Review Article

Dissecting toll-like receptor molecular regulatory network(s) in reproductive medicine, primarily infertility: A snapshot

Saumya Pandey

Ajanta Hospital and IVF Centre, Lucknow, U.P., India

Abstract

Introduction: Targeted manipulation of complex biochemical signaling pathways in unraveling the complexities associated with human reproductive disorders, including infertility, appears to be an attractive immunomodulatory therapeutic strategy in reproductive medicine and disease. Toll-like receptors (TLRs), a family of evolutionarily conserved pathogen recognition receptors, are emerging as pivotal players in the pathophysiology of a spectrum of human diseases, including infertility. Inflammation and infections in the female reproductive tract are common causes of infertility globally; TLR immune surveillance initiates inflammatory responses to foreign pathogens.

Materials and Methods: A comprehensive literature search using PubMed and Medline scientific database (s) (last accessed June 6, 2016) was performed, and accordingly the author included the selected articles in the present review; public health research studies for developing cost-effective infertility assessment programs in low-resource settings targeting North Indian couples are ongoing so as to reduce the burden of disease as well as psychosocial factors associated with infertility.

Results: Significant recent advances in microarray and next-generation sequencing technologies have enabled the application of whole-genome approaches to the study of infertility; successful implantation requires synchronization between the acquisition of implantation competency by the blastocyst and a receptive state in the uterine endometrium.

Conclusion: The author speculates that immunomodulation of cell-specific ligand–receptor interaction(s) is essential for initiating the subsequent intermediate/downstream events in signal transduction pathways, eventually leading to specific cellular/biological response(s); in this context, TLR-based therapeutics and subsequent TLR-based patient-centric biomarker research studies will certainly provide a more meaningful understanding of the pathophysiological basis of reproductive disorders, primarily infertility in the 21st century.

Keywords: Biomarker, immunomodulation, infertility, public health, Toll-like receptors

Address for correspondence: Dr. Saumya Pandey, MSc (Biochemistry), PhD (Life Science), Dy. Director (Academics, Clinical Research, Publications, Grants), Ajanta Hospital and IVF Centre, Lucknow, U.P., India (former).
E-mail: drsaumyapandey6@gmail.com

INTRODUCTION

Reproductive disorder(s), primarily infertility, are emerging as leading cause(s) of psychosocial intervention(s) in both the Western world as well as the Indian subcontinent in a relatively significant number of infertile couples.[1] Targeting biochemical signaling pathways in precisely dissecting the cellular/molecular complexities in

Access this article online	
Quick Response Code:	Website: www.fertilityscienceresearch.org
	DOI: 10.4103/2394-4285.196784

How to cite this article: Pandey S. Dissecting toll-like receptor molecular regulatory network(s) in reproductive medicine, primarily infertility: A snapshot. Fertil Sci Res 2015;2:95-101.

human reproductive disorders is emerging as one of the major thrust area(s) in reproductive medicine, including human reproductive physiology, pharmacology, pathophysiology, and therapeutics. Efficient cellular/biological response in the host cell/organism is mediated *via* "receptors" that recognize specific ligands at the membrane level.[2] Ligand–receptor interaction(s) are essential for initiating subsequent intermediate/downstream events in signal transduction pathways, eventually leading to specific cellular/biological response (s). Signal transduction sequential cascade(s) involve initial ligand–receptor pairing/interactions, structural changes/modifications in the receptor domains after ligand recognition, activation of intermediary components/kinases in cell signaling cascade(s), signal amplification, assembly of signaling mediators/components and/or gradient formation, nucleocytoplasmic shuttling, transcription factors, activation of target gene(s), and eventual cellular/biological host response. "Infertility" is defined as the inability to conceive following 12 months of regular unprotected sexual intercourse.[3] *Mycobacterium tuberculi* is the major etiological agent for infertility in women;[4] altered anti-Müllerian hormone/Müllerian inhibiting substance level(s) are associated with diminished ovarian reserve, thereby predisposing women of reproductive age to adverse pregnancy outcomes.[5,6] Toll-like receptors (TLRs) are a family of evolutionarily conserved transmembrane receptors that recognize distinct pathogen-associated molecular patterns.[7] Thirteen mammalian TLRs have been identified till date and the expression of 10 TLRs is known in humans.[8] Moreover, mammalian TLR proteins derive their name from the *Drosophila* "Toll" protein, with which they share sequence similarity; "Toll" was shown to be involved in dorsal–ventral patterning in fly embryos and was also implicated as a key component of host immunity against fungal infection.[9] A precise understanding of the immunobiological mechanisms and complex cellular/molecular signaling regulatory networks and biochemical signaling cross-talk events involved in developmental biology/human reproduction and disease is indeed essential in contemporary times for strategically dissecting the intricacies in pathophysiology of human reproduction, *viz.*, infertility, polycystic ovary diseases, hypothalamic amenorrhea, etc. Interestingly, female reproduction is underpinned by a complex series of cellular and molecular interactions orchestrated by autocrine, paracrine, intracrine, and endocrine actions of receptors, including TLRs, ovarian growth factors, chemokines, and hormones.[10]

SEARCH CRITERIA FOR SELECTION, EXTRACTION, AND SYNTHESIS OF DATA USING SCIENTIFIC DATABASE(S)

The author defined the initial search criteria for selection, extraction, assimilation, and synthesis of most relevant scientific data suitable for this review in the reproductive medicine field; she conducted a few initial rounds of brainstorming session(s) with her potential future research collaborators for rapid exchange of emerging scientific concepts in reproductive disorders, primarily receptor-based cell signaling in infertility. Thereafter, on the basis of her high-quality extensive research experience in States of Texas, New York, and Nebraska, USA, and India, she identified three clinically relevant burning questions that demand immediate attention of the scientific/clinical research community worldwide, and these questions (denoted by *Q) were as follows:

*Q1: Immunomodulaton of cell-specific ligand–receptor interaction(s) are essential for initiating the subsequent intermediate/downstream events in signal transduction pathways, eventually leading to specific cellular/biological response(s); in this context, could TLR-based therapeutics and subsequent TLR-based patient-centric biomarker research studies provide a meaningful understanding of the pathophysiological basis of reproductive disorders, primarily infertility in the 21st century?

*Q2: Could distinct TLR ligand-based molecular interactions and cross-talks among inter-related cell signaling cascades, *viz.*, ceramide and/or autophagy transmembrane regulatory network(s), be immuno-therapeutically manipulated using specific TLR agonists and/or antagonists so as to decipher the physiological and/or pharmacological basis of infertility in patients of diverse ethnicities, *viz.*, American, Asian Indian, and Danish cohorts, and thereby provide informative translational insights into reproductive success in clinically infertile patient cohorts worldwide?

*Q3: Oocyte maturational competence and integrity, sperm function, as well as quality, pre-embryo culture conditions, pre-embryo morphology, and ovarian age are emerging as significant physiological/immunobiological factors for determining a successful implantation and/or pregnancy outcome in a specific subset of infertile women of ethnically disparate population(s); will TLRs be the eventual cost-effective predictive and prognostic biomarkers in infertility management in the near future so as to reduce the increasing psychosocial and financial distress associated with infertility treatment among

infertile couples of differential socioeconomic status (e.g., US Dollars (USD), Indian Rupees (INR), etc.) at reproductive medicine laboratories/medical centers stringently managed by a highly qualified, scientific expert in the reproductive medicine healthcare arena?

Subsequently, after defining the above-mentioned questions (*Q1, *Q2, *Q3), she responsibly extracted a total of 34 most relevant articles related to TLRs in reproductive disorders/infertility by performing a timeline-based comprehensive literature search using the PubMed and Medline scientific database(s) (last accessed June 6, 2016), and accordingly included the selected articles (original research articles, reviews, commentaries, letter to editor, and clinical trials) in the present review; moreover, each of these 34 articles was thoroughly reviewed, and thereafter, the data were amalgamated for inclusion in this manuscript. Articles extracted were finally selected for inclusion in the review based on the novelty of research studies, careful assessment of well-defined objectives, stringent research methodologies, laboratory data, research outcomes, and broad-spectral translational and public health impact in a clinical research setting.

Overview of TLR signal transduction

The identification of similarity between the cytoplasmic domains of *Drosophila* Toll and mammalian Interleukin (IL)-1 receptor propelled the search for orthologous receptors, subsequently leading to the discovery of "human Toll" (later named TLR4) in humans.[9] The immunobiology of TLRs is indeed complex; TLRs are type 1 integral membrane glycoproteins comprising leucine-rich repeat (LRR) motifs in the pathogen-binding ectodomains (ECD), and cytoplasmic signaling domains known as Toll IL-1 receptor (TIR) domains, joined by a single transmembrane helix.[11] TLRs are expressed in diverse cell types (cervical, airway, gut epithelial cells, B cells, mast cells, NK cells, dendritic cells, regulatory T cells, macrophages, monocytes, neutrophils, basophils, and endothelial cells); few TLRs (1, 2, 4, 5, and 6) are expressed on the cell surface while other TLRs (3, 7, 8 and 9) are localized intracellularly.[8,9,11] TLRs can recognize a variety of pathogen-associated molecular patterns (PAMPs), including lipoprotein, lipopolysaccharide (LPS), peptidoglycan, zymosan, bacterial flagella, CpG deoxyribonucleic acid (DNA), and double strand and single strand ribonucleic acids (RNAs). Moreover, subcellular distribution of TLRs may be defined by their transmembrane and/or cytoplasmic domains.[12] Furthermore, deciphering the complexities associated with TLR signaling network(s) has been one of the primary objectives of molecular biology research-oriented experimental studies in recent years. TLR signal transduction involves myeloid differentiation factor (MyD)88-mediated pathway that leads to the activation of nuclear factor (NF)-kB and TIR domain-containing adaptor inducing Interferon (IFN)-beta (TRIF)-mediated pathway resulting in the activation of interferon regulatory factor (IRF)-3.[8] Two adaptor proteins, namely, the MyD88 and TRIF, also known as TIR-containing adaptor molecule, are the critical "molecular regulatory switches" of the TLR signal transduction pathway(s). MyD88 recruits members of the IL-1R-associated kinase (IRAK) family (IRAK-1 and IRAK-4) and TNF receptor-associated factor (TRAF-6).[8] Linker molecules, transforming growth factor beta (TGF-beta)-activated kinase (TAK-1), TAK-binding protein (TAB)-1, and TAB-2 activate downstream IkB kinase kinases (IKK); in a complex series of sequential cascade of subsequent signal amplification step(s), IKK-mediated phosphorylation of the inhibitory IkB family proteins leads to its ubiquitination and proteasomal degradation, enabling the nuclear translocation and/or shuttling of NF-kB, eventually resulting in activation of target genes encoding proinflammatory cytokines (TNF-α, IL-1, and IL-6) and chemokines (IL-8).[8] Interestingly, all TLRs, except TLR3, use the MyD88-dependent signaling pathway; on the contrary, the MyD88-independent pathway involves TRIF *via* the bridging adaptor TRIF-related adaptor molecule to activate NF-kB in either a TRAF-6-dependent fashion or a TRAF-6-independent manner, involving the kinase receptor-interacting protein (RIP)-1.[7-9,12] Moreover, TRIF interacts with TRAF family member-associated NF-kB activator (TANK)-binding kinase 1 (TBK-1) and inducible IKB kinase (IKK-e, also known as IKK-i), which phosphorylate IRF-3 and IRF-7, leading to their nuclear translocation and subsequent induction of type 1 IFN genes.[13] TRAF-3 is essential for the induction of type 1 IFN and the anti-inflammatory cytokine IL (IL)-10, and indispensable for expression of proinflammatory cytokines.[7] TRAF-3 adaptor molecule, therefore, appears to be a critical "cellular/molecular signaling rheostat" for TRIF-mediated IRF-3 activation and IFN-beta production. Moreover, C5a, the C5 cleavage fragment of the complement system, has a negative influence on TLR4-induced IL-12, IL-23, and IL-27 syntheses from murine macrophages *via* extracellular signal regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)-dependent pathways.[8] TLRs have been

implicated in inflammatory disorders/diseases, *viz.*, allergic airway inflammation, asthma, cancers (cervical, prostate, breast, gastric, and colorectal carcinomas), vasculoproliferative disorders including neointimal hyperplasia and restenosis, neurodegeneration, sepsis, and reproductive disorders.[14] The immunotherapeutic role of TLRs in reproductive physiology and pathophysiology is now beginning to be recognized; therefore, future research studies are warranted using *in vitro*, *in vivo*, and patient-centric research approaches with a strong clinical impact so as to reduce the increasing prevalence of infertility in ethnic cohorts of diverse geographic locations and varying lifestyles.

TLR signaling in infertility

Unraveling the intriguing receptor-mediated cell signaling in oogenesis, spermatogenesis, fertilization, placentation, and implantation in human reproduction and disease is the objective of advanced research investigations/studies in biomedical sciences, primarily reproductive medicine field in both the United States of America, including States of Texas, New York, and Nebraska, and the Asian subcontinent, including North India. TLRs may prove to be a boon in the treatment of reproductive disorders; high throughput, sophisticated, cell and molecular biology-based studies will be immensely valuable in the eventual identification and validation of TLRs as patient-specific predictive and/or prognostic biomarkers.

TLRs, a family of evolutionarily conserved pathogen recognition receptors, are emerging as pivotal players in the pathophysiology of a spectrum of human diseases, including infertility. Inflammation and infections in the female reproductive tract are common causes of infertility globally.[1] TLRs have significant roles in the immune system and initiate inflammatory response(s) to foreign pathogens including bacteria, viruses, and fungi, and are emerging as plausible susceptible markers in diverse human diseases, including reproductive disorders/diseases in both men and women.[7] Public health research studies for developing cost-effective infertility assessment programs in low-resource settings targeting North Indian couples are ongoing so as to reduce the burden of disease as well as psychosocial factors associated with infertility.[10] Significant recent advances in microarray and next-generation sequencing technologies have enabled the application of whole-genome approaches to the study of infertility. The physiology of human reproduction and disease is indeed complex, and scientific and clinical research efforts are warranted to provide a better understanding of the underlying cellular and molecular mechanisms involved in different phases of reproduction. Oogenesis involves a series of complex processes that produce a highly differentiated cell specialized for fertilization, with the distinct properties of a reduced chromosome complement, stored maternal mRNAs and energy precursors, pluripotency, and the ability to modify epigenetic marks on the paternal genome.[11] A plethora of growth factors, many belonging to the TGF-beta superfamily, are expressed by ovarian somatic cells and oocytes in a stage-specific developmental manner and function as intraovarian regulators of folliculogenesis; bone morphogenetic proteins, BMP-4 and BMP-7, are expressed by ovarian stromal cells and/or theca cells and have recently been implicated as positive regulators of the primordial-to-primary follicle transition.[15] Activin may play a positive role in oocyte maturation; in addition to its endocrine role to suppress Follicle Stimulating Hormone (FSH) secretion, increased output of inhibin by the selected dominant follicle(s) may upregulate Leutinizing Hormone (LH)-induced androgen secretion, which is required to sustain a relatively high level of estradiol secretion during the preovulatory phase.[15] A majority of physiological events in spermatogenesis are well coordinated *via* signal amplification at the cell–cell interface through cell junctions, illustrating the significance of cell junctions and adhesion in spermatogenesis.[16] Moreover, developing germ cells migrate across the seminiferous epithelium from the stem cell niche located in the basal compartment near the basement membrane of the tunica propria adjacent to the interstitium; additionally, synergistic and concerted actions of actin regulatory and polarity proteins may coordinate cyclic fluctuations of adhesion at Sertoli–Sertoli and Sertoli–germ cell interface(s) in the seminiferous epithelium during the epithelial cycle of spermatogenesis.[16] Receptor-mediated cell signaling, including calcium-mediated signaling regulating inositol triphosphate-induced release from internal stores in biological systems, is a key aspect in oocytes' response to fertilization in diverse species; initiation of embryo development depends on physiological $[Ca^{2+}]_i$ increase(s) in the egg, which is generally induced during fertilization.[17] The physiological increase in concentration of intracellular calcium level(s) and spatiotemporal patterns signal egg activation, which is the first stage in embryo development, with subsequent structural and biochemical modifications that transform eggs into zygotes.[17] Furthermore, the establishment of a successful pregnancy requires the implantation of a competent blastocyst into a "receptive" endometrium, facilitating the formation of a functional placenta.[18] One

of the major causes of infertility is inadequate implantation and placentation leading to first-trimester miscarriage(s), placental insufficiency, obstetric complications, etc.; embryo implantation comprises receptor-mediated and transmembrane cellular interactions between an implantation-competent blastocyst and a receptive uterus, which occurs in a limited time frame referred to as the window of implantation; aberrant physiological and/or metabolic defects originating during embryo implantation may lead to adverse consequences on later gestation events and unsuccessful pregnancy.[19] Following fertilization (union of an egg with a sperm), the zygote undergoes multiple divisions and morphogenesis to form the next embryonic stage, blastocyst, with two distinct cell lineages: the outer specialized trophectodermal epithelium and the inner cell mass; the blastocyst participates in the first biophysical/physiological receptor-based and/or cell signaling-mediated interaction(s) with the maternal endometrium for initiation of implantation. Thereafter, subsequent bidirectional biochemical cross-talk(s) are essential for normal implantation and successful pregnancy, because signaling perturbations may generate adverse outcomes for further development, including decidualization and placentation, with potential loss of pregnancy. Early pregnancy loss occurring during the peri-implantation period before pregnancy is a relatively common biological phenomenon in humans.[10]

Targeted manipulation of the TLR signaling pathway has immense practical/scientific utility; TLRs may be further validated as predictive biomarkers for diminished ovarian reserve, genital tuberculosis, and infertility in women of ethnically disparate populations, including North Indian women, thereby having wide economic implications in the future design of cost-effective public health infertility screening and disease management protocols in low-resource settings and accordingly stratifying susceptible individuals prior to *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) so as to have successful conception rates/pregnancy outcomes in women of developing countries. Inflammation and infections in the female reproductive tract are common causes of infertility globally; the activation of the innate immune system involving TLR2 and TLR2/6 in the uterus at the time of implantation has been demonstrated to interfere with the endometrial receptivity and thereby reduce the chances of implantation success.[20] TLRs have been implicated in endometriosis/adverse reproductive outcomes.[21] A recent study revealed that bacterial PAMPs initiate inflammation and perturb the endocrine function of bovine granulosa cells from dominant follicles *via* TLR2 and TLR4 pathways; supernatants of primary bovine granulosa cells accumulated IL-1β, IL-6, and IL-8 when treated for 24 h with Pam3CSK4 (PAM) that binds TLR2 or LPS that binds TLR4 but not flagellin that binds TLR5; granulosa cell responses to PAM or LPS were rapid, with increased phosphorylation of p38 and ERK1/2 within 30 min and increased abundance of IL6, IL1B, IL10, TNF, IL8, and CCL5 mRNA after 3 h of treatment, thereby strongly implicating TLR2 and TLR4 in endocrine-related disorders, including infertility.[22] Furthermore, bacterial endotoxins, LPS, and peptidoglycan can be detected in human semen; the activated TLRs, primarily TLR2 and TLR4, reduce sperm motility, induce sperm apoptosis, and significantly impair the potential for fertilization.[23] Although the cause of diminished ovarian reserve is multifactorial, it is possible that many cases attributed to an idiopathic cause may have a genetic component, including gene polymorphisms. A recent review on the genetic associations with diminished ovarian reserve implicated the emerging role of host genetic factors in infertility; one gene mutation (FMR1), three polymorphisms (GDF9, FSHR, and ESR1), and seven genes differentially expressed between women with diminished ovarian reserve and controls (AMH, LHCGR, IGF1, IGF2, IGF1R, IGF2R, and GREM1) were highlighted.[24] TLR4 and MBL2 polymorphisms have been implicated in receptiveness to pathogens causing genital tract infections and susceptibility to tubal factor infertility; the TLR4 Asp299Gly and Thr399Ile heterozygosity was associated with a decreased incidence of pathogens associated with genital tract infections in tubal factor infertility patients.[25] Furthermore, in hen ovarian follicles, theca cell layer expresses TLR2/4/5/7, while the granulosa cell layer expresses TLR4/55;[26] TLR4 was detected in porcine ovary,[27] and TLR5 is expressed primarily in human ovary, peripheral blood leukocytes, and prostate.[28] Interestingly, laboratory data in murine models have demonstrated the expression of TLRs 2, 4, 8, and 9 in mouse granulosa cells and cumulus cells; TLR2 and TLR4 are functional because their ligands, Pam3Cys and LPS, respectively induce expression of known TLR2/TLR4 target genes, *viz.*, Ptgs2, Il6, and TNF-α.[29] Microarray database on cumulus–oocyte-complex (COC) samples collected from preovulatory follicles at 0 h, 8 h, and 16 h post-hCG revealed the presence of a majority of key components of the complex TLR signaling pathway; these include the

genes encoding adaptor factors (CD14, Ly96, Myd88, Ticam1, Tirap, and Tollip), IRAKs, TNF-α receptor-associated factors (TRAFs), and IRFs, thereby strongly indicating the potential involvement of the TLR immune surveillance system in the ovulation process.[28,29] Obesity may enhance the risk of developing infertility because of physiologically altered and/or aberrant TLR spatiotemporal function(s) in adipocytes that are associated with the release of immune cell-related factors, cytokines, and other metabolic products that regulate the components of the reproductive system, including ovary.[30] Genetic studies are essential to identify/stratify disease-susceptible individuals in a population; host genetic differences that influence the primary immune response may determine those who are at increased risk for progression to clinical infertility. It is now becoming increasingly apparent that TLRs have considerable role in reproductive/endocrine diseases as well as gynecologic malignancies, including Human Papillomavirus-mediated cervical cancer.[31] In this context, association analyses in a population setting can be used to explore the role of genetic polymorphisms in susceptibility to various reproductive/endocrine disorders, including clinical infertility. Moreover, early identification of infertility-susceptible individuals at the population level may be successfully achieved by TLR-based therapeutics and TLR-based biomarker studies in an ethnically disparate cohort.[31]

SUMMARY/CONCLUSIONS

Infertility is a major public health problem in recent times;[32] despite significant research developments in IVF and embryo transfer (IVF-ET) technology that have significantly overcome many underlying causes of infertility, pregnancy success rates remain relatively low, primarily because of implantation failure. Molecular genetic evidence indicates that ovarian hormones together with locally produced signaling molecules, including transmembrane receptors, cytokines, growth factors, homeobox transcription factors, lipid mediators, and morphogen genes, function *via* autocrine, paracrine, and juxtacrine interactions to specify the complex process of implantation;[33] cross-talk between blastocyst and uterus mainly occurs during a brief time period, namely the "window of implantation".[34] Embryo implantation occurs toward the end of luteal phase of reproductive cycle; ovarian estrogen interacting *via* the nuclear Estrogen receptors (ERs) is required for the preparation of the receptive uterus, whereas its catechol metabolite 4-hydroxyestradiol produced locally in the uterus activates the blastocyst for subsequent implantation.[34] Moreover, the author speculate that TLR signaling may play a key role in the physiological regulation of the hypothalamo–pituitary–ovarian axis and neuroendocrine regulation of GnRH secretion and LH pulsatility states. To summarize, tailor-made personalized pharmacogenetic patient-centric immunotherapeutic treatment strategies by targeted manipulation of the TLR signaling regulatory network(s) may be highly beneficial in the eventual treatment of infertility in diverse population(s) of varying genetic landscape(s), thereby further enhancing our scientific/clinical expertise in reproductive medicine and research. Future TLR-based patient-centric cell/molecular biology translational research studies in pooled clinical samples of patients of diverse origins are warranted to provide a more comprehensive understanding of the immunobiological roles of TLRs and inter-related cell signaling pathways in clinical infertility worldwide.

Acknowledgements

Dr. Saumya Pandey, Dy. Director (Academics, Clinical Research, Publications, Grants), Ajanta Hospital and IVF Centre, Lucknow, U.P., India (former) acknowledges the initial collaborative research appreciation received from Drs. Anil and Gita Khanna of Ajanta Hospital-IVF Centre, Lucknow, U.P., India, and Dr. Sanjay Mehrotra of RML Path. Labs., Lucknow, U.P., India and Dr. Chandrawati of Krishna Medical Centre, Lucknow, U. P., India. Dr. Pandey is especially grateful to Dr. P.S. Schleigel (Chairman/Urologist-in-Chief) and Dr. D. Scherr of Department of Urology, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York City, NY, USA for providing an offer letter for a Visiting/Guest Faculty assignment including clinical observership, research and/or teaching in 2015.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Pandey S, Khanna G, Bajpai A, Khanna A. Emerging role of Color Doppler in infertility management: A public health perspective. Fertil Sci Res 2014;1:87-91.
2. Pandey S, Murphy RF, Agrawal DK. Recent advances in the immunobiology of ceramide. Exp Mol Pathol 2007;82:298-309.

3. Bojar I, Witczak M, Wdowiak A. Biological and environmental conditionings for a sperm DNA fragmentation. Ann Agric Environ Med 2013;20:865-8.
4. Homan G, Litt J, Norman RJ. The FAST study: Fertility ASsessment and advice Targeting lifestyle choices and behaviours: A pilot study. Hum Reprod 2012;27:396-404.
5. Davar R, Sekhavat L, Naserzadeh N. Semen parameters of non-infertile smoker and non-smoker men. J Med Life 2012;5:465-8.
6. Seifer DB, Baker VL, Leader B. Age-specific serum anti-Müllerian hormone values for 17,120 women presenting to fertility centers within the United States. Fertil Steril 2011;95:747-50.
7. Pandey S, Mittal B, Srivastava M, Mittal RD, Singh S, Srivastava K, *et al*. Evaluation of Toll-like receptors 3 (c.1377C/T) and 9 (G2848A) gene polymorphisms in cervical cancer susceptibility. Mol Biol Rep 2011;38:4715-21.
8. Pandey S, Agrawal DK. Immunobiology of Toll-like receptors: Emerging trends. Immunol Cell Biol 2006;84:333-41.
9. Akira S, Takeda K, Kaisho T. Toll-like receptors: Critical proteins linking innate and acquired immunity. Nat Immunol 2001;2:675-80.
10. Pandey S, Tyagi R. Risk factors for miscarriage from a prevention perspective: A nationwide follow-up study. BJOG 2014;121:1439.
11. Liu Z, Shimada M, Richards JS. The involvement of the Toll-like receptor family in ovulation. J Assist Reprod Genet 2008;25:223-8.
12. Schröder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. Lancet Infect Dis 2005;5:156-64.
13. Carneiro LC, Cronin JG, Sheldon IM. Mechanisms linking bacterial infections of the bovine endometrium to disease and infertility. Reprod Biol 2016;16:1-7.
14. Bhanothu V, Lakshmi V, Theophilus JP, Rozati R, Badhini P, Vijayalaxmi B. Investigation of Toll-like receptor-2 (2258G/A) and interferon gamma (+874T/A) gene polymorphisms among infertile women with female genital tuberculosis. PLoS One 2015;10: e0130273.
15. Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, *et al*. Physiological and molecular determinants of embryo implantation. Mol Aspects Med 2013;34:939-80.
16. Cheng CY, Wong EW, Lie PP, Mruk DD, Xiao X, Li MW, *et al*. Polarity proteins and actin regulatory proteins are unlikely partners that regulate cell adhesion in the seminiferous epithelium during spermatogenesis. Histol Histopathol 2011;26:1465-74.
17. Kinsey WH. Intersecting roles of protein tyrosine kinase and calcium signaling during fertilization. Cell Calcium 2013;53:32-40.
18. Zhao Y, Chegini N. The expression of granulocyte macrophage-colony stimulating factor (GM-CSF) and receptors in human endometrium. Am J Reprod Immunol 1999;42:303-11.
19. Sobinoff AP, Sutherland JM, Mclaughlin EA. Intracellular signalling during female gametogenesis. Mol Hum Reprod 2013;192:65-78.
20. Sanchez-Lopez JA, Caballero I, Montazeri M, Maslehat N, Elliott S, Fernandez-Gonzalez R, *et al*. Local activation of uterine Toll-like receptor 2 and 2/6 decreases embryo implantation and affects uterine receptivity in mice. Biol Reprod 2014;90:87.
21. Khan KN, Kitajima M, Fujishita A, Nakashima M, Masuzaki H. Toll-like receptor system and endometriosis. J Obstet Gynaecol Res 2013;39:1281-92.
22. Price JC, Bromfield JJ, Sheldon IM. Pathogen-associated molecular patterns initiate inflammation and perturb the endocrine function of bovine granulosa cells from ovarian dominant follicles *via* TLR2 and TLR4 pathways. Endocrinology 2013;154:3377-86.
23. Fujita Y, Mihara T, Okazaki T, Shitanaka M, Kushino R, Ikeda C, *et al*. Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. Hum Reprod 2011;26:2799-806.
24. Greene AD, Patounakis G, Segars JH. Genetic associations with diminished ovarian reserve: A systematic review of the literature. J Assist Reprod Genet 2014;31:935-46.
25. Laisk T, Peters M, Saare M, Haller-Kikkatalo K, Karro H, Salumets A. Association of CCR5, TLR2, TLR4 and MBL genetic variations with genital tract infections and tubal factor infertility. J Reprod Immunol 2010;87:74-81.
26. Subedi K, Isobe N, Nishibori M, Yoshimura Y. Changes in the expression of Toll-like receptor mRNAs during follicular growth and in response to lipopolysaccharide in the ovarian follicles of laying hens. J Reprod Dev 2008;53:1227-35.
27. Alvarez B, Revilla C, Chamorro S, López-Fraga M, Alonso F, Domínguez J, *et al*. Molecular cloning, characterization and tissue expression of porcine Toll-like receptor 4. Dev Comp Immunol 2006;30:345-55.
28. Chaudhary PM, Ferguson C, Nguven V, Nguven O, Massa HF, Ebv M, *et al*. Cloning and characterization of two Toll/interleukin-1 receptor-like genes TIL3 and Til4: Evidence for a multi-gene family in humans. Blood 1998;91:4020-7.
29. Shimada M, Hernandez-Gonzalez I, Gonzalez-Robanya I, Richards JS. Induced expression of pattern recognition receptors (PRRs) in cumulus oocyte complexes (COCs): Novel evidence for innate immune-like cells functions during ovulation. Mol Endocrinol 2006;20:3228-39.
30. Pevsner-Fischer M, Morad V, Cohen-Sfady M, Rousso-Noori L, Zanin-Zhorov A, Cohen S, *et al*. Toll-like receptors and their ligands control mesenchymal stem cell functions. Blood 2008;109:1422-32.
31. Pandey S, Chandravati. Homogeneity in case/control numbers and North Indian caste criteria in cervical cancer/female urology genetic-studies at a premier Medical Research Institute in Lucknow, India. Asian Pac J Cancer Prev 2013;14:6185-7.
32. Montazeri M, Sanchez-Lopez JA, Caballero I, Maslehat Lay N, Elliott S, López-Martín S, *et al*. Activation of Toll-like receptor 3 reduces actin polymerization and adhesion molecule expression in endometrial cells, a potential mechanism for viral-induced implantation failure. Hum Reprod 2015;30:893-905.
33. Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, *et al*. Molecular cues to implantation. Endocr Rev 2004;25:341-73.
34. Ma WG, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc Natl Acad Sci USA 2003;100: 2963-8.