Gonadal and nongonadal FSHR and LHR dysfunction during lipopolysaccharide induced failure of blastocyst implantation in mouse

Varkha Agrawal · Mukesh Kumar Jaiswal ·
Yogesh Kumar Jaiswal

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Abstract

Purpose The purpose of the present study was to investigate the impact of lipopolysaccharide (LPS) on follicle-stimulating hormone (FSH), luteinizing hormone (LH) and their receptors during preimplantation days of pregnancy.

Method The PBS or lipopolysaccharide (LPS) was injected intraperitoneally in the pregnant females on day 0.5 of pregnancy and serum, embryos, ovaries and uterine horns were collected on days 1.5, 2.5, 3.5, 4.0, 4.125, 4.33 and 4.42 of pregnancy.

Varkha Agrawal and Mukesh Kumar Jaiswal equally contributed to this work.

Capsule Altered level of FSH and LH in serum and expression of FSHR and LHR in gonadal and nongonadal tissues after LPS treatment may involve in blastocyst implantation failure in mouse.

V. Agrawal · M. K. Jaiswal · Y. K. Jaiswal (✉)
Molecular Biology and Reproductive Immunology Laboratory,
School of Studies in Biochemistry, Jiwaji University,
Gwalior 474 011 MP, India
e-mail: jaiswalmbri@gmail.com

V. Agrawal
e-mail: varkhaagrawal@gmail.com

M. K. Jaiswal
e-mail: mukeshjaiswal1980@gmail.com

V. Agrawal
Obstetrics and Gynecology, NorthShore University HealthSystem,
Evanston 60201 IL, USA

M. K. Jaiswal
Clinical Immunology laboratory,
Rosalind Franklin University of Medical and Science,
3333 Green bay Road,
North Chicago 60064 IL, USA

Y. K. Jaiswal
School of Studies in Biochemistry, Jiwaji University,
Gwalior 474 011 MP, India

Result(s) In the LPS-treated pregnant females, the secretion of FSH and LH is disturbed with respect to normal pregnancy. Furthermore, the expression of FSHR mRNA in embryos and ovaries, LHR mRNA in embryos and uterus get modulated in response to LPS during preimplantation days of pregnancy.

Conclusion(s) The disturbance in the serum level of FSH and LH in response to LPS leads implantation failure in mouse which suggests that these gonadotropins plays an integral role in the process of the successful implantation. This study also suggests a possible nongonadal role of FSHR and LHR in LPS-induced implantation failure in the mouse.

Keywords Embryo implantation · Follicle-stimulating hormone · Luteinizing hormone · Lipopolysaccharide · Pregnancy loss

Introduction

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 [35]. 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 [3, 14]. 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 [35]. With the rise in estrogens, LHRs are also expressed on the maturing follicle that produces an increasing

amount of 17β -estradiol (E_2). In the ovary, the LHR is necessary for follicular maturation and ovulation, as well as luteal function [8]. LH induces production of prostaglandins in endometrium, which contributes to the luteolysis of corpus luteum (CL) [35].

Several studies have shown a strong association between bacterial infections and pregnancy disorders such as implantation failure, abortion, preterm labor, intrauterine growth retardation (IUGR) and pre-eclampsia [13, 33]. Despite multiple strategies attempting to impede preterm delivery, the rate has increased from 10% to 12.7% of all pregnancy [18]. At least 40% of preterm births are associated with infection. The leading cause of infection-associated preterm labor is considered to be bacterial infections. Gram-negative bacterial lipopolysaccharide (LPS) response through toll-like receptor (TLR)-4 [24] and results in production of cytokines [5–7, 11]. TLR-4 stimulation is also known to induce fetal resorption when it occurs in early pregnancy. It has been established that gram-negative bacteria or its component trigger preterm labor in various animal models, and many attempts have been made to clarify this mechanism [10, 13]. We established a gram-negative bacterial infection model in the mouse by intraperitoneal (i.p.) injection of LPS [6]. LPS cause the embryonic cell death and induce the DNA damage in embryonic and uterine cells [12].

Many of the biological effects of LPS are mediated by stimulating macrophages to secrete cytokines, which in turn inhibit ovarian functions [28]. However, LPS also directly suppresses the ovarian cell functions [31]. Uterine secretions of many of these cytokines are under the control of ovarian steroids, confirming their close association with a distinct regulatory network [22], which may overlap or complement the FSH and LH. We have shown that LPS modulate the serum level of progesterone (P_4) and E_2 during preimplantation days of pregnancy [2], which are under the control of gonadotropins and their receptors.

Here we investigate the effect of LPS on FSH, LH and their complement receptors during preimplantation days of pregnancy. In this study, we used mouse as a model to test the hypothesis that LPS-induces the alterations in the serum level of FSH, LH and in the expression of their receptors during preimplantation days of pregnancy which may contribute to early pregnancy failure.

Materials and methods

Animal model

All animals were treated in accordance with institutional ethics committee guidelines for the care and use of animals in research. Park strain mice (6–8 weeks, 20–21 g) in proestrus were selected by measuring vaginal electrical resistance [1] and impregnated naturally by males. Mating was confirmed by the presence of vaginal plug and morning of plugging considered as day 0.5 of pregnancy.

Treatment and specimens

Conditions of gram-negative bacterial infection were induced by LPS (250 μ g/kg body weight i.e., 5 μ g/animal, *Salmonella enterica* serotype Minnesota Re 595; Sigma, St. Louis, MO) in pregnant animals on day 0.5 of pregnancy through i.p. route, in all experiments [6].

For measurement of FSH and LH blood was collected on days 1.5, 2.5, 3.5, 4.375 and 4.5 ($n=8$ each day) of pregnancy from animals received either PBS or LPS on day 0.5 of pregnancy. The blood was collected via saphenous vein present on the caudal surface of the thigh using Microvette CB 300 (Sarstedt, Germany). It was allowed to clot at room temperature and centrifuged at 10,000 $\times$ g for 15 min. The serum was collected and stored at -20°C in small aliquots. During blood collection extra care was taken to avoid any mishandling of females which may disturb the pregnancy.

For measurement of FSHR and LHR transcripts in embryos, animals were superovulated ($n=15$) with standard protocol [6] and, in reproductive tissues ($n=12$), un-superovulated animals were used. Animals received either PBS or LPS on day 0.5 of pregnancy were sacrificed on days 1.5, 2.5, 3.5, 4.0, 4.125, 4.33 and 4.42 of pregnancy and embryos or reproductive tissues (ovaries and uterus) were collected and processed for RNA extraction, immediately.

Measurement of FSH and LH

Serum level of FSH and LH were measured by enzyme immuno-assay kits (Bioserv diagnostics GmbH, Germany). Intra- and inter assay coefficients of variation were 6.10% and 5.90% for FSH, and 4.10% and 3.7% for LH, respectively. The limits of detection were 0.8mIU/ml and 2mIU/ml for FSH and LH, respectively.

RNA extraction

Embryos

Total 100 embryos were collected on each day of pregnancy (days 1.5 to 4.42), washed and resuspended in 50 μ l sterile PBS and used for extraction of total cellular RNA using High Pure RNA Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. Final washes of embryos were collected and used as a negative control. RNA was eluted in 60 μ l of elution buffer and RNA equivalent to 5 embryos were used for each reverse transcription (RT)-PCR reaction.

Tissues

Total cellular RNA was purified from tissue specimens after homogenization in trizol reagent (GIBCOBRL, USA) according

to the manufacturer's instructions. The quantity and quality of RNA was verified by spectrophotometry and 1.5% agarose gel electrophoresis, respectively.

Primer designing for RT-PCR

The RT-PCR primers for FSHR, LHR and β -actin were designed by using primer express software (Primer Express, Applied Biosystems, CA, USA). DNA and mRNA sequences of genes were taken from MGI 3.54 (The Jackson Laboratory, BarHarbor, Maine), World Wide Web (URL: <http://www.informatics.jax.org>). The approximate positions of primer pairs were checked by Spidey software (National Library of Medicine, USA). The oligonucleotide primers were designed to cross introns and exons boundaries for discrimination of the amplified products from genomic DNA and cDNA and synthesized by Sigma Genosys (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India). Sequences of primers, annealing temperature and amplicon lengths are shown in Table 1.

RT-PCR

Expression of FSHR, LHR and β -actin genes was analyzed by RT-PCR (with outer primer) followed by nested PCR (with inner primer) in embryos and by RT-PCR (with inner primer) in ovarian and uterine tissues. The reverse transcription followed by PCR was carried out using "Titan One Tube RT-PCR System" (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. Each RT-PCR reaction was performed in 50 μ l mixture containing 2 μ g RNA for tissues and for embryos RNA equivalent to 5 embryos with 0.4 μ M of each primer, 0.2 μ M dNTP mix, 5 mM DTT, 10U RNase inhibitor and 1U enzyme mix. Cycling conditions were followed according to manufacturer's instructions with annealing temperature

mentioned in Table 1 by using gradient in thermocycler (Bio-Rad Laboratories, Richmond, USA) and 35 cycles for each gene.

Nested PCR

The product of first round gene specific RT-PCR was used to carry out nested PCR for embryos. Nested PCR reaction was performed in 50 μ l reaction mixture containing 0.2 μ l of the first round PCR product, 0.4 μ M each primer, 0.2 μ M dNTP mix, 0.5U Taq DNA Polymerase (MBI Fermentas, UK). Cycling conditions were followed initial denaturation at 94°C 2 min; denaturation at 94°C, 10 s; annealing temperature as mentioned in Table 1, 30 s; elongation 72°C, 45 s; final elongation at 72°C, 7 min using gradient in thermocycler and 25 cycles for each gene.

After amplification, PCR products were sized fractionated by agarose gel electrophoresis (1.5%). 15 μ l of amplified product using inner primers from embryos, ovaries and uterus were loaded on the agarose gel and visualized by ethidium bromide staining under a UV-transilluminator (Bio-Rad Laboratories, Richmond, USA) and photographed. The semi-quantitative analysis of PCR products was done by densitometric scanning and using AlphaEaseFCTM software, version 3.0 (Alpha Innotech Corporation, San Leandro, CA, USA). The intensity of bands observed for different genes in both groups were normalized with the intensity of β -actin band observed under same experimental conditions. The identities of amplified PCR products for all cDNAs were verified by sequencing.

Statistical analysis

The results of each experiment were analyzed by using one way analysis of variance (ANOVA) with Duncan's multiple range test for comparison of the significance level (*P*)

Table 1 Sequences of primers, annealing temperature and amplicon lengths

mRNA (GenBank accession #)	PCR primer pair	Sequence of oligonucleotide 5'→3'	Annealing temperature (°C)	Product size (bp)
FSHR (AF095642.1)	Outer pair	5'TGCCCAACCATGGCTTAGA 3' 5'TGATGGCCAGGATGCTGATA3'	52.8	433
	Inner pair	5'GCGGCAAACCTCTGAACCTT3' 5'AAATGCATCTGGCTTTGGC3'	52.8	211
LHR (M81301.1)	Outer pair	5'ACCCGGTGCTTTTACAAACC3' 5'TGGCGATGAGCGTCTGAAT3'	53.8	407
	Inner pair	5'TGTAACACAGGCATCCGGA3' 5'CGTCCCATTGAATGCATGG3'	53.8	201
β -actin (AK152813.1)	Outer pair	5'AGGCTCTTTCCAGCCTTCCT3' 5'ACATCTGCTGGAAGGTGGACAG3'	54.2	291
	Inner pair	5'ATGGAATCCTGTGGCATCCA3' 5'TCCACACAGAGTACTTGCGCTC3'	53.8	217

between control and treated values. A $P<0.05$ value was considered as significant difference between the values compared.

Results

Effect of LPS on serum level of FSH and LH during preimplantation days

To determine whether serum level of gonadotropins are affected by LPS, ELISA was performed in serum collected from days 1.5 to 4.5 of pregnancy from animals injected with PBS or LPS on day 0.5 of pregnancy (Figs. 1 and 2).

Serum level of FSH increases from day 1.5 ($16.24\pm 1.46\text{mIU/ml}$) to 2.5 ($21.06\pm 0.58\text{mIU/ml}$) of pregnancy followed by a sharp decrease on day 3.5 ($6.24\pm 1.59\text{mIU/ml}$) and maintained till day 4.5 ($4.35\pm 0.81\text{mIU/ml}$) of pregnancy in control animals (Fig. 1). In LPS-treated animals, serum level of FSH was significantly higher ($P<0.01$) on day 1.5 ($22.01\pm 1.04\text{mIU/ml}$) and followed by a significant lower level on day 2.5 ($17.37\pm 0.44\text{mIU/ml}$) with a significant higher level on day 3.5 ($11.51\pm 0.51\text{mIU/ml}$) of pregnancy as compared to respective control. On day 4.375 ($7.14\pm 0.97\text{mIU/ml}$) of pregnancy, the serum level of FSH was same in both groups. However, it was significantly higher ($P<0.01$) on day 4.5 of pregnancy in LPS-treated animals ($18.72\pm 1.49\text{mIU/ml}$) as compared to respective control (Fig. 1).

Serum level of LH increases slightly from day 1.5 ($30.63\pm 3.42\text{mIU/ml}$) to 2.5 ($33.12\pm 2.90\text{mIU/ml}$) and maintained peak on day 3.5 ($70.31\pm 4.01\text{mIU/ml}$) of pregnancy followed by slight decrease on day 4.375 and maintained till day of implantation ($59.95\pm 2.64\text{mIU/ml}$) in control animals (Fig. 2). However, in LPS-treated animals level of LH

Fig. 1 Level of FSH in serum during different preimplantation days of pregnancy in control and LPS-treated animals ($n=8$ in each group). * $P<0.05$, ** $P<0.01$ versus counterparts (based on Duncan's multiple-range test). Values are expressed as the mean $\pm$ SEM

Fig. 2 Level of LH in serum during different preimplantation days of pregnancy in control and LPS-treated animals ($n=8$ in each group). * $P<0.05$, ** $P<0.01$ versus counterparts (based on Duncan's multiple-range test). Values are expressed as the mean $\pm$ SEM

was significant higher on day 2.5 ($58.74\pm 3.40\text{mIU/ml}$) of pregnancy followed by a significant lower level on day 3.5 ($38.67\pm 1.13\text{mIU/ml}$) and 4.375 ($22.09\pm 3.42\text{mIU/ml}$) ($P<0.01$) of pregnancy as compared to respective controls. The serum level of LH was almost same on day 4.5 ($79.18\pm 18\text{mIU/ml}$) of pregnancy in LPS-treated animals as compared to control (Fig. 2).

Effect of LPS on FSHR and LHR transcripts in embryos

To determine whether the nongonadal role of gonadotropins receptors i.e., FSHR and LHR is affected by LPS during the preimplantation period of pregnancy, the semiquantitative RT-PCR followed by nested PCR was performed in embryos obtained from days 1.5 to 4.5 of pregnancy from animals injected with PBS or LPS (Fig. 3).

FSHR and LHR mRNA expressed from day 1.5 to 3.5 of pregnancy in developing embryos recovered from both control animals and LPS-treated animals. The FSHR mRNA expression was very low in day 1.5 embryos, followed by its increased expression in day 2.5 embryos. The same level of expression was maintained in day 3.5 embryos recovered from control animals (Fig. 3a, g). In LPS-treated pregnant females, embryonic FSHR mRNA expression was not observed on day 1.5 with a lower expression on day 2.5 of pregnancy as compared to their respective controls. On day 3.5 its mRNA expression was same in both groups (Fig. 3b, g).

LHR mRNA expression was observed on day 1.5 followed by an increase on day 2.5 of pregnancy in embryos recovered from control animals. On day 3.5, its expression was sharply decreased. In LPS-treated pregnant females, embryonic LHR mRNA expression was significantly ($P<0.05$) higher on day 1.5 followed by same level of expression on day 2.5 with a slight increase on day 3.5 of pregnancy as compared to their respective controls (Fig. 3d,

Fig. 3 Expression of FSHR and LHR transcripts in the mouse embryos collected from PBS (control) or LPS-treated animals. The FSHR, LHR and β -actin transcripts were detected by RT-PCR followed by nested PCR. FSHR (a) control (b) LPS-treated, LHR (c) control (d) LPS-treated, β -actin (e) control (f) LPS-treated, depicted is a

representative figure from among three repeat experiments. Ratios of the relative signal intensities of (g) FSHR/ β -actin and (h) LHR/ β -actin are depicted ($n=15$ in each group). * $P<0.05$ versus counterparts (based on Duncan's multiple-range test). Values are expressed as the mean $\pm$ SEM

h). No FSHR and LHR mRNA was detected from days 4.0 to 4.42 of pregnancy in blastocyst recovered from both control and LPS-treated animals (Fig. 3c, d, h).

The expression of β -actin gene was uniform in embryos collected during preimplantation days of pregnancy studied from control and LPS-treated animals (Fig. 3e, f).

Effect of LPS on FSHR and LHR transcripts in reproductive tissues

To determine whether gene expression of FSHR and LHR is affected by LPS, semi-quantitative RT-PCR was performed in reproductive tissues (ovaries and uterus) obtained from days 1.5 to 4.5 of pregnancy from animals injected with PBS or LPS (Figs. 4 and 5).

The FSHR mRNA expression was not detected in uterus collected from control and LPS-treated animals during preimplantation days of pregnancy (Fig. 4). Only non-specific bands were observed in all the groups.

In ovaries, a gradual increase in FSHR mRNA expression was observed from days 1.5 to 3.5 of pregnancy, with a peak on day 3.5, followed by a gradual fall from day 4.0 to 4.125 of pregnancy in control animals. The low FSHR mRNA expression was maintained till day 4.42 of pregnancy in ovaries of control animals (Fig. 5a, e). While in LPS-treated pregnant females, on day 1.5 the level of ovarian FSHR mRNA expression was at peak and higher when compared to respective control. On day 2.5 of pregnancy, FSHR mRNA expression was slightly higher,

followed by significantly ($P<0.05$) lower level of expression on days 3.5 and 4.0 of pregnancy as compared to the respective controls. However, the same of expression was maintained on days 4.125 and 4.33 with respect to control (Fig. 5b, e). In contrast on day 4.42, the decreased expression of FSHR mRNA was observed in the LPS-treated ovaries as compared to the control. This result indicates that alteration of ovarian FSHR expression in the LPS-treated pregnant females could be reflective events of dysfunction of ovary. The expression of β -actin gene was uniform in ovaries collected from control and LPS-treated animals during preimplantation days of pregnancy studied (Fig. 5c, d).

The LHR mRNA expression was detected from day 1.5 and increase sharply on day 2.5 of pregnancy in the uterus recovered from control animals. No expression was detected on day 3.5 of pregnancy. However, its expression was increased near the window of implantation on day 4.0 and same level was maintained till day 4.33 of pregnancy in uterus recovered from control animals. Moreover, at the time of implantation (day 4.42), LHR mRNA was drastically increased in the uterus of control animals (Fig. 6a, e). In LPS-treated animals, the expression of LHR mRNA was same on day 1.5 of pregnancy followed by no detection on days 2.5 and 3.5 of pregnancy. The only LHR mRNA expression was observed on day 4.0 of pregnancy, but at the lower level than respective control. No expression of LHR was observed in uterus recovered from LPS-treated animals from day 4.125 to till the day of

Fig. 4 Expression of FSHR transcripts in the mouse uterus collected from PBS (control) or LPS-treated animals. The FSHR and β -actin transcripts were detected by RT-PCR. FSHR (a) control and LPS-treated, β -actin (b) control and LPS-treated, depicted is a representative figure from among three repeat experiments. ($n=12$ in each group)

Fig. 5 Expression of FSHR transcripts in the mouse ovaries collected from PBS (control) or LPS-treated animals. The FSHR and β -actin transcripts were detected by RT-PCR. FSHR (a) control (b) LPS-treated, β -actin (c) control (d) LPS-treated, depicted is a representative

figure from among three repeat experiments. Ratios of the relative signal intensities of (e) FSHR/ β -actin is depicted ($n=12$ in each group). * $P<0.05$ versus counterparts (based on Duncan's multiple-range test). Values are expressed as the mean $\pm$ SEM

Fig. 6 Expression of LHR transcripts in the mouse uterus collected from PBS (control) or LPS-treated animals. The LHR and β -actin transcripts were detected by RT-PCR. LHR (a) control (b) LPS-treated, β -actin (c) control (d) LPS-treated, depicted is a representative

figure from among three repeat experiments. Ratios of the relative signal intensities of (e) LHR/ β -actin is depicted ($n=12$ in each group). * $P<0.05$ versus counterparts (based on Duncan's multiple-range test). Values are expressed as the mean $\pm$ SEM

implantation (Fig. 6b, e). The expression of β -actin gene was uniform in uterus collected from control and LPS-treated animals during preimplantation days of pregnancy studied (Fig. 6c, d).

Discussion

In present study we report that LPS treatment leads to amend the serum level of FSH and LH and expression of

their receptors in nongonadal tissues studied during the preimplantation days of pregnancy in mouse.

The secretion of E_2 from the ovary is required for the growth of uterus and for the feedback regulation of gonadotropin secretion from the pituitary gland and hypothalamus [4]. The observed high serum level of FSH near the window of implantation due to LPS treatment may involve in the production of high amount of E_2 . We have previously shown that LPS treatment increase the serum level of E_2 during preimplantation days of pregnancy [2]. Higher estrogen levels promptly transform the uterus to a refractory state [16, 29]. It may be postulated that the receptive state of the uterus is altered at the molecular level in the presence of a higher E_2 in response to high serum level of FSH near the window of implantation, leading to implantation failure in LPS-treated animals.

LH has luteotrophic action and rise in its concentration increases the P_4 secretion during normal gestation in hamsters [25]. The observed high serum level of LH in control animals on day 3.5 of pregnancy may support the luteinization of CL and production of P_4 to maintain the uterine receptivity. We have reported that increased infiltration of macrophages in CL and its regression near the time of implantation cause the low serum level of P_4 in response to LPS [2]. Low serum level of LH in LPS-treated animals on days 3.5 and 4.375 of pregnancy may not support the luteinization of CL and inhibit the production of P_4 . This may keep the uterus non-receptive throughout the preimplantation days and inhibit the blastocyst implantation in the LPS-treated animals.

The observed significantly higher serum level of FSH and LH at the time of implantation (i.e., on day 4.5 of pregnancy) in the LPS-treated animals may trigger the maturation of new follicles followed by its luteinization which may induce a new reproductive cycle to begin. Our previous study has shown that these LPS-treated females start cycling just after the failure of implantation [1].

FSH is essential for folliculogenesis, ovulation and steroidogenesis in females [26]. FSHRs are responsible for the transduction of the biological actions of FSH to their target cells. In present study expression of FSHR mRNA was observed only from days 1.5 to 3.5 of pregnancy which gets altered in response to LPS. It was reported that FSH maintains cAMP concentrations in the oocyte and involves in its maturation and cleavage after fertilization [27]. The absence of FSHR mRNA on day 1.5 and lower expression on following days of pregnancy in embryos recovered from LPS-treated animals may not mediate these functions of FSH and cause the abnormal growth and differentiation of developing embryos and leads to early embryonic loss in mouse. Contrary to our in vivo study, an in vitro analysis shows that FSHR mRNA transcripts were present in unfertilized mouse oocytes, zygotes and in embryos at the two cell, four cell, morula and blastocyst stages of development [23].

In the present study, an inverse relation in pattern of FSHR mRNA expression was observed in the ovaries recovered from control and LPS-treated animals during preimplantation days of pregnancy. It is well reported that E_2 synergizes with the FSH to regulate the expression of FSHR on granulosa cells [17, 32]. We observed a high level of E_2 in serum during the early preimplantation days of pregnancy in LPS-treated animals [2]. This observation clearly suggests that higher FSHR mRNA expression in ovaries of LPS-treated animals during the initial preimplantation days (days 1.5 and 2.5) may be due to high serum level of E_2 . The lower expression of ovarian FSHR near the window of implantation in LPS-treated females could be reflective event of ovarian dysfunction and may not supportive for process of successful implantation.

In the present study, the expression of LHR mRNA was found from day 1.5 to 3.5 of pregnancy followed by its absence from day 4.0 to the day of implantation in embryos recovered from the animals of both groups. However, a higher level of LHR mRNA was expressed in embryos recovered from LPS-treated animals as compared to the controls. The expression of LHR mRNA was reported in an in vitro study in oocytes and preimplantation embryos in mouse [23]. LHRs are responsible for the transduction of biological actions of LH to their target cells, using cAMP as the main, although not the only, intracellular second messenger. In vivo maturation of oocytes is initiated by the LH surge, which results in an increase in the concentration of cAMP in the follicular cells which stimulate the production of ovarian steroids [30]. It has been reported that a tight regulation in the level of cAMP is critical for the normal embryonic development [21]. The observed early expression of LHR mRNA in the control embryos may enable them to respond to LH through intracellular cAMP and may control the early embryonic development and differentiation. The observed higher expression of LHR mRNA in response to LPS in the early preimplantation days embryos may disrupt the required level of cAMP which may disturb the normal embryonic development and ultimately lead to early embryonic loss in mouse. In an earlier study, we have reported an increased number of severely degenerated, fragmented and growth arrested embryos recovered during preimplantation days of pregnancy from LPS-treated animals [11]. Our current observation suggests that a higher expression of LHR mRNA in developing embryos in response to LPS may be responsible for these effects.

In present study LHR mRNA expression get altered in response to LPS in uterus during different preimplantation days of pregnancy. LHR is important in endometrium decidualization, implantation of embryos [9], regulation of uterine blood flow and maintenance of early pregnancy [19]. LH was shown to stimulate directly the uterine growth in mouse [15, 34]. The observed high level of LHR mRNA in

uterus on day 2.5 and from day 4.0 to 4.42 of normal pregnancy may enable it to respond to LH and support the implantation of embryos in uterus by regulating the blood flow in the uterus, survival of the early pregnancy, decidualization, regulation of enzymes within the uterus, and growth of uterus. The observed very low level of LHR mRNA throughout the preimplantation days of pregnancy in uterus recovered from LPS-treated animals may not enable it to respond to LH to support various events during preimplantation days and results in LPS-induced early pregnancy loss in mouse. Although in the future studies, it is important to study the ovarian LHR expression to emphasis its gonadal role during gram-negative infection.

A disturbance in the expression of gonadotropins receptors in response to LPS may trigger some pathways in these cells, which lead to early embryonic loss in mouse. 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 neat elimination of the developing embryos from the mother. The present study will contribute to our understanding of the mechanism of early pregnancy loss during gram-negative bacterial infection in pregnant mother. A thorough understanding of this mechanism may lead to develop effective strategies to either prevent or treat early embryonic loss.

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