GYNECOLOGY

First evidence of a menstruating rodent: the spiny mouse (*Acomys cahirinus*)

Nadia Bellofiore, BSc; Stacey J. Ellery, PhD; Jared Mamrot, BSc; David W. Walker, PhD, DSc; Peter Temple-Smith, PhD; Hayley Dickinson, PhD

BACKGROUND: Advances in research relating to menstruation and associated disorders (eg, endometriosis and premenstrual syndrome) have been hindered by the lack of an appropriate animal model. Menstruation, the cyclical shedding of the decidualized endometrium in the absence of pregnancy, is believed to be limited to 78 higher-order primates (human beings and Old World monkeys), 4 species of bat, and the elephant shrew. This represents only 1.5% of the known 5502 mammalian species and <0.09% of these are nonprimates. Thus, many aspects of menstruation remain poorly understood, limiting the development of effective treatments for women with menstrual disorders. Menstruation occurs as a consequence of progesterone priming of the endometrial stroma and a spontaneous decidual reaction. At the end of each infertile cycle as progesterone levels decline the uterus is unable to maintain this terminally differentiated stroma and the superficial endometrium is shed. True menstruation has never been reported in rodents.

OBJECTIVE: Here we describe the first observation of menstruation in a rodent, the spiny mouse (*Acomys cahirinus*).

STUDY DESIGN: Virgin female spiny mice (n = 14) aged 12–16 weeks were sampled through daily vaginal lavage for 2 complete reproductive cycles. Stage-specific collection of reproductive tissue and plasma was used for histology, prolactin immunohistochemistry, and enzyme-linked immunosorbent assay of progesterone (n = 4–5/stage of the menstrual cycle). Normally distributed data are reported as the mean ± SE and significant differences calculated using a 1-way analysis of variance. Nonnormal data are displayed as the median values of replicates (with interquartile range) and significant differences calculated using Kruskal-Wallis test.

RESULTS: Mean menstrual cycle length was 8.7 ± 0.4 days with red blood cells observed in the lavages over 3.0 ± 0.2 days. Cyclic endometrial shedding and blood in the vaginal canal concluding with each infertile cycle was confirmed in all virgin females. The endometrium was thickest during the luteal phase at 322.6 μm (254.8, 512.2), when plasma progesterone peaked at 102.1 ng/mL (70.1, 198.6) and the optical density for prolactin immunoreactivity was strongest (0.071 ± 0.01 arbitrary units).

CONCLUSION: The spiny mouse undergoes spontaneous decidualization, demonstrating for the first time menstruation in a rodent. The spiny mouse provides a readily accessible nonprimate model to study the mechanisms of menstrual shedding and repair, and may therefore be useful in furthering studies of human menstrual and pregnancy-associated disorders.

Key words: endometrium, menstruation, progesterone, spontaneous decidualization

Introduction

Menstruation, the cyclical shedding of the decidualized endometrium in the absence of pregnancy, is believed to be limited to 78 higher-order primates (human beings and Old World monkeys), 4 species of bat,[1,2] and the elephant shrew.[1,3,4] This represents only 1.51% of the known 5502 mammalian species[5]; <0.09% of menstruating species are nonprimates. Common to these species, and inherent to the process of menstruation, is spontaneous decidualization of the endometrial stroma without initiation from an implanting embryo. Under the control of progesterone from the ovary, the decidual reaction occurs in unison with a series of intricate structural changes to the uterine stratum functionalis, including extensive angiogenesis of maternal vasculature into spiral arterioles.[6] In the absence of pregnancy, degeneration of the corpus luteum results in progesterone withdrawal and endometrial shedding that, due to extensive vascularization of the endometrium, is accompanied by bleeding into the uterine cavity.[1] In nonmenstruating species, decidualization of the endometrium does not eventuate unless fertilization occurs and the process is signaled from the conceptus.

The spontaneous nature of the morphological changes that result in decidualization of the endometrial stroma are considered a preparatory maternal response to the impending invasion of the trophoblast and to aid in the adhesion of the attaching embryo.[1,7,8] The extent of trophoblastic invasion is greater in menstruating species; reaching as far as the inner third of the myometrium in women.[9] For successful implantation and pregnancy to occur, the maternal decidual reaction involves extensive remodeling of the myometrial and endometrial vascular beds, success of which ensures support of the invading trophoblast and development of the placenta.[1] Clinical diseases such as preeclampsia, currently one of the leading causes of fetal-maternal morbidity and mortality, are thought to be due to inadequate vascular remodeling and an impaired decidual reaction, resulting in shallow trophoblastic invasion and placental hypoxia.[10] Alternatively, if the trophoblast invades too deeply, women may experience placenta accreta, with abnormal placental-uterine adhesion. In extreme cases, this may only be able to be treated with peripartum

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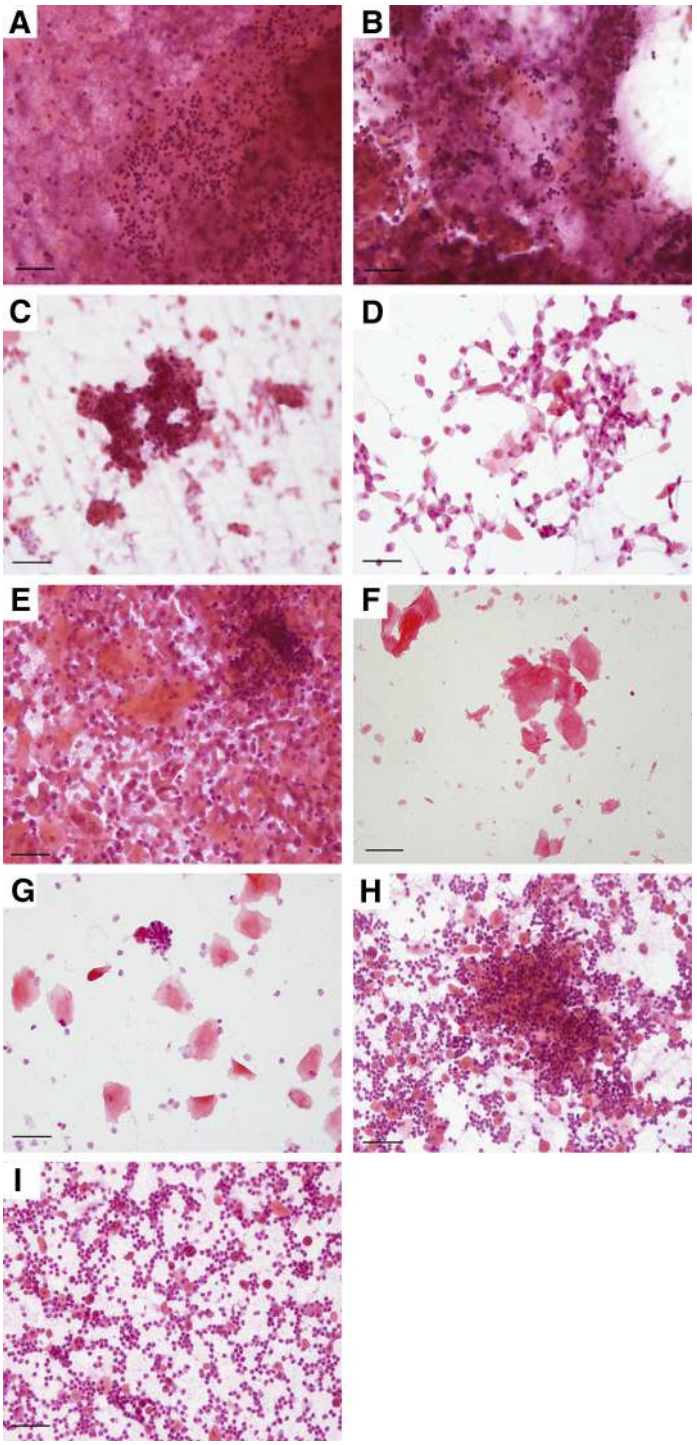
FIGURE 1
Vaginal cytology of female spiny mouse



A and **B**, Cytology showing female spiny mouse with 9-day cycle. Early menses at conclusion of previous infertile cycle. **C** and **D**, Proestrus, beginning of follicular phase, containing nucleated epithelial cells. **E**, Transition to estrus. **F**, Estrus, characterized by cornified epithelial cells. **G**, Metestrus; transitioning to luteal phase. **H** and **I**, Diestrus, luteal phase, containing high leukocytic infiltration. Menses will follow within 24–48 hours. Scale bars = 50 μm. Magnification ×200X. Hematoxylin-eosin stain.

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hysterectomy.[11] Endometriosis, resulting from the presence of endometrial tissue outside the uterine cavity, affects up to 10% of women with symptoms such as dysmenorrhea, chronic pelvic pain, dyspareunia, and infertility. As in preeclampsia and placenta accreta, the etiology of endometriosis is unknown and there is no cure, with treatments only targeting symptoms and not the underlying causes.

Although mouse models of artificial menstruation exist,[12,13] the limited research into menstruation and its related disorders is largely due to the absence of a cost-effective and practical laboratory model of natural menstruation. This study describes the first report of menstruation in a rodent: the common or Cairo spiny mouse (*Acomys cahirinus*), which is native to Northern Africa. The spiny mouse produces small litters (typically 2–3) of precocial pups, with most organogenesis completed in utero during the relatively long (for rodents) gestation of 39 days.[14] Observations of blood at the vaginal opening of nonpregnant female spiny mice in our breeding colony led to an investigation of the changes of endometrial structure and physiology during the reproductive cycle.

Materials and Methods

Animal care

All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and all experiments approved by the Monash University/Monash Medical Centre Animal Ethics Committee. These animals were sourced from our own research colony, where temperature is maintained at 25–27°C, humidity 30–40%, with a 12-hour light-dark cycle (lights on 7:00 AM).[14] Virgin female spiny mice (n = 18) aged 12–16 weeks, were housed in groups of 5–6 per cage. Cages were lined in wooden shavings and enrichment provided in forms of cardboard tunnels and climbing apparatuses. Food (rat and mouse cubes, Specialty Feeds, Glen Forrest, WA) and water were provided ad libitum and supplemental fresh vegetables (carrots and celery, up to 50 g per

TABLE
Characteristics of reproductive cycle of spiny mouse

	Follicular phase		Luteal phase		
	Proestrus	Estrus	Metestrus	Diestrus	Menses
Length of cycle stage, d[a]	1.80 ± 0.2	1.30 ± 0.2	0.71 ± 0.1	1.80 ± 0.3	3.00 ± 0.2
Uterine wet weight, g[b]	0.26 (0.22, 0.29)	0.32 (0.27, 0.33)[a,b]	0.15 (0.13, 0.16)	0.09 (0.07, 0.11)[a]	0.12 (0.09, 0.16)[b]
Endometrial thickness, μm[b]	64.7 (53.49, 144.3)[a]	112.7 (73.60, 181.4)[b]	225.9 (180.6, 375.7)[a]	322.6 (254.8, 512.2)[a,b]	213.3 (171.3, 300.1)[a]
Diameter of uterine lumen, μm[b]	671.1 (437.9, 1378.0)[a]	1334.0 (1158.0, 1868.0)[b]	345.9 (131.6,772.9)[b]	127.0 (110.3, 192.3)[a,b]	614.9 (228.4, 840.1)[b]
Endometrial optical density, arbitrary units (prolactin)[a]	0.029 ± 0.01[a]	0.046 ± 0.01	0.047 ± 0.01	0.071 ± 0.01[a]	0.061 ± 0.01
Plasma progesterone, ng/mL[b]	46.6 (29.4, 63.9)[a]	65.38 (32.9, 82.7)	42.9 (37.2, 54.6)	102.1 (70.9, 198.6)[a]	50.5 (30.6, 65.4)

Superscript letters correspond to groups significantly different from each other. Data subjected to normality tests. For normally distributed data, mean ± SE is reported.

[a] Median (25th percentile, 75th percentile) is reported for all other values; [b] Significant differences corresponded to *P* value of <.05 (1-way analysis of variance and Dunnett post-hoc for normally distributed data and Kruskal-Wallis and Dunn post-hoc for all other data).

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cage) were provided weekly. Animals were checked at least twice daily and cages were cleaned weekly. Female weights were monitored daily throughout the experiment.

Vaginal lavage

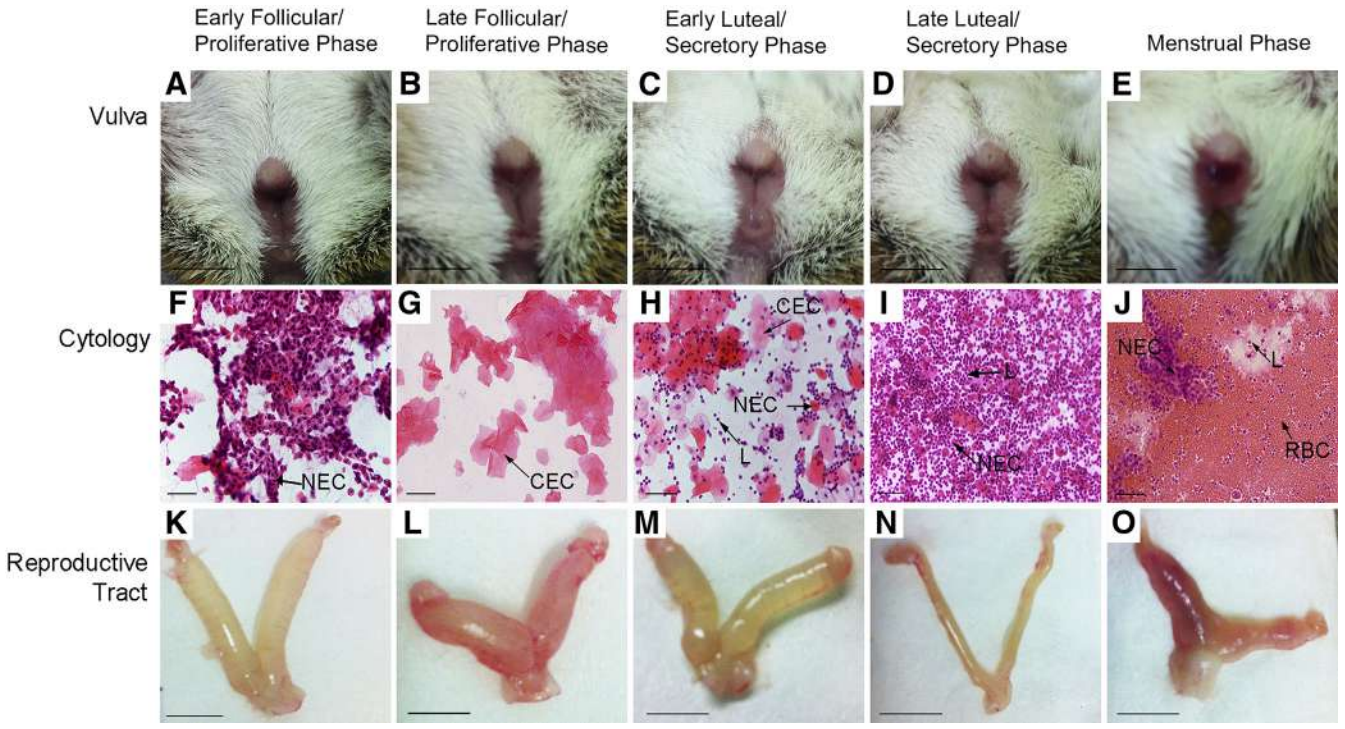
Vaginal lavages were performed daily (n = 18 females) between 12:30 PM and 2:30 PM using sterile saline solution (0.9% wt/vol), and the time of sampling recorded each day. Female spiny mice were restrained in the supine position with a small hand towel by gently scruffing behind the neck and shoulders (a hand towel is necessary due to the fragility of spiny mouse connective tissues and the propensity for skin to easily tear). The vulva was then lubricated with nonscented, water-based lubricant (Lifestyles, Ansell, Melbourne, Australia). A 1-mL plastic transfer pipette was used to draw up ~50 μL of saline, before gentle insertion into the vaginal canal. The length of the spiny mouse vaginal canal is 16.3 ± 1.1 mm based on measurements in our laboratory (unpublished). Hence, we inserted the pipette no deeper than 8 mm, and well before contact with the cervix to avoid possible stimulation of mechanoreceptors, which can cause alteration to the cycle stages in other rodents. The saline was flushed into the vaginal cavity twice, the solution redrawn into the pipette, the pipette removed and the sample expelled onto a glass histological slides (Menzel-Gläser Superfrost, Thermo-Scientific, Braunschweig, Germany). Samples were dried at 27°C for 5 minutes then sprayed with cytology fixative (Spray Fix, Surgipath Medical Industries, Melbourne, Australia). Smearing occurred consecutively for 18 days. Cycle stages were distinguished based on the dominant cell type(s) present in the smear.[15,16] If the smear appeared to be in a transitional phase, ie, between 2 consecutive stages, each of the stages was designated a time of 0.5 days to the total length. Otherwise, when a smear type was observed, it was designated a period of 1 day to the total length for that stage. Of the n = 18 females subjected to daily lavaging, 4 were omitted from cytological analysis due to non-cycling. All cytological data are based on data from n = 14 females.

For technical comparison, F_1 (C57BL/6J X CBA/J) hybrid mice (n = 5) sourced from the Monash Animal Research Platform weighing 17–20 g (40–60 days old) were smeared daily for 12 consecutive days. All aspects of animal handling, vaginal sample collection, and smearing technique were the same as described for the spiny mouse above, except use of a protective hand towel was not necessary.

Cytology staining

Slides were stained with hematoxylin-eosin (Harris hematoxylin and 1% aqueous eosin; Amber Scientific, Midvale, Australia). Briefly, slides were rehydrated in running tap water for 30 seconds −1 minute, prior to hematoxylin staining for 5–6 minutes. Slides were then washed 3 times in tap water to remove excess stain and differentiated in 5% acid ethanol. Following rinsing, slides were submerged repeatedly in a 5% ammonia solution to develop blue coloration. Slides were counterstained with eosin for 3 minutes. Slides were progressively dehydrated in graded ethanol solutions and underwent 3

FIGURE 2
Changes in reproductive tract morphology and cytology across spiny mouse menstrual cycle



Vulva prior to smearing showed **A** and **B**, gaping opening in follicular phase, cellular debris transitioning to **C**, luteal phase before **D**, closure. **E**, Bloody discharge was observed on vulva of 29% of females at time of smearing. Scale bar = 1 cm. Vaginal cytology (hematoxylin-eosin) consisted of **F**, nucleated epithelial cells (NEC), **G**, cornified epithelial cells (CEC), and **H** and **I**, leukocytes (L). **J**, Heavy onset of bleeding during first day of menses shows high numbers of red blood cells (RBC), some L and NEC. Scale bar = 50 μm. Uteri during proliferative phase were **K** and **L,** heavily distended with fluid before **M**, resorption and **N**, complete absence of fluid in secretory phase. **O**, Blood was clearly visible in uterine horns during menses. Scale bar = 1 cm.

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successive changes of clearing xylene for 2 minutes each. Each slide was coverslipped with DPX mounting medium.

Postmortem analysis and tissue collection

Female spiny mice (n = 20) were killed humanely at each distinct stage of the menstrual cycle (n = 4/cycle stage). Each female was heavily sedated using isoflurane before a cardiac puncture was performed for blood collection, followed immediately by cervical dislocation. Whole blood (0.3−1.2 mL per animal) was collected in a heparin-lined tube and plasma obtained after centrifugation (3000 RPM at 4°C for 10 minutes), which was then stored at −20°C for later analysis. The whole, intact uterus with both ovaries and the cervix attached was trimmed of fat and removed. Ovaries were then separated, and the uterus weighed (wet weight) before fixation in 10% buffered formalin for 48 hours, followed by immersion in 70% ethanol for 24−72 hours. Samples were processed to paraffin wax using a Leica ASP-300 processer and embedded in Paraplast paraffin medium; then 5 μm-thick sections were obtained, adhered to slides, and baked at 60°C for 20−30 minutes.

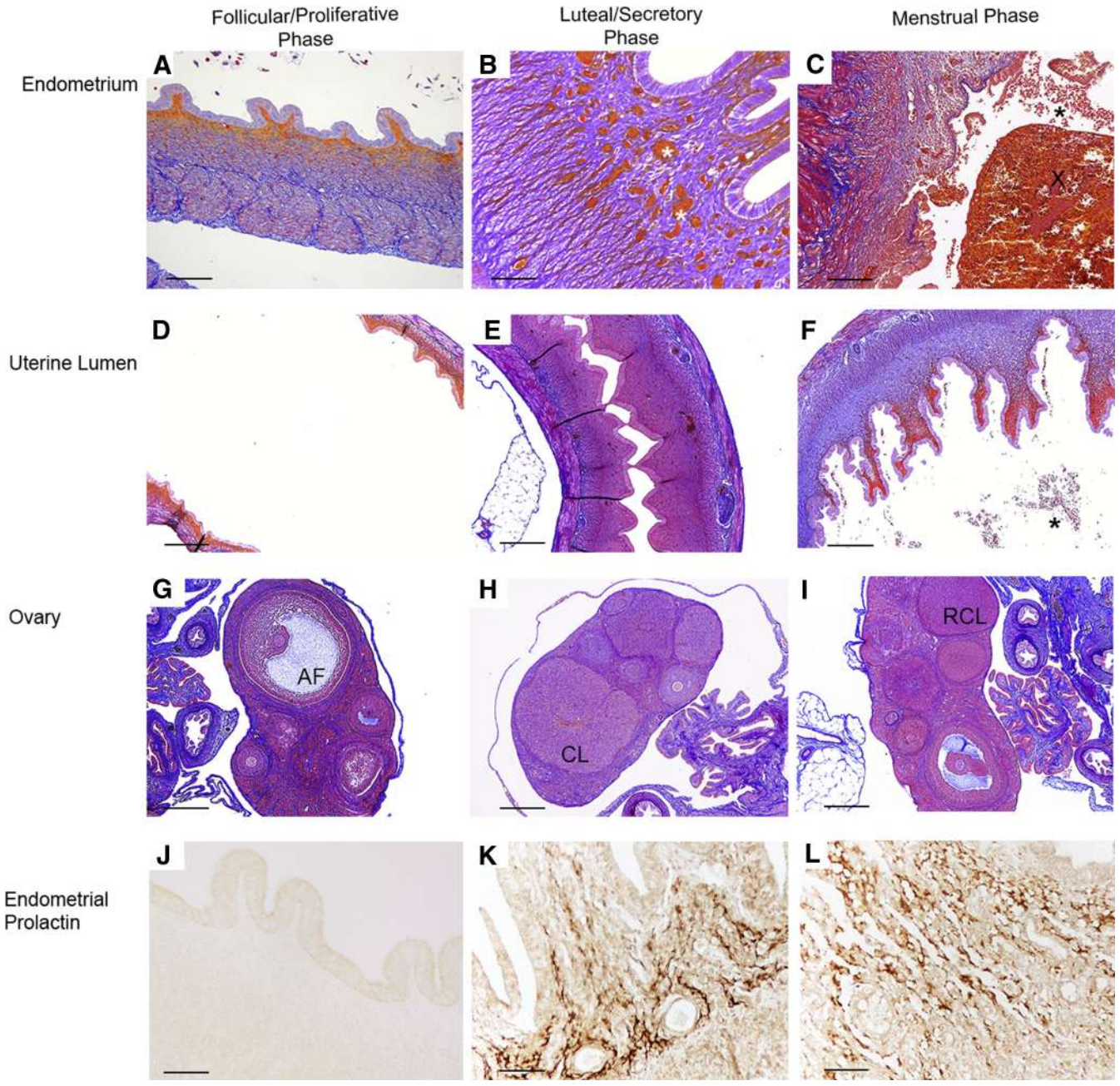
Histological staining

Samples were dewaxed through successive xylene changes, cleared in graded ethanol, and rehydrated in tap water. To visualize reproductive tissues, slides were stained with Mallory's trichrome. Briefly, sections underwent a secondary fixation in Bouin's solution at 60°C for 60 minutes prior to dewaxing. Following this, tissues were stained with acid fuchsin 1 g/100 mL dH_2O (distilled water) for 2 minutes, rinsed thoroughly in distilled water, and stained with phosphomolybdic acid 1 g/100 mL dH_2O for 2 minutes. Slides were rinsed before staining with orange G 2 g, methyl blue 0.5 g, and oxalic acid 2 g/100 mL dH_2O for 15 minutes. Following thorough rinsing, tissues were dehydrated and differentiated as for hematoxylin-eosin above. Red staining was indicative of nuclei and muscle, blue staining indicated collagen, and orange staining indicated anucleate cells (eg, erythrocytes).[17]

Progesterone assay

Plasma progesterone (P4) concentration was measured using a commercially available mouse/rat enzyme-linked immunosorbent assay kit (ALPCO, no. 55-PROMS-E01). All samples (n = 5 females per cycle stage) were measured

FIGURE 3
Histological changes of spiny mouse reproductive tract across menstrual cycle



Structural changes to endometrium (scale bar 100 μm), uterine lumen (scale bar 200 μm), and ovary (scale bar 200 μm). Endometrium during **A**, proliferative phase is thin before increasing in **B**, secretory phase, with presence of new blood vessels and vascular remodeling (white asterisks). During **C**, menses, endometrium is shed, visible in peeling of uterine epithelium, detachment of masses of old endometrial tissue (x), and **F**, red blood cell infiltration in uterine lumen (black asterisk). Uterine lumen is wide in diameter during **D**, proliferative phase and significantly reduced during **E**, secretory phase. Antral follicles (AF) are prominent in ovaries during **G**, follicular phase before collapse into **H**, corpora lutea (CL) and **I**, regressing copora lutea (RCL). Immunopositive endometrial prolactin is absent during **J**, proliferative phase, but secreted from decidualized stromal cells during **K**, luteal phase surrounding newly formed blood vessels before **L**, decidual cells are shed during menses.

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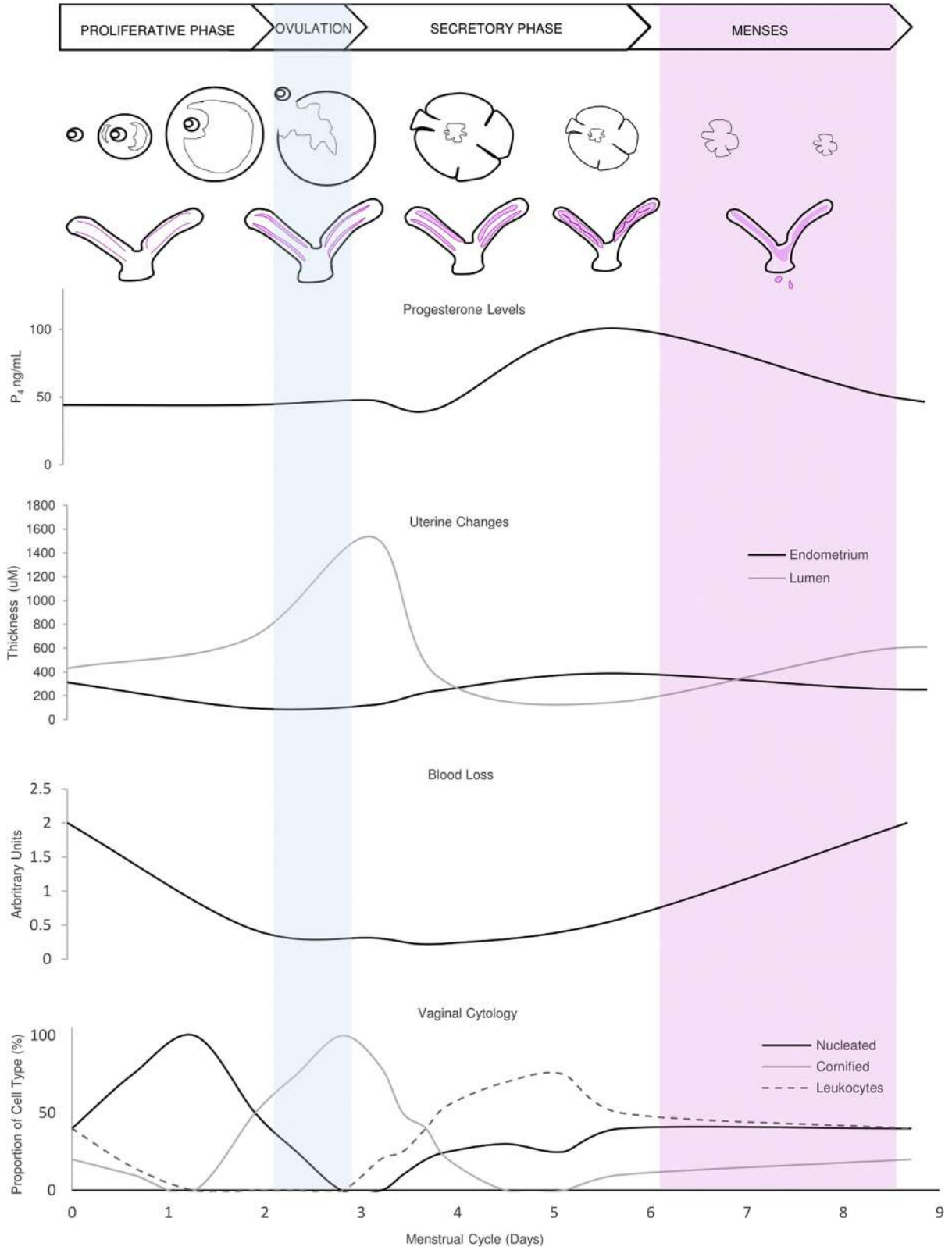
in duplicate and the median value reported. The validity of the assay for spiny mouse plasma was tested by spike and recovery and linearity of dilution procedures following the manufacturer's protocols. Selected samples from individual animals were spiked with 0, low (10 ng/mL), medium (25 ng/mL), or high (50 ng/mL) analyte (progesterone

FIGURE 4
Summary of spiny mouse menstrual cycle



Cyclical shedding of endometrium correlates with regression of corpus luteum and falling progesterone levels.

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powder, Sigma-Aldrich, Castle-Hill, Australia) and 1 sample diluted by a factor of 4, 8, and 16, and the expected vs observed recovery measured.

Immunohistochemistry

Fixed tissues were subjected to immunohistochemistry to detect presence of decidualization using prolactin as biomarker.[18,19] Samples were dewaxed through multiple changes of histolene, and rehydrated through decreasing concentrations of ethanol until submerged in running tap water for 1 minutes. All washing steps occurred in TBS-Tween 20 (0.1%) buffer solution. Slides were first washed for 3 × 5 minutes before exogenous peroxidase activity was blocked by applying 1% H_2O_2 solution for 20 minutes. Slides were washed 3 times in buffer and serum block was applied for 30 minutes at room temperature (X090930-2, Dako, Botany, Australia). Slides were incubated in primary polyclonal rabbit antihuman prolactin (A0569, Dako) at 1:400 dilution in 10% normal goat serum at 4°C overnight or 10% normal goat serum as a negative control. Slides were thrice washed and incubated in secondary biotinylated goat antirabbit IgG (Vector Laboratories, CA) at 1:200 dilution for 60 minutes at room temperature. Following washing, slides were incubated in avidin-biotin complex at room temperature for 60 minutes, then washed in buffer solution. 3,3'-Diaminobenzidine was then applied for 6 minutes for visualization. Slides were washed in buffer 3 times and washed again in running tap water before dehydration in increasing graded ethanol. Slides were cleaned and coverslipped as in the hematoxylin-eosin protocol outlined above. Uterine tissue from an early gestation (7 days postconception) pregnant spiny mouse was used as a positive control for prolactin staining (not shown).

Image acquisition

Images of whole organs were captured using an iPhone 4 (iPhone 4, Apple, Cupertino, CA). All histological images were captured using a Leitz Diaplan imaging bright-field microscope (Diaplan, Leitz, Baden-Württemberg, Germany), with light settings kept constant and analyzed with the programs Axio-Vision version 4.6.3 (Zeiss, Oberkochen, Germany) and Image Pro-Plus version 6 (Media Cybernetics, Tokyo, Japan). Images of the vaginal cytology of both the spiny mouse and F_1 mouse were taken at ×200 magnification. Histological images of the cervix and ovaries of the spiny mouse were taken at ×40 magnification, and the sections of the uterine horns taken at ×100 and ×200 magnification. Using Axio-Vision, the thickness of the endometrium and myometrium were measured, as was the diameter of the uterine lumen (n = 4 animals per stage receiving 3 random recordings per uterus to give n = 12 measurements per stage; μM), across all stages of the reproductive cycle in the spiny mouse. The degree of immunopositive staining was determined using Image Pro-Plus, as described by Atik[20] in 2014. Calibration of the light and dark fields was implemented by the use of a control image containing both incident light and infinite optical density; brought about by use of a scalpel blade placed on the stage to partially obscure the light. All images were captured in 1 session, with all program settings and light exposure kept exact for each section. Images were converted to grayscale and the mean optical density of positive prolactin-stained areas of the endometrium and myometrium was measured. Mean values were calculated for each section, with 3 fields of view at ×200 magnification per structure of interest (endometrium or myometrium) per animal. Regions containing background staining were measured for each image and subtracted from the mean optical density of each sample to adjust for nonspecific staining. The average optical density was calculated for each group. The investigators conducting measurements of the reproductive tract and optical density were blinded to the treatment groups at the time of analysis.

Image alterations

After completion of analysis, images were subjected to minor aesthetic alterations using Adobe Photoshop CC (Adobe Systems, San Jose, CA). Images were sharpened, and backgrounds brightened if required. Due to the variability in staining, color balancing was performed on some histological images to match specific sections of tissues for easier comparison.

Statistical analysis

All statistical analyses were conducted using SPSS (Version 22) (IBM, New York, NY) and GraphPad Prism (Version 6.01) (Graphpad Software, La Jolla, CA). Normality of the data was tested before statistical analysis. Uterine weight, endometrial and myometrial thickness, uterine lumen diameter, and plasma progesterone concentrations (n = 5 samples per stage) are displayed as the median values of replicates (with 25 and 75 percentile values) and significant differences were calculated using Kruskal-Wallis. Optical density and menstrual cycle length are reported as the mean ± SEM and significant differences (corresponding to a P value of $< .05$) were calculated using a 1-way analysis of variance. Tukey multiple comparisons test was used for comparing parameters between stages of the reproductive cycle during post-hoc analysis.

Results

We examined cytology of daily vaginal lavages from cycling virgin females (n = 14) and found an overall cycle length ranging from 6−10 days, with an average of 8.7 ± 0.4 days. Cytology showed all of the expected stages of a rodent estrous cycle: a follicular phase comprising proestrus and estrus and a luteal phase denoted by metestrus and diestrus. However, an additional stage was present in the spiny mouse, characterized by the presence of large numbers of red blood cells (Figure 1) over a period of 3.0 ± 0.2 days, consistent with menses. Blood was present in the vaginal lavage of all females (14/14) during the transition from the luteal to the follicular phase in both of the cycles studied. Blood was visible macroscopically on the external genitalia in 4 subjects (29%) during the lavage process. Reproductive tract dissections revealed significant differences in uterine weight during the cycle (Table), with

blood present in the uterine lumen corresponding to the time it was detected in the smears (Figure 2). To ensure that bleeding was not caused by vaginal lavage the technique was used in F_1 (C57BL/6J X CBA/J) hybrid mice. We found no evidence for the presence of red blood cells during any stage of the mouse estrous cycle (Supplemental Figure 1) and therefore concluded that the cyclical changes and uterine bleeding seen in the spiny mouse vaginal cytology were a natural phenomenon and not caused by trauma from the lavage technique.

Mallory trichrome stain was used to visualize histological changes in the reproductive tract (Figure 3). During the follicular phase of the spiny mouse cycle, the endometrial layer of the uterus was thin (64.77 [53.49, 144.3] μm) (Table) and the ovaries contained antral follicles, before rupture, and development of corpora lutea (Figure 3). During the luteal phase, angiogenesis and decidualization were observed in the uterus, endometrial thickness increased 4−5 fold (322.6 [252.8, 512.2] μm), and the diameter of the uterine lumen decreased (Table). Degeneration of the corpus luteum followed, coinciding with shedding of the endometrium (Figure 3 and Table).

Decidualization of the endometrium was confirmed with immunohistochemical staining for prolactin[18,21] (Figure 3). Endometrial prolactin staining was absent during ovulation. Immunopositive decidual cells in the endometrium were abundant during the luteal phase (Figure 3, A and B). To quantify prolactin immunostaining, we measured optical density. The strongest staining in the endometrium was during the luteal phase, which was followed approximately 2 days later by shedding of the uterine lining (Table).

Plasma progesterone was measured with an enzyme-linked immunosorbent assay kit (Supplemental Tables 1 and 2) to determine if histological and immunohistochemical findings were correlated with hormonal changes consistent with the process of menstruation. There was a significant increase in plasma progesterone concentrations to 102.1 (70.1, 198.6) ng/mL during the luteal phase compared to the follicular phase 46.6 (29.4, 63.9) ng/mL (Table).

Comment

The timing and recurrence of uterine bleeding is evidence for menstruation in the spiny mouse; indeed the onset of the cytological and hemorrhagic changes commenced at the time plasma progesterone concentrations begin to fall, a hallmark of spontaneous decidualization.[1,22]

The spiny mouse reproductive cycle is divided into uterine and ovarian phases consistent with other menstruating species (Figure 4), although this is the first report of such observations in a rodent. Recent hypotheses suggest that menstruation is a consequence of spontaneous decidualization, and that spontaneous decidualization evolved in those species with highly invasive trophoblast, deep penetration of the established placenta into the endometrium, and a high rate of embryonic chromosomal abnormality.[1,8]

Because the spiny mouse shares many reproductive features with other menstruating mammals, such as hemochorial placentation, spontaneous ovulation, and few, well-developed offspring,[16,23] this suggests that spontaneous decidualization and menstruation may be an example of parallel evolution of these traits.

The unprecedented discovery of menstruation in a rodent species suggests that this phenomenon was overlooked by previous investigators[16] because of the current dogma that rodents are not menstruating mammals. Studies are now required to determine if menstruation occurs in other species of spiny mice and in the related genera of *Uranomys*, *Deomys*, and *Lophuromys* and perhaps *Meriones*, *Gerbillus*, and *Tatera*.[24-26] The peripheral position of the *Acomyinae* in most rodent phylogenies[24,27] suggests that it may be useful and appropriate, in the light of these unusual characteristics of spiny mice, to broaden phylogenetic analysis of this and related genera.

Previous studies have established the spiny mouse as a more useful laboratory species for studies of various aspects of human reproduction and neonatal development than other rodents. The hormonal profile and precocial offspring greatly enhance the utility of this species as a research tool.[28,29] The spiny mouse should also now be regarded as an accessible and cost-effective laboratory model for research into menstruation and menstrual disorders of women, including premenstrual tension and endometriosis.[30] ■

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Author and article information

From the Ritchie Centre, Hudson Institute of Medical Research (Ms Bellofiore, Dr Ellery, Mr Mamrot, Dr Walker, and Dr Dickinson), and Department of Obstetrics and Gynecology, Monash University (all authors), Clayton, Australia.

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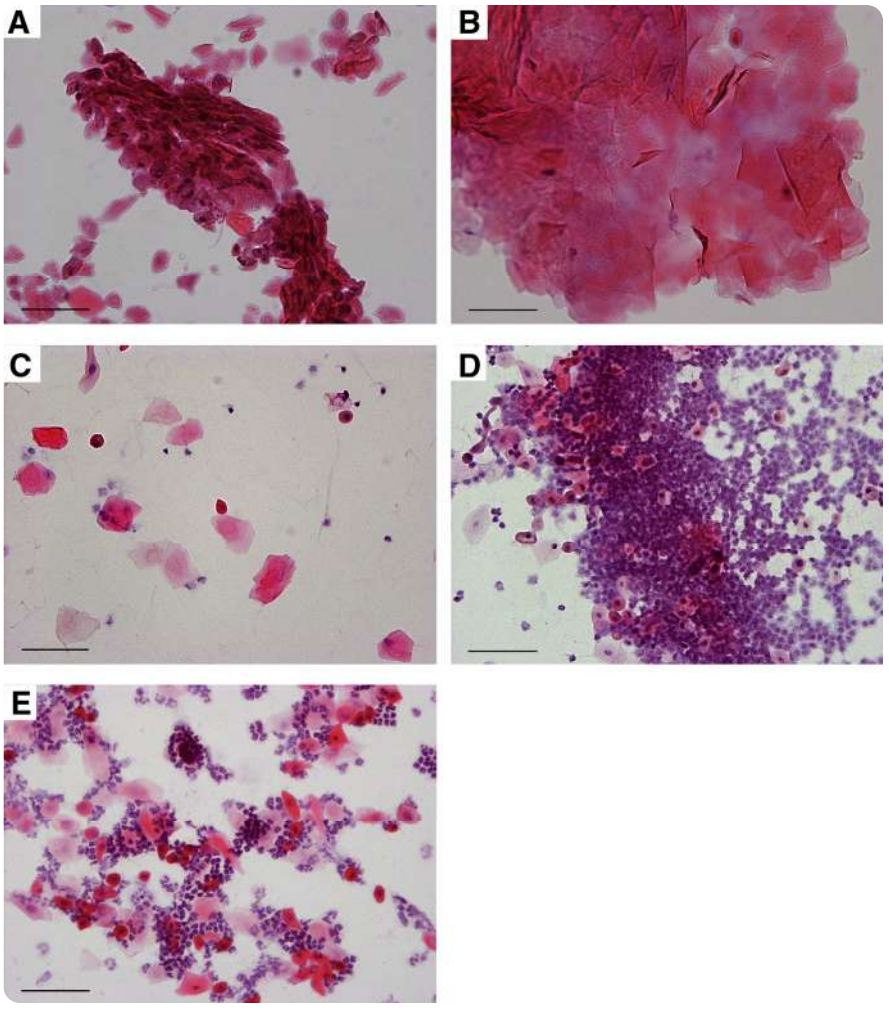
Corresponding author: Hayley Dickinson, PhD. hayley.dickinson@hudson.org.au

SUPPLEMENTAL FIGURE 1
Vaginal cytology of female F_1 mouse at ×200 magnification



A, Early follicular phase; transition from proestrus to estrus, containing both nucleated and cornified epithelial cells. **B**, Estrus characterized by cornified epithelial cells. **C**, Metestrus, transition to luteal phase. **D**, Early diestrus with large leukocytic infiltration. **E**, Transition to new fertile cycle. No red blood cells are observed during mouse estrous cycle. Scale bar = 50 μm.

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SUPPLEMENTAL TABLE 1
Progesterone spike and recovery for spiny mouse plasma

Sample, n	Level of analyte spike	Expected	Observed	Recovery, %
Plasma (4)	Low	16.7	18.8	112.5
	Medium	22.9	20.9	91.1
	High	70.4	103.7	147.4
Mean recovery				117.0

Recovery of >80% indicates sufficient detection of progesterone in spiny mouse plasma to consider derived values valid for analysis.

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SUPPLEMENTAL TABLE 2
Progesterone linearity of dilution for spiny mouse plasma

Sample	Dilution	Expected (neat)	Observed × dilution factor	Recovery, %
	Neat		26.6	
High P_4	1/4	26.6	46.6	174.8
Plasma	1/8		69.5	261.1
	1/16		28.9	108.3

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