Disruption of female reproductive function by endotoxins

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Disciplines
Agriculture | Animal Experimentation and Research | Animal Sciences | Cell and Developmental Biology

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Disruption of female reproductive function by endotoxins

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Short title: Endotoxemia-induced reproductive dysfunction

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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 to endotoxemia

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 Figure 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 track, uterine, 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, endotoxemia is a consequence of infection by LPS-producing bacteria. There are also a myriad of environmental exposures that can cause endotoxemia and these include non-steroidal anti-inflammatory drugs (Arakawa, et al. 2012, Van Wijck, et al. 2012), mycotoxins (Alizadeh, et al. 2015, Assuncao, et al. 2016, Marin, et al. 2015) and alcohol (Hartmann, et al. 2012, Hartmann, et al. 2015). Indeed “leaky gut”, and resultant metabolic endotoxemia, has been associated with many pathologies such as inflammatory

Heat stress is an abiotic stress that also induces endotoxemia. 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 endotoxemia (Pearce, et al. 2013a, Pearce, et al. 2012, Pearce, et al. 2013b, Pearce, et al. 2013c, 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 (Kvidera, et al. 2017, Rodriguez, et al. 1996). 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 versus 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 reproductive outcomes of LPS exposure, which have been examined in both traditional rodent and large animal models.

**The systemic response to endotoxemia**

*Lipoprotein-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

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 (Raetz 2008, Schumann 2011, Tobias, et al. 1989). 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 (Faisal, et al. 2016, Goris, et al. 2011). 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**


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 (NFκB) although the MyD88-dependent pathway activates pro-inflammatory cytokine genes while the MyD88-independent signaling activates Type I 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 NFKB 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 and Munford 1983). AOAH releases secondary acyl chains from LPS regardless of the acyl chain structure or location on the diglucosamine backbone of LPS (Erwin and 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 et al. 2000).
and 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 (Janelsins, et al. 2014) cells has been demonstrated.

**Effects of LPS on female reproduction and fertility**

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 and Peter 1987, Peter, et al. 1989a, Peter, et al. 1989b), abnormal or delayed folliculogenesis after parturition (Huszenicza and Kegl 1999), a longer postpartum anestrus period (Bosu and Peter 1987), and a lengthened luteal phase (Peter and 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 IL-6 and IL-8 in media collected after bovine granulosa cell or ovarian cortical strip culture was observed following LPS incubation, similar to the responsiveness of human immune cells (Bromfield and Sheldon 2013, Dentener, et al. 1993). Plasma LBP and follicular fluid IL-6 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 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 and 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 and Sheldon 2013) suggesting TLR4 in part regulates the ovarian LPS response. Phosphatase and tension 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 and 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 (Battaglia, et al. 2000, Kalra, et al. 1990, Peter, et al. 1990). 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 (E$_2$) increase during the preovulatory phase, thus delaying subsequent luteinizing hormone (LH) and follicle stimulating hormone (FSH) surges, culminating in delayed or inhibited ovulation (Battaglia, et al. 2000, Peter, et al. 1989a, Peter, et al. 1990, 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 (Coleman, et al. 1993, Ebisui, et al. 1992, Feng, et al. 1991, Kujjo, et al. 1995). In agreement with reduced E$_2$ compromising ovulation, when LPS was infused into the uterine lumen, the pre-ovulatory 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 the LH (LHβ) and LH receptor (LHR) (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 LHr, 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 LHr, CYP17, and CYP11A1 but no difference in steroidogenic acute regulatory proteins (StAR) or 3β-hydroxysteroid dehydrogenase (3β-HSD) 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 either 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 $E_2$ is needed to induce behavioral display of estrus, however the amount of $E_2$ 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 and 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 and Shimizu 2017). LPS also reduced blastocyst development of LPS-exposed oocytes and the trophoblast cell number of blastocysts (Magata and 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 $P_4$ was 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 (Hockett, et al. 2000, Moore, et al. 1991). Not only does LPS administration delay ovulation, it also lengthens the time to luteinization, CL formation and sufficient $P_4$ production (Lavon, et al. 2011, Suzuki, et al. 2001), 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 P4 receptor
abundance and regression of the CL, and these observations were absent in eCS deficient
mice (Schander, et al. 2016).

Administrating LPS to goats during their luteal phase did not affect steroid hormone
concentrations but did increase PGF2α metabolites (Fredriksson G 1985), and repeated
uterine LPS infusions in dairy cows every 6 h from 12 h prior to ovulation until 9 d post-
Culturing bovine luteal tissue in vitro with TNFα increased PGF2α in a dose-dependent
manner (Benyo and 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 P4 has been demonstrated, whereby P4 is
initially increased and then declines in LPS-treated, relative to control females (Herzog, et
LPS exposure initially decreased but then did not affect P₄ production in bovine granulosa cells in culture (Herath, et al. 2007). Further, P₄ concentrations were increased in large bovine follicles, and it has been proposed that less P₄ is being converted to E₂ (Magata, et al. 2014a, Magata, et al. 2014b). However, others demonstrated that LPS in vitro can inhibit steroid secretion, specifically P₄ and androstenedione in thecal-interstitial cells (Taylor and Terranova 1995) suggesting endotoxemia could alter P₄ production, representing an endocrine disrupting effect.

**Endotoxemia and pregnancy maintenance**

P₄ is essential for pregnancy maintenance, and LPS reduces the P₄ receptor in uteri of pregnant mice (Agrawal, et al. 2013). The effect of LPS on the ability of P₄ 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 pre-term labor in many species (Koga and Mor 2010) and in fact, intraperitoneal LPS injection is an established experimental model for inducing pre-term labor (Agrawal, et al. 2013, Deb, et al. 2004, (Elovitz and Mrinalini 2004)). In addition, infertility can be the result of reproductive tract infections in humans and production animals (Price, et al. 2013, Williams, et al. 2008). As mentioned earlier, LPS increases PGF2α release (Roberts, et al. 1975) leading to CL regression, a decline in P₄, and spontaneous abortion in goats (Fredriksson G 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 (Sheldon, 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 and 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 pre-term labor pregnancies were compared, there were increased amounts of TNFα in the pre-term 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

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 and Boudry 2016). Additionally, the bacterial source of LPS remains undefined in these assays and there are interactions that can alter the assay interpretation (Guerville and Boudry 2016). Thus the usefulness of measuring LPS directly has been questioned (Gnauck, et al. 2015, 2016, Stadlbauer, et al. 2007). 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, Nteeba, et al. 2013, Wu, et al. 2012), 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 2016) 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, 2017 #7016) 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 reproduction.
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*, 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 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.
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Figure 1. Structure of lipopolysaccharide (LPS).

LPS is found on the cell wall of gram-negative bacteria, such as *Escherichia coli*. The lipid A region, depicted in red, elicits the immune response.
Figure 1. Structure of lipopolysaccharide (LPS). LPS is found on the cell wall of gram-negative bacteria, such as Escherichia coli. The lipid A region, depicted in red, elicits the immune response.
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<td>0.1 - 10 mg</td>
<td>Newnham et al., 2005</td>
<td>Fetal death</td>
<td></td>
</tr>
<tr>
<td></td>
<td>intravenous 26 h</td>
<td>300 ng/kg</td>
<td>Battaglia et al., 2000</td>
<td>Decreased E2 and LH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2x (2 week interval)</td>
<td>40 ng/kg</td>
<td>Herman et al., 2010</td>
<td>Decreased LH, Increased prolactin, No effect on FSH</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>subcutaneous Daily injections for 2 or 6 d</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>
<td></td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>intravenous 2x daily for 5 d</td>
<td>150 μg</td>
<td>Xiao et al., 1999</td>
<td>Decreased P4</td>
<td></td>
</tr>
<tr>
<td>Trout</td>
<td>intraperitoneal Single injection</td>
<td>3 mg/kg</td>
<td>MacKenzie et al., 2006</td>
<td>Induced apoptosis No effects on germinal vesicle break down</td>
<td></td>
</tr>
<tr>
<td>Gilts</td>
<td>permanent cannulas Single injection</td>
<td>0.5, 1, 2, 3 μg/ kg</td>
<td>Cort, 1986</td>
<td>Abortions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5, 1, 2, 3 μg/ kg</td>
<td>Cort et al., 1986</td>
<td>No change in cycle length. Decreased P4, increased PGF2α</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50, 250, or 1,250 μg</td>
<td>Tuo et al., 1999</td>
<td>No effect on P4 plasma, fetal survival or development. Increased fetal weight and amniotic fluid volume</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 mg</td>
<td>Wrathall et al., 1978</td>
<td>Abortions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mixed intoation</td>
<td>40 mg</td>
<td>Cort et al., 1990</td>
<td>Increased PGF2α No change in P4</td>
<td></td>
</tr>
<tr>
<td>Animal Type</td>
<td>Route</td>
<td>Description</td>
<td>Dose</td>
<td>Reference</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------</td>
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<tr>
<td>Goats</td>
<td>intrauterine</td>
<td>injected 1 or 2x</td>
<td>0.1 - 5.2 μg/kg</td>
<td>Fredriksson et al., 1985</td>
<td>No hormonal changes</td>
</tr>
<tr>
<td></td>
<td>intravenous</td>
<td></td>
<td>0.1 - 5.2 μg/kg</td>
<td>Fredriksson et al., 1985</td>
<td>Increased PGF2α, decreased P4, abortions</td>
</tr>
<tr>
<td>Heifers</td>
<td>intrauterine</td>
<td>every 6 h for 10 trts</td>
<td>5 μg/kg</td>
<td>Peter et al., 1990</td>
<td>Decreased E2 production, inhibited LH surge, no change in P4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 μg/kg</td>
<td>Peter et al., 1989</td>
<td>Inhibited LH surge and ovulation, caused ovarian cysts</td>
</tr>
<tr>
<td></td>
<td>intrauterine</td>
<td>every 6 h for 9 d</td>
<td>3 μg/kg</td>
<td>Lüttgenau et al., 2016</td>
<td>Premature CL luteolysis, increased PGF2α metabolites, decreased P4, reduced luteal size and blood flow</td>
</tr>
<tr>
<td></td>
<td>intravenous</td>
<td>Single injection</td>
<td>5 μg/kg</td>
<td>Gilbert et al., 1990</td>
<td>Increased P4, PGF metabolites, cycle length was unchanged</td>
</tr>
<tr>
<td></td>
<td>intravenous</td>
<td>Single injection</td>
<td>0.01 μg/kg</td>
<td>Kujjo et al., 1995</td>
<td>Ovariectomized. Increased P4, decreased E2 and LH</td>
</tr>
<tr>
<td>Lactating Cows</td>
<td>intrauterine</td>
<td>2x @ 5 and 20 DIM</td>
<td>5 μg/kg</td>
<td>Peter et al., 1990a</td>
<td>Increased PGF2α metabolites</td>
</tr>
<tr>
<td></td>
<td>intravenous (iv) or intramuscular (im)</td>
<td>Single injection</td>
<td>0.5 μg/kg (iv) or 10 ug (im)</td>
<td>Lavon et al., 2008</td>
<td>No change in E2 yet delayed or inhibited ovulation</td>
</tr>
<tr>
<td></td>
<td>intramuscular</td>
<td>Single injection</td>
<td>200 μg</td>
<td>Lüttgenau et al., 2016b</td>
<td>No change in P4, luteal size or luteal blood flow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 μg</td>
<td>Lavon et al., 2011</td>
<td>Decreased follicular E2, P4</td>
</tr>
<tr>
<td>Non-lactating Cows</td>
<td>intravenous</td>
<td>Single injection</td>
<td>0.5 μg/kg</td>
<td>Herzog et al., 2012</td>
<td>Decreased luteal size and luteal blood flow, Increased P4 and PGE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 h</td>
<td>Giri et al., 1990</td>
<td>Abortions, increased PGF2α, decreased P4</td>
</tr>
<tr>
<td>Mice</td>
<td>intraperitoneal</td>
<td>Single injection</td>
<td>10 μg</td>
<td>Buhimschi et al., 2003</td>
<td>Preterm birth, stillborns</td>
</tr>
<tr>
<td>Dose and Route</td>
<td>Authors and Year</td>
<td>Effects</td>
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<td></td>
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<tr>
<td>50 μg/mouse</td>
<td>Fidel et al., 1994</td>
<td>Preterm birth</td>
<td></td>
<td></td>
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<tr>
<td>0.5 μg/g BW</td>
<td>Ogando et al., 2003</td>
<td>Resorptions</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>100 μg/mouse</td>
<td>Bromfield and Sheldon, 2013</td>
<td>Decreased primordial follicle pool, increased follicle atresia</td>
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<tr>
<td>1.0 μg/g</td>
<td>Aisemberg et al., 2013</td>
<td>Resorptions, decreased P4</td>
<td></td>
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<tr>
<td>0.4 - 2 mg/kg</td>
<td>Salminen et al., 2008</td>
<td>Preterm birth, stillborns</td>
<td></td>
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<tr>
<td>2.4 mg/kg</td>
<td>Rounioja et al., 2005</td>
<td>Fetal defects</td>
<td></td>
<td></td>
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<tr>
<td>0-100 mg</td>
<td>Kaga et al., 1996</td>
<td>Preterm birth</td>
<td></td>
<td></td>
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<tr>
<td>2x 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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<tr>
<td>10 μg</td>
<td>Harper and Skarnes, 1972</td>
<td>Abortions</td>
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<tr>
<td>7.5x10^6 E.coli</td>
<td>Coid et al., 1978</td>
<td>Resorptions</td>
<td></td>
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<tr>
<td>1.5 - 20 μg</td>
<td>Skarnes and Harper, 1972</td>
<td>Abortions</td>
<td></td>
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<tr>
<td>2 - 5 μg</td>
<td>Rioux-Darrieulat et al., 1978</td>
<td>Abortions</td>
<td></td>
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</tr>
<tr>
<td>0.1 μg</td>
<td>Zhong et al., 2008</td>
<td>Abortions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 μg</td>
<td>Rounioja et al., 2003</td>
<td>Fetal defects</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Reznikov et al., 2003</td>
<td>Resorptions</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Location</td>
<td>Route</td>
<td>Dose</td>
<td>Reference</td>
<td>Effect</td>
<td></td>
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<td>--------------</td>
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<tr>
<td>intrauterine</td>
<td>Single injection</td>
<td>250 μg</td>
<td>Elovitz et al., 2003</td>
<td>Preterm birth</td>
<td></td>
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<tr>
<td>subcutaneous</td>
<td>Single injection</td>
<td>0.5 mg/kg</td>
<td>Chua et al., 2006</td>
<td>Resorptions, lower fetal weight</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25 mg or 0.147 mg</td>
<td>Coid, 1976</td>
<td>Resorptions, lower fetal weight</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** iv = intravenous; im = intramuscular; E2 = 17β-estradiol; P4 = progesterone; LH = luteinizing hormone; FSH = follicle stimulating hormone; PGF2α = prostaglandin F2α; LPS = lipopolysaccharide; PGE = prostaglandin E