the endometrium and myometrium of the uterus peaking at 12 h of perfusion	Bulletti et al. [58]
Danazol	Reproductive age women with uterine leiomyomata	Oral (400 mg) and vaginal (100 mg) medication produced comparable ovarian and uterine tissue concentrations of the drug in spite of much lower serum danazol concentrations after vaginal administration	Mizutani et al. [60]

and has been demonstrated for few small molecule compounds. Direct access through the cervix has not been excluded as a contributor to this preferential distribution from the vagina to the uterus. The evidence for such preferential distribution is summarized in Table 2.

6. Empirical observations during mouse, rabbit, and monkey pregnancy

6.1. Small molecules

Possible alternative routes of exposure to chemicals in semen were evaluated in mice, rabbits, and monkeys. Because these animals will not mate when pregnant, test substances were administered vaginally rather than in semen.

Wild-type female mice mated to males carrying a transgene with a luciferase reporter produced embryos some of which carried the transgene and could be identified by exposure to D-luciferin. Intravaginal administration of D-luciferin during gestation resulted in a visible signal indicating access of the vaginal dose to the embryos [62]. The signal appeared within minutes of vaginal application, raising the possibility of direct transfer from the vagina to the uterus, but the time courses of the signals were similar whether dosing was intravenous, subcutaneous, or intraperitoneal. Ligation of the vagina near its apex did not prevent access of vaginally administered D-luciferin to the embryo, although transfer might have been delayed somewhat (Fig. 4). The experimental design could not quantify the amount of transfer to the embryo, only the timing. The findings were consistent with systemic absorption and distribution to the uterus or direct access from the vagina to the uterus through a countercurrent mechanism, because the vaginal ligation procedure was not believed to interfere with the vascular supply. Distribution by both a systemic and direct mechanism might also have been possible.

Pregnant rabbits were used to evaluate the transfer of intravaginal thalidomide to the embryo [26]. Time-mated New Zealand white rabbits were treated orally or intravaginally on GD 7–11, the sensitive period for thalidomide-induced embryopathy. Animals were monitored for vaginal leakage, which was minimal. In the pharmacokinetic portion of this study, the oral dose levels were 20 or 180 mg/kg body weight (bw)/day, representing known oral non-teratogenic and teratogenic dose levels, respectively. Vaginal

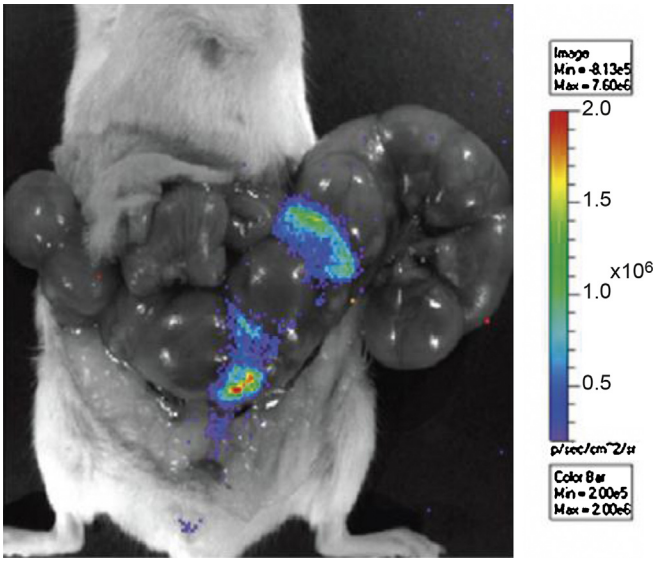

Fig. 4. Mouse carrying embryos with luciferase-reporter transgene ~6 min after vaginal treatment with D-luciferin. The vagina has previously been ligated near its apex. From Cao et al. [62], used by permission.

dose levels were 2, 20, or 180 mg/kg bw/day. The 2 mg/kg bw/day dose level was more than four orders of magnitude higher than the amount in human semen based on the known seminal distribution of this medication at up to 250 ng/g of ejaculate after a 100-mg dose [25]. Plasma exposures (area under the concentration-time curve) by the vaginal route were 30% compared with oral dosing, and maximum plasma concentrations were 3–7-fold lower by the vaginal route. Yolk sac fluid to plasma concentration ratios on GD 11 were similar (ranging from 0.49 to 0.66) across doses and routes of administration, suggesting the absence of preferential transfer from the vagina to the uterine contents.

In the embryo-fetal component of this study, pregnant rabbits were treated orally or intravaginally with thalidomide at 2 mg/kg bw/day on GD 7–19, and fetuses were evaluated on GD 29. A positive control group was given thalidomide at 180 mg/kg bw/day orally. Fetuses from does given 180 mg/kg bw/day orally had thalidomide-associated malformations typical for this species. There were no treatment-related

Table 3
Maternal and fetal metronidazole after vaginal dosing of cynomolgus monkeys (from Thompson et al. [64]).

		Metronidazole (ng/mL)			Hydroxymetronidazole (ng/mL)		
Dam ID	Gestation day	Maternal/fetal plasma	Ratio	Amniotic fluid	Maternal/fetal plasma	Ratio	Amniotic fluid
1501	70	94.4/105	0.9	145	3.66/4.17	0.9	2.01
1502	60	756/735	1.0	649	26.5/21.1	1.3	8.69
1503	71	494/539	0.9	817	19.6/21.3	0.9	13.4

adverse fetal effects at 2 mg/kg bw/day by either the oral or vaginal route. The data indicate that thalidomide exposure from seminal transfer in the rabbit does not represent an embryo-fetal risk. Inasmuch as the theoretical seminal dose of thalidomide in men would be four orders of magnitude lower than the tested vaginal dose in rabbits, thalidomide exposure from contact with semen would not be expected to pose an embryo-fetal risk in human pregnancy.

In another experiment, three pregnant cynomolgus monkeys were given 1 mL of 0.75% metronidazole gel intravaginally on GD 60, 70, or 71 using a commercial preparation formulated for use in women [63,64]. There was minimal leakage after administration. Cesarean sections were performed 7 h after dosing, and measurements of metronidazole and its hydroxylated metabolite were made in maternal and fetal blood samples and in amniotic fluid using tandem mass spectroscopy. Concentrations were similar between each mother and fetus, indicating no evidence of preferential transfer to the fetus. Therefore, modeling using systemic absorption by the mother and distribution across the placenta would have been predictive of fetal exposure (Table 3).

6.2. Monoclonal antibodies

Monoclonal antibody drugs are unlikely to be transferred to the embryo from semen in meaningful amounts based on the very low concentration of native immunoglobulins in semen and the lack of a placental receptor for immunoglobulin transfer early in pregnancy [29]. Empiric confirmation of this principle has been reported in rabbits and monkeys.

After intravenous administration of a therapeutic IgG4 product to male rabbits, semen concentrations were less than 1% of those in serum at all time points from 8 to 72 h after dosing [22]. Based on the concentration of IgG4 in semen and ejaculate volume, the total dose delivered vaginally by seminal transmission of the IgG4 would be 4–4.4 orders of magnitude lower than by intravenous dosing. Intravaginal administration of the antibody in nonpregnant females resulted in very low or undetectable serum concentrations. When equal doses were administered either vaginally or intravenously, the highest detectable serum concentration after intravaginal dosing was 3.5 orders of magnitude lower than the concentration in serum after intravenous dosing. On GD 29, intravenous IgG to pregnant rabbits resulted in fetal serum concentration 1.5-fold higher than maternal serum concentrations, suggesting ready placental transfer at term. Based on the concentrations attainable in semen, the estimated fetal exposure on GD 29 from intravaginal semen from a male given 100 mg/kg bw intravenously was 3.5×10^{-5} μg/mL. This dose level was estimated to result in fetal exposures 1.3×10^{-8} times lower than administration of 100 mg/kg bw intravenously to the dam, a dose level not expected to be biologically meaningful.

Pregnant cynomolgus monkeys were given an intravaginal dose (100 mg) of a fully human IgG2 every two weeks from GD 21 to GD 133 [28]. Maternal plasma concentrations of the immunoglobulin were undetectable at all time points in 3 of 5 animals. In the remaining two animals, concentrations were low but measurable at about half of the time points up to 120 h after dosing on GD 119 and GD 133. The maximum maternal plasma concentration represented 0.01% of the intravaginal dose, about 4 orders of magnitude lower than plasma concentrations after intravenous dosing. Cesarean section was performed 72 h after vaginal dosing on GD 133, but no detectable antibody was present in the plasma of any fetus. The low vaginal absorption in monkeys is consistent with the results described above for the rabbit. Collectively, these data indicate that seminal transfer does not represent an important exposure risk to the embryo–fetus.

6.3. Peptides

Although no literature was found on non-immunoglobulin peptide or protein drugs being excreted in semen, there is evidence that proteins of relevant size and structure are present in human semen. For example, it has been shown that insulin-like growth factor (IGF)-I and II, which share several structural and biological features with insulin, are present in significant amounts in seminal plasma (IGF-I ~0.1 μg/mL, IGF-II ~2 μg/mL), and the presence of IGF-1 is correlated with semen quality, suggesting a role in germ cell maturation [65,66]. Because albumin concentrations in seminal plasma are approximately half of those in blood plasma [11], corresponding to interstitial concentrations, peptides with albumin binding capacity will most likely not be up-concentrated in seminal plasma.

Absorption of vaginally administered peptides is possible, but bioavailability and potency are low compared to subcutaneous administration [67,68] unless absorption enhancers or oleaginous formulations are used as demonstrated with leuprolide, a gonadotropin-releasing hormone analog [69–71], and insulin [68–70,72,73]. Experimental administration of insulin per vaginam to ovariectomized rats resulted in clear reductions in blood glucose concentrations when insulin was administered in combination with an enhancer of absorption (sodium taurodihydrofusidate, polyoxyethylene-9-lauryl ether, lysophosphatidylcholine, palmitoylcarnitine chloride, or lysophosphatidylglycerol), while no effect on blood glucose levels was achieved when insulin was given without an enhancer [68]. In another study, it was shown that the effect on blood glucose concentrations in the rat was highly correlated with the thickness of the vaginal epithelium with a higher effect in metestrus and diestrus than estrus and pro-estrus [73]. Thickness of the human vagina does not vary appreciably across the menstrual cycle.

7. Conclusions

The hypothesis that vaginally administered medication will gain access to the conceptus through the cervical canal is not supported by available evidence. Transmission of chemicals to the oocyte by binding to spermatozoa is theoretically possible, but quantitative modeling does not suggest that clinically meaningful concentrations can be achieved in the conceptus by this mechanism. Transfer of medications absorbed *via* the vaginal epithelium into the venous or lymphatic drainage of the vagina to the arterial supply of the uterus has been demonstrated for few small molecule medications and largely in nonpregnant subjects or in perfused hysterectomy specimens. Additional studies on possible transfer to the embryo or fetus by this route would be welcome. Experimental animal models do not suggest that the theoretical alternative routes for

chemical transmission in semen will produce clinically meaningful exposures of the conceptus.

Because large molecules reach semen in only low concentrations, modeling assumptions used for small molecules will markedly over-estimate possible exposure. Monoclonal antibody transmission in semen results in negligible exposures of sexual partners and of the embryo and fetus. Vaginal peptidases and generally poor absorption across the vaginal epithelium will limit systemic access of seminal peptides. The available data on monoclonal antibody drugs indicate that vaginal absorption is extremely low.

The assumption that small-molecule pharmaceutical products in semen will be 100% systemically absorbed during intercourse permits a reasonable and conservative approach to human risk assessment. The low seminal excretion and low vaginal absorption of monoclonal antibodies, combined with the small ejaculate volume, would not result in biological meaningful exposure risk to the conceptus, and assuming 100% systemic absorption of seminal peptides would result in a gross overestimate of embryo/fetal exposure risk.

Transparency document

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