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REVIEW

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# Comprehending the role of LPS in Gram-negative bacterial vaginosis: ogling into the causes of unfulfilled child-wish

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**Abstract** *Introduction:* Intrauterine infection is frequently associated with pregnancy loss in pregnant women. *Discussion:* This article reviews the role of Gram-negative bacterial infection in various complications related to early pregnancy and subsequent pregnancy loss. Here we discus the pathways of ascending intrauterine infection, microbiology and the pathophysiology of such infections. The clinical impact, therapy, consequences, prevention and implications of Gram-negative bacterial infections in women during their reproductive life span is also discussed. This article also makes an attempt to discuss our studies and findings, related to the effect of the LPS component of the Gram-negative bacterial endotoxin on preimplantation stage embryonic development and implantation. This early phase of pregnancy remains mostly unnoticed by the mother as well as the health care provider, and therefore holds more threat to the life of the fetus and the mother. The molecular mechanisms of LPS-induced pregnancy losses through abnormal embryonic development, implantation failure, and preterm labor and birth with specific references to the role of proinflammatory cytokines like IL-1 and TNF are discussed. *Conclusion:* Once these inflammatory mediators have increased in the feto-maternal tissues, it may be too late or harmful to try and prevent the adverse outcomes of pregnancy.

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## Introduction

Child wish or desire for a child is a result of individual development, which does not exist from birth and usually increases with increase with time. In the past 20 years intrauterine infections have emerged as frequently associated with infertility and unfulfilled child wish [37]. It has been estimated that at least 40% of all preterm births occur to mothers with intrauterine infection, which is subclinical in nature. The molecular pathophysiology of this pathologic process has been established in the recent years [38]. This knowledge on the molecular mechanisms of pregnancy loss induced by pathogenic infections is considered important, since, by the time a woman is admitted in spontaneous preterm labour or birth, there may be irreversible changes in the uterine cervix, which renders futile the attempts to abrogate the process. It was found that the earlier in gestation at which pathogenic genital tract colonization is detected, the greater is the risk of an adverse outcome. Bacterial vaginosis is a polymicrobial condition associated with a number of adverse sequelae in both obstetrics and gynecology [52]. The presence of enteropharyngeal organisms, such as *Esterichia coli* or *Enterococus faecalis* are some times considered to be a part of the normal vaginal flora, but are sometimes considered pathological. However, bacterial vaginosis-related organisms such as anaerobes *Gardnerella vaginallis* or *Mycoplasma hominis* may be considered indicative of abnormal genital tract flora. Most of these microbes associated with bacterial vaginosis are Gram-negative organisms.

Beside genital tract infections systemic maternal infections of Gram-negative bacteria such as typhoid fever etc., has been associated with preterm labour and delivery. All Gram-negative bacteria possess endotoxins in the cell walls, which are a group of heat stable lipopolysaccharide

(LPS) molecules [13]. These toxins are constantly shed into the environment of the bacterium much like the daily shedding of the superficial layers of the human skin. Also, when bacterium lyse or disintegrate endotoxins are released from these organisms. Once free to act, endotoxins recruit particular host cells to secrete mediator molecules that act locally or float through the blood or both to elicit a wide variety of responses. In higher doses endotoxins have the capacity to activate the coagulation system, alter carbohydrate and lipid metabolism, modify hemodynamics, cause platelet aggregation, induces disseminated intravascular coagulation leading to shock and ultimately death [49].

In this review we describe how Gram-negative bacterial endotoxins have been found to extensively damage preimplantation stage embryos [27]. Endotoxins are known to decrease embryo cleavage rate, blastocyst formation and pregnancy rates in human. Infections, at this early phase of pregnancy are silent and remain mostly unnoticed by the mother as well as the health care provider, and therefore holds more risk to the life of the fetus and the mother. The presence of endotoxins during IVF of oocytes results in a high rate of polyspermy in human and bovine species [7]. Microorganisms such as *E. coli*, isolated from the uterine cervix, decrease sperm motility and possess spermicidal activity [24]. As a step towards understanding how endotoxins might achieve diverse pathological effects, researchers have concentrated on elucidating their chemical and three-dimensional structure [71].

Biological effects of LPS are mostly mediated by the proinflammatory cytokines like IL-1, IL-6, TNF, etc. Several of these cytokines have been implicated in maintenance of the delicate immune system balances that exist within the feto-maternal interface [46]. The presence of cytokines and growth factors in the endometrium and or embryos and the expression of their corresponding receptors on the implanting blastocyst and or endometrium, suggest that they play important role in the process of development and blastocyst invasion into the endometrium. Any disturbance of this delicate immune balance within the maternal-fetal tissues may result in pregnancy loss or other perinatal complications. It is likely that LPS brings about its antifertility effects by modulating the synthesis and secretion of the cytokines and growth factors from the lymphohaematopoetic and uterine cells [22]. These cytokines in turn could alter the embryonic expression of growth factors leading to preimplantation embryonic loss, implantation failure, or abortions. In this article, we have made an attempt to elucidate a probable mechanism of LPS-induced infertility, primarily focusing on the cytokine based molecular events involved in sustaining pregnancy and embryonic development. The understanding of these mechanisms are important for developing new therapeutic interventions to fulfill the wish for a child, which is a yet-not-existing third person, who cannot be included in the decision making processes and into the treatment of infertility.

## Overview of endotoxin structure and pharmacology

Lipopolysaccharides (LPS) are toxins associated with the cell walls of the Gram-negative bacteria and consists of mainly carbodydrates and lipids. The LPS molecule has two chemically dissimilar structural regions: the hydrophilic repeating polysaccharides of the core and O-antigenic structure and a hydrophobic domain known as Lipid-A [68]. The O-Specific chain of repeating polysaccharides show some structural diversity among the different Gram-negative bacteria. However, the structure of the core region is substantially conserved. Most of the LPS-induced biological responses are Lipid-A dependent and thus the recognition of the Lipid-A component of LPS by cells is the initial step in LPS-induced immune responses [16, 71].

Lipopolysaccharide is released when Gram-negative bacteria are ingested by phagocytes and degraded in vacuoles. When LPS enters the circulation, it is bound either to lipoproteins or LPS-binding proteins (LBP), which may stimulate macrophages by binding to a glycoprotein receptor (mCD14) present on the cell surface [77]. CD14 can also exist in a soluble form in the circulation (sCD14) [90] which can facilitate LBP mediated LPS transfer to high density lipoproteins (HDL) [97] and enable cells which do not express mCD14 to respond to low doses of LPS [32]. An understanding of the mechanism of action of LPS was greatly enhanced by the identification of Toll-like receptor (TLR) family, which is a family of innate immune signaling receptors. Recent discoveries suggest that CD14 interacts with toll-like receptors to transmit a signal [17, 50].

Activation of $TLR_4$, a type of toll like receptor on the cell surface which requires cooperation with at least CD14 and $MD_2$ [62] leads to the recruitment of adopter proteins (MyD88, IRAK, TRAF6). This recruitment ultimately couples the recognition of LPS to activation of the transcription factor complex NF-κB [1]. Translocation of this transcription factor complex in turn contributes to activation of the promoters of many inducible genes like that of the proinflammatory cytokines in macrophages (Fig. 1) [88]. These cytokines modulate the expression of cell surface adhesion proteins such as selectin and integrins (CD11/CD18) and their corresponding ligands (ICAM-1) on endothelial cells [94]. This initiates the recruitment of leukocytes at the site of infection. Many of these inducible genes like TNF, IL-1, IL-12, IL-6, M-CSF etc., not only effect the preimplantation embryonic growth and development, but also regulate the delicate inflammatory reactions at the site of implantation of the blastocyst. Any disturbance in the level of expression of these cytokines at the feto-maternal interface can lead to embryonic or pregnancy loss. Once these inflammatory mediators have increased in the feto-maternal tissues, it may be too late or harmful to try and prevent the adverse outcomes of pregnancy. Therefore, it is very important to detect the abnormal genital tract flora at the earliest during

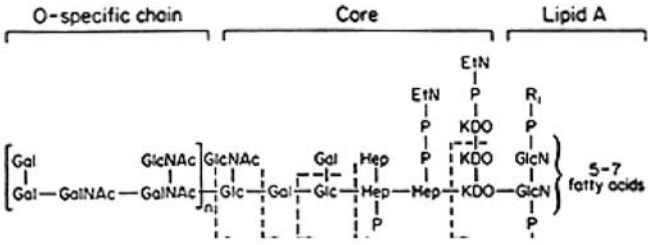


**Fig. 1** General structure of *Salmonella* LPS. *Glc* glucose, *GlcNac* N-acetyl-glucosamine, *Gal* galactose, *Hep* heptose, *P* phosphate, *Etn* ethanolamine

pregnancy, the preventive measures and antibiotics should be used as early as possible to prevent pregnancy loss.

## The vaginal microbial ecosystem: normal flora of the urinogenital tract

Bacterial vaginosis is a polymicrobial condition. Soon after birth the vagina gets colonized with *corynebacteria*, *staphylococci*, nonpyrogenic *streptococci*, *E. coli*, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*). During the woman's reproductive life span, from puberty to menopause, the vaginal epithelium contains glycogen due to the actions of circulating estrogens. Being able to metabolize the glycogen to lactic acid, Doderlein's bacillus predominates in this environment. The lactic acid and other products of metabolism inhibit colonization of all except Doderlein's bacillus and a selected number of lactic acid bacteria. The resulting low pH (which is usually below 4.5) of the vaginal epithelium discourages growth of most of the bacteria and also the potentially pathogenic yeast, *Candida albicans*. In an in vitro study it was demonstrated that Lactobacillus strains taken from normal vaginal flora show antagonistic activity against a variety of bacteria related to vaginal and urinary tract infections [87]. Under conditions of low pH, lactobacilli produce hydrogen peroxide ($H_2O_2$) which is toxic to bacteria, firstly by production of toxic hydroxic radicals and secondly by combining with the heavy pool of chlorine ions in the vagina to produce chloridinium ions. Thus the normal bacterial flora has a protective effect on their human host. Under increased alkaline conditions during vaginal douching, sexual intercourse or bleeding in pregnancy, lactobacilli are less efficient in producing $H_2O_2$ permitting the overgrowth of other microbes.

## Pathogenic flora of genital tract infections

Genital tract infection/vaginitis is one of the major causes of gynecologic morbidity, such as infertility, ectopic pregnancy, preterm labor and chronic pelvic pain [19]. These infections affect primarily the cervix, uterus, or fallopian tubes and during severe infections one or both ovaries may also get affected. The normal urinary tract is sterile and very resistant to bacterial colonization. However, urinary tract infection (UTI) in human is the most common bacterial infection in all age groups. Gram-negative aerobic bacteria (Table 1) cause most bacterial UTIs. A few UTIs are acquired hematogenously but about 95% occur when bacteria ascend from a colonized vaginal introitus and urethra to the bladder and in the case of acute uncomplicated pyelonephritis, up the ureter to the kidney. *Escherichia coli* is the most common bacterium isolated and accounts for about 80% of community-acquired infections, and *Staphylococcus saprophyticus* for about 10%. UTI, asymptomatic bacteriuria, and pyelonephritis are associated with an increased incidence of preterm labor and premature rupture of the membranes In hospitalized patients, *E. coli* accounts for about 50% of cases; the Gram-negative species *Klebsiella*, *Proteus*, *Enterobacter*, and *Serratia* for about 40%; and the Gram-positive bacterial cocci *Enterococcus faecalis* and *Staphylococcus sp* (*saprophyticus, aureus*) for the remainder.

**Table 1** Normal vaginal flora

| Type of bacteria | Name of the organism |
|---|---|
| Gram-negative aerobic | *Escherichia coli* |
| | *Proteus vulgaris* |
| | *Klebsiella* |
| | *Enterobacter* |
| Gram-negative anaerobes | *Bacteroides fragilis* |
| | *Bacteroides urolyticus* |
| | *Pervotella* |
| | *Mombiluncus spp.* |

Colonization of the vagina by *Lactobacillus sp* keeps the pH normal (3.8 to 4.2), preventing the overgrowth of bacteria and yeast [25]. Menstrual blood, certain infections, or semen often makes the vaginal pH alkaline. Among nuns bacteremia is significantly less frequent (0.4 to 1.6% from 15 to 54 years of age) than among sexually active women, suggesting a probable role for sexual intercourse in the development of acute uncomplicated UTI in women. Use of spermicide with a diaphragm is linked to an increased risk of UTI in women probably because spermicide-induced alterations in vaginal flora permit overgrowth of *E. coli*. Silent (subclinical) microbial infection in the genitourinary tract leads to preimplantation embryonic loss and is one of the major causes of reduced reproductive efficiency in most mammals. Various (aerobic and anaerobic) microorganisms (Table 1) found in the vagina have been associated with increased risk for early pregnancy loss [26] and or preterm labour but no single organism could be identified as having a causal relationship [35]. In the case of Gram-negative bacterial infections, fetal loss may result in part from host immune responses to the main antigenic component of the bacterial cell wall, endotoxin/lipopolysaccharide (LPS) [78].

## Vaginitis

Vaginitis is characterized by vaginal discharge or vulvar itching and irritation with a vaginal odor. The following three diseases most frequently associated with vaginal discharge:

1. Trichomoniasis: caused by *Tricomoniasis vaginalis*, a protozoa
2. Bacterial vaginosis (BV): caused by a replacement of the normal vaginal flora by an overgrowth of anaerobic Gram-negative microorganisms like *Gardnerella vaginalis*; and some Gram positive facultative anaerobe like *Mycoplasma hominis*
3. Candidiasis: usually caused by *Candida albicans*, a yeast

Other common causes of vaginal infections include such bacteria as *N. gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, streptococci, and staphylococci; foreign bodies; some viruses (e.g. herpes simplex); fistulas; radiation and genital tract neoplasms. BV represents 60% of all vaginal infections. Women with bacterial vaginosis also have more chance of getting pelvic inflammatory diseases (PID). PID can damage reproductive organs, cause infertility, pelvic pain and tubal pregnancy.

## Bacterial vaginosis

Bacterial vaginosis (BV) is the most common vaginal infection in women who have not gone through menopause. At least one-third of all vaginal infections are due to bacterial vaginosis.

Bacterial vaginosis was previously called nonspecific vaginitis, Haemophilus vaginitis, or Gardnerella vaginitis. Vaginitis is an inflammation of the vagina and vulva that usually causes itching, pain, and vaginal discharge. Bacterial vaginosis is not usually accompanied by inflammation. Nearly half of all women with BV have no symptoms or irritating or itchy vaginal discharge. The most distinctive symptom of BV, which helps distinguish it from vaginitis, is a thick, milky vaginal discharge with a strong fishy odor.

Bacterial vaginosis is an imbalance among the microorganisms that normally exist in the vagina. Those involved in BV include Gram-negative facultative anaerobes like *Gardnerella vaginalis*, *Chlamydia trachomatis* and other types of bacteria (*Mobiluncus*, *Bacteroides*, and *Mycoplasma*). For reasons not well understood, the numbers of these organisms increase with BV while the number of lactobacillus organisms decreases.

Most cases of bacterial vaginosis occur in sexually active women between the ages of 15 and 44. Pregnant women and women with a sexually transmitted disease (STD) are especially at risk for getting this infection. Bacterial vaginosis does not usually affect women after menopause. Pregnant women with BV are at risk for developing an infection of the amniotic fluid and placenta and are more likely to have a preterm labor and birth [40].

Bacterial vaginosis is, therefore, a change in the normal vaginal microbial ecosystem (Table 1) with three important features:

1. An overgrowth of anaerobic bacteria and the facultatives, *Gardnerella vaginalis* and *Mycoplasma hominis* (Table 2). These bacteria are frequently present in normal vaginal flora but quantitative culture demonstrates one to two log increases in BV. Anaerobes commonly identified in BV include the Gram-negative rods *Prevotella spp*. (especially *P.bivia*), *Porphyromonas* and *Bacteroides ureolyticus*, the curved *Mobiluncus spp*. and Gram-positive *Peptostreptococci*. Several observations demonstrate interdependence between these organisms [67]. For example treating BV with metronidazole frequently leads to the disappearance of *M. hominis* despite its resistance to metronidazole, suggesting it requires the presence of anaerobes. In vitro experiments demonstrate nutritional relationships between *Prevotella bivia* and other organisms. *P. bivia* produces ammonia which stimulates the growth of *G. vaginalis*, and amino acids which similarly promote growth of *Peptostreptococcus* and probably *G. vaginalis*
2. Biochemical changes [67]: the normal pH of 3.8 to 4.2 (acidic) is maintained by the *Lactobacillus sp*. in the vaginal flora. In bacterial vaginosis the vaginal pH becomes more than 4.5 (i.e. loss of normal acidity). Production of amines by the anaerobes (trimethylamine, putrescine, cadaverine) gives a fishy smell to the discharge, which is often a source of complaint. The succinate:lactate ratio increases, as the succinate-producing anaerobic Gram-negative rods outnumber lactobacilli. A number of enzymes, including sialidases and proteases, are produced which may interfere with vaginal defences against pathogens (discussed

**Table 2** Microbes associated with genitourinary tract infections and infertility

| Type of bacteria | Name of the organism |
|---|---|
| Gram-negative aerobic | *Escherichia coli* |
| | *Proteus vulgaris* |
| | *Klebsiella* |
| | *Acinetobacter calcoaceitus* |
| | *Enterobacter* |
| | *Pseudomonas aeruginesa* |
| | *Serratia* |
| | *Neisseria gonorrhoeae* |
| Gram-negative anaerobes | *Bacteroides fragilis* |
| | *Bacteroides urolyticus* |
| | *Pervotella* |
| | *Porphyromonas* |
| | *Mombiluncus spp.* |
| | *Chlamydia trachomatis* |
| | *Gardnerella vaginalis* |

below). The amines and proteases may act as tissue irritants, causing sloughing of epithelial cells and transudation, leading to discharge
3. Altered/absent lactobacilli: lactobacilli produce lactic acid, and many isolates produce hydrogen peroxide, both thought to inhibit the growth of some pathogens [41]. BV is most common in women without lactobacilli. Moreover, among those women with lactobacilli, it is more common in those whose lactobacilli produce no hydrogen peroxide. However, lactobacilli are frequently present in BV, but are greatly outnumbered by the overgrowth of BV organisms. Lactobacilli numbers may not be reduced [42], however, there is an inverse relationship between the presence of hydrogen peroxide-producers and BV

## Pathways and stages of ascending genital tract infections

Pathogenic microorganisms may gain access to the amniotic cavity and the fetus through many pathways which includes:

1. Ascending of the microbes from the vagina and cervix
2. Transplacental infection or haematogenous dissemination through the placenta
3. Retrograde seeding from the peritoneal cavity through the fallopian tubes
4. Accidental introduction at the time of invasive procedures, such as amniocentesis, percutaneous fetal blood sampling etc. [5, 75]

However, the most common pathway of intrauterine infection is the ascending route. Bacteria identified in cases of many congenital infections are found to be similar to that in the lower genital tract [5]. The ascending intrauterine infections progress through four stages [75]. Stage I is characterized by a change in the natural vaginal microbial flora or by the presence of pathogenic organisms. Stage II consist of the state when microorganisms gain access to the intrauterine cavity and start residing in the deciduas, causing a inflammatory reaction leading to deciduitis. In stage III the infection may invade the fetal vessels, or proceed through the amnion into the amniotic cavity (intra-amniotic infections). Once the infection reaches the amniotic cavity, the bacteria may gain access to the developing fetus through various modes like, the aspiration of the infected fluid by the fetus may lead to congenital pneumonia, and such a condition is considered as the Stage IV of infection. This condition might ultimately result in fetal bacteraemia and sepsis.

## Complications due to bacterial vaginosis

Complications due to bacterial vaginosis include:

1. Post-partum endometritis (particularly following Cesareans)
2. Post-hysterectomy vaginal-cuff cellulitis
3. PID following termination of pregnancy and there is weaker evidence linking BV with non-surgical PID

Bacterial vaginosis in pregnancy is strongly associated with:

1. Chorioamnionitis and amniotic fluid infection
2. Increased fetal loss at all stages of pregnancy—Ralph et al. [69] have confirmed this for first trimester miscarriage by studying women having IVF: those with BV had the same conception rate but roughly double the relative risk of miscarriage compared to those with normal flora:

1. Premature rupture of membranes
2. Low birth weight
3. Preterm birth—a body data demonstrate that the risk is higher if BV is diagnosed before 20 weeks' gestation, or even earlier

## Diagnosis for bacterial vaginosis

Bacterial vaginosis can be distinguished from vaginitis by the following methods:

1. Wet mount: a sample of vaginal discharge is mixed with a salt solution after placing it on a microscope slide. The prepared slide is examined to identify the bacteria present, to look for white blood cells that indicate an infection, and to look for unusual cells called clue cells. Clue cells are cells covered with Gardnerella bacteria. The bacteria give clue cells a characteristic granular look. Clue cells can flake off of the walls of the vagina and may be found in a vaginal smear. Chemicals released by the bacteria that cause bacterial vaginosis (BV) may damage vaginal wall cells, causing them to be flake off in greater numbers than usual. The presence of clue cells is the most reliable indicator of BV [61]
2. Whiff test: a strong fishy odor is produced after addition of several drops of a potassium hydroxide (KOH) solution to a sample of vaginal discharge. A fishy odor on the whiff test suggests BV
3. Vaginal pH: the normal vaginal pH is between 3.8 to 4.5. Bacterial vaginosis often raises the vaginal pH above 4.5
4. Gram stain: a sample of the vaginal discharge is placed on a microscope slide. A special dye (Crystal violet and Iodine as moderant) is applied to the slide, causing certain types of bacteria ("Gram-positive" bacteria) to turn a shade of purple while coloring others ("Gram-negative" bacteria) pink by counter staining with Saffranin. In bacterial vaginosis, Gram-negative bacteria, especially Gardnerella vaginalis, are most common
5. Oligonucleotide probes: this test detects the genetic material (DNA) of BV bacteria. Oligonucleotide probe

testing is very accurate but is not routinely available in most labs

6. Pap test: the Pap test (Papanicolaou test or Pap smear) is used to screen women for cancerous and precancerous cells of the opening of the uterus (cervix). The Pap test is very reliable for detecting early abnormal cell changes that could lead to cervical cancer. A Pap test may also detect infection (such as yeast infection), but this is not the main purpose of the test. During a Pap test, a small sample of cells from the surface of the cervix is collected by a health professional. The sample is then spread on a slide and sent to a lab for examination under a microscope. The cells are examined for abnormalities that may indicate cancer or changes that could lead to cancer. Any abnormal cells are classified according to their "degree" of abnormality. BV may be detected during routine Pap testing. However, Pap testing is not a standard test to diagnose BV
7. Culture: a culture of vaginal discharge may show heavy growth of *Gardnerella vaginalis*, which can be associated with BV [57].

The presence of clue cells, an increased vaginal pH, and a positive whiff test usually are enough evidence to treat for bacterial vaginosis [59]. The diagnosis can even be made without inserting a speculum, as there is good agreement between diagnoses made from wet preps from blindly-inserted swabs and those made with the aid of a speculum [11].

## Treatment for bacterial vaginosis

The principal goal of therapy for BV is to relieve vaginal symptoms and signs of infection. All women who have symptomatic disease require treatment, regardless of pregnancy status. BV during pregnancy is associated with adverse pregnancy outcomes. The results of several investigations indicate that treatment of pregnant women who have BV and who are at high risk for preterm delivery (i.e. those who previously delivered a premature infant) might reduce the risk for prematurity [12]. Therefore, high-risk pregnant women who do not have symptoms of BV may be evaluated for treatment.

Although some experts recommend treatment for high-risk pregnant women who have asymptomatic BV, others believe more information is needed before such a recommendation is made. The bacterial flora that characterizes BV has been recovered from the endometria and salpinges of women who have pelvic inflammatory diseases (PID). BV has been associated with endometritis, PID and vaginal cuff cellulitis after invasive procedures such as endometrial biopsy, hysterectomy, hysterosalpingography, placement of an intrauterine device, cesarean section, and uterine curettage. The results of one randomized controlled trial indicated that treatment of BV with metronidazole substantially reduced postabortion PID. On the basis of these data, consideration should be given to treatment of women who have symptomatic or asymptomatic BV before surgical abortion procedures are performed. However, more information is needed before recommending whether patients who have asymptomatic BV should be treated before other invasive procedures are performed.

## Recommended regimens for nonpregnant women

Metronidazole 500 mg orally twice a day for 7 days, or clindamycin cream 2%, one full applicator (5 g) intravaginally at bedtime for 7 days, or metronidazole gel 0.75%, one full applicator (5 g) intravaginally twice a day for 5 days.

Note: patients should be advised to avoid consuming alcohol during treatment with metronidazole and for 24 h thereafter. Clindamycin cream is oil-based and might weaken latex condoms and diaphragms.

## Alternative regimens

Metronidazole 2 g orally in a single dose, *or* clindamycin 300 mg orally twice a day for 7 days.

Metronidazole 2-g single-dose therapy is an alternative regimen because of its lower efficacy for BV. Oral metronidazole (500 mg twice a day) is efficacious for the treatment of BV, resulting in relief of symptoms and improvement in clinical course and flora disturbances. Based on efficacy data from four randomized controlled trials, overall cure rates 4 weeks after completion of treatment did not differ significantly between the 7-day regimen of oral metronidazole and the clindamycin vaginal cream (78% vs. 82%, respectively). Similarly, the results of another randomized controlled trial indicated that cure rates 7–10 days after completion of treatment did not differ significantly between the 7-day regimen of oral metronidazole and the metronidazole vaginal gel (84% vs. 75%, respectively). FDA has approved flagyl ER (750 mg) once daily for 7 days for treatment of BV.

Some health-care providers remain concerned about the possible teratogenicity of metronidazole, which has been suggested by experiments using extremely high and prolonged doses in animals. However, a recent meta-analysis does not indicate teratogenicity in humans. Some health-care providers prefer the intravaginal route because of a lack of systemic side effects (e.g. mild-to-moderate gastrointestinal disturbance and unpleasant taste). Mean peak serum concentrations of metronidazole after intravaginal administration are less than 2% the levels of standard 500 mg oral doses and the mean bioavailability of clindamycin cream is approximately 4%.

## Follow-up

Follow-up visits are unnecessary if symptoms resolve. Recurrence of BV is not unusual. Because treatment of BV

in high-risk pregnant women who are asymptomatic might prevent adverse pregnancy outcomes, a follow-up evaluation, at 1 month after completion of treatment, should be considered to evaluate whether therapy was successful. The alternative BV treatment regimens may be used to treat recurrent disease. No long-term maintenance regimen with any therapeutic agent is recommended.

## Management of sex partners

The results of clinical trials indicate that a woman's response to therapy and the likelihood of relapse or recurrence are not affected by treatment of her sex partner (s). Therefore, routine treatment of sex partners is not recommended.

## Special considerations

Allergy or intolerance to the recommended therapy

Clindamycin cream is preferred in case of allergy or intolerance to metronidazole. Metronidazole gel can be considered for patients who do not tolerate systemic metronidazole but patients allergic to oral metronidazole should not be administered metronidazole vaginally.

Treatment during pregnancy

Bacterial vaginosis has been associated with adverse pregnancy outcomes (e.g. premature rupture of the membranes, preterm labor, and preterm birth) and the organisms found in increased concentration in BV also are frequently present in postpartum or postcesarean endometritis. Because treatment of BV in high-risk pregnant women (i.e. those who have previously delivered a premature infant) who are asymptomatic might reduce preterm delivery, such women may be screened and those with BV can be treated [53]. The screening and treatment should be conducted at the earliest part of the second trimester of pregnancy. The recommended regimen is metronidazole 250 mg orally three times a day for 7 days [14]. The alternative regimens are:

1. Metronidazole 2 g orally in a single dose
2. Clindamycin 300 mg orally twice a day for 7 days

Low-risk pregnant women (i.e. women who previously have not had a premature delivery) who have symptomatic BV should be treated to relieve symptoms. The recommended regimen is metronidazole 250 mg orally three times a day for 7 days. The alternative regimens are:

1. Metronidazole 2 g orally in a single dose
2. Clindamycin 300 mg orally twice a day for 7 days
3. Metronidazole gel, 0.75%, one full applicator (5 g) intravaginally, twice a day for 5 days

Some experts prefer the use of systemic therapy for low-risk pregnant women to treat possible subclinical upper genital tract infections.

The lower doses of medication are recommended for pregnant women to minimize exposure to the developing fetus. Data are limited concerning the use of metronidazole vaginal gel during pregnancy. The use of clindamycin vaginal cream during pregnancy is not recommended because two randomized trials indicated an increase in the number of preterm deliveries among pregnant women who were treated with this medication.

## Effect of Gram-negative bacterial LPS on pregnancy

Pregnancy is a delicate condition where the receipt and tolerance of the allogenic fetus, must occur for a successful outcome. Any disturbance of the delicate immune balance at the interface of the mother and fetus may result in pregnancy loss or other perinatal complications. Dumoulin et al. [28] extensively studied the effect of Gram-negative bacterial endotoxin on gamet function, fertilization and embryo development. The presence of endotoxins during in vitro fertilization (IVF) results in a high rate of polyspermy in humans and bovine species [7, 24]. Such polyspermic fertilizations lead to chromosomal aneuploidy resulting in embryonic loss. Endotoxin-induced embryonic death, abortion, preterm labor and delivery and stillbirth have been reported in a number of species [29, 31, 36, 79]. Endotoxins decrease embryo cleavage rate, blastocyst formation and pregnancy rates in human [28]. Local and systemic presence of LPS also induces degeneration, fragmentation and developmental arrest of the preimplantation embryo, and is one of the causes of pregnancy loss and infertility in most mammals [27].

A large number of in vivo and in vitro studies suggests that many of the biological effects of LPS are indirectly mediated by the pro-inflammatory cytokines like, IL-6, interleukin-1alpha (IL-1$\alpha$), interleukin-1beta (IL-1$\beta$), tumor necrosis factor alpha (TNF-$\alpha$) etc. TNF and LPS show synergistic detrimental effects on the growth of preimplantation embryos in vitro [70]. High levels of TNF-$\alpha$ and other cytokines are known to appear in the amniotic fluid of LPS treated mice suggesting their probable role in abortions or fetal deaths [98]. These proinflammatory cytokines are produced primarily by activated monocytes and a variety of other cell types including gestational tissues and embryos. Romero et al. [73] reported that a high level of IL-6 concentration was present in the amniotic fluid of patients with pregnancy loss. Collectively these evidences indicate that a chronic intra-amniotic inflammatory process is associated with both spontaneous abortion and preterm delivery.

## Molecular mechanisms of LPS-induced embryonic loss and implantation failure

Studies show that lymphocytes and macrophages are present in the uterine endometrium throughout the pregnancy [58] and that they are present in a higher number in the case of uterine infections [51]. It is also known that uterine leukocytosis and leukocyte products (like cytokines) are toxic to the preimplantation embryos. Many of the cytokines and growth factors like IL-1, IL-6 TNF, CSF-1, TGF-β1 and LIF exist in both soluble and membrane associated or matrix bound forms, and are expressed in the uterus during pregnancy [20]. Receptors for many of these growth factors (like IL-1, TNF, CSF-1, PDGF, EGF etc.) are expressed on preimplantation embryos and are mostly transmembrane tyrosine kinases that are activated by ligand binding [33]. Presence of ligands for these receptors in the uterine environment suggests that signal transduction cascades involving complex phosphorylation patters might be activated in preimplantation embryos leading to proliferation and differentiation. Moreover, many of these cytokines like IL-1α, IL-1β, PDGF-A, TGF, TNF, CSF-1 etc. are expressed in a developmentally regulated manner in the preimplantation stage blastocyst [9, 21, 34]. This may be a general mechanism for the regulation of early embryonic development and for maintaining a synchrony between the embryo and uterine preparation for implantation.

Endotoxins activate the macrophages residing in the female reproductive tract, which leads to the production of several cytokines which target the embryos, and can adversely effect early embryonic development [2, 44] trophoblast proliferation, and fetal growth during later stages of gestation. High levels of interleukin-1 (IL-1α and IL-1β) and TNF-α are linked to fetal deaths [84, 85]. Colony stimulating factor-1 (CSF-1/ M-CSF) in the amniotic fluid is linked to positive fetal growth [99], however, Goldenberg et al. [37] demonstrated that high levels of G-CSF are associated with early preterm birth.

Morphological studies of the preimplantation embryos recovered from LPS treated pregnant mice, show developmental arrest, degeneration, and fragmentation of the blastocysts [45]. Expression studies carried out in our laboratory suggests that the pattern and level of expression of proinflammatory cytokines like IL-1α, IL-1β, TNF-α, and CSF-1 at various developmental stages of the mouse preimplantation embryos, get altered dramatically in presence of LPS in vivo (unpublished observations). We have seen that mRNA expression for IL-1α was found from day 1 to day 4.125 of pregnancy in the preimplantation mouse embryos, whereas, IL-1β expression started from day 4 and continued till implantation. Positive mRNA signals for TNF-α were found from day 4 of pregnancy and continued till implantation, and CSF-1 mRNA was found in the two-cell stage to the morula stage embryos [21]. However, after administration of LPS we observed an early expression of IL-1β and TNF-α mRNAs (i.e. from day 1.5 of pregnancy onwards) and IL-1α was found from day 1.5 of pregnancy and continued till implantation (unpublished observations). IL-1α is known to be responsible for the regulation of the various matrix metaloprotinases involved in tissue remodeling during the implantation event [81]. An observed change in the expression of this cytokine could, therefore, be responsible for implantation failure. The observed asynchronous cleavage and degeneration of the preimplantation embryos obtained from animals treated with LPS could be due to apoptosis triggered by the early expressions of TNF-α and IL-1β. This is further supported by the fact that, only the TNF-RI/p60 form of the TNF receptor is found in the mouse blastocyst, which has the death domain for triggering apoptotic pathway [64].

Liposaccharide has been known to inhibit steroidogenesis in steroidogenic tissues [92]. Also secretion of many of the cytokines from the uterus is under the control of ovarian steroids, confirming their close association with a distinct regulatory network. LPS is known to activate the macrophages and/or the corpora luteal cells in the corpus luteum of the ovaries [91], which produce a number of cytotoxic proinflammatory cytokines like TNF, IL-1, IL-6 etc. [3, 39]. These cytokines, exert autocrine and paracrine effects causing regression of the ovary, resulting in reduced steroidogenesis [4]. During pelvic bacterial infections IL-6 is expressed by the luteal cells of the corpora lutea, which may account for pregnancy loss, through the inhibition of progesterone production [93]. Reduction in the plasma progesterone (P): estradiol 17β (E) ratio decreases the number of normal blastocysts formed in many species. It has been shown that high levels of circulating estrogen can also inhibit normal embryonic development [18]. High level of progesterone in the circulation, during pregnancy has been shown to have an immunosuppressive effect, which suppresses the production of IL-1, IL-6 etc. [48]. Thus the modulation in the progesterone/estrogen ratio induced by LPS, could lead to early embryonic losses/abortions [54]. Thus in woman with immunologic reproductive failure the embryo may be killed as an innocent spectator to leukocyte activation in response to bacterial antigens.

The uterine endometrium is under the control of steroid hormones, particularly progesterone and thus control of cytokines by this steroid is very important [48]. The uterus becomes “receptive” on day 4 and is defined as the limited time ‘window’ when the uterine environment is conducive to blastocyst acceptance and implantation [65, 66]. In the absence of blastocyst the receptive uterus enters into the non-receptive phase. Although significant numbers of progesterone receptors are not found in endometrial leucocytes, however, it can modulate the expression of cytokines by acting on the uterine cells expressing its receptor. It has been reported that failure of implantation after IVF is sometimes associated with elevated estradiol: progesterone ratios. A disturbance in estrogen: progesterone ratio creates an uterine luminal milieu that suppresses embryonic metabolism [76]. It is supposed that effects could be reversed and implantation re-established by the correction of this ratio by exogenous progesterone. Studies

show that exogeneous progesterone administration could improve fertility rates in LPS treated animals.

Molecules like the cell adhesion molecules, cytokines and growth factors like EGF, TGF, TNF, IL-1, CSF-1,GM-CSF, PDGF-A etc. and their receptors [46], endometrial epithelial $\beta_3$ integrins, matrix metalloproteinases [10], prostaglandins and the inducible prostaglandin synthesis enzyme COX-2 are important for successful implantation [15]. They play a primary role in decidual tissue remodeling and regulation of trophoblast invasion. Expression of these molecules in the trophoblast and the uterine cells are strongly regulated by physiologically relevant cytokines and growth factors (e.g. IGF, TGF, IL-1, IL-6, TNF, etc.).

Recent studies in our laboratory suggest that the expression of IL-1α, IL-1β, TNF-α and CSF-1, in the uterus of pregnant mouse treated with LPS increased as compared to that of the control (unpublished data). Histopathological studies of the female reproductive organs in LPS treated mouse show marked morphological and physiological alterations in the uterus, ovaries and oviducts as compared to that of the normal. Degeneration of the luminal and glandular epithelium, reduction in the number of glands and heavy infiltration of macrophages in the stroma and hyperplasia were observed in the uterus of LPS-treated animals. The corpus luteum showed signs of regression in response to LPS and macrophage infiltration [45]. Therefore, it has been proposed that LPS-induced histopathological alterations mediated by elevated levels of proinflammatory cytokines, in the various reproductive organs of the pregnant mice would be one of the causes of embryonic loss during early stages of pregnancy in mouse.

Embryonic IL-1α and β, and their receptors on the uterine endometrium, are know to trigger the $\beta_3$ integrin expression in the uterine endometrium during implantation [86]. Endotoxin-induced changes in the levels of these implantation relevant cytokines can lead to implantation failure. TNF-α has been show to regulate the window of implantation in human [89]. The stimulation of TNF-α in response to endotoxins is well established and therefore high levels of TNF-α may possibly disturb the implantation "window", leading to infertility.

Liposaccharide strongly induces NF-κB activation and its activity can be inhibited by progesterone by either stimulating I kappa B; the molecule that sequester in the cytosol, or after binding to the nuclear receptor, competing with NF-κB for recognition sites on the relevant genes [48]. The NF kappa B (NF-κB) pathway is important in the regulation of cytokine synthesis and can modulate the production of chemokines, matrix metallopoteinases and COX-2. Therefore, LPS-induced infertility appears to operate mainly through a NF-κB mediated pathway. However, NF-κB is not the only transcription factor involved in the intracellular signaling events induced by LPS or the cytokines. It has been observed that TNF-α and IL-1 activate NF-κB whereas others like IFN-α, EGF and CSF-1 activate p91 (a subunit of interferon-stimulated gene factor-3) through its phosphorylation directly by a receptor-associated tyrosine kinase (JAK-STAT pathway). These observations indicate that several pathways exist for transduction of signals to the nucleus induced by the different cytokines and LPS, which may render the uterus non-receptive to the implanting embryo.

## Liposaccharide-induced preterm birth: molecular players

About 10–30% of woman with preterm labor have clinically evident or subclinical intrauterine infections. Mechanisms by which endotoxins cause abortions are recently being understood and appear to differ between groups of animals. In the goat [29], pig [100], horse [31], and cow [30, 36] pregnancy loss is attributed to altered maternal endocrine function, most notably, regression of the corpus luteum followed by abortion or onset of preterm labor. Abortion in pregnant rodents following endotoxin administration is accompanied by severe damage to the placenta [82] and endometrium [80], however, in other species no recognized lesions accompany abortion or preterm delivery has been observed. In sheep the duration of pregnancy is determined by the activity of the fetal hypothalamo-hypophyseal-adrenal-axis (HHAA) [55]. LPS-infused pregnant sheep experienced marked stimulation of HHAA as evidenced by the increase in adreno corticotrophic hormone (ACTH) and cortisol concentrations in both maternal and fetal circulation [79]. Endotoxin administration to pregnant ruminants also stimulates ACTH and cortisol production. Beside fetal stress and fetal hypoxia, maternal stress can also stimulate the fetal HHAA and induce secretion of corticotrophin-releasing-hormone (CRH) leading to prostaglandin $E_2$ ($PgE_2$) production. $PgE_2$ changes the dormant state of the uterus and induce softening of the cervix and the occurrence of synchronous contractions of the myometrium. Formation of gap-junctions is a prerequisite for myometrial contractions and is triggered by increased prostaglandins and oxytocin levels [47].

Microorganisms found in intrauterine infections exert phospholipase $A_2$ ($PIA_2$) activity [6]. Phospholipase $A_2$ is an enzyme necessary for the release of arachidonic acid from phospholipids. $PIA_2$ levels have been found to increase near the end of the term [60]. $PIA_2$ thus increases the level of arachidonic acid, which is converted to $PgE_2$ by COX-2. Also the production of prostaglandin dehydrogenase (PGDH), the enzyme which catabolises $PgE_2$ decreases near term, intriguingly, the production of this enzyme is regulated by the local estrogen: progesterone ratio [95]. Inflammatory mediators like leukotrienes, reactive oxygen species, nitric oxide etc. induced by LPS can also significantly signal the onset of labour.

During the course of ascending intrauterine infection, microorganisms may reach the decidua and an inflammatory reaction at the chorio-decidual interface is initiated. This inflammatory reaction of the decidua leads to a release of cytokines, mostly IL-1 and TNF. Decidual macrophages and polymorphonuclear leukocytes (PMN) can react to this inflammatory reaction and migrate to the

chorio-decidual interface. IL-1 and TNF initiate production and secretion of IL-6, IL-8, and $PgE_2$ from these decidual macrophages [60]. IL-8 stimulates migration of PMN [63] and these cells can release cytokines, collagenases and proteases. Finally, microorganisms that gain access to the fetus may elicit a systemic inflammatory response characterized by increased concentrations of interleukin-6 and other cytokines [74] as well as activation of neutrophil and monocyte activation [8].

TNF-α secreted by the macrophages in response to LPS bind to many tissues and alters their metabolism. It can damage blood capillaries and increase their permeability resulting in a drop in blood pressure that results in shock. TNF-α can stimulate IL-1 production [96] and this cytokine is known to cause fetal death in a dose-dependent fashion [82]. TNF-α can cause microvascular injury and thrombosis via the elaboration of numerous factors leading to vasoconstriction, platelet aggregation, and clotting. These events could potentially lead to ischemia and hemorrhagic necrosis in deciduas and trophoblast resulting in fetal death. Experimental i.v. administration of TNF-α causes plasma levels of cortisol and corticosterone to rise rapidly, however, it is unclear whether this rise is due to release of ACTH or to direct stimulation of the adrenal [43].

Production of prostaglandins (PG) is another possible mechanism of TNF-α-induced fetal death. Eicosanoids are known abortifacients and are produced by gestational tissues in response to TNF-α in vitro. Inhibition of prostaglandin production with indomethacin can decrease LPS-induced fetal death [85]. PGs are generated via cyclooxygenase (COX), the rate limiting enzyme for the conversion of arachidonic acid into $PgH_2$, the common substrate for various PGs. It has recently been shown that COX-2 derived $PgI_2$ is the primary PG that is essential for implantation and decidualization [56].

## Conclusions and future directions

Preterm labor and delivery continues to be the most important unsolved problem in obstetrics till today.

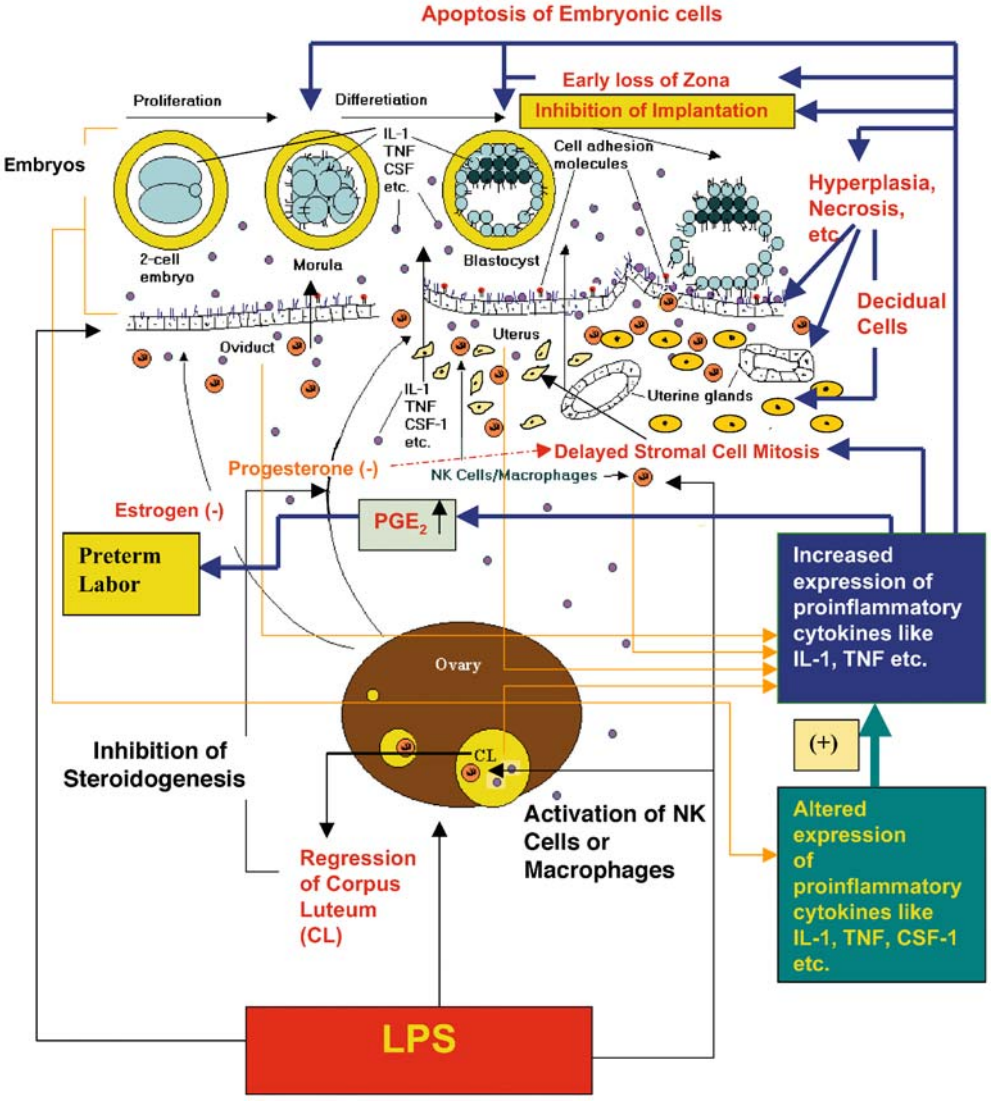


**Fig. 2** Model showing the mechanism of LPS induced failure of blastocyst implantation in mouse

Perhaps the primary reason for the little progress in the prevention and treatment of preterm labor is the lack of understanding of its pathophysiology. Understanding the nature of molecular signals that regulate implantation is of clinical relevance as they may lead to strategies to correct implantation failure and to develop novel contraceptive approaches. The pregnancy rate in IVF programs remains about 20–30% inspite of the high rate of successful fertilization. Several factors are known to exert a direct or indirect effect on the ability of the uterus to develop to a functionally receptive state. LPS-induced cytokines and growth factors, for instance, can significantly accelerate or delay the uterine transition to the receptive state. This would disrupt the normal coordination between embryonic and uterine development even though all molecular players may appear to be normal.

The sequels of fetal growth retardation/death by LPS may not only be similar to clinical intra uterine growth retardations (IUGR) caused due to Gram-negative bacterial infections but also to an extended clinical spectrum ranging from premature labor to fetal demise or still birth. LPS/endotoxins increases the production of immune and inflammatory mediators, like IL-1, and TNF and leads to a concentration dependent increase in the production of $PGE_2$ and IL-6. All these substances have been detected in the amniotic fluid of woman with infection associated preterm labor. IL-6 concentration in amniotic fluid are considered as a marker of intra-amniotic inflammation frequently associated with microbial infections. Systemic administration of recombinant TNF-α, IL-1α and IL-1β can induce pregnancy loss in pregnant animals [23, 72, 83].

On the basis of our experiments and findings it is evident that LPS induced embryonic loss or implantation failure results from a combined maternal and fetal response (Fig. 2). LPS may directly or indirectly activate peritoneal macrophages, and trigger the ovarian cells and or macrophages to secrete high levels of proinflammatory cytokines, which may act on the uterine cells, and activate the residing macrophages/lymphocytes. These cytokines may inversely affect the ovarian steroidogenesis inhibiting the synthesis and secretion of $E_2$ and or $P_4$, which may alter the level and pattern of expression of the cytokines in ovaries and uterus. Such changes in the cytokine based maternal signals may effect the embryonic expression of the developmentally important cytokines. The altered level and pattern of expression of the proinflammatory cytokines of the maternal and embryonic origins may:

1. Delay stromal cell proliferation during decidualization
2. Cause decidual cell necrosis and degeneration of the endometrial epithelium
3. Lead to early loss of the zona pellucida
4. Cause degeneration and degradation of the preimplantation embryos

Liposaccharide-induced proinflammatory cytokines may exert the effects mentioned above directly through their receptors, which are often ubiquitously expressed, or may act indirectly through the actions of various downstream molecules (like NO, COX-2, $PGE_2$ etc.) in the inflammatory pathways. Thus, LPS may act by altering the uterine receptivity for the implanting embryos and at the same time makes the developing blastocyst incompetent for invasion and successful implantation.

In view of the current status involving the interaction between the immune and reproductive systems we propose that swiping out of the LPS-induced embryotoxic TNF-α, or IL-1 from maternal circulation during the preimplantation period could improve fertility rates. Trials in this direction using anti-TNF-α monoclonal antibodies and or recombinant chimeric p75-TNF-α-receptor-Ig proteins to block LPS-induced circulating TNF-α is currently being carried out at our laboratory. In addition to TNF-α blockade the inhibition of other proinflammatory mediators/cytokines like IL-1 is also currently under investigation. There is considerable redundancy in the cytokine network and therefore it is not clear which particular cytokine is required to signal the onset of events related to LPS-induced pregnancy loss. Agents that down-regulate the inflammatory response such as anti-inflammatory cytokines (e.g. IL-10) and antioxidants may also play significant role.

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