INTRODUCTION
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Infertility is a subject of worldwide interest and important in both clinical practice and research, affecting both men and women of reproductive age, causing considerable personal suffering and disruption of family life. On the basis of the surveys made by WHO, 8-10% of couples experience some form of infertility problem during their reproductive lives. When this is extrapolated to the global population, it has been observed that 50-80 million people may be suffering from infertility.

According to WHO (1992) infertility is defined as "two years of exposure to the risk of pregnancy without conceiving". Female infertility could be primary or secondary. Its incidence varies from country to country and in India it is about 3% and 8% respectively (WHO, 1992). In an advanced country like the United States, 2.1 million couples (8.5% of all married couples) are affected by infertility (Anonymous, 1988).

Infertility could be explained or unexplained. In case of explained infertility the reasons are known and the treatment could be possible. For example, if tubal damage is diagnosed by means of hysterosalpingogram or laparoscopy, microsurgery could be done. Ovulation inducing agents improve ovulation in case of infertility due to ovulation disorders. Vaginitis and cervicitis, if related to infertility, could be treated. The criteria for the diagnosis of unexplained infertility includes,
infertility of at least two years, normal history and physical examination, adequate coital frequency, three normal semen analyses, regular monthly menstrual cycles with biphasic basal body temperature and luteal phase of more than 12 days, adequate cervical mucus and a normal postcoital test (PCT), normal hormonal profile, normal hysterosalpingogram (HSG) and laparoscopy (Pepperell and McBain, 1985; Jaffe and Jewelewicz, 1991).

Among the various reasons suggested for unexplained infertility, bacterial infections have long been postulated as having an association with infertility (Rosenthal, 1931; Wu, 1950; Kudsin et al., 1967; Brunham et al., 1985; Eggert-Kruse et al., 1990; Tuffrey et al., 1990; Spiegel, 1991). Infection related infertility due to *Mycoplasma hominis* (Gnarpe and Friberg, 1972), *Ureaplasma urealyticum* (Toth and Lesser, 1982), *Trichomonas vaginalis* (Tuttle et al., 1977b), *Chlamydia trachomatis* (Fedele et al., 1989; Brunham et al., 1985) and *Neisseria gonorrhoeae* (James-Holmquest et al., 1974) have been reported. According to WHO (1987), infection related causes of female infertility are more widespread in the world and this type of infertility is preventable.

Postinfectious infertility is also a big problem. The classic study by Westrom (1980) in Lund, Sweden, indicated that pelvic inflammatory disease develops in 6-60% patients, depending on the severity of the infection, the number of
episodes of infections and the age when the initial infection occurred. In the 20 years period from 1960-1980, the importance of infection as a cause of infertility doubled. This was true in Sweden as well as in the United States and in many other countries. After three episodes of pelvic inflammatory disease (PID), 75% of the patients are at risk for infertility. Following one episode, 10% are at risk for ectopic pregnancy.

The vaginal and cervical flora has been characterized in premenarcheal girls, postmenarcheal, pregnant and postmenopausal women in studies that have been reviewed by Ison (1990) and Hill et al. (1984). The vaginal microflora is greatly influenced by estrogens. In utero, the vagina of the fetus is microbiologically sterile. For the first 6 weeks of life, maternal estrogens are present in the vaginal epithelium, providing it with the morphology and microbiology of the adult vagina i.e. facultative lactobacilli predominate (Brown, 1978). After the estrogens have been metabolized, the flora contains skin organisms e.g., coagulase-negative staphylococci and faecal organisms like Escherichia coli. After menarche, the facultative lactobacilli and diphtheroidal forms, which include Gardnerella vaginalis, predominate. The other microorganisms of healthy vagina and cervix includes Staphylococcus aureus, Staphylococcus citreus, Streptococcus sp. (alpha and beta types), Proteus sp., Alcaligenes faecalis, Bacillus subtilis, Pseudomonas
aeruginosa, Escherichia coli, Gaffkya tetragena, Prevotella bivia, Prevotella disiens and other Prevotella sp. (Spiegel, 1991). It is quite clear that in the female genital tract, microorganisms are present which may be aerobes, anaerobes or facultative. However, limited data is available whether there are different types of microorganisms present in the genital tract of fertile and infertile women having clean cervices i.e. without any disease.

Microorganisms present in the cervix do come in contact with spermatozoa and the obvious question arises whether the microflora effect the functioning of spermatozoa in some way. In this regard since 1930’s (Rosenthal, 1931), there have been sporadic references in literature with respect to agglutination and immobilization of spermatozoa by various microorganisms (Teague et al., 1971; Del-Porto et al., 1975; Tuttle et al., 1977a, Gupta et al., 1978; Kaur et al., 1986), bacterial endotoxins or their metabolic end products (Dennis, 1962; Nowakowski, 1981), unidentified factor from E.coli (Paulson and Polakoshi, 1977) or proteases like elastase (Kaur et al., 1988a; Kaur et al., 1988b). However, on the basis of the data available so far, it is not possible to say which compound/s from microorganisms present in the cervical area specifically influence the functioning of spermatozoa. Antibiotic treatment of spermicidal organisms in women (Friberg, 1980; Cassell et al., 1983), men (Quesada et al., 1968; Toth and Lesser, 1982) and the ejaculates
itself has resolved the infections and resulted in pregnancies in many of the infertile couples.

The potential of a semen sample is evaluated by the motility, number and morphology of the spermatozoa. Motility is an essential character for the spermatozoal function (Hinting et al., 1988). The detailed chemical and biochemical structural information about the spermatozoa is available (Mann and Lutwak-Mann, 1981). In addition to other structures, the sperm tail has nine microtubular doublets in the periphery of flagellar axonemes which are held together at 96 nm intervals by inter doublet linkage consisting at least in part (2% of the total axoneme protein) of a protein called nexin. These interdoublet linkages are highly extensible to accommodate the sliding between microtubules that occur during flagellar bending. Brokaw (1980) suggested that nexin may be chemically similar to other elastic proteins like elastin and resilin and may be sensitive to digestion by proteases particularly elastase. Thus elastase may be playing a part in the immobilization of spermatozoa. In this direction, very limited work has been done. However, it has been shown that the elastase from infertile cervical isolates affect the sperm motility (Kaur et al., 1986; Kaur et al., 1988a; Kaur et al., 1988b).

Another factor which could be taken into consideration is the conversion of the chemical energy into mechanical energy by various enzymes in the spermatozoa tail.
Dynein ATPases play a central role in the motility of sperm flagella (Gibbons, 1988) and protein kinase C has also been reported to have a possible role in flagellar motility (Rotem et al., 1990). ATPases undergo conformational changes on digestion with trypsin (Inaba et al., 1990) and are also sensitive to antifertility compounds (Kalla and Vasudev, 1980). The possibility that certain microbial enzymes could be inhibiting the ATPases of spermatozoa and thereby affecting the motility, cannot be ruled out.

So far no literature is available on the presence of any specific microflora in the infertiles. However, the question arises that if there is no difference in the microflora of the fertile and infertile women, then what could be the reason that the microorganisms render some women infertile, whereas in others they are ineffective. Some reasons have been worked out. Casslen (1986) reported the presence of inhibitors of trypsin, chymotrypsin and elastase in human uterine fluid in fertiles. No mention is made if such inhibitors are present or absent in the infertiles. Proteolytic enzymes are known to be involved in the implantation process and these enzymes tend to be balanced by inhibitors since high concentrations of trypsin-like enzymes may adversely affect the process (Dabich and Andary, 1974 and 1976). Protease and elastase inhibitors are also reported to be present in respiratory tract mucus where they inhibit the leukocyte elastase and prevents lung destruction.
by the enzymes (Boudier and Bieth, 1989). The human plasma also contains protease and elastase inhibitors (Travis and Salvesen, 1982). Thus there is every scope to study the inhibitors in genital tract secretions against the microbial proteases responsible for malfunctioning of spermatozoa. These could be responsible for the balance or imbalance in the enzyme - enzyme inhibitor concentrations which in turn may be affecting the fertility status in women.

On the basis of the foregoing commentary on the subject of the role of microorganisms in infertility, following objectives were considered for study:

1. Cervical microflora of fertile and infertile (unexplained infertility) women and their effect on human spermatozoa in terms of:
   (i) Motility
   (ii) Agglutination
   under various conditions like the age of the culture, and different temperatures.

2. Production of enzymes like proteases and elastases by microorganisms isolated from cervices of infertile and fertile women.

3. Production of elastase by some selected isolates, its purification and study the effect of purified elastase on human and rat spermatozoa with respect to:
   (i) Motility
4. Inhibitors of elastase present in:
   (i) Cervical washings (mucus)
   (ii) Intra uterine fluid
   (iii) Seminal plasma

5. In vivo experiments using rat as an animal model
   (i) To establish elastase producing organisms in the genital tract of female rats and to study their effect on fertility.
   (ii) Reisolisation of established organism from the genital tract of animals and to study any change in their elastase producing capacity.
   (iii) Treatment of the infertile animals with antibiotics to eradicate the elastase producers and study the resumption of reproduction.