INTRODUCTION

Over the last few decades, the human population continues to soar up and maybe it will step at 8 billion by 2025 (worldpopulationreview.com). Main players, stringency of contraceptive methods and unintended pregnancies are striking shots for this increment and causing serious stress on the available resources; consequently, the whole world appears to be hanged by a thread. Worldwide 200 million cases of unintended pregnancies end at around 46 million abortion eventually in hostile physical and emotional health outcomes of both women and their young ones (Bahk et al., 2015) which finally affect social, economic and cultural parts of community health (Bishwajit et al., 2017). For a given couple the decision either to establish or avoid pregnancy is intensely personal and depends on both but, in a traditionally male-dominated society like India, the child-bearing responsibility of women and issues of trust of the male partners consistently articulate the situation of women. The problem does not end here, due to urbanization, social and economic development a woman typically spends more time to avoid pregnancies (Sedgh et al., 2014). Contraception is an important constituent of protective methods that allows women to have planned pregnancies. It is important for optimal health as well as social and economic consequences for women, families and society. Presently, contraceptive modalities available to women include hormonal contraceptives (birth control pills, contraceptive patch, and hormone injections), barrier methods, intrauterine devices (IUDs; copper IUD, vaginal rings, diaphragm, and spermicidal combinations), natural methods (abstinence near ovulation and early withdrawal before ejaculation) and sterilization. Combination oral contraceptive pills (OC) containing a balanced amount of estrogen and progestin are admired for their contraceptive effect and reversibility among rest of contraceptives. Oral contraceptives prompt an imbalance of reproductive hormones in the hypothalamus-pituitary-ovary axis, and prevent ovulation. Progestin also thickens the cervical mucus, thus creates hindrance in the way of sperm to move into the uterus and oviduct. Regretfully, these sex steroids are not unoffending; the surplus amount of either hormone in blood circulation can lead to nausea, weight gain and acne while major side effects may include thromboembolism,
hypertension, hyperlipidemia, cardiovascular disease, breast and cervical malignancies (Shoupe and Mishell, 2013). The intramuscular injections of progestin, depo-medroxyprogesterone acetate (DMPA) as contraceptive agents make vagina epithelial lining thin, and so increase the chances of microbial infection to vaginal area (Spevack, 2013). Intrauterine devices require a trained clinician to insert and the possibility of expulsion exists if not properly inserted. Their prolonged use may cause increased menstrual bleeding and pain in some cases (Speroff and Darney, 2005). Punching of cellular membrane and risk of infection following insertion of IUDs are additional adverse proceedings linked with them (Kaneshiro and Tod, 2010). Condoms if used properly and regularly are the only perceptible method for prevention of both unwanted pregnancies and transmission of potential pathogens of sexually transmitted infections (STIs) as sperm or STI-inducing microorganisms cannot infiltrate the polyurethane or polyisoprene condom layer. However, their use is intercourse dependent and requires mutualism from the male partner. The unwillingness of men to use condoms and women’s weakness in negotiation with the partner limits the use of this simple option of fertility control. Cultural preconceptions, breakage or slippage of condoms during intercourse are some other compromising issues that diminish the efficacy of condoms (Shoupe and Mishell, 2013). Female condoms represent an attempt to overcome the issues related to male condoms but most often are abandoned by women because of their efficacy and acceptability. Women who use the other barrier methods of contraception, e.g. diaphragm, cervical cap, contraceptive sponge, however, are at higher risk of vaginal infection, urinary tract infection and possibly toxic shock syndrome (Bharitkar et al., 2013). Natural family planning is a rhythmic method based on imprecise signs of women’s ovulation and many couples find it difficult to predict safe period (days in which unprotected intercourse won’t lead to pregnancy), particularly in case of women with irregular menstrual cycles for the copulation. Sterilization is an irreversible method with the inherent risks of surgery i.e. infection, blood loss and damage to internal organs (Gupta, 2005).

Various side effects, difficulties in using contraceptive methods consistently, a wide range of failure rates, cost, negotiation with male partner and cultural biases are some of the major leading factors that intercept contraceptive use frequently; accordingly need of around one hundred twenty million couples
remain unfilled each year and societies especially in developing countries meet high birth rates which consistently burden the earth (Ross and Winfrey, 2002; Shah and Ahman, 2009; Sitruk-Ware et al., 2013). The most important properties of an ideal contraceptive method desired by women are: safety (non-steroidal & non-surgical), efficacy, inexpensive, easily accessible, infrequent administration, long duration of action, fast reversibility and can be self-applied privately (Naz, 2006). Thus, an urgent need exists to develop a better method of contraception in women compliance.

Topical vaginal spermicides/contraceptives can address many of the issues associated with available chemical and physical contraceptive methods, may fit in women agreement and provide the power to defend themselves against unintended pregnancies. Vaginal contraceptives are the oldest method of fertility regulation. They are used topically only during the copulation hence can overcome the systemic consequences and are considered as one of the safest methods. They are more advantageous over other available contraceptives such as: being local agents do not cause side effects such as vomiting, headache and weight gain or others which are usually seen with the uptake of hormonal contraceptives, no bleeding or pain which is often accompanied with the insertion of IUDs. They can be self-applied just before intercourse in private omitting the need of any skilled personnel. Unlike condoms, the natural feeling persists during intercourse and overcome the negotiation with a male partner. Ease in availability and their reversible effect may add to their acceptance as contraceptive agents. However, even after these advantages the vaginal contraceptives had become the ‘ugly step-child’ of birth control methodology and given little research emphasis (Zaneveld, 1994). They received a setback with the introduction of oral contraceptive pills (OCPs) and intrauterine devices (IUDs) in the 1960s and remained virtually forgotten for a few decades. The exponential increase in unintended pregnancies and sexually transmitted diseases (STDs) like HIV infections worldwide renewed the researchers’ interest in this area. Commercially available vaginal contraceptive, such as nonoxynol-9 (N9), sodium dodecyl sulfate (SDS) and benzalkonium chloride rely on the cytotoxic action of detergents (Hughes et al., 2007). They are effective spermicides and their mode of action includes disruption of the plasma membrane of sperm. However, their act lack in cellular specificity so their frequent use impair the epithelial lining of
the lower female reproductive tract and hence, render the user to discomfort, vaginal irritation and increase susceptibility to STDs, including HIV (Fichorova et al., 2001; Van Damme et al., 2002). Furthermore, the non-specific action of these surfactants like N-9 and benzalkonium chloride adversely affects the viability of lactobacilli, the microflora of vaginal tissues (Richardson et al., 1998; Bélec et al., 2000), consequently, the disturbance in vaginal microflora increases the risk of vaginosis (Hay, 2005). Thus, the development of vaginal spermicidal agents without any cytotoxic effect may be a significant progress over the present spermicides.

Researchers are trying to develop non-detergent, safe, local contraceptives, and in this direction, several other alternatives constraining the sperm motility are being examined to be functional as non-invasive/non-cytotoxic vaginal contraceptives. Some of the synthetically prepared molecules such as acrylophenones, vanadocenes, gel microemulsions (GM-4, GM-44), anysylidines, thymols, and isoxazoles/oxazolines have non-detergent property and shown potent spermicidal activity (Singh et al., 2010). Amongst the natural products saponin, a surfactant obtained from the fruit pericarp of soap nut (Sapindus mukorossi) revealed effective spermicidal activity. Its cream formulation named “CONSAP” has been approved by the Drug Controller General of India (DCGI) and will be available in the market for use in near future (www.cdri.res.in/Consap.aspx). The acceptance and efficacy of ‘CONSAP’ by the users still remains to be seen. Spermicidal activity of emetine, verapamil and tartaric acid has been reported (Maikhuria et al., 2003). Neem tree aqueous extract and Mentha citrate oil also tested in a vaginal tablet. In Phase I study the tablet showed the contraceptive effect (Joshi et al., 2005).

Antimicrobial peptides (AMPs) are one of the natural defence systems of most living organisms against assailing pathogens. Besides their antimicrobial activities, they are being inspected for their contraceptive properties. An antimicrobial peptide, Magainin-A member of amphibian amphipathic α-helical family was isolated from the skin of frog Xenopus laevis. Reddy and co-workers demonstrated that magainin blocks contraception in rabbits effectively; it is a safe intravaginal compound since no alteration was seen in vaginal epithelial cells. Hematologic and serum biochemical profile of rabbits also remained normal after treatment with magainin (Reddy and Manjramkar, 2000; Aranha et al., 2004b).
Dermaseptins (DSs) are synthesized by amphibians (Zairi et al., 2005) and they exhibit spermicidal action by creating cation specific pores in the spermatozoa membrane. Nisin is an ionic antimicrobial peptide produced by bacteria Lactococcus lactis. It is spermicidal in vitro and leads to curtailment of fertility in rats and rabbits (Aranha et al., 2004a; Reddy et al., 2004; Gupta et al., 2009). Bacillus subtilis produces antimicrobial peptide subtilosin which has been reported to show its potential in jamming the movement of sperm. It inhibited the motility of human spermatozoa in a dose-dependent manner and can be considered as a preventive component aimed at fertility control (Sutayak et al., 2008). However, the cost of production of these peptides in pure form on a large scale has been a major obstacle for quite some time (Hughes et al., 2007; Zairi et al., 2009). Secondly, all these compounds are unripe and yet not be able to reach human trails. Therefore, the demand for a newer contraceptive agent that can clearly fulfill the contraceptive needs of developing countries is still on.

There is a growing interest towards the microbial armoury to dig out some intensive contraceptive agent, which exclusively averts pregnancy in women who have been exposed to unprotected sex. The negative influence of microorganisms on spermatozoa has been reported which is partly due to either direct bacterial attachment that might cause the agglutination/ immobilization of motile sperm or due to the production of some factors that cause morphological alterations and reduce the ability of sperm for acrosome reaction (Diemer et al., 2000). Chlamydia trachomatis block human sperm motility and cause premature death in vitro (Eley et al., 2005). Impairment in both sperm motility and its ultrastructure was seen with Candida albicans culture as well as its filtrates (Tian et al., 2007). The Ureaplasma urealyticum is associated with the production of reactive oxygen species that cause sperm membrane peroxidation and eventually loss of motility and impairment of acrosome reaction (Xiao-ping and Xiao-qin, 2017). Escherichia coli have been suggested to have the most negative influence on sperm motility and morphology (Golshani et al., 2006). Sperm motility impairing effect of E. coli can be by the action of soluble factors that stimulate cell death (Schulz et al., 2010). Further, Staphylococcus aureus mediated profound depression in motility of human spermatozoa in vitro was also reported (Liu et al., 2002; Kaur et al., 2010). Human sperm apoptosis induced by E. fecalis, E. coli and S. aureus was also demonstrated by Villegas et al. (2005).
Evidenced role of microorganisms in sperm impairment inflamed the engagement of microbial metabolites as a preventive armamentarium in the field of contraceptive research. Hence, various microbial spermicidal factors are being investigated and applauded by recent scientific horizon for their spermicidal properties (Tian et al., 2007; Prabha et al., 2009; Prabha et al., 2010; Schulz et al., 2010; Barbonetti et al., 2013; Cano-Chaves et al., 2017). During the extended research in the area of vaginal contraceptives, in our laboratory we isolated a *E. coli* strain producing a sperm immobilizing factor (SIF). The potential of SIF to inhibit sperm motility has strengthened its utility as spermicide that might be utilized for the development of an effective vaginal contraceptive. However, native factor’s limitations like impurities associated with wildtype *E. coli* protein fractions and low yield from the native source were hindering further progress with this factor in its present form. To fill these gaps we have done our work in the persuasion of recombinant DNA technology to produce a purified recombinant sperm immobilizing factor. We have identified, cloned and overexpressed the encoding gene for SIF, then, purified recombinant factor and finally checked its sperm immobilizing property of *in vitro* and contraceptive potential in the murine model.

Keeping the available literature, the ultimate goal and the previous research work performed in our laboratory we framed the objectives of our study.
Aim of the study
Identification of the gene encoding sperm immobilizing factor from *Escherichia coli*, cloning, over-expression of the gene and evaluation of the contraceptive efficacy of the recombinant factor.

Objectives
The present study was carried out with the following objectives:
1. Identification of the gene encoding sperm immobilizing factor.
2. Cloning and over-expression of gene encoding sperm immobilizing factor.
3. Standardization of growth conditions for the maximal production of the recombinant sperm immobilizing factor.
4. To determine the role of recombinant sperm immobilizing factor on sperm motility and morphology.
5. *In vivo* evaluation of the recombinant factor for its contraceptive efficacy.
6. Safety studies of recombinant sperm immobilizing factor in the murine model.