A review of the human vs. porcine female genital tract and associated immune system in the perspective of using minipigs as a model of human genital Chlamydia infection

Lorenzen, Emma; Follmann, Frank; Jungersen, Gregers; Agerholm, Jørgen S.

Published in:
Veterinary Research

Link to article, DOI:
10.1186/s13567-015-0241-9

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Lorenzen, E., Follmann, F., Jungersen, G., & Agerholm, J. S. (2015). A review of the human vs. porcine female genital tract and associated immune system in the perspective of using minipigs as a model of human genital Chlamydia infection. Veterinary Research, 46(1), Article 116. https://doi.org/10.1186/s13567-015-0241-9
A review of the human vs. porcine female genital tract and associated immune system in the perspective of using minipigs as a model of human genital *Chlamydia* infection

Emma Lorenzen¹²*, Frank Follmann², Gregers Jungersen³ and Jørgen S. Agerholm¹

**Abstract**

Sexually transmitted diseases constitute major health issues and their prevention and treatment continue to challenge the health care systems worldwide. Animal models are essential for a deeper understanding of the diseases and the development of safe and protective vaccines. Currently a good predictive non-rodent model is needed for the study of genital chlamydia in women. The pig has become an increasingly popular model for human diseases due to its close similarities to humans. The aim of this review is to compare the porcine and human female genital tract and associated immune system in the perspective of genital *Chlamydia* infection. The comparison of women and sows has shown that despite some gross anatomical differences, the structures and proportion of layers undergoing cyclic alterations are very similar. Reproductive hormonal cycles are closely related, only showing a slight difference in cycle length and source of luteolysing hormone. The epithelium and functional layers of the endometrium show similar cyclic changes. The immune system in pigs is very similar to that of humans, even though pigs have a higher percentage of CD4⁺/CD8⁺ double positive T cells. The genital immune system is also very similar in terms of the cyclic fluctuations in the mucosal antibody levels, but differs slightly regarding immune cell infiltration in the genital mucosa - predominantly due to the influx of neutrophils in the porcine endometrium during estrus. The vaginal flora in Göttingen Minipigs is not dominated by lactobacilli as in humans. The vaginal pH is around 7 in Göttingen Minipigs, compared to the more acidic vaginal pH around 3.5–5 in women. This review reveals important similarities between the human and porcine female reproductive tracts and proposes the pig as an advantageous supplementary model of human genital *Chlamydia* infection.
7. The vaginal flora and pH
8. Important differences between rodents and minipigs
9. Conclusions
10. List of abbreviations
11. Competing interests
12. Authors’ contributions
13. Authors’ information
14. References

1. Introduction
Animal models are essential for gaining new insight into disease mechanisms of human genital diseases and the development of new prophylactic strategies and treatments [1]. Predominantly rodents are used as models, within preclinical research, with mice often being the animal of choice [2,3]. Rodent models have clear advantages both regarding practical issues, by being small and easy to handle, and economically affordable [2]. Furthermore, several genetically modified knockout strains are easily accessible, creating a unique opportunity to study the role of specific mediators in the immune response [4,5].

However, when evaluating animal models, different parameters are important to consider depending on the purpose of the model [6]:

- Face validity; how well is the biology and symptoms of the human disease mimicked by the model.
- Predictive validity; how well is the effect of a drug/compound or treatment mimicked by the model.
- Target validity; how similar a role the target of interest plays in the model compared to humans.

Despite the many advantages of rodent models, rodents show a number of differences to humans in terms of size, anatomy, physiology and immunology that do not always allow them to mimic the human course of infection and immune response [4,5,7,8]. The face validity and predictive validity is therefore prone to be insufficient, leaving a strong need for an intermediate and reliable model for the study of female genital tract (FGT) infections and the development of appropriate vaccines against them [9,10]. Non-human primates (NHP) are the animals most closely related to humans and therefore likely to show the greatest face- and predictive validity. However, due to ethical concerns and costly experiments associated with studies in NHP, there is a need for an intermediate pre-clinical/advanced non-rodent animal model.

The pig has become an increasingly popular model, especially within the fields of atherosclerosis and diabetes research, because of its physiological and anatomical similarities to humans [11-13]. Pigs of reduced body size such as the Göttingen Minipigs offer a great advantage by having a smaller size at sexual maturity and a lower growth rate than conventional pigs [14]. Furthermore, such breeds are available as specific pathogen free from specialized breeding companies [15]. Wherever possible, this review will focus on the minipig, since this has been the experimental animal of choice in our research. Despite the physical size, there are no studies reporting any physiological differences between minipigs and conventional pigs. Furthermore, Göttingen Minipigs are partly derived from German Landrace pigs [15].

It has recently been shown that pigs are susceptible to Chlamydia trachomatis, the agent causing human genital Chlamydia, and that pigs are suitable models for the study of Chlamydia pathogenesis and evaluation of vaccine candidates [16]. To evaluate the pig as a model of human genital Chlamydia and to be able to interpret and extrapolate results critically and reliably, it is important to understand the morphological and functional similarities and differences between the human and porcine female reproductive systems. The purpose of this review is to provide the basis for this understanding.

2. Methods
The PubMed database [17], Google Scholar [18] and CAB ABSTRACTS database were searched, with the following keywords: Pig/swine/porcine, genital tract/ reproductive tract/vagina/cervix/uterus/uterine body/ uterine horn/Fallopian tubes, immunology/immune response/immunity, mucosal immunity/immune response, estrous cycle/menstrual cycle/ sex hormone regulation immunity, pig model/porcine model/animal model, sexually transmitted disease/genital infections, vaginal microbiota/ flora/ecosystem.

Due to the very limited numbers of original published papers within the search criteria no year limit was applied. The articles found were in the first line selected based on the abstract content, hereafter the selected articles were evaluated in detail and based on relevance for this review and on the quality of the study, articles were included in this review. Studies on pregnancy immunology/embryology were not included.

3. The female reproductive cycles
In women, the reproductive cycle (menstrual cycle) is described according to the gonadal activity or endometrial changes [19]. In pigs, the reproductive cycle (estrus cycle) is classified by the sexual behavior; estrus, where the pig is sexually receptive, or non-estrus [20]. Both of the cycles can be described with two phases; the luteal and the follicular phase, separated by ovulation (Figure 1).

In the pig, a significant follicle growth occurs during the luteal phase (i.e. the follicular phase overlaps the luteal phase), resulting in a slightly shorter cycle (19–21 days) than in women, where the two phases are more stringent separated and the cycle therefore lasts 28 days [19,20].
However, the mean length is very similar between pigs and women.

The menses/menstruation, a bloody uterine discharge, is specific for humans and some primates, usually lasts 3–7 days and is related to the beginning of the follicular phase [19]. Both women and pigs are spontaneous ovulators and continuously cycling [21]. A comparison of the changes in the reproductive hormones during the reproductive cycles is shown in Figure 1.

Both hormonal cycles are under control of the hypothalamic-pituitary-ovarian axis [19,20]. If no pregnancy occurs during an estrous cycle in the pig, the...
non-pregnant uterus secretes prostaglandin F2α (PGF-2α), which makes the corpus luteum regress (luteolysis) [20]. In women, the mechanism behind luteolysis is a bit more unclear, however, it is suggested that intraluteal PGF-2α plays a luteolyzing role [22]. The important differences between the porcine estrous and human menstrual cycles are summarized in Table 1 together with the same parameters in primates and mice, to show the level of similarity compared to these species.

4. The female genital tract in pigs and humans
4.1. Gross anatomy
The porcine uterus differs from the human by being bicornuate [23] (Figure 2). The bicornuate elongation of the uterine body into two uterine horns creates a longer distance from the porcine cervix to the entrance of the Fallopian tubes than in women. In women the uterine body is approximately 7 cm long [24] while in a 1-year-old sexually mature Göttingen Minipig gilt, each horn is an average 37.2 ± 5.9 cm long (mean ± SD, n = 12, unpublished data).

The cervix in Göttingen Minipigs is an average 7.5 ± 0.85 cm long, whereas the human cervix is around 2–3 cm [25]. The porcine cervix displays a characteristic feature, not found in women; the pulvini cervicales [23], which are a number of interdigitating prominent solid mucosal folds and protrusions throughout the length of the porcine cervix. Furthermore, the porcine urethra opens on the ventral surface of the vagina, creating a urogenital sinus that opens to the outside through the common urogenital orifice [11,14]. In women, the urethra and vagina have separate openings [19].

The vagina in women is approximately 7 cm along the anterior curvature and 9 cm along the posterior curvature [25]. In Göttingen Minipigs the vagina is an average 13.8 ± 0.9 cm (mean ± SD, n = 12, unpublished data).

The Fallopian tubes are 7–14 cm long and 0.5–1.2 cm in external diameter in women [26] and an average 17.3 ± 2.7 cm long and 0.4–0.5 cm in diameter (mean ± SD, n = 12, unpublished data) in 1-year-old Göttingen Minipigs.

4.2. Microscopic anatomy
Histology is a very important tool in the evaluation of pathological changes in animal models. Therefore, it is important to understand morphological differences between pigs and humans [12]. Generally, and common for both pigs and humans, the wall of the FGT consists of three layers: the mucosal, the muscular and the outer serosal layers [21]. The tunica mucosa facing the lumen (the endometrium), is built by the inner lamina epithelialis, lamina propria (connective tissue) and the tela submucosa. The muscular layer (tunica muscularis) is built by stratum circulare and stratum longitudinale. The outer tunica serosa (the perimetrium), facing the peritoneal and pelvic cavities, is built by a lamina propria and lamina epithelialis [21]. In the peritoneal cavity the lamina epithelialis of the tunica serosa has a simple squamous epithelium (visceral layer of the peritoneum) and in the pelvic cavity only loose connective tissue (adventitia) [21].

4.2.1. Vagina
The vagina is the entry site for most sexually transmitted diseases and therefore of great importance when comparing the pig model with humans [12]. The vaginal lamina epithelialis is made by non-keratinized stratified squamous epithelium and forms longitudinal folds called rugae in both women and pigs [12,27]. The porcine vaginal epithelium undergoes cyclic alterations reaching a maximum thickness in the late proestrus [21]. The lamina propria consists of vascularized fairly dense connective tissue with no glands or mucosal muscular layer in both pigs and humans [21].

The vaginal mucosa is moisturized with secretions from the cervix. Cranially the porcine vagina is covered by a typical tunica serosa (i.e. loose connective tissue covered by the mesothelium) while caudally, a tunica adventitia, consisting of loose connective tissue is present. Both tunica serosa and adventitia contain large blood vessels, extensive venous and lymphatic plexuses and numerous nerve bundles and ganglia [21,28]. In women, the vagina is externally covered by

| Table 1 Comparison of reproductive-cycle parameters in women, non-human primates, minipigs and mice |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Cyclicity                                      | Women (menstrual) [25,87]                      | Non-humane primates (menstrual) [88,89]        | Minipigs (estrous) [11,20,90]                  | Mice (estrous) [84,91]                        |
| Age of sexual maturity                        | Continuous cycling                             | Baboons: continuous cycling in captivity       | Continuous cycling                            | Continuous cycling                            |
| Length of cycle                               | 12.9 years                                     | Rhesus Macaque: seasonal poly-oestral.         | 4–6 months                                    | 6–8 weeks                                     |
| Follicular/fateal phase                        | 28 days                                        | 28–33 days (highly variable)                  | 19–21 days                                    | 3–5 days (highly variable)                   |
| Luteolysis inducer                            | 10–14 days/12–15 days                          | 8 days/19 days                                | 5–6 days/15–17 days                           | 2 days/2–3 days                              |
| Endometrial sloughing/menstruation            | Yes                                            | Yes                                           | Uterine PGF2α                                 | No                                           |


adventitia, primarily built with elastic fibers attaching the vagina to the surrounding connective tissues and organs [27]. *Tunica muscularis* is also similar for pigs and humans with an inner layer of circularly arranged smooth muscle cells and an outer longitudinal layer, however the pig can have a thin layer within the circular layer with longitudinally arranged fibers [21,27]. Studies have furthermore shown that the porcine vaginal permeability barrier, which is based on the lipid composition and intercellular lipid lamellae in the epithelium, closely resembles that of humans [12].

4.2.2. Cervix

The porcine cervix has a thick, muscular wall rich in elastic fibers [21,23], whereas the human only contains small amounts of smooth muscle and therefore mainly consists of dense connective tissue and elastic fibers [27].

The cervical *lamina epithelialis* differs between humans and pigs. In women the ectocervix has non-keratinized stratified squamous epithelium and the transformation zone separates it from the endocervix with a simple columnar epithelium [27]. In pigs, more
than 90% of the cervix may have a vaginal type of epithelium with stratified squamous epithelium that undergoes cyclic alterations. The porcine cervical epithelium changes between simple columnar, pseudostratified and stratified squamous epithelium, with primarily columnar in diestrus and primarily stratified in estrus [21].

Common for both species is the simple columnar epithelium, which is mucinous with mucus secreting goblet cells. The amount of mucus secreted depends on the cycle stage with an increased amount during estrus in pigs and midcycle in women (around ovulation). Much of the mucus passes to the vagina. Similarly the epithelium increase in thickness and edema develops during proestrus and estrus [21]. After ovulation the secretion decreases and the mucus becomes thicker [21].

4.2.3. Uterus
The human myometrium (tunica muscularis) is built by three muscular layers. The thick middle layer (stratum vasculare) contains many large vessels [27]. This highly vascularized and well-innervated stratum vasculare is, however, indistinct in the pig [21]. A tela submucosa, with dense irregular connective tissue, is not present in the uterus in women, where the epithelium with lamina propria lie closely applied to the myometrium [27].

The epithelium is simple columnar in both women and pigs, but in the pig it increases significantly in height during estrus and can turn into high pseudostratified columnar epithelium [21,29]. The endometrium and structure of the epithelial cells in women are also highly responsive to the hormonal changes and the thickness of the endometrium increases during the late proliferative phase [21,30].

The endometrium in pigs and women can be characterized by two zones or layers; the superficial functional layer (stratum functionale) and the deeper basal layer (stratum basale). The functional layer undergoes cyclic changes and degenerates partly or completely after pregnancy and estrus in the pig [21]. In humans, the degenerated tissue is shed during menstruation [27]. In contrast to women, the pigs’ basal layer is more cellular and fibrous. It remains during all cyclic stages and is the source for restoration of the functional layer [21,27].

The uterine epithelium in pigs and women contains both ciliated cells and non-ciliated secretory cells [21] and branched and coiled (endometrial) glands that extend into the lamina propria [28]. In women, these glands are short and straight in the proliferative (follicular) phase and long and coiled in the secretory (luteal) phase [30]. In the porcine endometrium, growth and branching of the glands are stimulated by estrogen and the coiling and copious secretion by progesterone [21,29].

4.2.4. Fallopian tubes
The Fallopian tubes are of special interest in genital Chlamydia research, as they represent the site of infection, where sterilizing pathology develops in women [31]. The mucosa at the Fallopian tubes is folded into longitudinal folds (plicae) and the epithelium has non-ciliated secretory cells and ciliated cells that aid in moving the sperm upwards and the ovum downwards. The mucosal plicae in the ampulla have secondary and sometimes tertiary folds creating a complex system of epithelial-lined spaces. The epithelial lining is made of a single layer of columnar epithelial cells which sometimes is pseudostratified in pigs [21,32]. The epithelium undergoes cyclic changes with the greatest height and ciliation in the late follicular phase, and atrophy together with loss of cilia in the luteal phase [30].

The Fallopian tube in both pigs and humans can be separated into three parts; the isthmus, which is communicating with the uterus, the ampulla (the middle thin walled part), and the infundibulum that has fimbriae to catch the oocyte, when it is released into the peritoneal cavity during ovulation. The human Fallopian tubes furthermore have an extra compartment called the intramural part. Fertilization will take place in the ampulla in both pigs (caudal ampulla) and humans [21,27].

4.3. Anatomical and histological differences of relevance for a Chlamydia model
The slight anatomical differences in the pig are important to consider when choosing the inoculation route and when evaluating the ascending capacity of an infection. The porcine cervical pulvini make the access from the vagina to the uterus complicated in pigs and should be considered when choosing the inoculation method. Furthermore, the longer uterine body, in terms of uterine horns, is an important factor for the face validity of the pig model in evaluating ascending infections reaching the Fallopian tubes. In sexually immature conventional pigs inoculation with C. trachomatis SvE resulted in an ascending infection with bacterial replication in the Fallopian tubes [16].

A clear benefit of the porcine anatomy is the human-like prominent Fallopian tubes in the pig that potentially allows studying the tubal pathology induced by a C. trachomatis infection.

Since the columnar epithelial cells are the target cells for the C. trachomatis [16,33] it is important to be aware of the slightly different localization of the target cells. In women the columnar epithelial cells are found together with the transitional cells found in the endocervix and upper FGT [34]. In the pig, the cervix is dominated by stratified squamous epithelium and columnar cells are only consistently found in the porcine uterus [21,35], and therefore not at the vagino-cervical transition as in
women. It is therefore recommended to inoculate pigs directly into the uterus.

5. Genetics
The majority of genes expressed in porcine female reproductive tissues are expressed in human FGT as well [36]. As further eluted to below, pigs share significantly more immune-system related genes and proteins with humans than mice do [37].

6. The porcine immune system compared to the human immune system
The porcine immune system is well characterized and highly resembles that of humans [11,36], although there are some differences. One of the differences is the anatomy of the lymph nodes, which are inverted in pigs [38]. The inverted lymph node structure only affects the lymphocyte migration through the lymph node. Porcine lymphocytes mainly leave the lymph node through high endothelial venules instead of efferent lymph vessels, as they do in humans [21,38,39]. Otherwise the physiology and immunologic reactions of the B and T cell areas in the lymph nodes do not differ [21,38].

Most of the protein mediators of the immune system are present with the same structure and function in humans and pigs and most of the immune cells identified in both species are similar [36,40]. The distribution of leukocytes in the blood is very similar in pigs and humans with a high percentage of neutrophils [41], how-ever, within the lymphocyte populations, pigs have a higher proportion of CD4+CD8+ double positive T cells and γδ T cells in the blood. Otherwise the distribution of the different lymphocyte populations in pigs and humans is quite similar [11,36,40,42] as summarized in Table 2.

The major histocompatibility complex (MHC) system in pigs, called the swine leukocyte antigen (SLA) system is very similar to the human leukocyte antigen system, in terms of polymorphic loci, haplotypes and differentiated expression on different cell populations [11,43]. However, resting porcine T lymphocytes can express MHCII before activation [11,43], whereas human T cells only express MHCII when activated [44].

All the cytokines in the human Th1/Th2/Th17/Treg paradigm have porcine orthologs [36], however, it is suggested that IL-4 might play a different role in pigs [45]. The expression and frequency of immunoglobulins are quite similar (Table 2) except that IgD has not been demonstrated in pigs. Similar to humans, pigs have at least five IgG subclasses: IgG1, IgG2a, IgG2b, IgG3 and IgG4 [11]. Humans have two IgA heavy constant region genes (Ca) and therefore two subtypes of IgA designated IgA1 and IgA2 [46], whereas pigs only have one Ca gene and therefore only one class of IgA [46-48]. Circulating IgA is mostly bone marrow derived and monomeric in humans [49], while circulatory IgA in pigs is half dimeric IgA and half monomeric IgA [50]. The dimeric proportion of circulating IgA in the pig is, however, primarily derived from the intestinal synthesis and lymph. Due to the hepatic plgR-mediated transcytosis of polymeric IgA (plgA) to the bile, the dimeric IgA is thought to be relatively short-lived in the circulation [50]. The hepatic polymeric immunoglobulin receptor (plgR)-mediated transcytosis of plgA happens in both humans and pigs [50].

In women, IgA2 is known to be the predominant isotype subclass in the genital secretions [51] while this distinction cannot be made in the porcine FGT secretions.

When modeling genital infections and evaluating vaccine responses, the toll-like receptors (TLR) play a crucial role in recognition of the pathogens and induction of and controlling/directing the immune response. It has been shown that the porcine TLR system is very similar to that of humans [41]. In terms of cytokines such as the neutrophil chemokine IL-8, the coding gene carried by humans and pigs is an ortholog [41]. Furthermore, human- and porcine macrophages produce indoleamine 2,3-dioxygenase (IDO) in response to lipopolysaccharide (LPS) and Interferon gamma (IFN-γ) stimulation [36,41].

6.1. The genital mucosal immune response
The genital mucosal immune responses are of specific importance when using the pig as a model of human genital C. trachomatis infections. The genital immune response is challenged in the sense that it has to tolerate sperm, the semi-allogeneic conceptus and the commensal vaginal flora, while it must mount defense responses against sexually transmitted pathogens in order to eliminate them [52].

The genital immune system consists of both innate and adaptive factors. The innate system is primarily built

| Lymphocytes [42,93,94] | Antibodies (in serum) [11,37,70] |
|------------------------|---------------------------------|
| **B cells** | **T cells (yδ)** | **T cells (αβ)** | **NK cells** | **IgM** | **IgG** | **IgA** | **IgE** | **IgD** |
| Humans | 18-47% | 2-8% | 28-59% (CD4+) | 13-32% (CD8+) | <3% (CD4+ CD8+) | 2-13% | 5-10% | 80% | 10-15% | <0,05% | 0,2% |
| Pigs | 8-18% | 9-19% | 25-27% (CD4+) | 27-32% (CD8+) | 10-13% (CD4+ CD8+) | 10-12% | 80-85% | 5-12% | <0,01% | Not described |
by the epithelial barrier, the production of antimicrobial agents and cytokines by the epithelial cells and the innate immune cells [40,53]. Both innate and adaptive humoral mediators and immune cells in the genital immune system are regulated by progesterone and estradiol and therefore fluctuate through the menstrual or estrous cycles [53].

The epithelial cells in the FGT with interconnecting tight junctions play an important role in the immune protection by providing a strong physical barrier, transporting antibodies to the mucosal surface, secreting antibacterial compounds and by recruiting immune cells [54,55]. The sex hormones regulate the structural changes in the epithelium during the cycle. Under the influence of estrogen, the integrity and strength of tight junctions in the epithelial barrier, is significantly weakened in women [54,56]. The secretion of antimicrobial compounds is also suppressed during the midcycle in women [53,57].

To preserve an intact protective barrier, the genital mucosal immune response is often non-inflammatory to avoid inflammation-mediated injuries usually caused by phagocytic activity and complement activation [55]. Most of the antigens in the FGT are therefore met with mucosal tolerance [55].

6.1.1. Distribution of immune cells in the genital tract tissue

The genital mucosa does not have immune inductive sites such as the nasal-associated lymphoid tissue or intestinal Peyer’s patches [55]. Thus, the genital mucosa lacks an organized center to disseminate antigen-stimulated B and T lymphocytes to the distinct sites of the mucosa. However, lymphoid aggregates (LA) are present in the female genital mucosa of both pigs [35] and humans [55] and leukocytes are dispersed throughout the mucosa of the FGT [58] as illustrated in Figure 2.

The LA are located in the basal layer of the endometrium close to the base of the uterine epithelial glands and built by a core of B cells surrounded by T cells and an outer layer of macrophages [58]. The T cells in the LA are primarily CD8+ T cells, however, CD4+ T cells are also present in variable numbers in the LA [58]. Both CD4+ and CD8+ T cells are found as intraepithelial lymphocytes and dispersed throughout the subepithelial tissue [58]. Aggregates of NK cells can also be found in the endometrium but they are placed in close contact with the luminal epithelium [58].

The leukocytes present in the FGT covers macrophages, dendritic cells, NK cells, neutrophils, B cells and T cells [53,59,60] with lymphocytes being the predominant immune cell type in both pigs and women [35,61,62]. The number of immune cells and the size of LA are under strong hormonal influence and fluctuate through the cycle [55,58] as summarized in Table 3.

6.1.2. The humoral genital immune response

The immunoglobulins found in the FGT either have been locally produced by subepithelial plasma cells, or derived from the circulation [63]. Although IgG producing plasma cells can be found in the FGT [64], genital IgG is mainly derived from the circulation [63,65-67] and transported to the mucosal surface by mechanisms such as passive leakage, paracellular diffusion or receptor-mediated transport [63,65]. In contrast, genital IgM and IgA are primarily derived from the subepithelial plasma cells [65,68-70] with up to 95% of the porcine IgA being locally produced [71] and up to 70% of the IgA being locally produced in women [55]. When produced locally, the polymeric secretory IgA (sIgA) is actively transported across the mucosal epithelia cells by the polymeric immunoglobulin receptor (pIgR) [65,66]. The secretion of sIgA primarily takes place in the cervix due to the focused pIgR localization in the cervix in women [72]. The pIgR is also expressed in the uterus, but to a lesser extent and in variable levels due to hormonal regulation [55].

Usually, sIgA is the predominant isotype found in mucosal secretions, such as the intestinal fluid. However, in the secretions from the FGT, there is a greater proportion of IgG compared to sIgA [65,73-75].

The FGT humoral immune response is under strong hormonal influence during the menstrual or estrous cycle [57,74]. The cyclic fluctuations in the antibody levels are compared in Table 3. The information on cycle-dependent variations in the level of antibodies in pigs is sparse and more knowledge is needed within this area.

6.1.3. Immunological differences of relevance for a Chlamydia model

The most important immunological difference with potential influence on Chlamydia models is the slightly different influx of immune cells in the porcine FGT, characterized by an increase in neutrophils during estrus. It should be kept in mind that this increased innate response during estrus could influence the establishment of infection.

7. The vaginal flora and pH

In women, the vaginal microflora is known to play an important role in the innate genital immune system by inhibiting the colonization of pathogens [76,77]. Lactobacilli and other lactic acid producing bacteria are particularly associated with equilibrium in the vaginal flora and inhibition of the growth of pathogens [76,78,79].

16S rRNA gene sequencing has allowed a thorough identification of the vaginal flora in women and the most common bacteria are: Lactobacillus spp., Staphylococcus spp., Ureaplasma urealyticum, Corynebacterium vaginale, Streptococcus spp., Peptostreptococcus spp., Gardnerella
Both 32 104 Escherichia coli 65 103 Bifidobacterium spp. [80]. Based on our Staphylococcus 29 upper genital tract 29 Enterococcus Fluctuations in immune cells and antibody levels in the female genital tract during the hormonal cycles. 57 constituted on average 3.9% of 57 Actinobacillus – 97 – 32 29 55 However, the species composition 97 – and spp. con- 29 55 96 – 5 102. Furthermore, we found that the spp., and 67 Mycoplasma 61 29 67 95 streptococcus spp., and 67 61 95 – 98 – 66 – 95 and the number of macrophages reaches maximum during menses [101]. The lymphoid follicles, in the subepithelial tissue develop during the proliferative phase, reach the largest size during midcycle, remain large during the secretory phase and almost disappear at the menses [58,102]. Activity of cytotoxic T cells in the mucosa is suppressed in the secretory phase [96]. The number of APC in the fallopian tubes is significantly higher after ovulation in the luteal phase compared to the preovulatory follicular phase [103]. Antigen response The uterine secretions display the highest levels of IgG around the ovulation/midcycle [57]. The Fallopian tubes show a response similar to the lower FGT with a lower level of antibodies around midcycle [57].

**Table 3 Fluctuations in immune cells and antibody levels in the female genital tract during the hormonal cycles.** Both women and pigs show regional differences in the hormonal regulation of the genital immune system. The antibody fluctuations seem similar in women and pigs but the influx of neutrophils during estrus is specific for pigs. It should be noted that the porcine studies are rather old and only including few animals. LGT – Lower genital tract, UGT – upper genital tract

| Immune cells | Pigs |
|--------------|------|
| LGT          | The number of plasma cells in the vaginal mucosa has been shown to increase during estrus [32,67]. |
| Antibody response | No significant changes was seen in the cervical mucosa [35], but a tendency was found, that the number of intraepithelial macrophages increased in estrus and that the number of lymphocytes, plasmacells and macrophages in the subepithelial tissue increased during estrous [32,35]. |
| UGT          | The cervix does not show infiltration by neutrophils during estrus [29,67,97]. |
| Antibody response | The amount of antibodies on the mucosa has been shown to decrease during estrus/ around ovulation [67]. |

**vaginalis, Bacteroides spp., Mycoplasma spp., Enterococcus spp., Escherichia coli, Veillonella spp., Bifidobacterium spp. and Candida spp.** However, the species composition can be very different between individuals and during the menstrual cycle [52,76,79]. In women, the lactic acid producing bacteria play an important role by contributing to an acidic environment with a pH of 3.5–5 [52].

In healthy pigs the vaginal flora has been characterized by culture dependent methods and was found to include both aerobic and anaerobic bacteria with the most prominent being the following: *Streptococcus* spp., *E. coli*, *Staphylococcus* spp., *Corynebacterium* spp., *Micrococcus* spp. and *Actinobacillus* spp. [80]. Based on our genetic screening of vaginal swabs from Göttingen Minipigs, it is evident that the above mentioned bacteria are present, but not dominating. *Streptococcus* spp. constituted on average 1.4% on the vaginal flora, *E. coli* 3.7%, and *Staphylococcus* 0.4%. Furthermore, we found that the vaginal flora was not dominated by lactobacillus as in humans. *Lactobacillaceae* constituted on average 3.9% of the total vaginal flora in Göttingen Minipigs. The vaginal flora in Göttingen Minipigs seemed to be dominated by
the following: unclassified genera belonging to Gamma-proteobacteria, unclassified genera from Clostridiales, *Yersinia*, *Paenibacillus*, *Listeria*, *Syntrophus*, *Helibacterium*, *Faecalibacterium*, *Kineococcus* and *Proteus* (unpublished data).

An old study showed that the FGT mean pH in estrus in pigs is 7.02 in the oviduct, 6.98 in the uterus, 7.49 in the cervix and 6.61 in the vagina [81]. Our own data, based on vaginal pH measurements with a pH electrode (Mettler-Toledo InLab® Surface Electrode, Sigma-Aldrich Broendby, Denmark), confirmed that the vaginal pH is just around neutral (~7) in both pre-pubertal and sexually mature Göttingen Minipigs.

8. Important differences between rodents and minipigs

The primary aim of this review was to compare the female reproductive physiology of humans and pigs, however, as a concluding section, we found it important to highlight where the minipig shows significant differences to the commonly used murine model in *Chlamydia* research. Similar comparisons of humans and mice has been done elsewhere [4,82,83], and only main points will be included here.

The reproductive cycle is significantly shorter in mice, having a 4–5 day cycle due to the lack of progesterone-producing *corpora lutea* and thereby a luteal phase, if no coital stimulation occurs [84]. Anatomically, the murine uterus is bicornuate and much smaller than the porcine and human ones [83]. Histologically, the vagina displays keratinized squamous epithelium during estrus, whereas porcine and human epithelium does not keratinize [83].

Within the immune system, the composition of circulating leukocytes is significantly different with a lower percentage of neutrophils and a corresponding higher abundance of lymphocytes in mice compared to pigs and humans [82]. Furthermore, and importantly for the *Chlamydia* model, murine macrophages do not produce IDO in response to LPS and IFN-γ stimulation, by contrast humans and porcines do [36,41]. Furthermore, murine macrophages produce nitric oxide (NO) in response to stimulation with LPS, whereas human and porcine macrophages do not [36]. There is also a great difference in the expression of cytokines such as IL-8, a strong neutrophil chemokine expressed in pigs and humans, but not in mice. In mice keratinocyte-derived chemokine and macrophage inflammatory protein-2 are considered to be the IL-8 counterpart [41].

In the FGT, the influx of immune cells happens slightly differently in mice, compared to pigs and humans. In the murine endometrium an influx of leukocytes is seen in the proestrus, during estrus the leukocytes are almost absent, during metestrus they are prominent and during diestrus an infiltration is seen [83]. The fluctuations in antibody levels in the murine FGT shows a similar pattern for IgG, with a lower level during estrus, while for IgA, it is opposite that of pigs and women, with mice having a higher level during estrus [85].

9. Conclusions

This comparison of the porcine and human FGT reveals clear similarities and gives an understanding of the differences between the species. Despite the bicornuate porcine uterus with a urogenital sinus and cervical pulvini, the anatomical and morphological construction and proportion of layers with cyclic alterations is very similar in humans and pigs. The hormonal cycles are closely related, only differing slightly in cycle duration, and origin of luteolysing hormone. The general immune system and the immune system associated with the FGT show great similarities. The antibody levels on the genital mucosa shows similar cyclic fluctuations in pigs and women, but the immune cell infiltration in the genital mucosa differs slightly between women and pigs, namely in the influx of neutrophils in the porcine endometrium during estrus. The porcine vaginal flora differs from the human by not being dominated by lactobacilli and the vaginal pH is slightly higher in pigs than in women.

It is difficult to tell the exact significance of the differences and similarities between the FGT in women and pigs and interpretation of data from animal models should always be done with caution. The similarities found in this review, however, suggest that the pig adds a greater predictive value to FGT studies than what can be achieved by studies in rodent models. Non-human primates is the species most closely related to humans, but ethical concerns and the relative ease of working with pigs propose the pig to be an advantageous model of human reproductive disorders such as *C. trachomatis* infection.

10. Abbreviations

APC: Antigen presenting cell; FGT: Female genital tract; IDO: Indoleamine 2,3-dioxygenase; IFN-γ: Interferon gamma; Ig: Immunoglobulin; LA: Lymphoid aggregates; LGT: Lower genital tract; LPS: Lipopolysaccharide; MHC: Major histocompatibility complex; NHP: Non-human primates; NO: Nitric Oxide; PGF-2α: Prostaglandin-F2α; pIgR: Polymeric immunoglobulin receptor; pIgA: Polymeric immunoglobulin A; sIgA: Secretory Immunoglobulin A; SLA: Swine leukocyte antigen; TLR: Toll-like receptor; UGT: Upper genital tract.

11. Competing interests

The authors declare that they have no competing interests.

12. Authors’ contributions

EL performed the literature study, drafted the structural design of the review and was responsible for writing the manuscript. FF, GJ and JSA contributed intellectually with a critical revision of the manuscript. All authors have read and approved the final manuscript.

13. Authors’ information

EL is DVM and currently a PhD student at University of Copenhagen and Statens Serum Institut, Denmark. For 2 years, EL has been working on a project, focusing on the development of a minipig model for human genital *Chlamydia*, for evaluation of vaccine candidates. FF is the Head of Chlamydia Vaccine Research at Statens Serum Institut, Denmark. FF is responsible for
pre-clinical antigen discovery, vaccine design and formulation. GJ is professor in Immunology and Vaccinology at the National Veterinary Institute with special expertise in porcine and bovine immune responses and immunological correlates of vaccine mediated protection. JSA is professor in Veterinary Reproduction and Obstetrics with a PhD in pathology. JSA has studied genital tract inflammation for several years and has supervised the development of a porcine model for genital Chlamydia in women since 2010.

Author details
1 Section for Veterinary Reproduction and Obstetrics, Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. 2 Chlamydia Vaccine Research, Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Copenhagen, Denmark. 3 Section for Immunology and Vaccinology, National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark.

Received: 7 May 2015 Accepted: 11 August 2015
Published online: 28 September 2015

14. References
1. Lanter F (2014) Animal models of emerging diseases: An essential prerequisite for research and development of control measures. Ani Front 4:7–12
2. De Clercq E, Kalmar L, Vanrompay D (2013) Animal models for studying female genital tract infection with Chlamydia trachomatis. Infect Immun 81:3060–3067
3. O’Weara CP, Andrew DM, Beagley KW (2014) The mouse model of Chlamydia genital tract infection: A review of infection, disease, immunity and vaccine development. Curr Mol Med 14:396–421
4. Mestas J, Hughes CCW (2004) Of mice and not men: differences between mouse and human immunology. J Immunol 172:2731–2738
5. Schautteet K, Stuyven E, Beeckman DSA, Van Acker S, Carlon M, Chiers K, Cox E, Vanrompay D (2011) Protection of pigs against Chlamydia trachomatis infection by administration of a MOMP-based DNA vaccine in the vaginal tract. Vaccine 29:1396–1407
6. Denayer T, Stöhr T, Van Roy M (2014) Animal models in translational medicine: Validation and prediction. New Horizons Trans Med 2:5–11
7. Hein WR, Griebel PJ (2003) A road less travelled: large animal models in immunological research. Nat Rev Immunol 3:79–85
8. Girard MP, Plotkin SA (2012) HIV vaccine development at the turn of the 21st century. Curr Opin HIV AIDS 7:4–9
9. Schautteet K, Stuyven E, Cox E, Vanrompay D (2011) Validation of the Chlamydia trachomatis genital challenge pig model for testing recombinant protein vaccines. J Med Microbiol 60:117–127
10. Dodet B (2014) Current barriers, challenges and opportunities for the development of effective STI vaccines: Point of view of vaccine producers, biotech companies and funding agencies. Vaccine 32:1624–1629
11. Bode G, Clasuing P, Gervais F, Loegsted J, Luft J, Nogues V, Sims J (2010) The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods 62:196–202
12. Squier CA, Mantz MJ, Schlievert PM, Davis CC (2008) Porcine vagina ex vivo as a model for studying permeability and pathogenesis in mucosa. J Pharm Sci 97:9–21
13. Turk JR, Henderson KK, Vanvickle GD, Watkins J, Laughlin MH (2005) Arterial endothelial function in a porcine model of early stage atherosclerotic vascular disease. Int J Exp Pathol 86:335–345
14. Swindle MW (2007) Swine in the Laboratory. CRC Press, Boca Raton
15. The Göteborg minipig [www.minipig.dk] Accessed 5 May 2015
16. Vanrompay D, Hoang TQT, De Vos L, Verminnen K, Harkinezhad T, Chiers K, Mørre SA, Cox E (2002) Specific-pathogen-free pigs as an animal model for studying chlamydia genital infection. Infect Immun 73:8317–8321
17. The PubMed Database [http://www.ncbi.nlm.nih.gov/pubmed/] Accessed 20 Oct 2014
18. Google Scholar Database [https://scholar.google.dk/] Accessed 20 Oct 2014
19. Silverthorn DU (2007) Human Physiology. Pearson Benjamin Cummings, United States of America
20. Senger PL (2005) Pathways to Pregnancy and Parturition. Current Conceptions Inc., Washington
21. Eurell JA, Frappier BL (2006) Dellmann’s Textbook of Veterinary Histology. Blackwell Publishing, United States of America
22. Corpus Luteum [http://www.glown.com/section_view_heading/CorpusLuteum/item290] Accessed 5 May 2015
23. König HE, Liebich HG (2009) Anatomie Der Haussäugetiere. Schattauer, Stuttgart
24. Konar H (2014) DC Dutta’s Textbook of Obstetrics. Jaypee Brothers Medical Publishers Ltd., New Delhi
25. Konar H (2014) DC Dutta’s Textbook of Gynecology. Jaypee Brothers Medical Publishers Ltd, New Delhi
26. Ledger WL, Tan SL, Bahathiq ADS (2010) The Fallopian Tube in Infertility and VF Practice. Cambridge University Press, Cambridge
27. Krause WJ (2005) Krause’s Essential Human Histology For Medical Students. Universal Publishers, United States of America
28. Bacha WJ, Bacha LM (2000) Color Atlas of Veterinary Histology. Lippincott Williams & Wilkins, United States of America
29. Kaeoket K, Persson E, Dali AM (2002) Corrigendum to “The sow endometrium at different stages of the oestrous cycle: studies on morphological changes and infiltration by cells of the immune system” [Anim. Reprod. Sci. 65 (2001) 95–114]. Ani Reprod Sci 73:899–107
30. Strauss JB, Barbieri RL (2014) Yen and Jaffe’s Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management. Saunders Elsevier, Philadelphia
31. Danville T, Hlitke TJI (2010) Pathogenesis of genital tract disease due to Chlamydia trachomatis. J Infect Dis 201(Suppl 2):114–125
32. Husein AM, Newby TJ, Bourne FJ (1983) Immunohistochemical studies of the local immune system in the reproductive tract of the sow. J Reprod Immunol 5:11–15
33. Brunham RC, Rey-Ladonis J (2005) Immunology of Chlamydia infection: implications for a Chlamydia trachomatis vaccine. Nat Rev Immunol 5:149–161
34. Howard C, Friedman DL, Leete JK, Christensen ML (1991) Correlation of the percent of positive Chlamydia trachomatis direct fluorescent antibody detection tests with the adequacy of specimen collection. Diagn Microbiol Infect Dis 14:233–237
35. The porcine cervix [http://ev-eision.slu.se:8080/archive/00003222/01/EEF_Karin_Edstrom.pdf] Accessed May 6, 2015
36. Meurers F, Summerfield A, Nauwychyn H, Salf L, Gerds V (2012) The pig: a model for human infectious diseases. Trends Microbiol 20:55–57
37. McNulty PA, Dayan AD, Ganderup N-C, Hastings KL (eds) (2011) The Minipig in Biomedical Research. CRC Press, United States of America
38. Brins RPM, Pabst R (1994) Lymphoid tissue structure and lymphocyte trafficking in the pig. Vet Immunol Immunopathol 43:79–87
39. Rothkötter H-J (2009) Anatomical particularities of the porcine immune system—a physician’s view. Dev Comp Immunol 33:267–272
40. Mair KH, Schedl K, Rätscher P, Tasnády L, Bögerer B, Germain W, Saalmüller A, Summerfield A, Gerds V, Wilson HL, Meurers F (2014) The porcine innate immune system: An update. J Reprod Immunol 95:1–13
41. Fairbairn L, Kapetanovic R, Sester DP, Hume DA (2011) The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. J Leukoc Biol 89:855–871
42. Zuckermann FA, Gaskins HR (1996) Distribution of porcine CD4 / CD8 double-positive T lymphocytes in mucosa-associated lymphoid tissues. Immunology 87:493–499
43. Saalmüller A, Maurer S (1994) Major histocompatibility antigen class II expression in resting porcine T lymphocytes are potent antigen-presenting cells in mixed leukocyte culture. Immunobiology 190:23–34
44. Holling TM, Schooten E, van Den Elsen PJ (2004) Function and regulation of MHC class II molecules in T-lymphocytes: of mice and men. Hum Immunol 65:282–290
45. Murtha MP, Johnson CR, Xiao Z, Scannuma RW, Zhou Y (2009) Species specialization in cytology biology: Is interleukin-4 central to the Th1-Th2 paradigm in swine? Dev Comp Immunol 33:344–352
46. Snoeck V, Peters IR, Cox E (2006) The IgA system: a comparison of structure and function in different species. Vet Res 37:455–467
47. Gibbons DL, Spencer J (2011) Mouse and human intestinal immunity: same ballpark, different players; different rules, same score. Mucosal Immunol 4:148–157
48. Mills FC, Harindranath N, Mitchell M, Max EE (1997) Enhancer complexes located downstream of both human immunoglobulin Calpha genes. J Exp Med 186:845–858
49. Van der Boog PJM, van Kooten C, de Fijter JW, Daha MR (2005) Role of macromolecular IgA in IgA nephropathy. Kidney Int 67:813–821
50. Vaerman J, Langendries A, Reinhardt P, Rothköter H (1997) Contribution of serum IgA to intestinal lymph IgA, and vice versa, in minipigs. Vet Immunol Immunopathol 58:301–308
51. Cerutti A (2008) The regulation of IgA class switching. Nat Rev Immunol 8:421–434
52. Quayle AJ (2002) The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. J Reprod Immunol 57:61–79
53. Hickey DK, Patel MV, Fahey Jv, Wira CR (2011) Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: stratification and integration of immune protection against the transmission of sexually transmitted infections. J Reprod Immunol 88:185–194
54. Ochiel DO, Fahey Jv, Ghosh M, Haddad SN, Wira CR (2008) Innate immunity in the female reproductive tract: Role of sex hormones in regulating uterine epithelial cell protection against pathogens. Curr Women’s Heal Rev 4:112–117
55. Russell MW, Mestecky J (2002) Humoral immune responses to microbial infections in the genital tract. Microbes Infect 4:667–677
56. Wira CR, Fahey Jv, Ghosh M, Patel MV, Hickey DK, Ochiel DO (2013) Sex hormone regulation of innate immunity in the female reproductive tract: the role of epithelial cells in balancing reproductive potential with protection against sexually transmitted pathogens. Am J Reprod Immunol 65:544–565
57. Stanberry LR, Rosenthal SL (Eds) (2012) Sexually Transmitted Diseases: Vaccines, Prevention, and Control. Academic, Oxford
58. Yeaman GR, Wirat R, Guyre PM, Gonzalez J, Collins JE, Stern JE (1997) Unique CD8+ T-cell-rich lymphoid aggregates in human uterine endometrium. J Leukoc Biol 61:427–435
59. Booker SS, Jayanetti C, Karalak S, Hsiu J-G, Archer DF (1994) The effect of hormonal influences. Infect Dis Res Treat 3:1–9
60. Kamat BR, Isaacson PG (1987) Immunocytochemical distribution of leukocytes in the human endometrium. Am J Pathol 127:66–73
61. Dalin A-M, Kaeoket K, Persson E (2004) Immune cell infiltration of normal mucosal sites. Am J Reprod Immunol 65:248–252
62. Bischof RJ, Brandon MR, Lee C-S (1994) Studies on the distribution of lymphoid aggregates in human uterine endometrium in pregnancy. J Pathol 182:485–494
63. Mestecky J, Alexander RC, Wei Q, Moldoveanu Z (2011) Methods for evaluation of humoral immune responses in human genital tract secretions. Am J Reprod Immunol 65:361–367
64. Rebello R, Green F (1975) A study of secretory immune system in the female reproductive tract. Br J Obstet Gynaecol 82:812–816
65. Wright PF (2011) Inductive/effecter mechanisms for humoral immunity at mucosal sites. Am J Reprod Immunol 65:248–252
66. Kutheth WH, Prince SJ, Hammond KR, Kutheth CC, Mestecky J (1996) Variations in immunoglobulins and IgA subclasses of human uterine cervical secretions around the time of ovulation. Clin Exp Immunol 104:538–542
67. Hussein AM, Newby TJ, Stokes CR, Bourne FJ (1983) Quantiﬁcation and origin of immunoglobulins A, G, M and in the secretions and fluids of the reproductive tract of the sow. J Reprod Immunol 5:17–26
68. Woof JM, Mestecky J (2005) Mucosal immunoglobulins. Immunol Rev 206:64–82
69. Kutheth WH, Prince SJ, Mestecky J (1982) Tissue origins of human polymeric and monomeric IgA. J Immunol 128:990–995
70. Murphy K (2011) Janeway’s Immunobiology. Garland Science, United States
71. Butler JE, Brown WR (1994) The immunoglobulins and immunoglobulin genes of swine. Vet Immunol Immunopathol 43:5–12
72. Iwasaki A (2010) Antiviral immune responses in the genital tract: clues for vaccines. Nat Rev Immunol 10:699–711
73. Naz RK (2012) Female genital tract immunity: distinct immunological challenges for vaccine development. J Reprod Immunol 93:1–8
74. Mestecky J, Raska M, Novak J, Alexander RC, Moldoveanu Z (2010) Antibody-mediated protection and the mucosal immune system of the genital tract: relevance to vaccine design. J Reprod Immunol 85:81–85
75. Häfner LM, Wilson DP, Timms P (2014) Development status and future prospects for a vaccine against Chlamydia trachomatis infection. Vaccine 32:1563–1571
76. Farage MA, Miller KW, Sobel JD (2010) Dynamics of the vaginal ecosystem – hormonal influences. Infect Dis Res Treat 3:1–15
77. Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR, Fomey LJ (2004) Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. Microbiology 150:2565–2573
78. Mastromarino P, Di Pietro M, Schiavoni G, Nardis C, Gentile M, Sessa R (2014) Effects of vaginal lactobacilli in Chlamydia trachomatis infection. Int J Med Microbiol 304:654–661
79. Larsen B, Monif GRG (2001) Understanding the bacterial flora of the female genital tract. Clin Infect Dis 32:669–77
80. Bara M, McGowan M, O’Boyle D, Cameron R (1993) A study of the microbial flora of the anterior vagina of normal sows during different stages of the reproductive cycle. Aust Vet J 70:256–259
81. Mather EC, Day BN (1977) “In vivo” pH values of the estrous reproductive tract of the gilt. Theriogenology 8:323–327
82. Haley PJ (2003) Species differences in the structure and function of the immune system. Toxicology 188:49–71
83. Treuting PM, Dintzis SM (eds) (2012) Comparative Anatomy and Histology - a Mouse and Human Atlas. Academic, Oxford
84. Goldman JM, Murr AS, Cooper RL (2007) The rodent estrous cycle: Characterization of vaginal cytology and its utility in toxicological studies. Birth Defects Res 80:84–97
85. Gallichan WS, Rosenthal KL (1996) Effects of the estrous cycle on local humoral immune responses and protection of intranasally immunized female mice against herpes simplex virus type 2 infection in the genital tract. Virology 224:487–497
86. Manipulation of the estrous cycle in swine [http://www2.ca.uky.edu/agc/pubs/asc/asc152/asc152.htm] Accessed May 6, 2015
87. Prostaglandins and the reproductive cycle [http://www.glowm.com/section_view/heading/Prostaglandins_and_the_Reproductive_Cycle/item/313] Accessed May 5, 2015
88. H’Oggoe TM, Nyachieo A, Chai DC, Kyama CM, Spiessens C, Mwenda JM (2008) Reproductive research in non-human primates at Institute of Primate Research in Nairobi, Kenya (WHO Collaborating Center): a platform for the development of clinical infertility services? Hum Reprod 2008:102–107
89. Wolfe-Coote S (ed) (2005) The Laboratory Primate. Elsevier Academic Press, San Diego
90. Swindle MM, Makin A, Herron AJ, Clubb FJ, Frazier KS (2012) Swine as models in biomedical research and toxicology testing. Vet Pathol 49:344–356
91. Coleman DL, Kaliss N, Dagg CP, Russell ES, Fuller JL, Staats J, Green MC, Russell CPDES, States JLFJ (1966) Biology of the Laboratory Mouse. Dover Publications inc., New York
92. D’Cruz DJ, Erbeck D, Uckun FM (2005) A study of the potential of the pig as a model for the vaginal irritancy of benzalkonium chloride in comparison to the nonirritant microbicide PHI-443 and the spermicide vanadocene dithiocarbamate. Toxicol Pathol 33:465–476
93. Benington JE, Barge D, Fenton AC, Cant AJ, Spickett GP (2005) Lymphocyte subsets in term and significantly preterm UK infants in the first year of life analysed by single platform flow cytometry. Clin Exp Immunol 140:289–292
94. Pomorska MI, M, Markowska-Daniel I (2011) Age-dependent changes in relative and absolute size of lymphocyte subsets in the blood of pigs from birth to slaughter. Bull Vet Inst Pulawy 55:305–310
95. Ludney J, Quayle AJ, Anderson DJ (2003) Immunological microenvironments in the human vagina and cervix: Mediators of cellular immunity are concentrated in the cervical transformation zone. Biol Reprod 71:1253–1263
96. White HD, Crassi KM, Givan A, Stern JE, Memoli VA, Green WR, Wirat CR (1997) CD3 + CD8+ CTL activity within the human female reproductive tract. J Immunol 158:3017–3027
97. Jiwanakon J, Persson E, Kaeoket K, Dalin A-M (2005) The sow endosalpinx at different stages of the oestrous cycle and at anoestrus: Studies on morphological changes and infiltration by cells of the immune system. Reprod Domest Anim 40:28–39
98. Utsa S, Usata F, Hott J, Schumacher G (1989) IgG and IgA content of vaginal fluid during the menstrual cycle. J Reprod Med 34:202–204
99. Nordell-Haefliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F, De Grandi P, Ponci F, De Grandi P (2003) Specific antibody levels at the cervix during different stages of the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J Natl Cancer Inst 95:1128–1137
100. Keller MJ, Guzman E, Hazrati A, Kasowitz N, Wallenstein S, Cole AL, Cole AM, Proft AT, Wira CR, Hogyati K, Herold BC (2007) PRO 2000
elicits a decline in genital tract immune mediators without compromising intrinsic antimicrobial activity. AIDS 21:467–476

101. Thiruchelvam U, Dransfield I, Saunders PTK, Critchley HOD (2013) The importance of the macrophage within the human endometrium. J Leukoc Biol 93:217–225

102. Yeaman GR, Collins JE, Fanger MW, Free R (2001) CD8+ T cells in human uterine endometrial lymphoid aggregates - evidence for accumulation of cells by trafficking. Immunology 102:434–440

103. Shaw JLV, Fitch P, Cartwright J, Entrican G, Schwarze J, Critchley HOD, Homea AW (2011) Lymphoid and myeloid cell populations in the non-pregnant human Fallopian tube and in ectopic pregnancy. J Reprod Immunol 89:94–91

104. Kaeoket K, Dalin A-M, Magnusson U, Persson E (2002) Corrigendum to “The sow endometrium at different stages of the oestrous cycle: studies on the distribution of CD2, CD4, CD8 and MHC class II expressing” cells. [Anim. Reprod. Sci. 68 (2001) 99-109]. Anim Reprod Sci 73:109-119