Female sex hormones regulate the Th17 immune response to sperm and Candida albicans

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STUDY QUESTION: What role do female sex hormones play in the antisperm immune response?

SUMMARY ANSWER: We found that sperm induce a Th17 immune response and that estradiol down-regulates the antisperm Th17 response by dendritic cells.

WHAT IS KNOWN ALREADY: Estradiol down-regulates the immune response to several pathogens and impairs the triggering of dendritic cell maturation by microbial products.

STUDY DESIGN, SIZE, DURATION: Ex vivo and in vivo murine models of vaginal infection with sperm and Candida albicans were used to study the induction of Th17 and its hormonal regulation.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We analyzed the induction of Th17 cytokines and T cells in splenocytes obtained from BALB/c mice challenged with sperm and C. albicans. For the in vivo vaginal infection models, we used ovariectomized mice treated with vehicle, estradiol or progesterone, and we assessed the effect of these hormones on the immune response in the lymph nodes.

MAIN RESULTS AND THE ROLE OF CHANCE: Th17 cytokines and T cells were induced by sperm antigens in both ex vivo and in vivo experiments. Estrus levels of estradiol down-regulated the Th17 response to sperm and C. albicans in vivo.

LIMITATIONS, REASONS FOR CAUTION: This study was conducted using murine models; whether or not the results are applicable to humans is not known.

WIDER IMPLICATIONS OF THE FINDINGS: Our results describe an adaptive mechanism that reconciles immunity and reproduction and further explains why unregulated Th17 could be linked to infertility and recurrent infections.

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Key words: estradiol / spermatozoa / Th17 / dendritic cells

Introduction

The mucosal immune system of the female reproductive tract (FRT) must be uniquely prepared to maintain the balance between the presence of commensal microbiota, sexually transmitted pathogens and allogeneic spermatozoa. Semen is a foreign material for the female FRT and, upon contact with the mucosa, it induces a series of immunological reactions to eliminate it. For instance, insemination is followed by...
an influx of neutrophils to the mucosa of the FRT (Rozeboom et al., 1999; Gorgens et al., 2005; Schuberth et al., 2008). In humans, semen was shown to induce a local recruitment of leukocytes, detected only after coitus without a condom (Sharkey et al., 2012a). Moreover, the presence of antisperm antibodies in females has been widely reported (Clarke, 2009). However, the passage of sperm through the FRT must be regulated in order to maximize the probability of fertilization. In some species, sperm may be stored for days (horses) or even months (bats) before the arrival of the oocyte. In humans, fertilization occurs when intercourse takes place up to 5 days before ovulation (Wilcox et al., 1995). Female sex hormones regulate ovulation and have a potent effect on the mucosal immune system of the FRT and the survival of microbiota (Dunbar et al., 2012). Thus, a transient increase in vaginal microbiota is observed during the follicular phase (proestrus) of the ovarian cycle (Keane et al., 1997; Eschenbach et al., 2000). This increase peaks during the ovulatory phase (estrus) and decreases during the luteal phase (diestrus) (Fidel et al., 2000).

Dendritic cells (DCs) are professional antigen-presenting cells in the mucosa of the FRT (De Bernardis et al., 2006; LeBlanc et al., 2006) that sample the environment in search of antigens and danger signals. Upon contact with exogenous ligands or inflammatory cytokines, DCs migrate to draining lymph nodes, thereby promoting T-cell activation and polarization (Buelens et al., 1997; Sallusto et al., 1998; De Bernardis et al., 2006). DCs primed with exogenous materials can polarize CD4+ T cells to induce a highly efficient immune response that is specific to each kind of pathogen (Murphy and Stockinger, 2010). For example, intracellular pathogens promote Th1 responses, and extracellular fungi and bacteria are very efficient at promoting Th17 responses (Conti et al., 2009). The designated CD4+ Th17 T-cell lineage is characterized by production of the proinflammatory cytokines, IL-17A and IL-22, and the lineage-specific transcription factor, RORC (RAR-related orphan receptor gamma or ROR-γt) (Kim et al., 2011). IL-17 is required for host defense against fungal infections (Conti et al., 2009), because IL-17 recruits neutrophils to the site of infection through induction of granulocyte colony-stimulating factor; neutrophil-induced chemokines (Liang et al., 2007) and neutrophils are the cells that clear fungal infections (Thompson and Wilton, 1992).

Sperm are considered weakly immunogenic because they induce low IFN-γ levels (Witkin and Chaudhry, 1989), and the association between antibodies against sperm and infertility is controversial (Clarke, 2009; Naz, 2011). Therefore, most research on factors associated with infertility/fertility has investigated seminal plasma-induced immune responses and cytokines (Politch et al., 2007; Remes Lenicov et al., 2012; Robertson et al., 2013). On the other hand, an association has been established between celiac disease in female patients and infertility (Choi et al., 2011), and it is well documented that these patients have higher numbers of Th17 cells (Fernandez et al., 2011) and higher levels of IL-17A expression (Monteleone et al., 2010). Similarly, male and female fungal infections are closely linked with infertility, which also generate high numbers of Th17 cells and high levels of IL-17A expression (Nagy and Sutka, 1992; Ulcova-Gallova, 1997). Furthermore, a strong association has been observed between Chlamydia antibodies and anti-sperm antibodies (Blum et al., 1989). We reasoned that the Th17 response could be likened to antispem immunity. Therefore, we investigated the Th17 antispem response and found that sperm-primed DCs induce a Th17 response in the same way as do Candida albicans antigens. Furthermore, we hypothesized that female sex hormones can modulate the induction of the Th17 antispem response to allow sperm to survive during ovulation. We observed that pretreatment with estradiol (E2) diminished the Th17 response, whereas pretreatment with diestrus hormones (progesterone [P] and lower E2) restored the pathogen-host equilibrium.

## Methods

### Mice, sperm collection and sperm immunization

Experiments were performed using 4- to 6-week-old female BALB/c (H-2d) mice and male CD1 Crl:CD1 (ICR) (outbred) mice. The animals were maintained under specific pathogen-free conditions in the Animal Facility of IISGM. The vasa deferentia were removed and transferred to a 35-mm petri dish in phosphate-buffered saline (PBS). Sperm were expelled carefully from the vas deferens with a set of sterile fine-tipped forceps. Sperm were allowed to ‘swim out’ from the tissues for 10 min at room temperature before being washed in PBS. Females were immunized by peritoneal injection [5 times with 500 μl of PBS containing 5 × 10⁶ sperm mixed with adjuvant (200 ng of lipopolysaccharide (LPS) Sigma-Aldrich, USA)] every 20 days (Basten, 1988; Yokochi et al., 1990).

### C. albicans: strain, culture and immunization protocol

The SC5314 C. albicans (ATCC MYA-2876) strain was grown on Sabouraud dextrose chromomycophil agar plates (Conda, Spain) overnight at 30°C prior to the experiments. Before staining, cells were collected, washed and labeled with Oregon Green 488 or FITC (Rellosao et al., 2012). Females were immunized (peritoneal injection) twice with 500 μl of PBS containing 5 × 10⁵ yeast every 20 days.

### Splenocytes, spleen DCs and CD4+ T-cell purification

Five days after the last immunization, the spleens were dissected and the splenocytes were isolated. Five million cells/ml were plated on a 24-well plate on RPMI 1640 without phenol red medium [10% heat-inactivated fetal calf serum (FCS), 1 mM sodium pyruvate, 1% amino acids, 2 mM l-glutamine, 50 μM 2-ME and 15 mM HEPES] complemented with amphotericin B (Duchefa Biochemie, Netherlands) at 0.09 μg/ml (Rellosao et al., 2012). Splenocytes were treated with 17β-E2 (E2) or E2 and P (Calbiochem, Germany) and dissolved in ethanol at physiological range concentrations (Paharkova-Vatchkova et al., 2004; Mao et al., 2005) for 3 h. Control cells were treated with hormone-free ethanol (vehicle). Splenocytes were pulsed with C. albicans at an MOI of 10 or with 1 sperm for every 5 splenocytes. After 20–24 h, the medium was removed and the cytokine concentration was measured using enzyme-linked immunosorbent assay (ELISA) (Quantikine R&D Systems, USA). Splenic DCs were purified using a DC isolation kit (CD11c MicroBeads, Miltenyi Biotec, Germany). CD4+ spleen T cells were purified using the CD4+ T Cell Isolation Kit (Miltenyi Biotec, Germany) following the manufacturer’s instructions, and the live cells were counted in a hemocytometer using trypan blue exclusion.

### Ovariectomization and hormonal treatment

Four-week-old mice were bilaterally ovariectomized under anesthesia (Rellosao and Esporda, 2000) and given 2 weeks to recover. Females were then injected subcutaneously with 0.01 mg of E2 or E2 and P (Calbiochem, Germany) dissolved in 50 μl of sesame oil (Sigma-Aldrich, USA) every 4 days (Day −2, 1, 4 and 7). Control animals were injected with sesame oil
without hormone (vehicle). Hormone treatment was based on a previous report, and the dose was sufficient to maintain hormone concentrations in female mice (Fidel et al., 2000; LeBlanc et al., 2006; Relloso et al., 2012). To test the effect of P, bilaterally ovariectomized were treated with E2 on Day 2 and treated with E2 or E2 and P or vehicle at Day 1 after the infection. Mice were sacrificed and samples were analyzed at Day 3.

Vaginal infection and insemination
At 72 h after the first hormonal treatment (Day 2), mice were inoculated with \(2 \times 10^6\) of SC5314 C. albicans blastococnia or \(5 \times 10^6\) sperm in 20 \(\mu\)l of PBS into the vagina (Day 0). Samples were analyzed on the indicated day.

Single cell suspension from lymph nodes
Inguinal lymph nodes (ILN)s were aseptically removed, and tissues were disrupted by forcing them through a 70-\(\mu\)m cell strainer (BD Biosciences) in PBS. Cells were stained for flow cytometry analysis with anti-CD3, anti-B220, anti-MHCII and anti-CD11c antibodies (BD Pharmingen).

RORC staining
Cells were stained for flow cytometry analysis with anti-CD3 (BD Pharmingen, USA), anti-CD4 (BD Pharmingen, USA) and anti-RORC antibody (eBioscience, USA). We used the BD Cytork/Cytoperm Fixation/Permeabilization kit (BD Biosciences, USA) following the manufacturer’s instructions.

Flow cytometry
Cellular phenotypic analysis was carried out by indirect immunofluorescence. The antibodies used for cell surface staining were obtained from BD Biosciences (USA) and included CD3 (clone 17A2), CD4 (clone GK1.5), CD11c (clone HL3), CD45R (B220; clone RA3–6B2) and MHC II (clone 2G9). All incubations were in the presence of 50 \(\mu\)g/ml mouse IgG to prevent binding through the Fc portion of the antibodies. The same isotype control antibody was always included as a negative control. Dead cells were excluded by staining with 0.125 mg/ml of 7-amino-actinomycin D (Sigma, USA). Flow cytometry was performed with a Gallios device (Beckman Coulter, USA) and data were analyzed using the Flowjo software (Tree Star, Inc., USA). Cells were counted using Flow-Count fluorospheres (Beckman Coulter, USA) following the manufacturer’s instructions.

Vaginal fungal burden and PAS staining
After mice were inoculated in the vagina with \(2 \times 10^6\) of C. albicans, vaginal tissue was excised under sterile conditions and the fungal burden was analyzed. Vaginal samples were weighed under sterile conditions and homogenized in 0.5 ml of sterile water, and 1–10 serial dilutions were plated on YED agar media to assay the C. albicans concentration per milligram of organ (in CFU). For the histology analysis, we performed the PAS staining. Tissue was fixed in 10% formalin and embedded in paraffin. Sections of 5 \(\mu\)m were incubated in 0.5% of periodic acid, washed, incubated with Schiff reagent for 15 min and stained with Mayer’s hematoxylin solution.

Statistical analysis
The test used to determine significance between the treatments in each experiment can be found in the figure legends. GraphPad Prism 5 (GraphPad Software, Inc., USA) was used to determine statistical significance. A P-value of \(<0.05\) was considered significant.

Ethical approval
Procedures involving animals and their care complied with national and international laws and policies. The ISGM Animal Care and Use Committee approved all the procedures.

Results
Sperm induce a Th17 immune response
Extracellular pathogens are very efficient at promoting Th17 responses, which are characterized by production of the cytokines, IL-17A and IL-22, and recruitment of neutrophils to the site of infection (Liang et al., 2007). An influx of neutrophils is also observed after insemination (Schuberth et al., 2008). Therefore, we hypothesized that sperm could induce a Th17 immune response. To investigate the role of Th17 in antisperm immunity, we performed a sperm mixed with LPS challenge into the peritoneal cavity to mimic the human physiological route of female immunization (Friberg et al., 1987; Suarez and Pacey, 2006). We chose LPS because it is an IFN-\(\gamma\)/Th1 inducer and in our experiments, splenocytes from LPS-challenged mice produced IFN-\(\gamma\) (Th1) when they were pulsed with LPS (Supplementary data, Fig. S1A). We also used seminal plasma-free sperm to avoid the effect of semen cytokines on the outcome of experiments (Politch et al., 2007; Remes Lenicov et al., 2012).

Mice were challenged with PBS or sperm or sperm mixed with LPS as an adjuvant. Splenocytes were isolated, plated and pulsed with seminal plasma-free sperm, and the cytokine content in the medium was determined. We detected a strong increase in IL-17A production in the cultures of splenocytes from mice challenged with sperm mixed with LPS. Our experiment indicated that the adjuvant is necessary for correct immunization of the mice (Supplementary data, Fig. S1C). Therefore, splenocytes released IL-17A as a result of the presence of sperm in the culture. We also detected significant production of IL-22 and IFN-\(\gamma\) but not of IL-4, IL-10 or TGF-\(\beta\) (Fig. 1A). We compared the cytokine expression profile with C. albicans, a well-known Th17 inducer. We challenged the mice by injecting C. albicans into the peritoneal cavity to ensure the same means of delivery in both cases. We detected a similar range of IL-17A and IL-22 (Fig. 1B). However, IFN-\(\gamma\) secretion which was very low or undetectable in sperm-pulsed splenocytes, was robust and consistent in C. albicans-pulsed splenocytes.

To test the specificity of the antisperm immune response, mice were challenged several times with PBS, C. albicans, LPS or sperm mixed with LPS (Fig. 1C–G). Splenocytes were then isolated and pulsed with PBS, LPS, C. albicans or seminal plasma-free sperm without LPS, and cytokine IL-17A content in the media was determined using ELISA. We detected release of IL-17A in all the cultures pulsed with either LPS or C. albicans, regardless of the original challenge. However, only sperm-challenged splenocytes produced significant amounts of IL-17A when they were pulsed with sperm (Fig. 1C–F). Therefore, we can conclude that several encounters with sperm mixed with microbial products in the peritoneal cavity are necessary to produce an antisperm-specific adaptive immune response. Furthermore, the fact that sperm antigens induce a Th17 response shed new light on the female antisperm immune response.
E2 delays the vaginal-induced Th17 immune response

To further investigate the in vivo effect of female hormones on the Th17 response, we set up C. albicans vaginal infections in hormonally treated ovariectomized mice (Fig. 4A). Three days after the inoculation, vaginal tissue was examined to detect C. albicans by periodic acid Schiff staining (PAS) and CFU counts. We did not detect C. albicans in vehicle- or P-treated animals (Supplementary data, Fig. S2A and B). However, E2-treated mice showed numerous filaments of C. albicans, and we detected the same number of CFUs at Day 3 as at 1 h (Supplementary data, Fig. S2C). Therefore, E2-treated mice were not able to reduce the infection. To further investigate the role of P, we pretreated the mice with E2 and after the infection, we treated them with vehicle, E2 or P. Two days after the second hormone treatment, the tissue was examined to detect C. albicans. We detected a higher number of cells in E2-treated mice after infection than in the vehicle-treated mice. However, E2-pretreated mice treated with P after the infection were able to clear the fungal infection, since filaments or CFUs were not detected (Supplementary data, Fig. S2D and E). Our data are consistent with those of a previous report (Fidel et al., 2000), thus validating our vaginal infection model.

DCs are responsible for triggering the Th17 immune response

After contact with exogenous antigens or products, DCs migrate from the vagina to the draining lymph nodes (LeBlanc et al., 2006). Therefore, we studied their kinetics of entry into the ILNs after infection. As shown in Fig. 4B, DCs recruited from vehicle- and P-treated mice reached their maximum count at Day 3 after infection. However, DCs from E2-treated mice were at their maximum count at Day 5. Therefore, E2 treatment induced a delay in recruitment kinetics. To investigate the role of P, we pretreated the animals with E2, P or vehicle and 4 days later we treated them with E2, P or vehicle (Fig. 4A). Higher numbers of DCs were detected in P-treated and vehicle-treated animals after infection compared with those treated with E2. However, E2-pretreatment and P treatment after infection revealed increased DC numbers in the ILNs (Fig. 4C). These experiments suggest that treatment with E2 reduces the ability of the DCs to migrate to the ILNs but that treatment with P reverts that effect. Next, we analyzed inflammation in the ILNs.

E2 impairs the Th17 antisperm immune response

After ovulation, a normal ovarian cycle has a window of vulnerability during which females are more susceptible to infection, since the immune response is weakened to allow sperm survival (Dunbar et al., 2012). It has been demonstrated that E2 diminishes the pathogen-induced immune response (Kauschic et al., 2000; Gillgrass et al., 2005; Escribese et al., 2008; Relloso et al., 2012); however, the effect of E2 on antisperm immunity is unknown. In order to test the hormones controlling the Th17-induced antisperm immune response, we treated splenocytes with estrus levels of E2 (10\(^{-10}\) M) or diestrus levels of E2 (10\(^{-11}\) M) and/or diestrus levels of P (10\(^{-8}\) M) and pulsed them with sperm. Estrus levels of E2 treatment significantly reduced the production of IL-17A and IL-22 by the splenocytes after sperm pulsing, whereas diestrus levels of E2 and/or P had no effect on the release of cytokines (Fig. 2A). We obtained similar results in the same experiments performed with C. albicans-pulsed splenocyte cultures (Fig. 2B). Therefore, E2 reduces secretion of IL-17 and IL-22 in response to sperm and in C. albicans antigens. Next, we investigated the presence of Th17 positive T cells by RORC expression detection in CD4 cells, and detected a significant reduction in RORC+ cells in sperm-pulsed cultures (Fig. 3A and B) and C. albicans-pulsed cultures (Fig. 3C) after treatment with estrus levels of E2. Our data suggest that the estrus E2 concentrations weaken the Th17 response. Therefore, the effect of E2 on the Th17 response could enable sperm antigens to survive and at the same time diminish the defense against pathogens. Furthermore, diestrus hormones could restore the Th17 response to maintain the equilibrium.
We detected significant differences in T-cell numbers at Day 3 after infection in vehicle- and P-pretreated animals compared with non-infected animals. In contrast, E2-treated animals showed significant differences in T-cell numbers at Day 5 (Fig. 5A), an observation that is consistent with DC recruitment in the ILNs (Fig. 4B). Moreover, when E2-pretreated animals were treated with P after infection, the T-cell count recovered (Fig. 5B). Furthermore, we detected lower RORC$^+$ CD4 T-cell expansion in the ILNs of the E2-pretreated animals, but when E2-pretreated animals were treated with P after infection, the RORC$^+$ T-cell count recovered (Fig. 5B).

We also investigated the in vivo role of female hormones in the Th17 antisperm response. We inoculated sperm into the vagina of ovariectomized mice treated with E2, Pg or vehicle (Fig. 4A). We did not detect significant differences in T-cell numbers in the ILNs of the mice that were vaginally inoculated with sperm alone (data not shown). In contrast, when mice were injected with sperm mixed with LPS in the peritoneal cavity a week before the hormone treatment and vaginal inoculation, E2-treated animals showed a significant reduction in T-cell counts at Day 3 compared with P- and vehicle-treated mice (Fig. 5C). Moreover, we detected lower RORC$^+$ T-cell expansion in the ILNs of the E2-pretreated animals than in the Pg- and vehicle-treated mice (Fig. 5D). Our data suggest that sperm-pulsed DCs are potent Th17 inducers. Furthermore, during ovulation, the E2 concentration down-regulates DC antigen presentation and cellular polarization toward the antisperm and anticandida Th17.

**E2 impairs triggering of the Th17 antisperm immune response by DCs**

To investigate the effect of E2 on T-cell activation by DC, we treated CD11c$^+$ spleen DCs with hormones and pulsed them with seminal plasma-free sperm. Next, we added CD4$^+$ T cells obtained from sperm-challenged mice. We detected lower levels of IL-17 and IL-22 in the sperm-pulsed co-culture supernatants after treatment with estrus levels of E2 compared with that after treatment with vehicle, P or diestrus levels of E2 (Fig. 6A). In line with our previous observations, we detected low levels of IFN-$\gamma$ in the sperm-pulsed co-cultures, in contrast to the *C. albicans*-pulsed co-cultures (Fig. 6B). However, reductions in the expression of Th17 cytokines were consistent in both the sperm- and *C. albicans*-pulsed E2-treated cells. Our data suggest that sperm-pulsed DCs are potent Th17 inducers. Furthermore, during ovulation, the E2 concentration down-regulates DC antigen presentation and cellular polarization toward the antisperm and anticandida Th17.

**Discussion**

The female reproductive mucosa recognizes semen as a foreign material and induces reactions to eliminate it (Sharkey et al., 2007). The generation of immune responses against sperm during ovulation must be controlled in order to allow the sperm to survive and fertilize. Therefore, there may be a window of vulnerability after ovulation during which females are more susceptible to infection (Dunbar et al., 2012; Relloso et al., 2012). Sperm are considered weakly immunogenic because they induce low IFN-$\gamma$ levels (Clarke, 2009; Naz, 2011). As a result,
We did not use fungal adjuvant, because it could lead to a Th17 response, thus shedding new light on female antisperm immunity. Although the nature of the antisperm immune response remains unknown (Clarke, 2009), we demonstrate that sperm can induce a Th17 immune response during pregnancy (Yang et al., 2006a; Segerer et al., 2009) and during estrus (Escribese et al., 2007; Uemura et al., 2008) and even induction of the tolerogenic DC phenotype (Remes Lenicov et al., 2012). In addition, we and others have reported that E2-treated DCs fail to mature and promote an immune response during pregnancy (Yang et al., 2006a; Kyurkchiev et al., 2007; Uemura et al., 2008; Segerer et al., 2009) and during estrus (Escribese et al., 2008; Kovats, 2012; Relloso et al., 2012), thus explaining the window of vulnerability after ovulation during which females are more susceptible to infection (Dunbar et al., 2012).

Researchers have focused on seminal plasma-induced immune responses to identify factors associated with infertility/fertility (Fichorova and Boulanov, 1996; Yang et al., 1998; Moldoveanu et al., 2005). However, antisperm immune responses have been documented (Witkin and Chaudhry, 1989; Fichorova et al., 1995; Ulcova-Gallova, 1997), and sperm have even been sought as vaccine candidates (Naz, 2011). Although the nature of the antisperm immune response remains unknown (Clarke, 2009), we demonstrate that sperm can induce a Th17 immune response, thus shedding new light on female antisperm immunity.

To investigate the role of Th17 in antisperm immunity, we performed a sperm challenge using sperm mixed with LPS injected into the peritoneal cavity. Given that human fallopian tubes open into the peritoneal cavity, sperm can be found in the peritoneal cavity of women (Maathuis, 1974; Asch, 1976; Stone, 1983; Aref et al., 1984; Suarez and Pacey, 2006), together with microbes and microbial products (Friberg et al., 1987) that probably were in the vagina. It has been suggested that this could be one of the ways females acquire antisperm immunity (Cunningham et al., 1991; Naz and Menge, 1994; Hazumdar and Levine, 1998). We used intraperitoneal injections of sperm combined with LPS as an adjuvant to mimic the human physiological route of female immunization. We did not use fungal adjuvant, because it could lead to a Th17 response, and we did not use Freund’s adjuvant, because it can affect mouse reproduction (Naz, 2011) and is made out of Mycobacterium tuberculosis products, which can also lead to a Th17 response. We chose LPS because it is a bacterial product that promotes general activation to the immune system; it has been described as an IFN-γ/Th1 inducer, and our experiments corroborated it.

DCs are present on the mucosal surfaces of the sites at which sperm have to coexist with commensal microbiota and pathogens (lijima et al., 2007), while semen deposition results in DC recruitment (Berlier et al., 2006) in the same fashion as C. albicans (De Bernardis et al., 2006; LeBlanc et al., 2006; lijima et al., 2007), since DC membrane molecules, such as CD209 (also called DC-sign), are able to recognize both semen and microbial products, which can also lead to a Th17 response. We chose LPS because it is a bacterial product that promotes general activation to the immune system; it has been described as an IFN-γ/Th1 inducer, and our experiments corroborated it.
Our data suggest that sperm-pulsed DCs are potent inducers of Th17. Furthermore, the E2 concentration during ovulation down-regulates DC antigen presentation and delays T-cell activation and polarization toward the antisperm Th17 immune response, while diestrus hormones restore the equilibrium. Moreover, the effect of E2 on Th17 is not sperm antigen-specific, because the anti- C. albicans immune response is also diminished by E2. The mechanism that we propose is consistent with our in vivo data. E2-treated mice showed a delay in DC recruitment kinetics and T-cell activation in draining lymph nodes. Furthermore, E2-treated mice had lower numbers of CD4+/RORC+ T-cell levels and reduced induction of IL-17A and IL-22. As a result, survival of foreign material in the vagina improved. Therefore, a diminished Th17 response would enable the survival of allogeneic sperm, which thus acquire the ability to fertilize during estrus (with high E2 concentrations). During diestrus (with low E2 and high P concentrations), DCs recover their antigen presentation efficiency (Liang et al., 2006) and T cell stimulation efficiency (Yang et al., 2006b). In vivo evidence supporting this hypothesis is that mice treated with P cleared the infection, and when P was administered after E2, the inhibitory effect of E2 on induction of Th17 was reversed.

In conclusion, while the role of fungal infections and Th17-related diseases in female infertility is well documented (Ulcova-Gallova, 1997; Choi et al., 2011), regulation of the Th17 response in antisperm immunity is unknown. Our data show that sperm induce expression of Th17 cells and cytokines and that E2 treatment diminishes it, since E2-treated DCs are less efficient at triggering the adaptive immune response (Relloso et al., 2012). Diestrus hormones restore the equilibrium; in the case of hormone imbalance, pathogens such as C. albicans could use this diminished immune system reaction to grow and promote infection. However, a strong Th17 response that is not controlled during estrus could lead to infertility. Diagnosis of the implication of the Th17 immune response in infertility or recurrent candidiasis could be greatly improved if more were known about hormonal regulation of the balance between reproduction and infection.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.
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Authors’ roles

M.R. conceived the study, S.L., D.E., M.G.-G., R.R.-M. and M.R. carried out the experiments. M.R. wrote the manuscript and S.S.-R., P.E. and M.A.M.-F. revised the manuscript. All the authors approved the submitted version.

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Conflict of interest

None declared.

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