

## Review Article

# LPS-Induced Implantation Failure: One of the Causes of Female Infertility

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Submitted: 28 August 2013

Accepted: 30 October 2013

Published: 01 November 2013

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**OPEN ACCESS****Keywords**

- Lipopolysaccharide
- Uterus
- Progesterone
- Corpus luteum
- Cytokines

**Abstract**

A fundamental feature of implantation is synchronized development of the embryo to the blastocyst stage and differentiation of the uterus to the receptive state. Lipopolysaccharide (LPS), a bacterial endotoxin, is a component of the outer membrane of Gram-negative bacteria and its detection in biologic fluids is an evidence of microbial invasion of a sterile compartment, such as the amniotic cavity. A gram-negative bacterial infection model has been established in the mice. LPS alters the expression of tumor necrosis factor- $\alpha$ , interleukin-1, and colony-stimulating factor. LPS treatment disturbs serum P<sub>4</sub>, E<sub>2</sub> and their receptors. Expression of Hsp90, Hsp70, Hsp60, and Hsp25 also altered due to LPS treatment. LPS disturbs the level of FSH and LH and, FSHR in embryos and ovaries, LHR in embryos and uterus. Disturbance in the embryonic and uterine expression of cytokines, growth factors, steroid hormones and its receptors, gonadotropins and its receptors and heat shock proteins in response to LPS probably trigger the multiple factors in the embryonic and uterine cells, which are responsible for implantation failure. The present review will contribute to our understanding of the mechanism of early pregnancy loss during gram-negative bacterial infection in pregnant mother.

**INTRODUCTION**

A fundamental feature of implantation is the synchronized development of embryo to the blastocyst stage and differentiation of the uterus to the receptive state [1,2]. The transition of a receptive to a non-receptive uterus leads to failure of blastocyst implantation. Progesterone (P<sub>4</sub>) regulates the uterine receptivity for blastocyst attachment and coordinates uterine and embryonic interactions [3]. Estrogen is essential for on-time uterine receptivity and activates the dormant blastocysts to make them adhesion competent for implantation in the mouse [1].

Lipopolysaccharide (LPS), a bacterial endotoxin, is a component of the outer membrane of Gram-negative bacteria [4] and its detection in biologic fluids is an evidence of microbial invasion of a sterile compartment, such as the amniotic cavity [5]. An increased concentration of endotoxin in non-sterile body fluid, such as cervical mucus or vaginal fluid is an indication of an enlarged Gram-negative microbial population and has been reported in women with bacterial vaginosis. LPS is a major cause of gynecologic morbidity, such as infertility, ectopic pregnancy,

chronic pelvic pain and pre-term labor [6-9]. LPS has been cited as a potential cause of reduced success in IVF- ET and it may bring its effect by reducing the rate of fertilization of ova, by declining the quality of fertilized ova and by lowering the rate of pregnancy [10].

**CYTOKINES**

We established a gram-negative bacterial infection model in the mouse in which the intraperitoneal injection of LPS (250 mg/kg body weight on day 0.5 of pregnancy) causes embryonic cell death [11] and leads to implantation failure [11,12]. Many of the biological effects of LPS are mediated by the proinflammatory cytokines (such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF) etc.). Several of these cytokines have been implicated in the delicate immune system balances that exist within the fetomaternal interface [13]. 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 may play an important role in the process of development and blastocyst invasion into the

endometrium. Previous observations support the involvement of IL-1 in implantation. IL-1 stimulates the production of endometrial leukemia inhibitory factor (LIF), which may be crucial for implantation. Uterine secretions of many of these cytokines are under the control of ovarian steroids, confirming their close association with a distinct regulatory network that may overlap or compliment the immune system represented by the lymphohematopoietic cells residing in the uterine endometrium [14]. This may be a general mechanism for the regulation of early embryonic development and for maintaining a synchrony between the embryo and the uterine preparation for implantation. Any disturbance of this delicate immune balance within the maternal-fetal interface may result in pregnancy loss or other perinatal complications [15].

Lipopolysaccharide causes an anti-fertility effect by altering expression of tumor necrosis factor- $\alpha$  [16], interleukin-1 [15,17], and colony-stimulating factor [18]. Disturbance in embryonic and uterine secretion of these cytokines may trigger DNA damage in embryos [12] and uterine cells [12] during preimplantation days and leads to implantation failure in the mouse.

## STEROID HORMONES

Progesterone ( $P_4$ ) and estrogen are the ovarian steroids that play a vital regulatory role in development and implantation of blastocyst.  $P_4$  regulates uterine receptivity for blastocyst attachment, induces stromal cell proliferation and differentiation, regulates epithelial cell proliferation, and coordinates uterine and embryonic interactions at a morphologic and molecular level [19]. The effects of  $P_4$  are mediated by the progesterone receptor (PR).  $17\beta$ -Estradiol ( $E_2$ ) is the most potent estrogen and acts as a growth hormone for the tissues of the reproductive organs by supporting the lining of the vagina, cervical glands, endometrium and lining of the fallopian tubes.  $E_2$  enhances the growth of the myometrium. The rising estrogen levels during the first part of the reproductive cycle enhance endometrial cell proliferation. Following ovulation,  $P_4$  secreted by the luteinized follicles lead to the differentiation of these cells. At this point, the endometrium is mature and primed for embryo implantation. During pregnancy,  $E_2$  increases due to placental production [20].  $E_2$  mediates its effect through two intracellular estrogen receptors (ER) i.e. ER- $\alpha$  and ER- $\beta$ .

Lipopolysaccharide stimulates macrophages to secrete cytokines, which induces ovarian dysfunctions. Cytokine rich environments cause the generation of superoxide radicals in corpus luteum (CL) [21] and its regression. LPS induces ovarian dysfunction which decreases serum  $P_4$  and increases serum  $E_2$  during the preimplantation days of pregnancy [1]. This alteration in the levels of ovarian steroids may be due to LPS-induced changes in ovarian tissues. The increased infiltration of macrophages in CL near implantation days causes its regression and leads to low serum level of  $P_4$  in LPS-treated mice. The CL secretes the adequate amounts of  $P_4$  which maintains the uterine endometrium for blastocyst implantation. However, in LPS-treated animals, high  $E_2$  and low  $P_4$  level in serum during preimplantation days may transform the uterine receptivity to a refractory state that results in unsuccessful pregnancy in the mouse. A distinct  $E_2/P_4$  ratio is required during preimplantation days to maintain the uterine receptivity and to help developing

blastocysts in reaching an implantation-competent state. The significantly higher  $E_2/P_4$  ratio in LPS-treated animals keeps the uterus non-receptive during implantation and not prepare the developing blastocysts for implantation [1]. LPS treatment also alters the mRNA expression of progesterone receptor (PR) and estrogen receptor (ER) in embryos and uterus during preimplantation days of pregnancy. PR and ER play an important role in Gram-negative bacteria infection and induced implantation failure in mouse [22].

## HEAT SHOCK PROTEINS

LPS treatment significantly increased the percentage of abnormal embryos and DNA damage in the embryos [12, 23] which is correlated with the lower expression of anti-apoptotic Heat shock proteins (Hsp), Hsp90, Hsp70, and Hsp60 in preimplantation embryos [23]. Pandey et al, (2000) showed that Hsp90 blocks caspase-3 activation and apoptosis [24]. Hsp70 expression began with first zygotic cleavage [25,26] and it exerts a protective effect against apoptosis for the preimplantation embryos [27]. Ravagnan et al, (2001) have reported that Hsp70 is a well-known inhibitor of apoptosis. Cytochrome *c* triggers the oligomerization of Apaf-1, which in turn recruits pro-caspase-9 and pro-caspase-3 into the apoptosome (i.e., caspase activation multiprotein complex). Hsp70 interacts with Apaf-1, and prevent its interaction with procaspase-9 [28]. Hsp60 is also a potent inhibitor of apoptosis by binding to Bax and Bak proteins in the cytosol of cardiac myocytes and inhibits apoptosis [29-31]. LPS-treated preimplantation embryos had extensive DNA damage and significant lower expression of Hsp90, Hsp70 and Hsp60. This lowered gene expression of Hsps may be key mediator for the induction of extensive DNA damage in the preimplantation embryos, which leads to degeneration and degradation of the preimplantation embryos. This extensive DNA damage in the preimplantation embryos is positively correlated with down-regulation of Hsp90, Hsp70 and Hsp60 gene expression in LPS-treated group.

The expression of Hsp90, Hsp70, Hsp60, and Hsp25 was altered in uterus of LPS-treated mice when compared to their respective controls. At the time of implantation Hsp90 and Hsp60 were decreased in stromal cells of LPS-treated uterus. Hsp25 was highly expressed in the endometrium and stromal cells of LPS-treated uterus. Higher expression of Hsp25 in the endometrium disturbs endometrial receptivity and contributes to inhibition of implantation in mice [23]. The altered uterine expression of Hsps may not prepare the uterus for implantation, which may ultimately lead to the early pregnancy loss in mouse.

## GONADOTROPINS

Follicle stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) are expressed in both gonadal and nongonadal cells. Nongonadal cells of reproduction associated tissues including mainly the uterus, oviduct, cervix, blood vessels and mammary glands have the cycle-dependant expression of these receptors [32]. FSH maintains the normal function of ovaries to produce oocytes and hormones in all mammalian species. FSH acts exclusively on granulosa cells of the follicles through specific FSHRs [33,34]. A distinct level of FSH is required for the maintenance of arresting state of follicles during the

preimplantation days of pregnancy [20]. The main role of LH in the myometrium is stimulation of growth and hyperplasia, and relaxation of uterine motility through LHR [32]. With the rise in estrogens, LHRs are also expressed on the maturing follicle that produces an increasing amount of  $17\beta$ -estradiol ( $E_2$ ). In the ovary, the LHR is necessary for follicular maturation and ovulation, as well as luteal function [35]. LH induces production of prostaglandins in endometrium, which contributes to the luteolysis of corpus luteum (CL) [32].

LPS treatment also disturbs the non-gonadal function of FSH and LH and leads to implantation failure in mice. The expression of FSHR in embryos and ovaries, LHR in embryos and uterus also get altered in response to LPS treatment during preimplantation days of pregnancy [36] which suggests that these gonadotropins and its receptors play an integral role in the process of the successful implantation.

## CONCLUSION

A disturbance in the embryonic and uterine expression of cytokines, growth factors, steroid hormones and its receptors, gonadotropins and its receptors and heat shock proteins in response to LPS probably trigger the multiple factors in the embryonic and uterine cells, which are responsible for implantation failure. However, the exact mechanism underlying embryonic loss is not clearly understood. It may be induced by numerous factors which trigger different pathways that may culminate with elimination of the developing embryos from the mother.

The present review will contribute to our understanding of the mechanism of early pregnancy loss during gram-negative bacterial infection in pregnant mother. These studies on effect of LPS on the various factors involve in different pathway related to the normal development and differentiation of preimplantation-stage embryonic and uterine cells may help us to find a good regimen to prevent the early embryonic loss.

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**Cite this article**

Agrawal V, Jaiswal MK, Jaiswal YK (2013) LPS-Induced Implantation Failure: One of the Causes of Female Infertility. *Med J Obstet Gynecol* 1(2): 1014.