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# Disruption of female reproductive function by endotoxins

K. L. Bidne  
*Iowa State University*

M. J. Dickson  
*Iowa State University*

J. W. Ross  
*Iowa State University, jwross@iastate.edu*

L. H. Baumgard  
*Iowa State University, baumgard@iastate.edu*

A. F. Keating  
*Iowa State University, akeating@iastate.edu*

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# Disruption of female reproductive function by endotoxins

## **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.

## **Disciplines**

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

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1                    **Disruption of female reproductive function by endotoxins**

2                    \*Bidne, K.L., \*Dickson, M.J., Ross, J.W., Baumgard, L.H., †Keating, A.F.

3                    Department of Animal Science, Iowa State University, Ames, IA 50014

4

5   **Short title:** Endotoxemia-induced reproductive dysfunction

6   \* Co-first authors.

7   † Corresponding Author: Aileen F. Keating, 2356J Kildee Hall, Iowa State University, Ames

8   IA 50014. [akeating@iastate.edu](mailto:akeating@iastate.edu); telephone 515-294-3849

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13 **Abstract**

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

25

## 26 **Introduction to endotoxemia**

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

34  
35 LPS consists of a core oligosaccharide, O-antigens, and a lipid A moiety (depicted in Figure  
36 1). The lipid A moiety portion of LPS is responsible for inducing the cellular response  
37 (Loppnow, et al. 1989). Systemic endotoxemia (increased circulating LPS) reflects either  
38 bacterial infection or compromised epithelial (skin, lung, gastrointestinal track, uterine,  
39 and mammary) barrier function. Metabolic endotoxemia is described as the physiological  
40 state when circulating LPS is 10–50 times lower than that observed during septic shock;  
41 (Cani, et al. 2007).

42  
43 Unsurprisingly, endotoxemia is a consequence of infection by LPS-producing bacteria.  
44 There are also a myriad of environmental exposures that can cause endotoxemia and these  
45 include non-steroidal anti-inflammatory drugs (Arakawa, et al. 2012, Van Wijck, et al.  
46 2012), mycotoxins (Alizadeh, et al. 2015, Assuncao, et al. 2016, Marin, et al. 2015) and  
47 alcohol (Hartmann, et al. 2012, Hartmann, et al. 2015). Indeed “leaky gut”, and resultant  
48 metabolic endotoxemia, has been associated with many pathologies such as inflammatory

49 bowel syndrome (Michielan and D'Inca 2015), cirrhosis (Fukui 2015, Lutz, et al. 2015), and  
50 cancer (Saggiaro 2014). In addition, evidence that gut barrier function becomes  
51 compromised during obesity, resulting in metabolic endotoxemia, is firmly established (Al-  
52 Attas, et al. 2009, Amar, et al. 2008, Hawkesworth, et al. 2013). Although the etiology is not  
53 clear, low-grade, chronic inflammation caused by obesity-induced endotoxemia is thought  
54 to play a key role in the development of obesity-related disorders (Cani, et al. 2007,  
55 Hawkesworth, et al. 2013) including female reproductive dysfunction.

56

57 Heat stress is an abiotic stress that also induces endotoxemia. In an attempt to maximize  
58 radiant heat dissipation, heat-stressed animals redistribute blood to the periphery, and in  
59 order to maintain blood pressure, blood flow to the splanchnic tissues, including the  
60 gastrointestinal tract, is markedly reduced. The intestinal epithelial cells are extremely  
61 sensitive to oxygen and nutrient restriction (Rollwagen, et al. 2006). Heat stress thus  
62 causes marked hypoxic-induced conformational changes which ultimately reduces  
63 intestinal barrier integrity. Depending upon the severity and magnitude, heat stress can  
64 cause intestinally-derived endotoxemia (Pearce, et al. 2013a, Pearce, et al. 2012, Pearce, et  
65 al. 2013b, Pearce, et al. 2013c, Sanz Fernandez, et al. 2014). The duration of leaky gut is  
66 variable and transitory, for example, intestinal integrity is reduced as early as two hours  
67 after the onset of heat stress in pigs (Pearce, et al. 2014) and with removal of heat stress,  
68 intestinal integrity returned within days. Additionally, leaky gut can be caused by reduced  
69 nutrient intake and this has been demonstrated in multiple models (Kvidera, et al. 2017,  
70 Rodriguez, et al. 1996). Further, psychological and emotional stress also increases  
71 gastrointestinal tract barrier permeability (Vanuytsel, et al. 2014). Thus, endotoxemia is

72 relatively common and arises due to a variety of frequent initiators, but the severity of it  
73 depends on the source (epithelial barrier endotoxin infiltration versus bacterial infection)  
74 and duration of the inducing agent(s).

75  
76 The major purpose of this review is to collectively describe experiments that have either  
77 directly tested the female reproductive effects of endotoxemia through *in vitro* culture  
78 models or *in vivo* experiments in which animals are administered LPS. Additionally, we will  
79 highlight research that has identified associations between physiological scenarios that  
80 compromise intestinal integrity (and concomitantly increase circulating endotoxin) with  
81 detrimental impacts on female reproduction. Studies evaluating the impact of metabolic  
82 and acute endotoxemia are included. Typically, controlled experiments to evaluate  
83 endotoxemia's impact on female reproduction have utilized the acute approach (i.e. an I.V.  
84 or I.M. LPS bolus). Further, we will describe how specific cells recognize and respond to  
85 LPS, characterize the systemic response to endotoxemia and the reproductive outcomes of  
86 LPS exposure, which have been examined in both traditional rodent and large animal  
87 models.

88

## 89 **The systemic response to endotoxemia**

### 90 *Lipopolysaccharide-binding protein*

91 Hepatic acute phase proteins (APP), which are produced as a secondary (non-local)  
92 response to a toxic stimuli, have been widely utilized as indicators of systemic and  
93 metabolic inflammation, including metabolic endotoxemia (Ceciliani, et al. 2012).  
94 Lipopolysaccharide-binding protein (LBP) is an APP, primarily produced in hepatocytes

95 (Grube, et al. 1994, Kirschning, et al. 1997), that interacts directly with the lipid A moiety of  
96 LPS (Schumann 2011, Tobias, et al. 1986, 1989). Interaction between LBP and LPS results  
97 in an LBP conformational change promoting recognition and transfer of LPS to  
98 macrophages (Wright, et al. 1989). Interleukin (IL)-6 (Grube, et al. 1994, Kirschning, et al.  
99 1997), IL-1 $\beta$  and dexamethasone (Schumann, et al. 1996) stimulate hepatic LBP production  
100 but LBP can also be produced in lung epithelial cells (Dentener, et al. 2000, Klein, et al.  
101 1998), gastrointestinal tract cells (Vreugdenhil, et al. 1999), kidney (Wang, et al. 1998), and  
102 the epididymis (Malm, et al. 2005). LBP acts as a soluble receptor and transports LPS to the  
103 appropriate toll-like receptor (TLR) to initiate intracellular signal cascades to elicit an  
104 immunological response (Schumann 2011). In humans, circulating LBP and plasma C-  
105 reactive protein (another broad biomarker of inflammation) are positively correlated  
106 (Tremellen, et al. 2015), thus providing rationale for using LBP as an inflammatory  
107 biomarker (Opal, et al. 1999).

108

### 109 **The cellular response to endotoxemia**

110 The lipid A moiety of LPS is highly conserved among species and it stimulates an  
111 inflammatory response because it is recognized by membrane bound TLR4 (Raetz 2008,  
112 Schumann 2011, Tobias, et al. 1989). Utilizing TLR4-deficient mice, it has been shown that  
113 TLR4 is required for LPS recognition and the subsequent cellular response (Hoshino, et al.  
114 1999). However, other TLRs can also mediate a cellular response to LPS, dependent on the  
115 bacterial strain of origin. As an example, the LPS produced by *Leptospirosis* can instigate an  
116 intracellular response via TLR2, TLR4 or TLR5 (Faisal, et al. 2016, Goris, et al. 2011). In  
117 addition, host species can also differ in their response to LPS with some having variable



118 sensitivity to a specific LPS which impacts both the physiological response and  
119 development of mitigation strategies such as vaccine production (Werling, et al. 2009).

#### 120 *Toll-like Receptor 4*

121 TLR4 is a membrane spanning protein bearing similarity to the interleukin 1 (IL-1)  
122 receptor (Aderem and Ulevitch 2000, Greenfeder, et al. 1995, Medzhitov and Janeway  
123 2000). LPS binds to cluster of differentiation 14 (CD14) and is then transferred to a  
124 complex between TLR4 and myeloid differentiation factor 2 (MD-2) to initiate a cellular  
125 response (da Silva Correia, et al. 2001, Triantafilou and Triantafilou 2002). The MD-2  
126 protein is a crucial component of LPS recognition as an extracellular piece of the TLR4  
127 complex (Shimazu, et al. 1999). Soluble CD14 (sCD14) is integral for serum- and cell-  
128 mediated responses to LPS (Pugin, et al. 1993, Wright, et al. 1989) while the membrane-  
129 bound form (mCD14) is a glycosylphosphatidyl-inositol anchored protein (Haziot, et al.  
130 1988, Simmons, et al. 1989) and works with TLR4 to transmit the LPS signal across the  
131 lipid bilayer to initiate a cellular response (Poltorak, et al. 1998). LBP was originally  
132 thought to be necessary for CD14 to bind LPS (Wright, et al. 1992), however, other studies  
133 suggest LPS directly activates CD14 or the MD-2-TLR4 complex (da Silva Correia, et al.  
134 2001, Dentener, et al. 2000, Triantafilou and Triantafilou 2002), and LBP increases the rate  
135 of LPS binding to CD14 (Hailman, et al. 1994).

136

137 Following LPS recognition, TLR4 recruits proteins including TIR domain-containing  
138 adaptor protein (TIRAP), myeloid differentiation primary response gene 88 (MyD88), TIR  
139 domain-containing adaptor inducing interferon beta (TRIF), and TRIF-related adaptor  
140 molecule (TRAM) via its Toll-interleukin-1 receptor (TIR) domain causing downstream

141 pathway activation. TIRAP and MyD88 mediate MyD88-dependent signaling whereas TRIF  
142 and TRAM mediate MyD88-independent signaling. Both pathways involve phosphorylation  
143 of the REL-associated protein (RELA) subunit of nuclear factor kappa B (NFκB) although  
144 the MyD88-dependent pathway activates pro-inflammatory cytokine genes while the  
145 MyD88-independent signaling activates Type I interferon genes (Kawai, et al. 1999,  
146 Shimazu, et al. 1999). Phosphorylated RELA increases concomitant with increased LPS  
147 exposure demonstrating the ability of LPS to drive TLR4-mediated NFκB activation (Chow,  
148 et al. 1999). Interestingly, single nucleotide polymorphisms (SNPs) in the *TLR4* gene affects  
149 immune function and reproductive ability in dairy cows (Shimizu, et al. 2017), though the  
150 importance of *Tlr4* SNPs in humans remains vague (Gowin, et al. 2017, Hajjar, et al. 2017)  
151 and is an area of future interest regarding the biological response(s) to endotoxemia.

152

### 153 **Detoxification of LPS by Acyloxyacyl Hydrolase**

154 Acyloxyacyl hydrolase (AOAH) is a lipase that deacylates and detoxifies LPS within cells  
155 and (Hall and Munford 1983). AOAH releases secondary acyl chains from LPS regardless of  
156 the acyl chain structure or location on the diglucosamine backbone of LPS (Erwin and  
157 Munford 1990). AOAH is primarily produced in macrophages, neutrophils, and dendritic  
158 cells (Ojogun, et al. 2009) and converts hexaacylated LPS to pentaacylated or tetraacylated  
159 LPS rendering it unable to stimulate a response through TLR4 complex formation  
160 (Teghanemt, et al. 2005). AOAH activity increased in murine serum and hepatocytes  
161 following a 25 µg bolus of LPS (Ojogun, et al. 2009). In these mice, AOAH activity peaked  
162 after three days and returned to normal levels by day nine post LPS-injection (Ojogun, et al.  
163 2009). Deacylated LPS (dLPS) can compete with LPS for LBP or CD14 binding (Kitchens

164 and Munford 1995a, b), however, binding of dLPS does not stimulate a cellular response  
165 (Kitchens, et al. 1992). Interestingly, LBP alone or in coordination with CD14 increases the  
166 susceptibility of LPS to AOA<sub>H</sub> detoxification (Gioannini, et al. 2007). *Aoah*-deficient mice  
167 have increased pulmonary damage in response to intranasal LPS exposure corroborating  
168 AOA<sub>H</sub>'s protective role against LPS (Zou, et al. 2017). Thus, the chemical modification of  
169 LPS by AOA<sub>H</sub> partly regulates the immune response by decreasing the capacity of LPS to  
170 stimulate an intracellular signal cascade (Lu, et al. 2005).

171  
172 AOA<sub>H</sub> cannot act on LPS when the fatty acyl chains are orientated to the inside of LPS  
173 aggregates or when LPS is anchored on the outer membrane of bacteria (Gioannini, et al.  
174 2007). AOA<sub>H</sub> can act on LPS-LBP complexes as well as monomeric LPS-sCD14 complexes,  
175 suggesting a model where LBP and sCD14 transfer of LPS exposes fatty acyl chains to AOA<sub>H</sub>  
176 (Gioannini, et al. 2007). However, when LPS is transferred and bound to MD-2, the fatty  
177 acyl chains are less accessible, decreasing AOA<sub>H</sub>'s ability to deacylate LPS and reduce TLR4  
178 activation (Gioannini, et al. 2007). Whether the female reproductive tract has the capacity  
179 to locally detoxify LPS remains unknown though recently, the importance of AOA<sub>H</sub> in the  
180 lung (Zou, et al. 2017), urinary tract (Yang, et al. 2017) and colonic dendritic (Janelins, et  
181 al. 2014) cells has been demonstrated.

182

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

184 Understanding the effects of LPS exposure on ovarian function is of interest in humans and  
185 production livestock species, since increased circulating LPS is associated with heat stress  
186 (Pearce, et al. 2013a, Pearce, et al. 2012, Pearce, et al. 2013b, Pearce, et al. 2014, Sanz

187 Fernandez, et al. 2014), obesity (Cani, et al. 2007), and bacterial infection. Uterine  
188 infections have been associated with various negative impacts on bovine fertility, including  
189 cystic ovaries (Bosu and Peter 1987, Peter, et al. 1989a, Peter, et al. 1989b), abnormal or  
190 delayed folliculogenesis after parturition (Huszenicza and Kegl 1999), a longer postpartum  
191 anestrus period (Bosu and Peter 1987), and a lengthened luteal phase (Peter and Bosu  
192 1988). Interestingly, follicular fluid that surrounds and nourishes the maturing oocyte  
193 contains LPS levels reflective of the systemic circulation (Herath, et al. 2007). An  
194 accumulation of IL-6 and IL-8 in media collected after bovine granulosa cell or ovarian  
195 cortical strip culture was observed following LPS incubation, similar to the responsiveness  
196 of human immune cells (Bromfield and Sheldon 2013, Dentener, et al. 1993). Plasma LBP  
197 and follicular fluid IL-6 concentrations were also positively correlated, suggesting that  
198 systemic endotoxemia is associated with ovarian inflammation (Tremellen, et al. 2015).  
199 Thus, LPS can locate the ovary and potentially interact directly with the oocyte, though  
200 remains to be determined.

201

### 202 *Impacts of endotoxemia on folliculogenesis*

203 Bovine ovarian cortical explants exposed to LPS had reduced number of primordial follicles  
204 due to hyperactivation (Bromfield and Sheldon 2013). Similarly, mice exposed to LPS *in*  
205 *vivo* had reduced primordial follicle number which was described as TLR4-mediated, since  
206 *Tlr4*<sup>-/-</sup> mice are refractory to LPS-mediated primordial follicle depletion (Bromfield and  
207 Sheldon 2013) suggesting TLR4 in part regulates the ovarian LPS response. Phosphatase  
208 and tension homolog (PTEN) and Forkhead box O3 (FOXO3), both proteins involved in  
209 regulating primordial follicle activation, were translocated out of the oocyte nucleus of

210 primordial and primary follicles in cultured bovine cortical strips after LPS exposure  
211 (Bromfield and Sheldon, 2013). The aforementioned indicate premature primordial follicle  
212 activation, potentially leading to depletion of the ovarian follicular reserve. In rodent  
213 studies, altered protein abundance due to LPS exposure in neonatal rodents has been  
214 observed (Sominsky, et al. 2013). Furthermore, a diminished follicular reserve and earlier  
215 onset of ovarian senescence occurs in female rats neonatally exposed to LPS, raising  
216 concern about reproductive outcomes of bacterial infections early in life (Sominsky, et al.  
217 2012).

218

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

220 Immune challenges can disrupt the follicular phase in multiple species (Battaglia, et al.  
221 2000, Kalra, et al. 1990, Peter, et al. 1990). LPS suppresses the hypothalamic-pituitary  
222 gonadal axis by decreasing pulsatile gonadotrophin-releasing hormone (GnRH) secretion  
223 (Hoshino, et al. 1999). LPS also blunts the  $17\beta$ -estradiol ( $E_2$ ) increase during the  
224 preovulatory phase, thus delaying subsequent luteinizing hormone (LH) and follicle  
225 stimulating hormone (FSH) surges, culminating in delayed or inhibited ovulation (Battaglia,  
226 et al. 2000, Peter, et al. 1989a, Peter, et al. 1990, Suzuki, et al. 2001). Using gonadectomized  
227 animals, it has been demonstrated that LPS suppresses GnRH release, thus disrupting the  
228 LH surge amplitude, frequency, and concentration (Coleman, et al. 1993, Ebisui, et al. 1992,  
229 Feng, et al. 1991, Kujjo, et al. 1995). In agreement with reduced  $E_2$  compromising ovulation,  
230 when LPS was infused into the uterine lumen, the pre-ovulatory LH surge was attenuated  
231 (Peter, et al. 1989a). Furthermore, LPS-treated females had delays in the time to the LH  
232 surge (Fergani, et al. 2012) and lower ovulation rates (Williams, et al. 2008). Recently,

233 ovine kisspeptin/neurokinin B/dynorphin (KNDy) neuron activation has been  
234 demonstrated to be disrupted by LPS exposure, thus altering the hypothalamic-pituitary-  
235 ovarian axis (Fergani, et al. 2017).

236

237 LPS alters anterior pituitary hormones in circulation, through direct or indirect  
238 mechanisms. LPS infusion decreased LH but stimulated systemic prolactin (PRL) and  
239 cortisol levels in anestrous ewes and reduced mRNA abundance of the LH (LH $\beta$ ) and LH  
240 receptor (LHr) (Herman, et al. 2010). Further, mRNA encoding FSH and the FSH receptor  
241 (FSHr), PRL and the PRL receptor were increased by LPS infusion (Herman, et al. 2010).  
242 Granulosa cells exposed to high levels of LPS had reduced mRNA expression of *LHr*, *FSHr*,  
243 and cytochrome P450 (CYP) 19A1 (*CYP19A1*) (Magata, et al. 2014a). Theca cells isolated  
244 from follicles exposed to high levels of LPS also had decreased mRNA abundance of *LHr*,  
245 *CYP17*, and *CYP11A1* but no difference in steroidogenic acute regulatory proteins (*StAR*) or  
246 3 $\beta$ -hydroxysteroid dehydrogenase (*3 $\beta$ -HSD*) levels compared to theca cells from follicles  
247 exposed to low levels of LPS (Magata, et al. 2014b). LPS exposure did not impact cell  
248 number or androstenedione production from cultured theca cells from either small,  
249 medium or large ovarian follicles, but it did reduce E<sub>2</sub> production from cultured granulosa  
250 cells isolated from all three follicular sizes (Williams, et al. 2008). In addition, bovine  
251 follicles with high levels of LPS (> 0.5 EU/ml) had lower E<sub>2</sub> but elevated progesterone (P<sub>4</sub>)  
252 levels, relative to follicles with lower LPS concentrations (Magata, et al. 2014a). In an *in*  
253 *vitro* system where bovine granulosa cells were cultured with LPS and provided with FSH  
254 and androstenedione, E<sub>2</sub> and P<sub>4</sub> conversion were reduced potentially due to decreased  
255 expression of *Cyp19a* mRNA and protein (Herath, et al. 2007). During the *in vivo* LH surge,

256 a threshold of E<sub>2</sub> is needed to induce behavioral display of estrus, however the amount of  
257 E<sub>2</sub> actually required for the behavioral estrus is thought to be at lower level than that  
258 required to induce ovulation (Saifullizam, et al. 2010) and LPS negatively impacts female  
259 estrus behavior and frequency (Battaglia, et al. 2000).

260 Post-ovulation impacts of LPS have also been demonstrated. Bovine oocytes subjected to *in*  
261 *vitro* maturation with LPS were less likely to successfully complete meiosis with intact  
262 meiotic structures (Bromfield and Sheldon 2011). In addition, increased levels of reactive  
263 oxygen species and apoptotic genes, and altered methylation patterns were observed in  
264 bovine oocytes as a result of LPS (Zhao, et al. 2017). Further, LPS negatively affected bovine  
265 oocyte nuclear maturation by compromising meiotic progression, mitochondrial  
266 membrane potential and mitochondrial cytoplasmic redistribution (Magata and Shimizu  
267 2017). LPS also reduced blastocyst development of LPS-exposed oocytes and the  
268 trophoblast cell number of blastocysts (Magata and Shimizu 2017). These studies support  
269 the potential for LPS to negatively impact oocyte developmental competence.

270

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

272 Endotoxemia can compromise P<sub>4</sub> production and lead to decreased luteal function. Corpus  
273 luteum (CL) formation and the expected increase in P<sub>4</sub> was delayed in heifers exposed to  
274 LPS (Suzuki, et al. 2001). During a normal estrous cycle, in the absence of fertilization and  
275 pregnancy, prostaglandin F<sub>2</sub>α (PGF<sub>2</sub>α) causes CL regression and LPS can cause CL  
276 regression by inducing PGF<sub>2</sub>α production (Hockett, et al. 2000, Moore, et al. 1991). Not  
277 only does LPS administration delay ovulation, it also lengthens the time to luteinization, CL  
278 formation and sufficient P<sub>4</sub> production (Lavon, et al. 2011, Suzuki, et al. 2001), thus LPS has

279 numerous targets within the luteal phase. Additionally, CL size is reduced by LPS perhaps  
280 due to activation of pro-apoptotic pathways (Herzog, et al. 2012). The cannabinoid  
281 receptor type 1 (eCS) has recently been discovered to be involved in LPS-induced CL  
282 regression in mice as wild-type mice had increased uterine prostaglandin-endoperoxide  
283 synthase (PTGS2) and PGF2 $\alpha$  expression, which resulted in reduced ovarian P<sub>4</sub> receptor  
284 abundance and regression of the CL, and these observations were absent in eCS deficient  
285 mice (Schander, et al. 2016).

286

287 Administrating LPS to goats during their luteal phase did not affect steroid hormone  
288 concentrations but did increase PGF2 $\alpha$  metabolites (Fredriksson G 1985), and repeated  
289 uterine LPS infusions in dairy cows every 6 h from 12 h prior to ovulation until 9 d post-  
290 ovulation resulted in CL regression much sooner than controls (Luttgenau, et al. 2016).  
291 Culturing bovine luteal tissue *in vitro* with TNF $\alpha$  increased PGF2 $\alpha$  in a dose-dependent  
292 manner (Benyo and Pate 1992). Additionally, porcine luteal tissue, when cultured *in vitro*  
293 with PGF2 $\alpha$ , exhibits a feedback mechanism in which more PGF2 $\alpha$  is produced (Guthrie, et  
294 al. 1979). Normally, the porcine CL acquires capacity to undergo luteolysis around day 13  
295 of the luteal phase (Guthrie, et al. 1979), but multiple administrations of PGF2 $\alpha$  can induce  
296 luteolysis in the porcine CL at an earlier time (Diaz, et al. 2000) suggesting LPS may  
297 accelerate luteolysis via TNF $\alpha$  and PGF2 $\alpha$  induction in pigs, though this remains to be  
298 confirmed.

299

300 A temporal pattern of LPS affecting circulating P<sub>4</sub> has been demonstrated, whereby P<sub>4</sub> is  
301 initially increased and then declines in LPS-treated, relative to control females (Herzog, et



302 al. 2012). LPS exposure initially decreased but then did not affect P<sub>4</sub> production in bovine  
303 granulosa cells in culture (Herath, et al. 2007). Further, P<sub>4</sub> concentrations were increased in  
304 large bovine follicles, and it has been proposed that less P<sub>4</sub> is being converted to E<sub>2</sub>  
305 (Magata, et al. 2014a, Magata, et al. 2014b). However, others demonstrated that LPS *in vitro*  
306 can inhibit steroid secretion, specifically P<sub>4</sub> and androstenedione in thecal-interstitial cells  
307 (Taylor and Terranova 1995) suggesting endotoxemia could alter P<sub>4</sub> production,  
308 representing an endocrine disrupting effect.

309

### 310 *Endotoxemia and pregnancy maintenance*

311 P<sub>4</sub> is essential for pregnancy maintenance, and LPS reduces the P<sub>4</sub> receptor in uteri of  
312 pregnant mice (Agrawal, et al. 2013). The effect of LPS on the ability of P<sub>4</sub> to sustain  
313 gestation could cause spontaneous abortion, a phenotypic event frequently associated with  
314 physiological conditions in which LPS is elevated. Infection from gram negative bacteria or  
315 their outer wall components (including LPS) triggers pre-term labor in many species (Koga  
316 and Mor 2010) and in fact, intraperitoneal LPS injection is an established experimental  
317 model for inducing pre-term labor (Agrawal, et al. 2013, Deb, et al. 2004, (Elovitz and  
318 Mrinalini 2004)). In addition, infertility can be the result of reproductive tract infections in  
319 humans and production animals (Price, et al. 2013, Williams, et al. 2008). As mentioned  
320 earlier, LPS increases PGF<sub>2</sub>α release (Roberts, et al. 1975) leading to CL regression, a  
321 decline in P<sub>4</sub>, and spontaneous abortion in goats (Fredriksson G 1985). LPS and bacterial  
322 infection also increase PGF<sub>2</sub>α in the mare (Fredriksson, et al. 1986) and the cow  
323 (Fredriksson, et al. 1985). Uterine epithelial and stromal cells express TLR4 and both  
324 produced PGF<sub>2</sub>α and prostaglandin E<sub>2</sub> (PGE) after LPS exposure, a response abrogated by

325 using a TLR4 antagonist in bovine endometrial explants (Herath, et al. 2006). Endometrial  
326 epithelial and stromal cells can respond to LPS exposure via the TLR4- and MYD88-  
327 dependent pathways (Cronin, et al. 2012) and cows experiencing endometritis had  
328 increased endometrial expression of TLR4 and pro-inflammatory mediators in the first  
329 week post-partum (Herath, et al. 2009). TLR4 also mediates the local immune response in  
330 human (Hirata, et al. 2005, Rashidi, et al. 2015), feline (Jursza, et al. 2015) and canine  
331 (Silva, et al. 2012) endometrial cells. Recent evidence supports that metabolic stress, such  
332 as negative energy balance in lactating dairy cows, may alter the endometrial response to  
333 LPS (Sheldon, et al. 2017), a concern for animals experiencing the transition from gestation  
334 to lactation or for animals (and humans) who have metabolic perturbations.

335  
336 Bovine embryos exposed *in vitro* to both LPS and PGF2 $\alpha$  had reduced survival indicating  
337 the potential for LPS to alter pregnancy success (Soto, et al. 2003). Human trophoblast cells  
338 cultured with LPS increase pro-inflammatory macrophage production (Li, et al. 2016) and  
339 as mentioned earlier, there are fewer trophoblast cells in blastocysts that develop from LPS  
340 exposed oocytes (Magata and Shimizu 2017). Additionally, human decidual cells exposed  
341 to LPS produced TNF $\alpha$  and PGF2 $\alpha$  which negatively affected cell growth. Further, when  
342 human amniotic fluid from normal relative to pre-term labor pregnancies were compared,  
343 there were increased amounts of TNF $\alpha$  in the pre-term samples, and LPS was detectable in  
344 50% of preterm labor amniotic fluids (Casey, et al. 1989). Furthermore, as evidence that  
345 LPS can alter the maternal capacity to support pregnancy, LPS-induced changes to human  
346 and bovine endometrial epithelial cell protein abundance (which could affect implantation

347 at the critical time of maternal recognition of pregnancy) has been demonstrated (Cronin,  
348 et al. 2012, Jensen and Collins 2012)(Piras, et al. 2017).

349

350 **Additional considerations:**

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

362

363 Of additional interest and concern is that LPS causes hyperinsulinemia; either directly as an  
364 insulin secretagogue or indirectly by increasing glucose stimulated insulin secretion  
365 (Baumgard et al, 2016). Reasons why a catabolic signal like LPS increases an acutely  
366 anabolic hormone like insulin are not clear but reports suggest that insulin has potent anti-  
367 inflammatory effects (Chalmeh et al., 2013) and that immune cells are insulin sensitive  
368 (Maratou et al., 2007). Whether the ovary responds to hyperinsulinemia is unclear  
369 (Akamine, et al. 2010, Brothers, et al. 2010, Nteeba, et al. 2013, Wu, et al. 2012), however,

370 elevated insulin levels have been reported in both serum and follicular fluids of obese  
371 females (Robker, et al. 2009, Valckx, et al. 2012). Primordial follicle hyperactivation  
372 (similar to that caused by LPS exposure) has been documented in neonatal rat ovaries due  
373 to insulin administration (Kezele, et al. 2002). The negative effects of hyperinsulinemia and  
374 insulin resistance on female reproduction have been well-documented, largely as  
375 pertaining to obesity and polycystic ovary syndrome (Goodarzi, et al. 2011, Ogden 2016)  
376 and while not described herein in the interest of brevity, hyperinsulinemia could be a  
377 secondary consequence of endotoxemia with the potential to negatively influence female  
378 reproduction, though studies to specifically investigate this have not yet been performed.  
379 Hyperinsulinemia is not the sole secondary metabolic alteration observed due to  
380 endotoxemia: reduced circulating high density lipoprotein (HDL)-cholesterol was observed  
381 in dairy cows subjected to an acute exposure to LPS {de Campos, 2017 #7016} and, as  
382 discussed herein, LPS-induces an inflammatory response and inflammatory mediators  
383 could also impact reproduction as an indirect secondary consequence of elevated LPS.

384

### 385 **Conclusion**

386 In summary, endotoxemia negatively affects female fertility and fecundity and has many  
387 points of action within the reproductive tract. Endotoxemia originates from a variety of  
388 stressors and also during times of bacterial infection. Several studies investigating  
389 reproductive impacts of endotoxemia have used acute, bolus exposures, as summarized in  
390 Table 1, which may not accurately represent the temporal pattern of bacterial infection, or  
391 “leaky gut”, thus more continuous chronic low-level LPS experiments are warranted in  
392 order to identify mitigation strategies to protect and/or improve mammalian female

393 reproductive function. *In vitro* experiments also are largely reflective of acute exposures  
394 since these levels are likely to be much higher than those that occur *in vivo* or those LPS  
395 concentrations that reach the follicular fluid and/or the oocyte. Additionally, endotoxemia  
396 that results from compromised intestinal integrity is accompanied by systemic exposure to  
397 additional intestinal components, many of which have not been characterized and  
398 identified and which may also be dynamic in response to the initiating stressor. Thus  
399 greater understanding of resident microbial populations and shifts to these populations  
400 will ultimately improve our understanding of the gut-hypothalamic-pituitary-ovarian-  
401 uterine axis.

402

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

415

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421

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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.

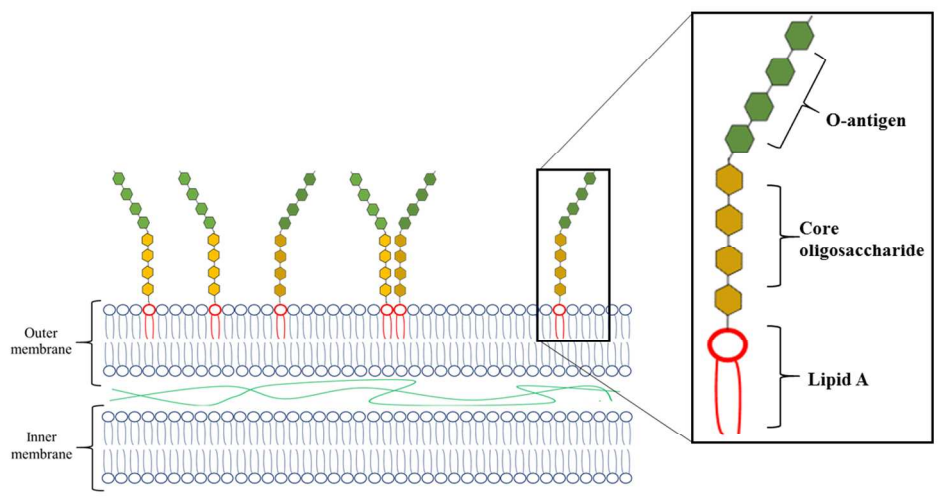


Figure 1. Structure of lipopolysaccharide (LPS). LPS is found on the cell wall of gram-negative bacteria, such as *Escherichia coli*. The lipid A region, depicted in red, elicits the immune response.

338x190mm (96 x 96 DPI)

<b>Species</b>	<b>Route</b>	<b>Duration</b>	<b>Dose</b>	<b>Citation</b>	<b>Findings</b>	
<b>Ewes</b>	intraamniotic	Single injection	0.1 - 10 mg	Newnham et al., 2005	Fetal death	
			400 ng/kg	Battaglia et al., 1997	Ovariectomized. Increased P4, Decreased LH	
	intravenous	26 h	300 ng/kg	Battaglia et al., 2000	Decreased E2 and LH	
		2x (2 week interval)	40 ng/kg	Herman et al., 2010	Decreased LH, Increased prolactin, No effect on FSH	
<b>Rats</b>	subcutaneous	Daily injections for 2 or 6 d	2 mg/kg or 20 µg/kg	Shakil et al., 1994	Decreased P4 and E2, Fewer large preovulatory follicles	
<b>Rhesus monkey</b>	intravenous	2x daily for 5 d	150 µg	Xiao et al., 1999	Decreased P4	
<b>Trout</b>	intraperitoneal	Single injection	3 mg/kg	MacKenzie et al., 2006	Induced apoptosis No effects on germinal vesicle break down	
<b>Gilts</b>	permanent cannulas	Single injection	0.5, 1, 2, 3 µg/kg	Cort, 1986	Abortions	
			0.5, 1, 2, 3 µg/kg	Cort et al., 1986	No change in cycle length. Decreased P4, increased PGF <sub>2</sub> α	
			50, 250, or 1,250 µg	Tuo et al., 1999	No effect on P4 plasma, fetal survival or development. Increased fetal weight and amniotic fluid volume	
	intrauterine		36 mg	Wrathall et al., 1978	Abortions	
	intravenous					
	mixed into ration			40 mg	Cort et al., 1990	Increased PGF <sub>2</sub> α No change in P4

<b>Goats</b>	intrauterine	injected 1 or 2x	0.1 - 5.2 µg/kg	Fredriksson et al., 1985	No hormonal changes
			0.1 - 5.2 µg/kg	Fredriksson et al., 1985	Increased PGF <sub>2</sub> α, decreased P4, abortions
	intravenous				
<b>Heifers</b>	intrauterine	every 6 h for 10 trts	5 µg/kg	Peter et al., 1990	Decreased E2 production, inhibited LH surge, no change in P4
			5 µg/kg	Peter et al., 1989	Inhibited LH surge and ovulation, caused ovarian cysts
		every 6 h for 9 d	3 µg/kg	Lüttgenau et al., 2016	Premature CL luteolysis, increased PGF <sub>2</sub> α metabolites, decreased P4, reduced luteal size and blood flow
	intrauterine or intravenous	Single injection	5 µg/kg	Gilbert et al., 1990	Increased P4, PGF metabolites, cycle length was unchanged
	intravenous	Single injection	5 µg/kg	Suzuki et al., 2001	Decreased LH pulse frequency, E2, increased LH pulse amplitude, P4. Delayed LH surge
			0.01 µg/kg	Kujjo et al., 1995	Ovariectomized. Increased P4, decreased E2 and LH
<b>Lactating Cows</b>	intrauterine	2x @ 5 and 20 DIM	5 µg/kg	Peter et al., 1990a	Increased PGF <sub>2</sub> α metabolites
	intravenous (iv) or intramuscular (im)	Single injection	0.5 ug/kg (iv) or 10 ug (im)	Lavon et al., 2008	No change in E2 yet delayed or inhibited ovulation
			200 µg	Lüttgenau et al., 2016b	No change in P4, luteal size or luteal blood flow
	intramuscular	Single injection	10 ug	Lavon et al., 2011	Decreased follicular E2, P4
<b>Non-lactating Cows</b>	intravenous	Single injection	0.5 µg/kg	Herzog et al., 2012	Decreased luteal size and luteal blood flow, Increased P4 and PGE
		6 h	1.0 or 2.5 µg/kg	Giri et al., 1990	Abortions, increased PGF <sub>2</sub> α, decreased P4
<b>Mice</b>	intraperitoneal	Single injection	10 µg	Buhimschi et al., 2003	Preterm birth, stillborns

			50 µg/mouse	Fidel et al., 1994	Preterm birth
			0.5 µg/g BW	Ogando et al., 2003	Resorptions
			100 µg/mouse	Bromfield and Sheldon, 2013	Decreased primordial follicle pool, increased follicle atresia
			1.0 µg/g	Aisemberg et al., 2013	Resorptions, decreased P4
			0.4 - 2 mg/kg	Salminen et al., 2008	Preterm birth, stillborns
			2.4 mg/kg	Rounioja et al., 2005	Fetal defects
		Single injection or multiple at 1 - 6 h intervals d 12 - 17	0-100 mg	Kaga et al., 1996	Preterm birth
		2x	10 µg/kg then 120 µg/kg	Xu et al., 2007	Pre-treatment of LPS saved embryonic resorption
	intravenous	Single injection	10 µg	Harper and Skarnes, 1972	Abortions
			7.5x10 <sup>6</sup> E.coli	Coid et al., 1978	Resorptions
			1.5 - 20 µg	Skarnes and Harper, 1972	Abortions
			2 - 5 ug	Rioux-Darrieulat et al., 1978	Abortions
			0.1 µg	Zhong et al., 2008	Abortions
	intraamniotic	Single injection	0.25 µg	Rounioja et al., 2003	Fetal defects
	intracervical	Single injection		Reznikov et al., 1999	Resorptions



	intrauterine	Single injection	250 µg	Elovitz et al., 2003	Preterm birth
	subcutaneous	Single injection	0.5 mg/kg	Chua et al., 2006	Resorptions, lower fetal weight
			0.25 mg or 0.147 mg	Coid, 1976	Resorptions, lower fetal weight

**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