Disruption of female reproductive function by endotoxins

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Abstract

Endotoxemia can be caused by obesity, environmental chemical exposure, abiotic stressors and bacterial infection. Circumstances that deleteriously impact intestinal barrier integrity can induce endotoxemia, and controlled experiments have identified negative impacts of lipopolysaccharide (LPS; an endotoxin mimetic) on folliculogenesis, puberty onset, estrus behavior, ovulation, meiotic competence, luteal function and ovarian steroidogenesis. In addition, neonatal LPS exposures have transgenerational female reproductive impacts, raising concern about early life contacts to this endogenous reproductive toxicant. Aims of this review are to identify physiological stressors causing endotoxemia, to highlight potential mechanism(s) by which LPS compromises female reproduction and identify knowledge gaps regarding how acute and/or metabolic endotoxemia influence(s) female reproduction.

Introduction

Gram-negative bacteria protect themselves using two phospholipid membranes. The outermost facing membrane contains glucosamine-based phospholipid known as lipopolysaccharide (LPS), which is a recognized endotoxin, meaning it has toxic effects to the host after being shed from lysed bacteria (Raetz 1990, Rietschel et al. 1994). Endotoxin elicits a well-characterized robust immune response in animals, but there is recent appreciation for its marked alteration of host metabolism (independent of overt immune modulation) in multiple laboratory models and humans.

LPS consists of a core oligosaccharide, O-antigens and a lipid A moiety (depicted in Fig. 1). The lipid A moiety portion of LPS is responsible for inducing the cellular response (Loppnow et al. 1989). Systemic endotoxemia (increased circulating LPS) reflects either bacterial infection or compromised epithelial (skin, lung, gastrointestinal tract, uterus and mammary) barrier function. Metabolic endotoxemia is described as the physiological state when circulating LPS is 10–50 times lower than that observed during septic shock (Cani et al. 2007).

Unsurprisingly, endotoxia is a consequence of infection by LPS-producing bacteria. There are also a myriad of environmental exposures that can cause endotoxia and these include non-steroidal anti-inflammatory drugs (Arakawa et al. 2012, Van Wijck et al. 2012), mycotoxins (Alizadeh et al. 2015, Marin et al. 2015, Assuncao et al. 2016) and alcohol (Hartmann et al. 2012, 2015). Indeed ‘leaky gut’, and resultant metabolic endotoxemia, has been associated with many pathologies such as inflammatory bowel syndrome (Michielan & D’Inca 2015), cirrhosis (Fukui 2015, Lutz et al. 2015) and cancer (Saggioro 2014). In addition, evidence that gut barrier function becomes compromised during obesity, resulting in metabolic endotoxemia, is firmly established (Amar et al. 2008, Al-Attas et al. 2009, Hawkesworth et al. 2013). Although the etiology is not clear, low-grade, chronic inflammation caused by obesity-induced endotoxia is thought to play a key role in the development of obesity-related disorders (Cani et al. 2007, Hawkesworth et al. 2013) including female reproductive dysfunction.

Heat stress is an abiotic stress that also induces endotoxia. In an attempt to maximize radiant heat dissipation, heat-stressed animals redistribute blood to the periphery, and in order to maintain blood pressure, blood flow to the splanchnic tissues, including the gastrointestinal tract, is markedly reduced. The intestinal epithelial cells are extremely sensitive to oxygen and nutrient restriction (Rollwagen et al. 2006). Heat stress thus causes marked hypoxic-induced conformational changes, which ultimately reduces intestinal barrier integrity. Depending upon the severity and magnitude, heat stress can cause intestinally derived endotoxia (Pearce et al. 2012, 2013a,b,c, Sanz Fernandez et al. 2014). The duration of leaky gut is variable and transitory, for example, intestinal integrity is reduced as early as two hours after the onset of heat stress in pigs (Pearce et al. 2014) and with removal of heat stress, intestinal integrity returned within days. Additionally, leaky gut can be caused by reduced nutrient intake, and this
has been demonstrated in multiple models (Rodriguez et al. 1996, Kvidera et al. 2017). Further, psychological and emotional stress also increases gastrointestinal tract barrier permeability (Vanuytsel et al. 2014). Thus, endotoxemia is relatively common and arises due to a variety of frequent initiators, but the severity of it depends on the source (epithelial barrier endotoxin infiltration vs bacterial infection) and duration of the inducing agent(s).

The major purpose of this review is to collectively describe experiments that have either directly tested the female reproductive effects of endotoxemia through in vitro culture models or in vivo experiments in which animals are administered LPS. Additionally, we will highlight research that has identified associations between physiological scenarios that compromise intestinal integrity (and concomitantly increase circulating endotoxin) with detrimental impacts on female reproduction. Studies evaluating the impact of metabolic and acute endotoxemia are included. Typically, controlled experiments to evaluate endotoxemia’s impact on female reproduction have utilized the acute approach (i.e. an I.V. or I.M. LPS bolus). Further, we will describe how specific cells recognize and respond to LPS, characterize the systemic response to endotoxemia and the reproductives outcomes of LPS exposure, which have been examined in both traditional rodent and large animal models.

The systemic response to endotoxemia

**Lipopolysaccharide-binding protein**

Hepatic acute phase proteins (APP), which are produced as a secondary (non-local) response to a toxic stimuli, have been widely utilized as indicators of systemic and metabolic inflammation, including metabolic endotoxemia (Ceciliani et al. 2012). Lipopolysaccharide-binding protein (LBP) is an APP, primarily produced in hepatocytes (Grube et al. 1994, Kirschning et al. 1997), that interacts directly with the lipid A moiety of LPS (Tobias et al. 1986, 1989, Schumann 2011). Interaction between LBP and LPS results in an LBP conformational change promoting recognition and transfer of LPS to macrophages (Wright et al. 1989). Interleukin (IL)-6 (Grube et al. 1994, Kirschning et al. 1997), IL-1β and dexamethasone (Schumann et al. 1996) stimulate hepatic LBP production but LBP can also be produced in lung epithelial cells (Klein et al. 1998, Dentener et al. 2000), gastrointestinal tract cells (Vreugdenhil et al. 1999), kidney (Wang et al. 1998) and the epididymis (Malm et al. 2005). LBP acts as a soluble receptor and transports LPS to the appropriate toll-like receptor (TLR) to initiate intracellular signal cascades to elicit an immunological response (Schumann 2011). In humans, circulating LBP and plasma C-reactive protein (another broad biomarker of inflammation) are positively correlated (Tremellen et al. 2015), thus providing rationale for using LBP as an inflammatory biomarker (Opal et al. 1999).

The cellular response to endotoxemia

The lipid A moiety of LPS is highly conserved among species, and it stimulates an inflammatory response because it is recognized by membrane-bound TLR4 (Tobias et al. 1989, Raetz & Whitfield 2008, Schumann 2011). Utilizing TLR4-deficient mice, it has been shown that TLR4 is required for LPS recognition and the subsequent cellular response (Hoshino et al. 1999). However, other TLRs can also mediate a cellular response to LPS, dependent on the bacterial strain of origin. As an example, the LPS produced by *Leptospirosis* can instigate an intracellular response via TLR2, TLR4 or TLR5 (Goris et al. 2011, Faisal et al. 2016). In addition, host species can also differ in their response to LPS with some having variable sensitivity to a specific LPS, which impacts both the physiological response and development of mitigation strategies such as vaccine production (Werling et al. 2009).

**Toll-like receptor 4**

TLR4 is a membrane spanning protein bearing similarity to the interleukin 1 (IL1) receptor (Greenfeder et al. 1998).
LPS binds to cluster of differentiation 14 (CD14) and is then transferred to a complex between TLR4 and myeloid differentiation factor 2 (MD-2) to initiate a cellular response (da Silva Correia et al. 2001, Triantafilou & Triantafilou 2002). The MD-2 protein is a crucial component of LPS recognition as an extracellular piece of the TLR4 complex (Shimazu et al. 1999). Soluble CD14 (sCD14) is integral for serum- and cell-mediated responses to LPS (Wright et al. 1989, 1990, Pugin et al. 1993) while the membrane-bound form (mCD14) is a glycosylphosphatidylinositol anchored protein (Haziot et al. 1988, Simmons et al. 1989) and works with TLR4 to transmit the LPS signal across the lipid bilayer to initiate a cellular response (Poltorak et al. 1998). LBP was originally thought to be necessary for CD14 to bind LPS (Wright et al. 1992), however, other studies suggest LPS directly activates CD14 or the MD-2-TLR4 complex (Dentener et al. 2000, da Silva Correia et al. 2001, Triantafilou & Triantafilou 2002), and LBP increases the rate of LPS binding to CD14 (Hailman et al. 1994).

Following LPS recognition, TLR4 recruits proteins including TIR domain-containing adaptor protein (TIRAP), myeloid differentiation primary response gene 88 (MyD88), TIR domain-containing adaptor-inducing interferon beta (TRIF) and TRIF-related adaptor molecule (TRAM) via its Toll-interleukin-1 receptor (TIR) domain causing downstream pathway activation. TIRAP and MyD88 mediate MyD88-dependent signaling, whereas TRIF and TRAM mediate MyD88-independent signaling. Both pathways involve phosphorylation of the REL-associated protein (RELA) subunit of nuclear factor kappa B (Nfkb) although the MyD88-dependent pathway activates pro-inflammatory cytokine genes while the MyD88-independent signaling activates type 1 interferon genes (Kawai et al. 1999, Shimazu et al. 1999). Phosphorylated RELA increases concomitant with increased LPS exposure demonstrating the ability of LPS to drive TLR4-mediated NFκB activation (Chow et al. 1999). Interestingly, single-nucleotide polymorphisms (SNPs) in the TLR4 gene affects immune function and reproductive ability in dairy cows (Shimizu et al. 2017), though the importance of Tlr4 SNPs in humans remains vague (Gowin et al. 2017, Hajjar et al. 2017) and is an area of future interest regarding the biological response(s) to endotoxemia.

### Detoxification of LPS by acyloxyacyl hydrolase

Acyloxyacyl hydrolase (AOAH) is a lipase that deacylates and detoxifies LPS within cells and (Hall & Munford 1983). AOAH releases secondary acyl chains from LPS regardless of the acyl chain structure or location on the diglucosamine backbone of LPS (Erwin & Munford 1990). AOAH is primarily produced in macrophages, neutrophils and dendritic cells (Ojogun et al. 2009) and converts hexaacylated LPS to pentaacylated or tetraacylated LPS rendering it unable to stimulate a response through TLR4 complex formation (Teghanemt et al. 2005). AOAH activity increased in murine serum and hepatocytes following a 25 μg bolus of LPS (Ojogun et al. 2009). In these mice, AOAH activity peaked after three days and returned to normal levels by day nine post LPS injection (Ojogun et al. 2009). Deacylated LPS (dLPS) can compete with LPS for LBP or CD14 binding (Kitchens & Munford 1995a, b); however, binding of dLPS does not stimulate a cellular response (Kitchens et al. 1992). Interestingly, LBP alone or in coordination with CD14 increases the susceptibility of LPS to AOAH detoxification (Gioannini et al. 2007). Aoah-deficient mice have increased pulmonary damage in response to intranasal LPS exposure corroborating AOAH’s protective role against LPS (Zou et al. 2017). Thus, the chemical modification of LPS by AOAH partly regulates the immune response by decreasing the capacity of LPS to stimulate an intracellular signal cascade (Lu et al. 2005).

AOAH cannot act on LPS when the fatty acyl chains are orientated to the inside of LPS aggregates or when LPS is anchored on the outer membrane of bacteria (Gioannini et al. 2007). AOAH can act on LPS-LBP complexes as well as monomeric LPS-sCD14 complexes, suggesting a model where LBP and sCD14 transfer of LPS exposes fatty acyl chains to AOAH (Gioannini et al. 2007). However, when LPS is transferred and bound to MD-2, the fatty acyl chains are less accessible, decreasing AOAH’s ability to deacylate LPS and reduce TLR4 activation (Gioannini et al. 2007). Whether the female reproductive tract has the capacity to locally detoxify LPS remains unknown though recently, the importance of AOAH in the lung (Zou et al. 2017), urinary tract (Yang et al. 2017) and colonic dendritic (Janelins et al. 2014) cells has been demonstrated.

### Effects of LPS on female reproduction and fertility

Understanding the effects of LPS exposure on ovarian function is of interest in humans and production livestock species, since increased circulating LPS is associated with heat stress (Pearce et al. 2012, 2013a, b, 2014, Sanz Fernandez et al. 2014), obesity (Cani et al. 2007) and bacterial infection. Uterine infections have been associated with various negative impacts on bovine fertility, including cystic ovaries (Bosu & Peter 1987, Peter et al. 1989a, b), abnormal or delayed folliculogenesis after parturition (Huszenicza et al. 1999), a longer postpartum anestrous period (Bosu & Peter 1987) and a lengthened luteal phase (Peter & Bosu 1988). Interestingly, follicular fluid that surrounds and nourishes the maturing oocyte contains LPS levels reflective of the systemic circulation (Herath et al. 2007). An accumulation of IL6 and IL8 in media collected after a bolus of LPS (Ojogun et al. 2009) reflects of the systemic circulation (Reproduction 2018 155 R169-R181).
responsiveness of human immune cells (Dentener et al. 1993, Bromfield & Sheldon 2013). Plasma LBP and follicular fluid IL6 concentrations were also positively correlated, suggesting that systemic endotoxemia is associated with ovarian inflammation (Tremellen et al. 2015). Thus, LPS can locate the ovary and potentially interact directly with the oocyte, though it remains to be determined.

**Impacts of endotoxemia on folliculogenesis**

Bovine ovarian cortical explants exposed to LPS had reduced number of primordial follicles due to hyperactivation (Bromfield & Sheldon 2013). Similarly, mice exposed to LPS in vivo had reduced primordial follicle number, which was described as TLR4 mediated, since Tlr4−/− mice are refractory to LPS-mediated primordial follicle depletion (Bromfield & Sheldon 2013) suggesting TLR4 in part regulates the ovarian LPS response. Phosphatase and tensin homolog (PTEN) and Forkhead box O3 (FOXO3), both proteins involved in regulating primordial follicle activation, were translocated out of the oocyte nucleus of primordial and primary follicles in cultured bovine cortical strips after LPS exposure (Bromfield & Sheldon 2013). The aforementioned indicate premature primordial follicle activation, potentially leading to depletion of the ovarian follicular reserve. In rodent studies, altered protein abundance due to LPS exposure in neonatal rodents has been observed (Sominsky et al. 2013). Furthermore, a diminished follicular reserve and earlier onset of ovarian senescence occurs in female rats neonatally exposed to LPS, raising concern about reproductive outcomes of bacterial infections early in life (Sominsky et al. 2012).

**Effects on the follicular stage of the estrous cycle, including ovulation**

Immune challenges can disrupt the follicular phase in multiple species (Kalra et al. 1990, Peter et al. 1990, Battaglia et al. 2000). LPS suppresses the hypothalamic-pituitary-gonadal axis by decreasing pulsatile gonadotrophin-releasing hormone (GnRH) secretion (Hoshino et al. 1999). LPS also blunts the 17β-estradiol (E2) increase during the preovulatory phase, thus delaying subsequent luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surges, culminating in delayed or inhibited ovulation (Peter et al. 1989a, 1990, Battaglia et al. 2000, Suzuki et al. 2001). Using gonadectomized animals, it has been demonstrated that LPS suppresses GnRH release, thus disrupting the LH surge amplitude, frequency and concentration (Feng et al. 1991, Ebisui et al. 1992, Coleman et al. 1993, Kuijo et al. 1995). In agreement with reduced E2 compromising ovulation, when LPS was infused into the uterine lumen, the preovulatory LH surge was attenuated (Peter et al. 1989a). Furthermore, LPS-treated females had delays in the time to the LH surge (Fergani et al. 2012) and lower ovulation rates (Williams et al. 2008). Recently, ovine kisspeptin/neurokinin B/dynorphin (KNDy) neuron activation has been demonstrated to be disrupted by LPS exposure, thus altering the hypothalamic-pituitary-ovarian axis (Fergani et al. 2017).

LPS alters anterior pituitary hormones in circulation, through direct or indirect mechanisms. LPS infusion decreased LH but stimulated systemic prolactin (PRL) and cortisol levels in anestrous ewes and reduced mRNA abundance of LH (LHβ) and luteinizing hormone/choriogonadotropin receptor (LHCGR) (Herman et al. 2010). Further, mRNA-encoding FSH and the FSH receptor (FSHR), PRL and the PRL receptor were increased by LPS infusion (Herman et al. 2010). Granulosa cells exposed to high levels of LPS had reduced mRNA expression of LHCGR, FSHR and cytochrome P450 (CYP) 19A1 (CYP19A1) (Magata et al. 2014a). Theca cells isolated from follicles exposed to high levels of LPS also had decreased mRNA abundance of LHCGR, CYP17 and CYP11A1, but no difference in steroidogenic acute regulatory protein (STAR) or 3β-hydroxysteroid dehydrogenase (HSD3B1) levels compared to theca cells from follicles exposed to low levels of LPS (Magata et al. 2014b). LPS exposure did not impact cell number or androstenedione production from cultured theca cells from small, medium or large ovarian follicles, but it did reduce E2 production from cultured granulosa cells isolated from all three follicular sizes (Williams et al. 2008). In addition, bovine follicles with high levels of LPS (>0.5 EU/mL) had lower E2 but elevated progesterone (P4) levels, relative to follicles with lower LPS concentrations (Magata et al. 2014a). In an in vitro system where bovine granulosa cells were cultured with LPS and provided with FSH and androstenedione, E2 and P4 conversion were reduced potentially due to decreased expression of Cyp19a mRNA and protein (Herath et al. 2007). During the in vivo LH surge, a threshold of E2 is needed to induce behavioral display of estrus; however, the amount of E2 actually required for the behavioral estrus is thought to be at lower level than that required to induce ovulation (Saifullizam et al. 2010) and LPS negatively impacts female estrus behavior and frequency (Battaglia et al. 2000).

Post-ovulation impacts of LPS have also been demonstrated. Bovine oocytes subjected to in vitro maturation with LPS were less likely to successfully complete meiosis with intact meiotic structures (Bromfield & Sheldon 2011). In addition, increased levels of reactive oxygen species and apoptotic genes and altered methylation patterns were observed in bovine oocytes as a result of LPS (Zhao et al. 2017). Further, LPS negatively affected bovine oocyte nuclear maturation by compromising meiotic progression, mitochondrial membrane potential and mitochondrial cytoplasmic redistribution (Magata & Shimizu 2017). LPS also reduced blastocyst development of LPS-
exposed oocytes and the trophoblast cell number of blastocysts (Magata & Shimizu 2017). These studies support the potential for LPS to negatively impact oocyte developmental competence.

**Impact of LPS on luteal phase of the estrous cycle**

Endotoxemia can compromise $P_4$ production and lead to decreased luteal function. Corpus luteum (CL) formation and the expected increase in $P_4$ were delayed in heifers exposed to LPS (Suzuki et al. 2001). During a normal estrous cycle, in the absence of fertilization and pregnancy, prostaglandin F2α (PGF2α) causes CL regression and LPS can cause CL regression by inducing PGF2α production (Moore et al. 1991, Hockett et al. 2000). Not only does LPS administration delay ovulation, it also lengthens the time to luteinization, CL formation and sufficient $P_4$ production (Suzuki et al. 2001, Lavon et al. 2011); thus, LPS has numerous targets within the luteal phase. Additionally, CL size is reduced by LPS perhaps due to activation of pro-apoptotic pathways (Herzog et al. 2012). The cannabinoid receptor type 1 (eCS) has recently been discovered to be involved in LPS-induced CL regression in mice as wild-type mice had increased uterine prostaglandin-endoperoxide synthase (PTGS2) and PGF2α expression, which resulted in reduced ovarian $P_4$ receptor abundance and regression of the CL, and these observations were absent in eCS-deficient mice (Schander et al. 2016).

Administering LPS to goats during their luteal phase did not affect steroid hormone concentrations but did increase PGF2α metabolites (Fredriksson & Edqvist 1985), and repeated uterine LPS infusions in dairy cows every 6 h from 12 h prior to ovulation until 9 day post-ovulation resulted in CL regression much sooner than controls (Luttenau et al. 2016). Culturing bovine luteal tissue in vitro with TNFα increased PGF2α in a dose-dependent manner (Benny & Pate 1992). Additionally, porcine luteal tissue, when cultured in vitro with PGF2α, exhibits a feedback mechanism in which more PGF2α is produced (Guthrie et al. 1979). Normally, the porcine CL acquires capacity to undergo luteolysis around day 13 of the luteal phase (Guthrie et al. 1979), but multiple administrations of PGF2α can induce luteolysis in the porcine CL at an earlier time (Diaz et al. 2000) suggesting LPS may accelerate luteolysis via TNFα and PGF2α induction in pigs, though this remains to be confirmed.

A temporal pattern of LPS affecting circulating $P_4$ has been demonstrated, whereby $P_4$ is initially increased and then declines in LPS-treated, relative to control females (Herzog et al. 2012). LPS exposure initially decreased but then did not affect $P_4$ production in bovine granulous cells in culture (Herath et al. 2007). Further, $P_4$ concentrations were increased in large bovine follicles, and it has been proposed that less $P_4$ is being converted to $E_2$ (Magata et al. 2014a,b). However, others demonstrated that LPS in vitro can inhibit steroid secretion, specifically $P_4$ and androstenedione in thecal-interstitial cells (Taylor & Terranova 1995) suggesting endotoxemia could alter $P_4$ production, representing an endocrine-disrupting effect.

**Endotoxemia and pregnancy maintenance**

$P_4$ is essential for pregnancy maintenance, and LPS reduces the $P_4$ receptor in uteri of pregnant mice (Agrawal et al. 2013). The effect of LPS on the ability of $P_4$ to sustain gestation could cause spontaneous abortion, a phenotypic event frequently associated with physiological conditions in which LPS is elevated. Infection from gram-negative bacteria or their outer wall components (including LPS) triggers preterm labor in many species (Koga & Mor 2010) and in fact, intraperitoneal LPS injection is an established experimental model for inducing preterm labor (Deb et al. 2004, Elovitz & Mrinalini 2004, Agrawal et al. 2013). In addition, infertility can be the result of reproductive tract infections in humans and production animals (Williams et al. 2008, Price et al. 2013). As mentioned earlier, LPS increases PGF2α release (Roberts et al. 1975) leading to CL regression, a decline in $P_4$ and spontaneous abortion in goats (Fredriksson & Edqvist 1985). LPS and bacterial infection also increase PGF2α in the mare (Fredriksson et al. 1986) and the cow (Fredriksson et al. 1985). Uterine epithelial and stromal cells express TLR4 and both produced PGF2α and prostaglandin E2 (PGE) after LPS exposure, a response abrogated by using a TLR4 antagonist in bovine endometrial explants (Herath et al. 2006). Endometrial epithelial and stromal cells can respond to LPS exposure via the TLR4- and MYD88-dependent pathways (Cronin et al. 2012) and cows experiencing endometritis had increased endometrial expression of TLR4 and pro-inflammatory mediators in the first week post-partum (Herath et al. 2009). TLR4 also mediates the local immune response in human (Hirata et al. 2005, Rashidi et al. 2015), feline (Jursza et al. 2015) and canine (Silva et al. 2012) endometrial cells. Recent evidence supports that metabolic stress, such as negative energy balance in lactating dairy cows, may alter the endometrial response to LPS (Sheeldon et al. 2017), a concern for animals experiencing the transition from gestation to lactation or for animals (and humans) who have metabolic perturbations.

Bovine embryos exposed in vitro to both LPS and PGF2α had reduced survival indicating the potential for LPS to alter pregnancy success (Soto et al. 2003). Human trophoblast cells cultured with LPS increase pro-inflammatory macrophage production (Li et al. 2016) and as mentioned earlier, there are fewer trophoblast cells in blastocysts that develop from LPS-exposed oocytes (Magata & Shimizu 2017). Additionally, human decidual cells exposed to LPS produced TNFα and PGF2α, which negatively affected cell growth. Further, when human amniotic fluid from normal relative to
preterm labor pregnancies were compared, there were increased amounts of TNFα in the preterm samples, and LPS was detectable in 50% of preterm labor amniotic fluids (Casey et al. 1989). Furthermore, as evidence that LPS can alter the maternal capacity to support pregnancy, LPS-induced changes to human and bovine endometrial epithelial cell protein abundance (which could affect implantation at the critical time of maternal recognition of pregnancy) has been demonstrated (Cronin et al. 2012, Jensen & Collins 2012, Piras et al. 2017).

Additional considerations
Measuring circulating LPS should be interpreted with caution, since the limulus amebocyte lysate assay measures endotoxin biological activity and not LPS that is bound to inflammatory mediators such as soluble CD14 or LBP (Guerville & Boudry 2016). Additionally, the bacterial source of LPS remains undefined in these assays, and there are interactions that can alter the assay interpretation (Guerville & Boudry 2016). Thus, the usefulness of measuring LPS directly has been questioned (Stadlbauer et al. 2007, Gnauck et al. 2015, 2016). Also, most assays do not distinguish between LBP bound to LPS or that which is unbound; thus, LBP data must also be appropriately interpreted and within context. Taken together, a lack of an effective and convenient LPS assay is limiting the immune-reproduction field and a collective approach in defining the physiological endotoxemia response is required.

Of additional interest and concern is that LPS causes hyperinsulinemia, either directly as an insulin secretagogue or indirectly by increasing glucose stimulated insulin secretion (Baumgard et al. 2016). Reasons why a catabolic signal like LPS increases an acutely anabolic hormone like insulin are not clear, but reports suggest that insulin has potent anti-inflammatory effects (Chalmeh et al. 2013) and that immune cells are insulin sensitive (Maratou et al. 2007). Whether the ovary responds to hyperinsulinemia is unclear (Akamine et al. 2010, Brothers et al. 2010, Wu et al. 2012, Nteeba et al. 2013); however, elevated insulin levels have been reported in both serum and follicular fluids of obese females (Robker et al. 2009, Valckx et al. 2012). Primordial follicle hyperactivation (similar to that caused by LPS exposure) has been documented in neonatal rat ovaries due to insulin administration (Kezele et al. 2002). The negative effects of hyperinsulinemia and insulin resistance on female reproduction have been well documented, largely as pertaining to obesity and polycystic ovary syndrome (Goodarzi et al. 2011, Ogden 2015) and while not described herein in the interest of brevity, hyperinsulinemia could be a secondary consequence of endotoxemia with the potential to negatively influence female reproduction, though studies to specifically investigate this have not yet been performed. Hyperinsulinemia is not the sole secondary metabolic alteration observed due to endotoxemia: reduced circulating high-density lipoprotein (HDL) cholesterol was observed in dairy cows subjected to an acute exposure to LPS (De Campos et al. 2017) and, as discussed herein, LPS induces an inflammatory response and inflammatory mediators could also impact reproduction as an indirect secondary consequence of elevated LPS.

Conclusion
In summary, endotoxemia negatively affects female fertility and fecundity and has many points of action within the reproductive tract. Endotoxemia originates from a variety of stressors and also during times of bacterial infection. Several studies investigating reproductive impacts of endotoxemia have used acute, bolus exposures, as summarized in Table 1, which may not accurately represent the temporal pattern of bacterial infection, or ‘leaky gut’, thus, more continuous chronic low-level LPS experiments are warranted in order to identify mitigation strategies to protect and/or improve mammalian female reproductive function. In vitro experiments also are largely reflective of acute exposures since these levels are likely to be much higher than those that occur in vivo or those LPS concentrations that reach the follicular fluid and/or the oocyte. Additionally, endotoxemia that results from compromised intestinal integrity is accompanied by systemic exposure to additional intestinal components, many of which have not been characterized and identified and which may also be dynamic in response to the initiating stressor. Thus greater understanding of resident microbial populations and shifts to these populations will ultimately improve our understanding of the gut-hypothalamic-pituitary-ovarian-uterine axis.

Numerous questions remain to be clarified in our understanding of the impacts of endotoxemia on female fertility include but are certainly not limited to: (1) the level and/or duration required to impact fertility; the initiating insult to the reproductive tract, (2) the immune response within the reproductive tract that responds to endotoxemia, (3) the potential for tolerance to elevated LPS to develop, (4) the actual impact of LPS on the quality of the germ line, (5) potential effects on offspring (trans- and multi-generational) exposed to endotoxemia in utero and (6) the contribution or lack thereof of LBP on data derived from in vitro experiments. In addition, it is difficult to surmise the duration of metabolic endotoxemia, which is likely to vary dependent on the physiological situation, but which ultimately has a potential to impact physiological outcomes. Each of these areas are worthy of investigation with relevance to many facets of public health and production animal agriculture.
Table 1  Summary of LPS studies with effects on reproductive outcomes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Duration</th>
<th>Dose</th>
<th>Citation</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewes</td>
<td>IA</td>
<td>Single injection</td>
<td>0.1–10 mg 400 ng/kg</td>
<td>Newnham et al. (2005) Battaglia et al. (1997)</td>
<td>Fetal death Ovariecotomized, increased P4, decreased LH</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>26 h</td>
<td>300 ng/kg</td>
<td>Battaglia et al. (2000) Herman et al. (2010)</td>
<td>Decreased E2 and LH Decreased LH, increased prolactin, no effect on FSH</td>
</tr>
<tr>
<td></td>
<td>S.C.</td>
<td>Daily injections for 2 or 6 day</td>
<td>2 mg/kg or 20 μg/kg</td>
<td>Shakil et al. (1994)</td>
<td>Decreased P4 and E2, fewer large preovulatory follicles</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>IV</td>
<td>2x daily for 5 day</td>
<td>150 μg</td>
<td>Xiao et al. (1999)</td>
<td>Decreased P4 and E2, fewer large preovulatory follicles</td>
</tr>
<tr>
<td>Trout</td>
<td>IP</td>
<td>Single injection</td>
<td>3 mg/kg</td>
<td>MacKenzie et al. (2006)</td>
<td>Induced apoptosis, no effects on germinal vesicle break down Abortions</td>
</tr>
<tr>
<td>Gilts</td>
<td>PC</td>
<td>Single injection</td>
<td>0.5, 1, 2, 3 μg/kg</td>
<td>Cort (1986) Cort et al. (1986)</td>
<td>No change in cycle length, decreased P4, increased PGFα, no effect on P4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single injection</td>
<td>50, 250, or 1250 μg</td>
<td>Tuo et al. (1999)</td>
<td>No effect on P4 plasma, fetal survival or development, increased luteal size and amniotic fluid volume Abortions</td>
</tr>
<tr>
<td></td>
<td>IU or IV</td>
<td>Single injection</td>
<td>5 μg/kg</td>
<td>Gilbert et al. (1990)</td>
<td>Increased P4, PGF2α metabolites, cycle length was unchanged</td>
</tr>
<tr>
<td></td>
<td>LV</td>
<td>Single injection</td>
<td>0.01 μg/kg</td>
<td>Kuijko et al. (1995)</td>
<td>Increased P4, PGF2α metabolites, cycle length was unchanged</td>
</tr>
<tr>
<td>Lactating cows</td>
<td>IU</td>
<td>2x @ 5 and 20 DIM</td>
<td>5 μg/kg</td>
<td>Peter et al. (1990)</td>
<td>Increased PGF2α metabolites</td>
</tr>
<tr>
<td></td>
<td>IV or IM</td>
<td>Single injection</td>
<td>0.5 μg/kg or IM: 10 μg</td>
<td>Laven et al. (2008)</td>
<td>No change in E2 yet delayed or inhibited ovulation</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>Single injection</td>
<td>200 μg</td>
<td>Lüttgenau et al. (2016)</td>
<td>No change in P4, luteal size or luteal blood flow</td>
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<tr>
<td>Non-lactating cows</td>
<td>IV</td>
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<td>10 μg</td>
<td>Lavon et al. (2011)</td>
<td>Decreased luteal size and luteal blood flow, increased P4 and PGE</td>
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<tr>
<td></td>
<td>IV</td>
<td>6 h</td>
<td>1.0 or 2.5 μg/kg</td>
<td>Giri et al. (1990)</td>
<td>Abortions, increased PGF2α, decreased P4</td>
</tr>
<tr>
<td>Mice</td>
<td>IP</td>
<td>Single injection</td>
<td>50 μg/mouse</td>
<td>Fidel et al. (1994)</td>
<td>Preterm birth, stillborns</td>
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<tr>
<td></td>
<td>IP</td>
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<td>0.5 μg/g BW</td>
<td>Ogando et al. (2003)</td>
<td>Resorptions</td>
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<td>100 μg/mouse</td>
<td>Bromfield and Sheldon (2013)</td>
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<td>Aisemberg and Salminen (2013)</td>
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<td>2.4 mg/kg</td>
<td>Rouxioja et al. (2005)</td>
<td>Fetal defects</td>
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<tr>
<td></td>
<td>IP</td>
<td>Single or multiple injections at 1–6 h intervals, 12–17 day</td>
<td>0–100 mg</td>
<td>Kaga et al. (1996)</td>
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<td>IP</td>
<td>2x</td>
<td>10 μg/kg then 120 μg/kg</td>
<td>Xu et al. (2007)</td>
<td>Pre-treatment of LPS saved embryonic resorption</td>
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<td>Abortions</td>
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<td>7.5 x 10⁶ E.coli</td>
<td>Coid et al. (1978)</td>
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<td>Skanes et al. (1972)</td>
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<td>Rioux-Darrioul et al. (1978)</td>
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(Continued)
Table 1

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<th>Findings</th>
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<td>Zhong et al. (2008)</td>
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<td>Reznikov et al. (1999)</td>
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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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