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Review

Characteristics of the endometrium in menstruating species: lessons learned from the animal kingdom[†]

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Abstract

Here we have summarized what is currently known about menstruating animal species with special emphasis on non-primate species: length of their menstrual cycle, ovulation, implantation, placentation, decidualization, and endometrial characteristics. Having an overview of all the possible animal models that can be used to study menstruation and the menstrual cycle could be useful to select the one that better matches the needs of the individual research projects. The most promising species to study menstruation seems to be the spiny mouse *Acomys cahirinus*. It is a rodent that could be easily held in the existing laboratory facilities for rats and mice but with the great advantage of having spontaneous menstruation and several human-like menstrual cycle characteristics. Among the species of menstruating bats, the black mastiff bat *Molossus ater* and wild fulvous fruit bat *Rousettus leschenaultii* are the ones presenting the most human-like characteristics. The elephant shrew seems to be the less suitable species among the ones analyzed. The induced mouse model of menstruation is also presented as an adaptable alternative to study menstruation.

Summary Sentence

A summary of the menstrual cycle and endometrial characteristics of human-like menstruating species.

Key words: menstrual cycle, endometrium, implantation, ovulation, placentation.

Introduction

Menstruation can be defined as the periodic shedding of the superficial functional layer of the endometrium in the absence of embryonic implantation. This endometrial shedding occurs only in a restricted number of mammals. Indeed, the endometrium, in most mammals, instead of being shed is resorbed and replaced to start a new uterine cycle [1]. Most of the menstruating species, including humans, are primates [2]. Besides in primates, menstruation has been observed

only in the spiny mouse [3], 3–5 species of bats [4–6], and the elephant shrews [7] (Figure 1).

Here, we summarize what is currently known about their menstrual cycle. We briefly summarize the characteristic of primates' menstrual cycle because it has been widely studied [2, 8]. The order in which the different species will be described will follow the lineage reconstruction (Figure 2) created using the NCBI taxonomy database [9]. They will be listed from the closest to *Homo sapiens* to the most distant one.

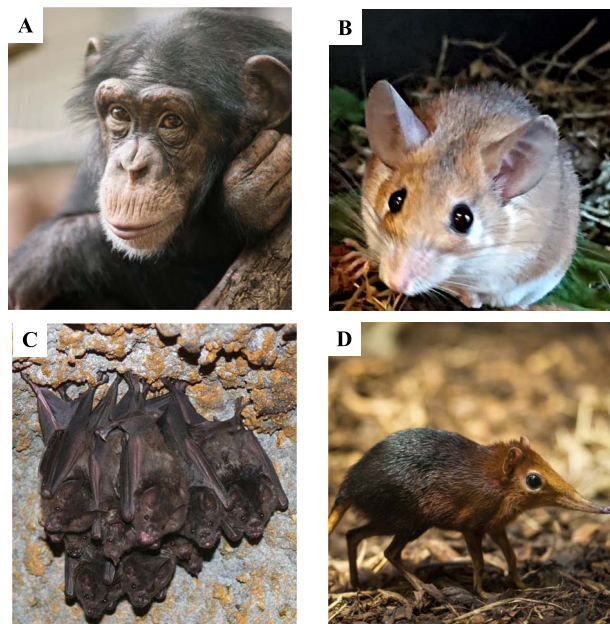


Figure 1. Reference photo of (A) chimpanzee, credit to Copenhagen Zoo; (B) spiny mouse, own picture; (C) short-tailed fruit bat *C. perspicillata*, credit to Zoo New England; (D) Elephant shrew *Rhynchocyon petersi* credit to ZOO Antwerpen/Jonas Verhulst.

The use of one or more animal models that could illustrate human reproductive processes as close as possible and at the same time being easily maintained in captivity is fundamental to better understand all the physiological processes which occur during menstrual cycle bringing new light on the mechanisms behind infertility and other pathologies related to the female reproductive system.

In our review, we decided to include also an additional animal model: the induced mouse model of menstruation [10]. This model does not represent physiological menses, but it is commonly used as a model of menstruation thanks to its adaptability [11–13].

Menstruating animals

Primates

Non-human primates, such as the great apes (Figure 1A), the Old World monkeys, the lesser apes, and the New World monkeys, have similar menstrual cycles with some minor difference related to the different species. They are spontaneous ovulators, and most of them are continuous breeders with some exception, for example, macaques [14–16]. They have a gestation period that ranges from 6 to 9 months, and they usually give birth to a single pup [16–18]. Embryo implantation is less invasive than in human although their placenta is hemochorial [19, 20]. The length of their menstrual cycle ranges from 21 to 37 days [14, 15, 18, 21–24]. Menses duration is 2–4 days and the hormonal pattern is similar between species [16]. Progesterone pattern is almost identical, and it is characterized by an increased concentration during the luteal phase. Major differences can be appreciated in the estrogen pattern. Indeed, in some species, for example, in baboons, macaques, or capuchin monkeys, there is only one peak of estrogen during ovulation, while in others, such as chimpanzees and spider monkeys, it rises again during luteal phase [16, 23–27]. Decidualization is not extensive as in humans, and in some species, like in baboons, it is not spontaneous but embryo dependent [28, 29].

Spiny mouse

The common or Cairo spiny mouse *Acomys cahirinus* (Figure 1B) is the only known rodent that undergoes spontaneous endometrial decidualization and menstruation [3]. It is native to Africa and the Middle East and has several characteristics in common with other menstruating mammals. Indeed, this spiny mouse has a spontaneous ovulation [30], an intrauterine endocrine milieu similar to humans [31], and a hemochorial placenta [32], and it has a long gestation (for rodents) of 39 days with production of few well-developed, precocial pups (2–4) [33].

According to Bellofiore et al. [3], who have been the first to observe and describe the spiny mouse menstrual cycle in detail, the length of the menstrual cycle is 8–9 days. The estrous cycle is similar to other rodents [30] but includes an extra phase, lasting more or less 3 days, in which blood is detected either macroscopically, both on external genitalia and inside the lumen of both uterine horns, or microscopically, in vaginal lavage smears [3]. The endometrium goes through modifications following the different stages of the menstrual cycle. Its thickness increases 4–5-fold during the luteal phase with a consecutive decrease of uterine lumen diameter. Moreover, at the same time, angiogenesis and decidualization are observed in the uterus. This phase, in the absence of ovum fertilization, is followed by the shedding of superficial layers of the endometrium after the degeneration of the corpus luteum. The presence of spontaneous decidualization during the luteal phase is confirmed by the increased endometrial presence of prolactin [3] and interleukin 11 (IL11) [34] which are two well-known markers of decidualization [35, 36]. These endometrial changes are also correlated with hormonal changes typical of menstruating mammals. In particular, it has been observed a substantial increase of plasma progesterone concentration during the luteal phase compared to the follicular phase and the occurrence of menstrual bleeding due to the decrease in progesterone concentration [3].

Bats

There are at least three species of bats in which menstruation has been observed:

The short-tailed fruit bat *Carollia perspicillata* (Figure 1C) is a microchiropteran bat native to the Neotropical realm. Females are spontaneous ovulators and seasonal breeders in the wild. They usually have two pregnancies per year and give birth to one single pup after a gestation of 4 months [5]. The implantation is limited to the superior end of the simplex uterine cavity with the formation of a hemochorial placenta [37]. The timing between ovulation and menstruation is short; therefore, in these bats, endometrial cellular proliferation is post-ovulatory. The endometrium appearance after coitus is different depending upon whether the female is pregnant or not. If the oocyte is fertilized, 1–3 days after coitus, the endometrium appears thin but with an elevated glandular and stromal mitotic activity. Moreover, a well-developed corpus luteum can be observed. Endometrial mitotic activity proceeds until initiation of implantation which results in a very thick endometrium close to implantation. However, there is no clear evidence of a spontaneous decidual reaction before embryo implantation. If the oocyte is not fertilized, 1–3 days after coitus, the female presents a regressing corpus luteum and a thick and necrotic endometrium shed with associated bleeding in a menstruation-like process [5]. It seems that the cycle length is 21–27 days. This interval has been measured by Rasweiler et al. [38] as the time necessary to establish a pregnancy after a pregnancy failure followed by a new breeding period.

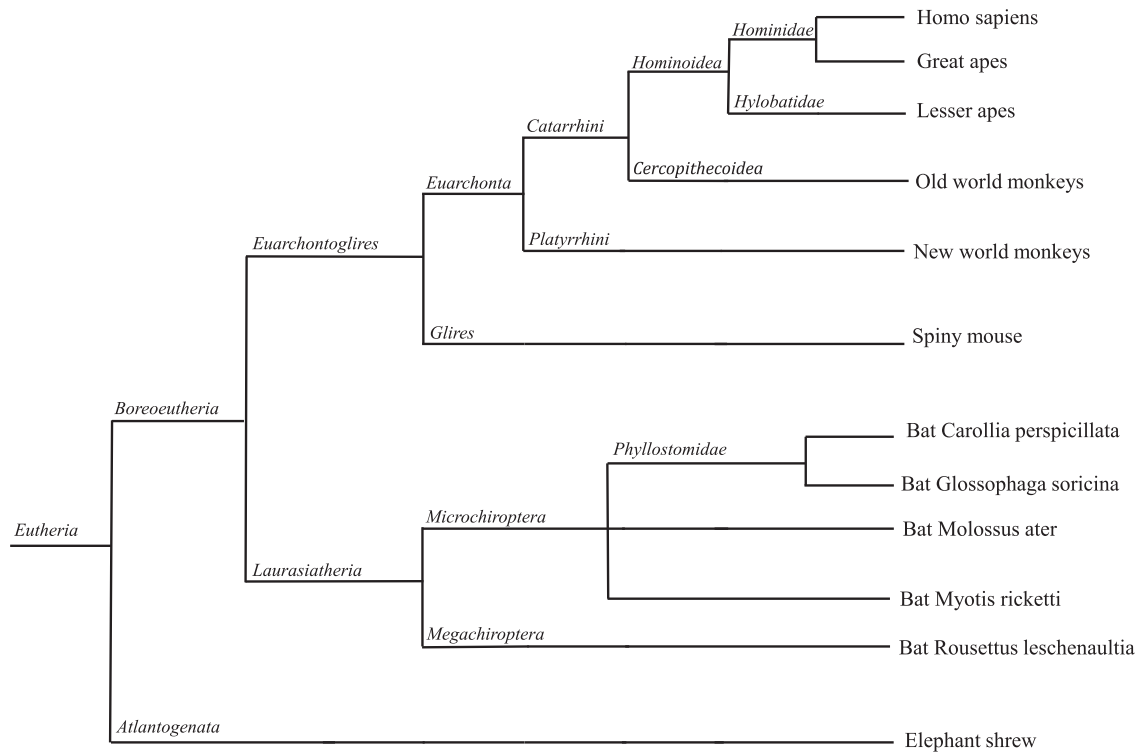


Figure 2. Graphic representation of the cited menstruating species' lineage. Lineage information obtained from NCBI taxonomy database [9].

The long-tongued bat *Glossophaga soricina* is also a microchiropteran bat native to the Neotropical realm. Females are polyestrous, and their ovulation is cyclical and spontaneous during the year [39]. The implantation site is restricted to a tubular segment of their simplex uterus, called intramural uterine cornu, at the uterotubal junction, and their placentation is hemochorial as the species described above [40]. They have a menstrual cycle of 22–26 days [39]. The endometrium is thin before ovulation and starts significantly growing afterward in concomitance with the formation of the corpus luteum. The endometrium becomes especially thick at the fundus where implantation usually occurs [41].

In cycles not resulting in pregnancy or in case of embryo loss, the hypertrophied endometrium break downs and is shed with associated bleeding [41]. Ovulation and menstruation occur very close to each other. For this reason, in this species, the ovum remains for a long time in the oviduct, and embryo development is very slow, giving the endometrium the time to regenerate [39]. The endometrium does not present a spontaneous decidualization, but it appears after the trophoblastic penetration of the endometrial stroma [40].

The black mastiff bat, *Molossus ater*, is another microchiropteran bat native to the Neotropical realm. In this species, ovulation is spontaneous and occurs only from the right ovary [42]. Embryo implantation is superficial and restricted to the right horn of the bicornuate uterus [43]. In the wild, they are seasonal breeders and usually give birth to only one pup after a gestation of 3–4 months [43]. Their hemochorial placenta always develops at the superior end of the right uterine horn [43]. Its creation is preceded by the formation of a vascular tuft at the same endometrial site [4]. The preovulatory endometrium is thin, while soon after ovulation it begins to hypertrophy especially at the superior end of both uterine horns where vascular tufts start to develop. During the luteal phase,

the endometrium, especially the area around the vascular tuft, undergoes a spontaneous and extensive decidualization. In nonpregnant cycles, this decidua becomes necrotic and is shed with associated bleeding [4].

Menstruation has been also observed in other two species of bats: the wild fulvous fruit bat *Rousettus leschenaultii* and the piscivorous vespertilionid bat *Myotis ricketti*. However, data from these two species require confirmation since other researchers have risen doubts about the accuracy and reliability of the shown data [38].

The wild fulvous fruit bat *R. leschenaultii* is a megachiropteran bat native to the Indomalayan/Oriental realm. Females have spontaneous ovulation and usually have two pregnancies per year with the production of a single pup after a long gestation of 4 months [6]. The implantation is restricted to the superior end of either uterine horn and is followed by the formation of a hemochorial placenta [37]. They have a menstrual cycle of 33 days that can be divided into a proliferative, a secretory, and a menstrual phase. The proliferative phase is characterized by endometrial proliferation, ovarian follicle development, and increased production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which reach their maximum concentration at day 18, coinciding with ovulation. In the secretory phase, there is a formation of the corpus luteum in the ovary, an increased production of estradiol and progesterone, and an initial thickening of the endometrium that is followed by decidualization. In the event of unsuccessful fertilization or implantation, the corpus luteum regresses, concentration of progesterone falls, and the upper part of the endometrium is shed with visible vaginal bleeding. This menstrual phase lasts only 1 day [6]. Zhang et al. [6] have been able to observe two consecutive menstruation events in female bats in the absence of male bats demonstrating that this species cyclically menstruates independently of coitus.

The Rickett big-footed bat *M. ricketti* is a piscivorous vespertilionid bat native to China. It seems to be a spontaneous ovulator. It has a bicornuate uterus and it usually gives birth to one single pup. Ovulation of one single oocyte occurs during spring. In this species, delayed ovulation has been observed with one single Graafian follicle present in one ovary for 5–7 months during hibernation. They usually copulate in autumn, before hibernation; for this reason, they store spermatozoa in the uterotubal junction till spring waiting for the ovulatory period. Menstruation has been observed during the preovulatory period in concomitance with an increased production of estradiol [44].

Elephant shrew

Another example of non-primate menstruating animals is elephant shrews (Figure 1D) [7]. They are a small group of mammals (15 species) native to Africa and belonging to the family of Macroscelididae [45]. They have a spontaneous polyovulation, but, in all except one species, only a maximum of two precocial pups are born for each gestation (42–75 days long) [46, 47]. The implantation is restricted to the inferior end of each uterine horn [46], and also their placentation is hemochorial [47]. The average length of their estrous cycle is 12 days [48, 49].

Most of the information regarding their menstrual cycle derives from the work of Professor C. J. van der Horst that, in the 1940s, did an incredible amount of research on this topic examining histological specimens of female reproductive organs from the Eastern rock elephant shrew *Elephantulus myurus* [50]. Menstruation, in the wild, is not a cyclical event in elephant shrews but occurs only at the end of the breeding season. Indeed, females that ovulate during the height of breeding season are most of the time fertilized and become pregnant, while at the end of the breeding season, there is a higher possibility to have ovulation without fertilization that is followed by menstruation [51]. The initial endometrial modifications in fertilized and unfertilized cycles are similar and characterized by an extensive increase of endometrial thickness. After ovulation, there is an edema formation at the site of implantation in each uterine horn. In fertilized cycles, this edema further develops to create the embryo chamber with an initial decidual reaction which requires embryo presence and development to be completed [52]. In unfertilized cycles, from the edema, there is a polyp-like outgrowth of stromal cells. After degeneration of the corpus luteum, this polyp is shed in a process similar to menstruation [7, 51].

Induced mouse model of menstruation

Even though it is not a physiological model of menstruation, the induced mouse model of menstruation is still worthy of mention considering its frequent use in research and its adaptability.

The commonly used laboratory mouse *Mus musculus* has spontaneous ovulation and a hemochorial placenta [53] and gives birth to a litter of 2–14 pups after a gestation of more or less 20 days [54]. As said before, rodents, except the abovementioned spiny mouse, do not naturally menstruate, but they go through an estrous cycle. This cycle lasts 4–5 days, and if there is no fertilization, after an initial thickening, the endometrium is reabsorbed to start a new cycle. Decidualization is embryo dependent and occurs only after embryo implantation [55].

Nevertheless, researchers have been able to induce menstruation in mice. To produce this menstrual-like endometrial shedding, female mice have to be hormonally stimulated. Female mice can

be ovariectomized and then stimulated by estrogen injections, followed by a subcutaneous insertion of a progesterone implant, or progesterone production can be stimulated, in intact female mice, by pseudopregnancy induction. After this first hormonal stimulation, decidualization is mechanically induced with an intrauterine injection of sesame or arachis oil. After the decidualization induction, the progesterone implant is removed, and menstruation can be observed. Decidualization in this model is rapid, extensive, and very destructive [10, 55, 56].

Human-like characteristics

Decidual immune cells

One characteristic that has been observed in all these menstruating species is the presence of decidual immune cells. In recent years, it became clear that the presence of immune cells at the maternal–fetal junction is important to promote pregnancy and develop tolerance to fetal antigens [57]. In primates, as in humans, only a few leukocytes are present in the endometrium during the first stages of the menstrual cycle. After ovulation, there is a substantial increase in endometrial leukocytes, especially natural killers and macrophages [58, 59].

In the spiny mouse decidualized stroma, the presence of neutrophils, macrophages, and several pro-inflammatory cytokines, such as interleukin 11 (IL11), interleukin 8 (IL8), and macrophage migration inhibitory factor (MIF), has been observed. Their presence was especially pronounced during the late secretory phase and menstruation supporting the idea that endometrial inflammatory response plays an important role in preparation for implantation and during uterine shedding [34].

In the decidua of elephant shrews and three of the abovementioned bats (*M. ater*, *G. soricina*, and *C. perspicillata*), only the presence of large granulated cells has been reported. These cells have periodic acid-Schiff (PAS)-positive granules, and it has been suggested that they could be similar to the uterine natural killer cells observed in humans. They have been detected both in pregnant and nonpregnant cycles especially at the basal lamina of the endothelial cells close to the generally predetermined implantation site [4, 5, 37, 47, 50].

In the induced mouse model of menstruation, an increased presence of inflammatory mediators and leukocytes, mainly neutrophils, has been also observed after progesterone removal and beginning of bleeding [60].

Spiral arteries

The presence of spiral arteries, a key structure in human and primate endometrial vasculature [61, 62], is a feature present in a few of the species analyzed here. Indeed, the presence of these arteries has been observed only in the spiny mouse, in the elephant shrew, and in one species of bat: the black mastiff bat *M. ater*.

In the spiny mouse, the formation of spiral arteries has been observed during the late secretory phase. Moreover, in this species, also the presence of the vascular endothelial growth factor (VEGF) has been investigated. VEGF has been detected especially in the stroma surrounding these spiral arteries suggesting its involvement in endometrial vascular regulation [34]. While in the spiny mouse, the presence of these spiral arteries has been detected also in nonpregnant cycles, in the elephant shrew, they have been observed only in the decidua of pregnant females. Data about nonpregnant cycles are not available [47].

In bats, spiral arteries have been observed only in the black mastiff bat. However, it seems that they are not essential for menstruation in this species. Indeed, they are mostly located in the myometrium and in the endometrial lamina basalis, becoming straighter in their terminal segments in the lamina functionalis. Moreover, they do not show any increase in coiling during the progression of the menstrual cycle as observed in primates and humans [4, 63]. In the long-tongued bat *G. soricina* and the short-tailed fruit bat *C. perspicillata*, the endometrium is vascularized only by capillaries, and no presence of spiral arteries have been detected even in the late phases of the menstrual cycle close to endometrial shedding [4, 5]. In the wild fulvous fruit bat *R. leschenaultii*, there is evidence of an increased distribution of capillaries and arterioles in the endometrium during the proliferative phase, but the presence of spiral arterioles has not been investigated [6].

Premenstrual spiral arteries remodeling has not been observed in the induced mouse model of menstruation [34].

Implantation and trophoblast invasion

Most of the examined menstruating animals in this study, as humans and apes, are characterized by invasive implantation, while one of them, the black mastiff bat *M. ater*, has superficial implantation, like Old World monkeys [64]. Interestingly, the implantation site, in all the examined species, is predetermined and restricted to a specific uterine area, while in humans and primates there is not a restriction in this regard. However, in humans, it preferentially occurs in the upper and posterior end of the simplex uterus [65]. Information about implantation on the spiny mouse is not available since until now it has not been investigated.

In elephant shrews, implantation occurs only at the inferior end of the uterine horns. The presence of the embryo at the implantation site stimulates an extensive decidual reaction and a substantial expansion of the surrounding uterine glands [46]. In this species, implantation is invasive. Initially, the syncytiotrophoblast invasion is restricted to the basement membrane of the uterine epithelium. Then, after the amniotic cavity formation, the trophoblast invades the decidua to create anchoring points and remodel the maternal capillaries in preparation for the following placenta formation [50, 52].

As said before, in bats, implantation is restricted to a specific portion of the uterus, but there are some species-specific differences. For example, in *C. perspicillata* implantation is interstitial. Interestingly, in the case of post-implantation delays in embryo development, trophoblastic invasiveness highly increases, penetrating also the myometrium, oviducts, and other extrauterine tissues [66]. It has been observed that *C. perspicillata* modifies gestation length, delaying embryo development, in response to stress in captivity or seasonally in the wild [67]. In the closely related long-tongued bat *G. soricina*, implantation is also interstitial. Decidualization is not spontaneous and occurs only at day 15 *post-coitus* after the trophoblastic penetration of the endometrial stroma, characterized by extensive destruction of maternal epithelium and trophoblast penetration of maternal basement membrane [40]. The black mastiff bat, *M. ater*, is the only menstruating bat with superficial implantation. The implantation site is restricted to the central area of the right uterine horn. After an initial spontaneous decidualization around the vascular tuft, during implantation, the decidual reaction continues to expand along the endometrial superficial lamina functionalis. However, no trophoblastic invasion of the endometrial stroma has been observed. The trophoblast is characterized by a single layer of flattened cells connecting the blastocyst and the endometrium [43].

Mice have rapid and invasive implantation. After embryo apposition, decidualization is stimulated, and the trophoblastic cells start penetrating through the uterine luminal basal lamina entering in contact with the maternal blood to form the hemochorial placenta [68].

Summary

Looking at the summary table (Table 1) is clear that some species have more human-like characteristics than others, making them more suitable animal models. Primates, being the closest related species to humans (Figure 2), share most of their features with them, and they would be the easiest choice as animal models to study menstrual cycle and reproductive processes. However, the use of non-primate animal models in research is often preferable due to ethical considerations and the possibility of maintaining big colonies with less effort in terms of space, money, and handling.

Using non-primate animal models developing spontaneously pathologies of the reproductive system like endometriosis would be advisable. It will be important for drug testing and pathology treatments but also for improvement of assisted reproductive technologies, ovarian stimulation, and endometrial receptivity.

Bats could be good animal models in reproductive research especially in their native countries where they are present in a considerable amount. They can also be caged and maintained in captivity. However, even though they have spontaneous ovulation and true menstruation for which they could be used to study menstrual dysfunction, they lack some other important features of human physiology. For example, the short-tailed fruit bat *C. perspicillata* and the long-tongued bat *G. soricina* do not present a clear spontaneous decidualization or spiral arteries, and no data are available for what concerns their endocrine profile, while very few information is available for the Rickett big-footed bat *M. ricketti*. The black mastiff bat *M. ater* and wild fulvous fruit bat *R. leschenaultii* are the ones that most resemble humans. However, the bat *M. ater* has superficial implantation, and we do not have any endocrine profile data, while for the bat *R. leschenaultii* we do not know anything about the presence of decidual immune cells, spiral arteries, or implantation. More research should be done in these two last species of bats to further determine their reproductive physiology and their use as animal models.

The elephant shrew seems to be less suitable than the bats as an animal model. Decidualization starts spontaneously, but the embryo presence is necessary for its further development. Decidualization and endometrial shedding are localized in the polyp-like outgrowth of the uterus, and there is no information about its endocrine profile. Moreover, the lack of cervix and true vagina and the consequent inability to detect estrus changes by vaginal smear technique add an additional problem to the use of this animal in research [46]. It is also the most distantly related species to humans analyzed here (Figure 2), adding extra concerns in its selection as a preferable animal model.

The most promising animal to be used as non-primate animal model is the spiny mouse *A. cahirinus*. It could be easily bred and kept in captivity, and it has several human-like characteristics even though no data are available regarding implantation. The fact of being a rodent, like the rat and the mouse, gives this species an additional advantage to be considered as a good animal model. There is plenty of literature about rat and mouse physiology that could be used as a starting point to further investigate the spiny mouse characteristics. Moreover, most of the laboratories will have the facilities to host this new rodent species already in place, being

Table 1. Overview of the menstrual cycle characteristics of the cited species in comparison with the human menstrual cycle.

	Human	Primates	Spiny mouse	Elephant shrews	Short-tailed fruit bat <i>C. perspicillata</i>	Long-tongued bat <i>G. soricina</i>	Black mastiff bat <i>Molossus ater</i>	Wild fulvous fruit bat <i>R. leschenaultii</i>	Rickett big-footed bat <i>M. ricketti</i>	Induced mouse model of menstruation
Menstrual cycle	28 days	21–37 days	8–9 days	12 days	21–27 days	22–26 days	NA	33 days	NA	No
Spontaneous ovulation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Spontaneous decidualization	Yes	Only in some species	Yes	No	No	No	Yes	Yes	NA	No
Decidual immune cells	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	NA	Yes
Spiral arteries	Yes	Yes	Yes	Yes	No	No	Yes	NA	NA	No
Implantation	Invasive	Invasive	NA	Invasive	Invasive	Invasive	Superficial	NA	NA	Invasive
Implantation site	Not specific	Not specific	NA	Inferior end of uterine horn in bicornuate uterus	Superior end of simplex uterus	Uterotubal junction of simplex uterus	Right horn in bicornuate uterus	Inferior end of uterine horn in bicornuate uterus	NA	Not specific
Hemochorial placenta	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Estrogen peak	During ovulation and luteal phase	During ovulation and in some species also during luteal phase	NA	NA	NA	NA	NA	During luteal phase	During ovulation	Induced
Progesterone peak	During luteal phase	During luteal phase	Yes	NA	NA	NA	NA	During luteal phase	During ovulation	Induced
Offspring	1	1	2–4	1–2	1	1	1	1	1	2–14
Gestation period	6 months	6–9 months	39 days	42–75 days	4 months	NA	3–4 months	4 months	NA	20 days

Table 2. Advantages and disadvantages of using the cited species as animal models.

	Advantages	Disadvantages
Primates	<ol style="list-style-type: none"> 1. Good to study spontaneous decidualization, menstruation and endometrial regeneration, inflammatory processes, spiral artery remodeling, implantation, hormonal modulation 2. Morphological and molecular data already available 3. Techniques, reagents, and antibodies already developed 	<ol style="list-style-type: none"> 1. Ethical limitations 2. Expensive handling and husbandry
Spiny mouse	<ol style="list-style-type: none"> 1. Good to study spontaneous decidualization, menstruation and endometrial regeneration, inflammatory processes, spiral artery remodeling, hormonal modulation 2. Some morphological and molecular data already available 3. Information available for handling and husbandry 	<ol style="list-style-type: none"> 1. Techniques, reagents, and antibodies have to be improved
Elephant shrew	<ol style="list-style-type: none"> 1. Good to study menstruation and endometrial regeneration, inflammatory processes, spiral artery remodeling, implantation 2. Some morphological data already available 3. Information available for handling and husbandry 4. Not expensive handling and husbandry 	<ol style="list-style-type: none"> 1. No observed menstruation in captivity 2. Few molecular data available 3. Techniques, reagents, and antibodies have to be improved
The short-tailed fruit bat <i>C. perspicillata</i>	<ol style="list-style-type: none"> 1. Good to study menstruation and endometrial regeneration, inflammatory processes, implantation 2. Some morphological and molecular data already available 3. Information available for handling and husbandry 4. Not expensive handling and husbandry 	<ol style="list-style-type: none"> 1. Techniques, reagents, and antibodies have to be improved
Long-tongued bat <i>G. soricina</i>	<ol style="list-style-type: none"> 1. Good to study menstruation and endometrial regeneration inflammatory processes, implantation 2. Some morphological data already available 3. Information available for handling and husbandry 4. Not expensive handling and husbandry 	<ol style="list-style-type: none"> 1. Few molecular data available
Black mastiff bat <i>M. ater</i>	<ol style="list-style-type: none"> 1. Good to study menstruation and endometrial regeneration, spontaneous decidualization, inflammatory processes, spiral artery remodeling, implantation 2. Some morphological data already available 3. Information available for handling and husbandry 4. Not expensive handling and husbandry 	<ol style="list-style-type: none"> 2. Techniques, reagents, and antibodies have to be improved
Wild fulvous fruit bat <i>R. leschenaultii</i>	<ol style="list-style-type: none"> 1. Good to study menstruation and endometrial regeneration, spontaneous decidualization, inflammatory processes, spiral artery remodeling, implantation 2. Some morphological data already available 3. Information available for handling and husbandry 	<ol style="list-style-type: none"> 1. Few molecular data available
Rickett big-footed bat <i>M. ricketti</i>	<ol style="list-style-type: none"> 1. Good to study spontaneous decidualization, menstruation and endometrial regeneration 	<ol style="list-style-type: none"> 1. Few morphological data already available 2. No molecular data available 3. Techniques, reagent, and antibodies have to be improved 4. No information available for handling and husbandry
Induced mouse model of menstruation	<ol style="list-style-type: none"> 1. Good to study decidualization, menstruation and endometrial regeneration, inflammatory processes 2. Techniques, reagent and antibodies already developed 3. Information available for handling and husbandry 4. Not expensive handling and husbandry 	<ol style="list-style-type: none"> 1. Few morphological data already available 2. No molecular data available 3. Techniques, reagent, and antibodies have to be improved 4. No information available for handling and husbandry

similar to other rodents. However, further studies have to be done in this species to better characterize its menstruation. The colony at the Hudson Institute of Medical Research in Australia is the only colony in which menstruation, in this species, has been reported in scientific journals.

The induced mouse model of menstruation is easily maintained, menstruation induction is reproducible, and the immune response during menses seems to be similar to humans. However, it does not present spiral arteries remodeling, and the induced decidualization reaction is too destructive and extensive and does not resemble the physiological process observed in humans.

Anyway, the absence of detailed genetic information, techniques, and reagents, particularly antibodies, that would allow routine use of the abovementioned menstruating animal models still renders the induced mouse model of menstruation a valid alternative.

The use of naturally menstruating animal models would be recommendable because, in addition to true menstruation, they present several other human-like characteristics like long gestation with the production of one single pup, invasive placentation, and spontaneous ovulation. Their use and more detailed characterization will allow a more comprehensive overview of all the interconnected mechanisms that lead to the evolution of these features. Moreover, the study of factors involved in severe menstrual bleeding, endometriosis, or premenstrual syndrome will be more easily studied in animal models with physiological menstruation. Unfortunately, until now, comparative studies of different models of menstruation have been done only between the spiny mouse and the induced mouse model of menstruation [34]. Indeed, in bats, most of the studies have been observational or morphological studies, and there is almost no molecular information about them. However, there is already information about care and handling and histology descriptions since most of them have used animal models in the studies of reproductive biology especially ovarian and placentation studies [69–71]. Information on handling and husbandry is available also for elephant shrews, but it seems that females do not menstruate in captivity but enter in an anestrus state [49, 51].

The animals listed in this review are the ones that so far are known to menstruate. It seems menstruation appeared several times during the speciation of mammals, and it is not related to just one lineage (Figure 2). Several hypotheses have been proposed during the years for menstruation evolution, but no definitive answer has been found. The first theory suggested that menstruation evolved to clean the uterus from sperm-borne pathogens introduced during coitus [72]. Then it has been argued that menstruation was necessary to conserve energy, claiming that the cyclical renewal of the endometrium would be less costly than the maintenance of a continuous metabolically active endometrium which is required for implantation [73]. However, these first theories have been criticized in favor of a new theory that defines menstruation as a nonadaptive consequence of uterine–embryo coevolution and the development of decidualization. Indeed, to balance the increased invasiveness of the embryo, the uterus evolved increasing its cellular growth and differentiation (decidualization) to protect itself [74]. Since in non-menstruating species decidualization is embryo induced while in most of the menstruating species it is spontaneous, the key to menstruation seems to be the evolution of spontaneous decidualization. In the most recent theory, spontaneous decidualization evolved by genetic assimilation of the decidualization reaction, which is induced by the fetus in non-menstruating species, driven by two possible selective forces: invasive embryos and high incidence of impaired embryos [2]. The reason why only a few species and not closely

related to each other seems to have spontaneous decidualization and menstruation is still unknown but recently has been suggested that nutrition (omnivorous diet and food availability) could have played a role in species selection [75].

Researchers should keep investigating the possible presence of other menstruating animals that maybe have been overlooked not being closely related to known menstruating species or for their lack of clear macroscopical bleeding.

As can be deduced from this review, there is a need for more basic and comparative studies to better characterize these menstruating species. The information available for most of the different non-primate menstruating species is still not enough to render these species a competitive alternative to the use of primate or mouse models (Table 2).

The spiny mouse seems to be the one with more potential to become a common and valid alternative to the models. Nevertheless, other menstruating species also deserve further studies to develop a more comprehensive view of menstruation and its evolution. Indeed, the presence of robust animal models will allow further understanding of why menstruation occurs, what controls menstrual blood loss, and the mechanism behind the endometrial cyclical “injury” and “repair” system [76]. Moreover, comparative studies will be necessary to test the theory of genetic assimilation. Several experiments could be set up in the lab to test this theory comparing data from menstruating and non-menstruating species bringing new knowledge on how menstruation and spontaneous decidualization evolved [2]. The availability of genetically known menstruating animal models would be also very important to test the role and the importance of genes and proteins discovered thanks to the advancement of genomics and proteomics. Indeed, to develop new therapeutic targets would be important to understand the cellular and phenotypic changes that the selected genes may mediate, their interactions, and signaling pathways [77].

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Conflict of Interest

The authors have no conflicts of interest to declare.

Author contribution

Laura Catalini: literature review, manuscript drafting. Jens Fedder: work conception, critical manuscript revision, final approval of the manuscript.

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