2. REVIEW OF LITERATURE

Infertility is a health problem with very definite physiological, psychological and social implications. Individuals experiencing infertility express marked emotional distress, feelings of depression, isolation, anger, shame, inadequacy and personal failure (Leader et al. 1984; Domar and Siebel, 1990). These reports are hardly surprising because infertility evaluation and treatment involve tremendous physical, social and financial sacrifice. The total annual cost on infertility treatment in the U.S. alone is 1 billion (Sciarrà, 1991). Most couples experience monthly cycles of hope and optimism followed by despair at menstruation. Depressive symptoms are more prevalent in infertile women than in the fertile (Freeman et al. 1983; Dennerstein and Morse, 1985; Lalas et al. 1986; Domar et al. 1992a). Behavioural treatment has also been recommended for infertile patients (Garcia et al. 1985; Domar et al. 1992b). Because of the psychological factors associated with infertility, the infertility investigations and its treatment becomes very important.

2.1 PREVALENCE OF INFERTILITY

A study reported in 1987 indicated that infertility in the United States affects 2.1 million married couples; this is 8.5% of all married couples in the U.S. If those couples in whom a sterilization operation has already been performed are excluded, 13.9% of the married potentially
fertile couples in the U.S. will have infertility problem at some time during their lives. This figure of approximately 14% is similar to reported figures from other developed countries for a combination of primary and secondary infertility. The annual incidence of infertility in England was approximately 2 couples for every 1000 of the population, with the lifetime incidence of 17% (Hull et al. 1985). In under developed countries like Cameroon, the rate of primary and secondary infertility is 12% and 33% respectively and in developing countries like India, it is 3% and 8% (WHO, 1992).

Rowe at the WHO, made some estimates as to the number of men and women of reproductive age throughout the world affected by infertility (Rowe, 1988; Rowe and Farley, 1988). On the basis of this study and other reports it has been conservatively assumed that 8% of couples experience some form of an infertility problem during their reproductive lives. 50-80 million people in the world may be experiencing either primary or secondary infertility at the present time. From the perspective of health care delivery, approximately 2 million additional infertile couples appear per year (Sciarra, 1991).

The most comprehensive study of infertility was conducted by the WHO between 1978 and 1984 (Cates et al. 1985; WHO, 1987). Investigation in 33 centres in 25 countries throughout the developed and developing world, over 5800 couples were investigated and several important conclusions
relating to infertility patterns worldwide emerged, particularly in Africa, Asia and Latin America. This study demonstrated that bilateral tubal occlusion and infection related diagnosis were clearly important factors among infertility patients worldwide. Up to 64% of female patients in Africa and 28% to 35% of patients in other areas of the world had infertility that could be traced to prior infection. Specifically, an infectious etiology for infertility could be directly related to a woman’s history of sexually transmitted disease (STD), pelvic inflammatory disease (PID), and pregnancy complications, the latter including complications following both normal child birth and abortion. The degrees of infection differed among the four regions investigated. African centers had a pattern of infertility different from those in other developing countries (Cates et al. 1985). African couples were more likely, than those from elsewhere, to have secondary infertility, a history of STDs or pregnancy complications and infertility diagnosis (such as bilateral tubal occlusion or pelvic adhesions) suggestive of previous genital infections. 49% of the female patients studied in Africa showed infectious tubal disease and in other areas of the world 11% to 15% of patients presented this problem.

A male factor is responsible in 40% to 50% of infertile couples; some form of ovulatory dysfunction in 30%, uterine or tubal disease in 20%; cervical problems,
Numerous factors may be responsible for low conception rates. Conception and pregnancy depend on complex physiological, anatomic and immunological factors. The male needs normal spermatogenesis, reproductive anatomy and sexual function to deposit adequate number of morphologically normal, motile spermatozoa in the upper vagina. The female needs a functionally intact hypothalamic-pituitary-ovarian axis to regulate the menstrual cycle and provide normal folliculogenesis, ovulation and luteal phase hormonal milieu. For the ovum and spermatozoa to meet in the fallopian tube, the spermatozoa must initially penetrate periovulatory cervical mucus and the fallopian tube must be adequately motile and functional to pick up and transport the ovum. Once fertilization has occurred the pre-embryo is transferred to the uterus, where successful implantation depends on a hormonally stimulated endometrium maintained by a functional corpus luteum. A disruption in any aspect of these processes can result in infertility.
2.2 TYPES OF INFERTILITY

Infertility could be due either or both of the partners. In males it could be due to anatomical disorder, aspermia, azoospermia, teratozoospermia, asthenozoospermia, oligozoospermia (WHO, 1987b). In females the system is more complex and infertility could be divided into two types:

(i) **Primary infertility**: when the never pregnant woman have had uninhibited sexual intercourse without using contraception for two years without conceiving.

(ii) **Secondary infertility**: when the women who having had at least one pregnancy and have been exposed to the risk of pregnancy (have had uninhibited sexual intercourse without using contraception) for two years without conceiving.

In both cases, the etiology could be explained or unexplained. If the cause of infertility is explained, it is curable depending on the site and extent of damage in the reproductive system.

2.2.1 **Explained infertility**

The etiology of infertility is of major importance if any therapeutic or preventive measures are to be implemented, but assessment of the cause of infertility at the level of the individual couple is often difficult and time-consuming. While various factors that contribute to a couple’s infertility can be identified following a full and comprehensive investigation of both partners, it is often
difficult to attribute the infertility to any one factor and thus clearly identify a "cause of infertility" (WHO, 1992).

In women, the percentage of diagnoses that could be attributed to infectious causes varied between 28% and 64% in different parts of the world. The most common specific causes of infertility are bilateral tubal occlusion (Brunham et al. 1985), pelvic adhesions in female partner (Sciarrà, 1991), acquired tubal abnormalities, ovulatory disorders, endometritis (Khatamee and Sommers, 1989), hyperprolactinaemia, cervical factor, accessory gland infection and immune infertility. Other etiological factors in tubal obstruction or pelvic adhesions include postpartum and postabortal infection, tuberculosis, acute appendicitis, filariasis, schistosomiasis, iatrogenic causes and traditional practices involving the female genital tract such as female circumcision (WHO, 1992).

2.2.2 Immunological Etiology

Immunological factors have been found in 5% to 17% of infertile couples (Dodson and Joshi, 1989) and in up to 40% of couples with unexplained infertility (Wallach, 1985). In the male, infection, vasectomy, testicular torsion or trauma may result in breakdown of the blood testis barrier, resulting in an immunological reaction and subsequent formation of antisperm antibodies. In the female, sexual activity results in constant exposure to the antigenic
stimuli of spermatozoa or seminal plasma and may cause development of sperm antibodies in genital tract fluids or serum. During infections, pathogens may elicit a localized genital tract immune response against the bacterial membrane carbohydrate that cross-react with carbohydrates on the sperm surface (Sarkar, 1974). Men with genital tract infections have a high incidence of antibodies, reactive with spermatozoa which is associated with reduced fertility (Witkin and Toth, 1983).

Repeated pregnancy wastage throughout gestation has been reported in the presence of abnormal autoantibodies (Lubbe et al. 1984; Branch et al. 1986). The autoantibodies are causally related to infertility (Suresh et al. 1987; Gleicher et al. 1989). These may also pass into the follicular fluid and affect pregnancy rates in in vitro fertilization process (El-Roeiy et al. 1987). Acrosin, a serine protease on the inner acrosomal membrane of spermatozoa, has been identified as having a potential role in immunological infertility in both animal and human studies.

2.2.3 Fertilization capacity

The sperm penetration assay evaluates the interaction of sperm with hamster egg after the removal of zona pellucida by digestion of trypsin (Ürehara and Yanagimachi, 1976). Standards have not been established for this assay and its clinical significance remains controversial because human IVF
(in vitro fertilization) and pregnancy have occurred with a poor or negative zona-free hamster egg penetration test (Coulam et al. 1988; Dodson and Joshi, 1989).

2.2.4 Histocompatibility Leukocyte Antigens

It has been suggested that the major histocompatibility complex controls selective mechanisms that interfere with gametic interactions (Kaminodo et al. 1980). Stolp et al (1973) reported a significantly high incidence of circulating anti human leukocyte antigen (HLA) antibodies in infertile women. However, the value of HLA tests in an infertility investigation is unknown (Jaffe and Jewelewicz, 1991).

2.2.5 Luteinized Unruptured Follicle

The syndrome has also been reported to occur in 5-11% of cycles of fertile women (Ritchie, 1985; Janssen-Caspers et al., 1986). There is an increased incidence of luteinized unruptured follicle syndrome in women with unexplained infertility, endometriosis and pelvic adhesions (Kerin et al., 1983; Janssen-Caspers et al., 1986).

2.2.6 Endometrial Factor

The endometritis due to mycoplasma could be a possible cause of infertility and early fetal loss (Taylor-Robinson et al., 1975; Witkin and Toth, 1983; Khatamee and Sommers, 1989). Graham et al. (1990) studied a group of women
with unexplained infertility to see whether there was a defect that was intrinsic to the endometrium, in presence of normal hormonal profiles and suggested that a primary dysfunction of the endometrium might be associated with unexplained infertility.

2.2.7 Cervical Factor

Abnormalities of the cervix or cervical secretions are responsible for infertility in 5% to 10% of women (Moghissi, 1972). Therefore the assessment of the interaction of sperm and cervical mucus is an important component of infertility evaluation and is tested early in the work-up of the infertile couple (Overstreet, 1986). PCT is the most extensively used method to monitor the interaction of sperm and cervical mucus. In vitro tests such as slide test (Miller and Kurzok, 1932), sperm-cervical mucus contact test (Kremer and Jager, 1976), the Kremer capillary penetrability test (Kremer, 1965) and the sperm-mucus cross match test (Overstreet, 1986), are often recommended when the PCT shows poor results.

Barros et al. (1988) reported that human spermatozoa can undergo certain modifications during their interaction with human cervical mucus and this interaction can have an important effect upon the process of fertilization. It was suggested that cervical mucus prevent the spermatozoa from undergoing the acrosome reaction, thus prolonging the fertile life of the spermatozoan. 3 component of complement has been

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detected in human cervical mucus (Schumacher, 1970a). In women with complement-dependent sperm immobilizing antibodies in the cervical mucus, infertility is caused because of the impairment of motility of spermatozoa (Price and Boettcher, 1979).

Several workers (Matthews and Buxton, 1951; Buxton et al., 1954; Hok et al., 1967; De Louvois et al., 1978; Kaur et al., 1986) have given extensive consideration to the bacteriology of the mucus. The spermicidal organisms in the cervical mucus is suspected to be one of the causes of infertility.

2.2.8 Unexplained Infertility

When conception fails to occur after a period of 1-2 years and no major or minor abnormality can be detected in either partner, then infertility is unexplained or idiopathic. The prevalence of unexplained infertility in various studies ranges from 6% to 60% with an average of 20% (Coulam et al. 1988). Criteria for diagnosis of unexplained infertility includes:

(1) infertility of at least 2 years; (2) normal history and physical examination; (3) adequate coital frequency; (4) three normal semen analysis; (5) regular monthly menstrual cycles with biphasic BBT and luteal phase >12 days; (6) adequate cervical mucus and a normal post coital test (PCT); (7) normal hormones and normal hysterosalpingogram and
Haxton and coworkers in 1987 investigated 95 couples with long standing unexplained infertility fully assessed according to a fixed protocol. The secondary investigations showed that abnormal sperm mucus interaction is a significant factor in strictly defined unexplained infertility. In some abnormal ovarian function was an important factor. By definition, the cause of infertility is unknown, although the results of numerous studies lead to the conclusion that unexplained infertility may have multifactorial causes (Fisch et al., 1989).

2.3 BACTERIOLOGY OF THE FEMALE GENITAL TRACT MICROFLORA FROM HEALTHY FEMALES

The vaginal flora is greatly influenced by estrogens. In utero, the vagina of the fetus is microbiologically sterile. Organisms are first acquired from the infant’s feces. 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 (Kotcher, 1967; Brown, 1978). After the estrogens have been metabolized, the flora contains skin organisms, e.g. coagulase - negative staphylococci and fecal organisms like Escherichia coli. After the menarche, the facultative microflora of the healthy vagina is dominated by lactobacilli and diphtheroidal forms.
(Spiegel, 1991). Most women are also colonized by anaerobic organisms including but not limited to lactobacilli, peptostreptococci, *Prevotella bivia*, *Prevotella disiens*, other *Prevotella* sp. (Shah and Collins, 1990) and *Porphyromonas* sp. (Shah and Collins, 1988). Menstrual cycle and pregnancy affect the vaginal flora (Ison, 1990). The flora is more homogeneous during pregnancy (Lindner et al. 1978). After menopause, facultative lactobacilli can be cultured from 65% of women (Larsen et al. 1982).

The microflora examined from the vagina and cervix of human females show that in general there are less microbial types isolated from the cervix than from vagina (Corbishley, 1977). The human cervix and vagina are regarded as bearing a complex flora of interacting and competing microorganisms. The conditions of low pH and oxygen tension, the production of inhibitory substances by other organisms and by the tract itself and the competition for nutrients will all affect the survival and density of any organism in the female genital tract.

Hok et al. (1967) reported that there was no significant qualitative or quantitative difference in the bacterial flora grown from endocervical mucus of women who desired to be fitted with an intrauterine contraceptive device and women who required tubal insufflation because of infertility.
Eschenbach et al. (1989) examined the role of hydrogen peroxide-producing vaginal lactobacilli in the control of other vaginal flora. Most anaerobic bacteria lack catalase peroxide and therefore cannot eliminate toxic hydrogen peroxide from the environment. The authors hypothesized that hydrogen peroxide producing lactobacilli would be present in women without bacterial vaginosis.

Various microorganisms have been isolated from the genital tract secretions of fertile and infertile women. When their effect was studied on spermatozoa in vitro, it was found that several of the species were spermicidal while others had little or no effect (Matthews and Buxton, 1951; Kaye et al., 1954). Gepfert and Davis (1953) also studied the spermicidal organisms in infertile females and treated them with broad spectrum antibiotics and found that 71.4% of patients became pregnant after treatment and the number of spermicidal organisms were reduced in them.

The presence of pathogens in the female genital tract with the absence of clinical symptoms is suggestive of the fact that there are more factors involved in the pathogenicity of these organisms than their ability to survive and multiply at a site (Corbishley, 1977). Because many pathogens have been isolated from the vagina of healthy women (Archer, 1968; De Louvois et al. 1975), the normal flora has been considered as the endogenous source for the infections.
2.4 ROLE OF MICROORGANISMS IN INFERTILITY

The association of pathogenic bacteria in the male and female genito-urinary tract with lowered fertility has been noted (Lane-Roberts, 1948; Henry-Suchet et al., 1987; Eggert-Kruse et al., 1990; Soffer et al., 1990). Specific and nonspecific infections usually occur in the reproductive system that play an important role in the reproductive failure (Matthews and Buxton, 1951; Buxton et al., 1954; Gordon et al., 1966; Rogers et al., 1972; Horne et al., 1974; De Louvois et al., 1975; Paavonen et al., 1983; Miettinen et al., 1990; Spiegel, 1991). The most studied of these organisms are the pathogens involved in infertility, like mycoplasmas and Chlamydia trachomatis.

2.4.1 Mycoplasmas

Mycoplasmas have been associated with abnormalities of pregnancy since Slingerland and Morgan described a case of postpartum septicemia with pleuro-pneumonia like organisms in 1952. T-strain mycoplasmas have been isolated from decidual and placental membranes (Kundsin et al., 1967) and a role in reproductive failure has been suggested for these organisms. It could cause habitual, spontaneous or infectious abortion (Horne and Rock, 1952; Jones, 1967; Taylor-Robinson, 1971; Gnarpe and Friberg, 1972; Horne et al., 1974). Mycoplasmas have been isolated from the human endometrium at a significantly higher rate from patients with a history of
reproductive failure than from normal controls (Stray-Pedersen et al., 1978; Khatamee and Sommers, 1989). The more common human genital mycoplasmas are *Ureaplasma urealyticum* and *Mycoplasma hominis*. Both of these species have been implicated in certain diseases like nongonococcal urethritis and pelvic inflammatory disease (Taylor-Robinson and MacCormark, 1980). In 1991, A.C. Hill reported a new species, *M. spermatophilum* isolated from human spermatozoa and cervix.

2.4.2 **Chlamydia trachomatis**

It is the most frequently sexually transmitted microorganism in industrial countries (Westrom, 1980; Sachachter, 1978) and has been shown to be associated with a number of diseases like urethritis, cervicitis, conjunctivitis, salpingitis, Reiter’s and Fitz-Hugh-Curtis syndromes (Brunham et al., 1984; Fraiz, 1988; McGregor, 1989). 20% to 50% of female nongonococcal salpingitis is caused by *C. trachomatis* (Sachachter, 1978). It is also suspected to be interfering with human fertility at the level of the endometrium (Fedele et al., 1989). *C. trachomatis* has been isolated from the cervix of 5% to 12% of healthy nonpregnant women (McCormark et al., 1979; Wager et al., 1980; Osser and Persson, 1982). It has also been isolated from the cervix of up to 36% of women with acute PID and positive cervical culture for *C. trachomatis* (Mardh, 1980).

Brunham et al. (1985) reported evidence of the causality of the relationship between *C. trachomatis* infection
and tubal occlusion by demonstrating that the prevalence of antibodies to *C. trachomatis* was dependent on sexual activity in women with tubal infertility. The percentage of seropositivity is high in women with both a higher number of lifetime sexual partners and tubal abnormalities (Ruijs, 1991).

Severe mucus congestion accompanied by tubal oedema and loss of ciliated epithelia play a major role in the aetiology of chlamydial-induced tubal damage. Infertility following chlamydial salpingitis could be associated with failure of egg transportation to the oviduct (Tuffrey et al., 1990). The high prevalence of antibodies in women with infertility as well as patients with ectopic pregnancy compared to healthy pregnant women indicate that *C. trachomatis* plays an important role in the pathogenesis of these conditions (Kihlstrom et al., 1990).

2.4.3 *Vibrio fetus*

Curtis (1913) was the first to record the isolation of an anaerobic vaginal vibrio which was isolated in pure culture from the vagina and cervix of a case of purpurial infection. Moore (1954) observed highly motile anaerobic vaginal vibrios that were capable of progressing through cervical mucus in an in vitro test of sperm motility and reported its possible causal association with certain cases of sub-fertility. *Vibrio fetus* has been isolated from a
number of cases of infertility (Donaldson et al., 1967; Donaldson and Clark, 1970; Rogers et al., 1972).

2.4.4 Etiology of Pelvic Inflammatory Disease

As infertility is so intimately linked with pelvic infection, the etiologic factors contributing to the development of pelvic inflammatory disease (PID) is of important consideration (Sciarrà, 1991).

The classic theory of causation of PID was popularized in the U.S. in the early part of this century. The concept was that bacterial pathogens progressed up the female reproductive tract, starting at the cervix. Progression to the tubes or indirectly through the lymphatic system. During subsequent years, a host of endogenous and exogenous factors that either facilitate or impair the progression of cervical infection to tubes has been described.

Recently, Keith and colleagues (Keith et al., 1984; Keith et al., 1986) proposed an alternative hypothesis for the entry of major sexually transmitted pathogens into the upper female reproductive tract, particularly Neisseria gonorrhoae and Chlamydia trachomatis. The alternative hypothesis involves the concept of bacterial attachment to the surface of motile organisms in the human reproductive tract, especially trichomonads and sperm. These organisms thus may be additional facilitating etiologic factors in the development of PID. Evidence from the in vitro studies
suggests that trichomonads and sperm can transport bacteria (Toth et al., 1981; Wolner-Hanssen and Mardh, 1984). Cocci and coliforms have been shown to attach to trichomonads and sperm. Sperm, therefore, when exposed to an environment where pathogenic bacteria are present, such as the vagina and cervix, may transport bacteria to the target areas of the upper female genital tract. Thus both sperm and bacteria may be additional symbiotic ingredients for the development of many cases of PID.

Other supporting evidence for the alternative hypothesis proposed by Keith and colleagues may be found in the classic infections. Evidence exists that women married to men with high sperm counts are more likely to develop pelvic infections and to have decreased fertility. Also, there is evidence that wives of azoospermic men rarely develop PID (Sciarra, 1991).

There are many similarities between the bacterial flora of the male and female genital tracts. Swenson et al. (1980) and Toth and Lesser (1981) have shown that the seminal fluid of asymptomatic infertile males is colonised with a wide variety of both aerobic and anaerobic bacteria. This wide variety of bacteria has been recovered from the vagina and cervix of asymptomatic women (Corbishley, 1977). Microorganisms such as *N. gonorrhoeae* (James-Holmquest et al., 1976), *E. coli* (Teaque et al., 1971), *Chlamydia trachomatis* (Friberg et al., 1985) have the ability to adhere
to spermatozoa. Howard (1971) reported that microorganisms attached to spermatozoa might be transported from the seminal vesicles to the epididymis, thus giving rise to epididymitis. Wolner-Hassen and Mardh (1984) observed the attachment of serovars D, H and I of C. trachomatis to human spermatozoa in vitro.

Eschenbach et al. (1973) first reported the association of anaerobic bacteria with pelvic inflammatory disease. In the pregnant women, bacterial vaginosis (BV) has been associated with preterm birth, premature rupture of membranes, and chorioamnionitis (Martius and Eschenbach, 1990). The organisms recovered from the amniotic fluid represented vaginal flora i.e. Gardnerella vaginalis, Fusobacterium nucleatum, Prevotella melaninogenica, Ureaplasma urealyticum, Candida albicans of BV puts the patient at increased risk of upper genital tract infections, with several consequences to fertility and outcome of pregnancy (Spiegel, 1991). Vaginitis in premenarcheal girls is not common and may be due to yeast, Neisseria gonorrhoeae, pinworm, Shigella sp., Streptococcus pyogenes or bacterial vaginosis (Kotcher, 1967; Rein, 1990). Bacterial vaginosis is the most common of the three categories of vaginitis (T. vaginalis vaginitis, yeast vaginitis and bacterial vaginosis), accounting for 40-50% of all cases (Gardner and Dukes, 1955; Balsdon et al., 1980). In non-specific vaginitis, Gardnerella vaginalis and anaerobic bacteria
become predominant in comparison to *Lactobacillus* sp. in normal vaginal flora (Spiegel et al., 1980). Moreover, anaerobic bacteria increase in the vaginal flora of all IUCD users (Von-Feichér et al., 1979; Watt et al., 1981). Actinomycetes colonization of female genital tract is frequently found in connection with use of inert IUCD and likelihood of harbouring these organisms increase with time (Aubert et al., 1980; Duguid et al., 1980).

### 2.5 Effect of Microorganisms on Sperm Function

The important components of human sperm function are sperm transport, sperm egg recognition, the acrosome reaction and sperm oocyte fusion (Aitken, 1990). Sperm motility forms one of the most important parameters in assessing the fertility potential of a semen specimen, as immotile human sperm cannot penetrate cervical mucus (Amelar et al., 1980). The type of movement also influences fertilizing capacity as the vigorous beating of the sperm tail is necessary for penetration of the sperm head through the corona radiata to fertilize ovum (Nelson, 1985). The involvement of the dynein arms in the sliding microtubule mechanism of ciliar and flagellar movement is now well established (Mann and Lutwak-Mann, 1981; Guraya, 1987). According to this hypothesis, the sperm tail moves when the microtubule, powered by dynein arm ATP hydrolysis, slide past one another (Gibbons, 1988). For a better understanding of the molecular mechanisms involved in
the motility, it is important to determine the three-dimensional arrangement of the various structural components associated with the doublet microtubules (e.g. dynein arms, radial spokes and nexin fibres), as the interactions between them are of great significance in the development of bending waves (Guraya, 1987). Brokaw (1980) studied the effect of elastase on demembranated spermatozoa and suggested that elastase digests the nexin molecule in the sperm tail which is chemically similar to elastin and thereby changes the flagellar motility. In the female genital tract, the spermatozoa come in direct contact with the vaginal and cervical microflora and these microorganisms might have some detrimental effect on the sperm motility and/or sperm function. In the male genital tract also, the microorganisms have some role to play in infertility.

2.5.1 Sperm Motility

The effect of microorganisms on spermatozoal motility could be direct (by agglutination) or indirect by the production of extracellular metabolic products that affect sperm motility and viability. Experiments of Kazda (1963) on the effect of culture filtrates of Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa on bull spermatozoa indicated that extracellular products of bacteria (found in semen of some animals) influence the viability of spermatozoa. Similar studies have been done by Nowakowski et al. (1981) using purified Staphylococcal and Streptococcal
toxins. Bacterial endotoxin (lipopolysaccharide) complex from *Vibrio fetus*, a common pathogen in ram and bulls, have been shown to immobilize spermatozoa (Dennis, 1962). In women with bacterial vaginosis, it has been reported that the concentration of endotoxin is higher than that in women with a normal vaginal exam (Sjoberg and Hakansson, 1991).

A low molecular weight spermatozoal immobilization factor has been isolated from *E. coli* (Paulson and Polakoshi, 1977). Various species of vaginal anaerobes produce enzymes, including collagenase and proteases. The role of these products in causing bacterial vaginosis is unknown (Spiegel, 1991) but it is known that proteases like trypsin might change the conformation of ATPases thereby affecting the sperm motility (Inaba et al., 1990). Some authors have shown the effect of extracellular products of aerobic cervical microflora, particularly elastase on the motility of spermatozoa of man and animals (Gupta et al., 1978; Kaur et al., 1986; Kaur et al., 1988a).

Several different organisms isolated from the genital tracts of infertile couples have been shown to be spermicidal to spermatozoa in vitro and/or to have a direct etiological role in infertility (Horne et al., 1974; Buxton et al., 1954; Kaye et al., 1954; Peleg and Ianconescu, 1966; Teague et al., 1971; Tuttle et al., 1977a and Tuttle et al., 1977b; Cohen et al., 1977; Kaur et al., 1986). However, the factor responsible for this activity has not been characterised and the mechanism involved has not been discussed.
2.5.2 Spermagglutination

Spermatozoa have been found to be agglutinated by various bacteria and endotoxins of various bacteria. It was reported that live pathogenic *E. coli* obtained from urinary or cervical cultures produced profound depression in the motility and viability of human spermatozoa (Teague et al., 1971). Immediately after mixing the ejaculate with the live bacteria, clumping of spermatozoa was noted. Sperms of different animal species have been found to be agglutinated by *Pseudomonas aeruginosa*, *E. coli*, *Bacillus subtilis* (Teague et al., 1971; Gupta et al., 1978; Kaur et al., 1986), *Mycoplasma* (Taylor-Robinson and Manchee, 1967), *Candida albicans* (Tuttle et al., 1977a), Myxoviruses (Peleg and Ianconsco, 1966) and gonococci (James-Holmquest et al., 1974).

Certain organisms have been shown to be capable of adhering to human spermatozoa (Rosenthal, 1931; Peleg and Ianconescu, 1966; Taylor-Robinson and Manchee, 1967; Gupta et al., 1978; Gomez et al., 1979; Busolo et al., 1984; Wolner-Hassen and Mardh, 1984; Friberg et al., 1985) and in a way decrease the motility of these cells. Taylor-Robinson and Manchee (1967) showed adsorption of bovine and human spermatozoa to colonies of mycoplasmas as well as spermagglutination by an avian mycoplasma. These phenomena showed some similarity to haemadsorption and haemagglutination by mycoplasma. Similarly, studies by Tuttle
et al. (1977a and 1977b) showed the interference of human spermatozoal motility by Trichomonas vaginalis and Candida albicans and suggested their possible role in human infertility.

Swenson (1982) reported that Mycoplasma pulmonis adversely affects sperm transport through the female reproductive tract in mouse by adhering to the heads and tails of spermatozoa. The findings of Toth and Lesser (1982) suggested that antibiotic therapy could be valuable in improved semen quality (motility as well as morphology of the spermatozoa) when Ureaplasma is isolated.

Several studies have polarized attention on Ureaplasma urealyticum (formerly known as T-Mycoplasma) in both human and animal semen (Erno and Blom, 1972; Gnarpe and Friberg, 1972; De Louvois et al., 1974; O'Leary and Frick, 1975; Goffaux et al., 1976). Epidemiological work on Ureaplasma urealyticum provided a pointer that the positive results obtained in infertility with tetracycline derivatives might be due to a direct interaction of these drugs with the sperm plasma membrane and the mycoplasma.

2.2 PROTEOLYTIC SYSTEMS IN REPRODUCTIVE TRACT

2.6.1 Proteinases

The association of proteinases and various activators and inhibitors with male and female genital tract secretions has been known. The sperm transport through the cervical

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mucus is aided by plasminogen activator in the seminal plasma (Propping et al., 1974; Zaneveld et al., 1974).

The acrosomal protein acrosin is capable of dissolving the zona pellucida of the ovum and is probably essential for penetration of the zona pellucida by spermatozoa during the fertilization process (Stambaugh and Bukley, 1969; Zaneveld et al., 1970; Stambaugh et al., 1969; Zaneveld et al., 1971). The addition of certain synthetic or lower molecular weight natural proteinase inhibitors to spermatozoa decreases the fertilization rate significantly (Stambaugh et al., 1969; Zaneveld et al., 1971). Green and Summers (1980) demonstrated the subcellular location of trypsin like protease in acrosomes of sea urchin sperm. Trypsin and chymotrypsin-like enzymes are believed to be involved in sperm penetration of the vitelline coat.

In the female genital tract, proteolytic enzymes are involved in the implantation process. At the initiation of implantation, trypsin - like and chymotrypsin like enzymes appears in the uterus. They have been closely studied in the rabbit (Denker, 1977) and mouse (Dabich and Andary, 1976). The function which has been suggested for these enzymes is that of proteolytic modification of the pre-implantation blastocyst, modification which probably promotes the adhesion of the blastocyst to the uterine epithelium (Hoversland and Weitlauf, 1982). These enzymes tend to be balanced by inhibitors, since high concentrations of trypsin like enzymes
may adversely affect cell-to-cell interaction (Deman et al., 1974).

The microorganisms isolated from the cervicess of human females have been shown to produce proteases and elastase (Kaur et al., 1988a and Kaur et al., 1988b). It is possible that these organisms increase the protease content in the genital tract, creating an imbalance in the proteolytic system which is otherwise essential in the process of fertilization and implantation.

2.6.2 Proteinase Inhibitors

2.6.2.1 Proteinase inhibitors in male genital tract fluids and spermatozoa

(a) Acrostatin: It is the acrosin inhibitor which has been proposed to be either a protein or one of the steroid sulphates, such as cholesteryl or demosteryl sulphate, which normally occur in spermatozoa. The acrosin inhibitor present within the spermatozoa is not identical with the acrosin-inhibiting proteins of the seminal plasma (Guraya, 1987). The acrosin (or proteinase) inhibitor in seminal plasma of fertile and infertile men is naturally occuring inhibitor and is produced by the epithelial cells of the male genital tract. It is bound to the outer acrosomai membranes of spermatozoa and later on removed sperm migration in the female genital tract (Hirschhauer
and Baudner, 1972). The removal of the inhibitor appears to coincide with capacitation (Guraya, 1987). p-nitrophenyl-p-guanidinobenzoate (NPGB) has been found to find the sperm surface as an acrosin inhibitor and appears to be an effective contraceptive when tested in the mouse either in vitro or after vaginal application (Bhattacharya and Zaneveld, 1978).

(b) -Macroglobulin ($\alpha_2M$) is a broad spectrum proteinase inhibitor inactivating mammalian and even prokaryotic endopeptidases of all known classes (serine, cysteine, aspartic, metallo type proteases; Van-Leuven, 1982). When $\alpha_2M$ interacts with a proteinase, a peptide bond localized within the so-called bait region of the inhibitor molecule is cleaved and is followed by trapping of the enzymes (Barrett and Starkey, 1973). Because of their different electrophoretic mobilities, the noncomplexed $\alpha_2M$ molecule is termed the slow form of $\alpha_2M$ ($\alpha_2M^S$) and the $\alpha_2M$/proteinase complex the fast form of $\alpha_2M$. The trapped proteinases retain their enzymatic activity but because of steric hindrance they are able to cleave substrates of lower (<20,000) but not higher molecular weight (Werb et al., 1974; Barrett et al., 1979).
The biological and, in particular, the possible pathogenic role of $\alpha_2$M in seminal fluid is not known at present. Kramer et al. (1992) has considered the possibility that $\alpha_2$M could control the activity of physiologically relevant proteolytic enzymes normally operative in seminal fluid. Complex formation of the respective proteinases with the broad specific inhibitor $\alpha_2$M could interfere with these processes and could thus be one factor adding to male infertility. Also, the $\alpha_2$M/proteinase complexes might interfere with local immune responses in the male genital tract (Kramer et al., 1992). Because cellular immunity plays an important role in the control of genital infections, local suppression of cellular immune responses by $\alpha_2$M/proteinase complexes may promote infection with the sexually transmitted pathogens.

Five proteinase inhibitors have been purified from boar seminal plasma, which have molecular weights varying between 1500 and 13,000 (Polakoshi and Williams, 1974). These inhibit acrosin, trypsin and plasmin, but not chymotrypsin, thrombin or pancreatic kappaliprein. Besides the low molecular weight inhibitors, high molecular weight inhibitors are also present in human seminal plasma (Schumacher, 1970b; Tauber et al., 1973; 1975; Zaneveld et al., 1974).
These are immunologically identical to the serum inhibitors $\alpha_1$-antitrypsin and $\alpha_{1\text{x}}$-antichymotrypsin.

2.6.2.2 Proteinase inhibitors of female genital tract fluids

Similar to the proteinase inhibitors of seminal plasma, the inhibitors of the female genital tract could be divided into two general classes: inhibitors with a molecular weight below 15,000, and those with a higher molecular weight. Most of the latter are immunologically identical to serum inhibitors (Zaneveld et al., 1975).

The low molecular weight inhibitor of cervical mucus was first reported to possess a molecular weight of 2200 (Haendle et al., 1970) and was later corrected to 11,500 (Wallner and Fritz, 1974). It appears to be present in a free and in a masked form, probably as an enzyme - inhibitor complex. The cervical mucus inhibitor inhibits trypsin, chymotrypsin and neutral proteinases from human leukocytes, but has no effect on plasmin, thrombin, kallikrein and acrosin. The inhibitor shows a decrease during the ovulatory phase of the cycle.

The presence of serum inhibitors in cervical mucus has been well documented (Schumacher et al., 1965; Schumacher, 1970a, 1973a,b; Schumacher and Zaneveld, 1972 and 1974). These include $\alpha_1$-antitrypsin, inter-$\alpha$-trypsin inhibitor. $\alpha_{1\text{x}}$-antichymotrypsin, antithrombin III and the $C_1$-esterase inhibitors. The last two are only occasionally detectable in cervical mucus, but appear most frequently in
specimens obtained from women under treatment with hormonal contraceptives. The first three inhibitors show their lowest concentrations at the ovulatory period.

Proteinase inhibitors were also shown to be present in the uterus, fallopian tubes and follicular fluid and even in amniotic fluid (Woraschk and Kressner, 1962; Hirschhauser et al., 1971; Stambaugh et al., 1974). In the rat, inhibitors of trypsin-like and chymotrypsin-like enzymes are diminished in the uterus on the day of implantation (Blackwood et al., 1968). Dabich and Andary (1974) showed that blastocysts implantation could be prevented in mice by exogenous proteinase inhibitors.

Casslen (1986) studied the inhibitors of trypsin, chymotrypsin and elastase in luteal phase uterine fluid in humans. Inhibitory activity was present in the fractions containing $\alpha_2$-macroglobulin, $\alpha_1$-antitrypsin, $\alpha_1$-antichymotrypsin and antileukoprotease. It was suggested that these may have physiological implications in the control of proteinases released by the blastocyst during implantation, since proteinases are invariably balanced by inhibitors under physiological conditions.

A progesterone-induced protease inhibitor from uterine secretions of pigs was purified and characterized by Fazleabas et al. (1982). This inhibitor was of low molecular weight ($\sim$14500) which inhibited trypsin, plasmin, chymotrypsin. The inhibitor was found to be associated with the glandular and surface epithelium in the uterus.
Chymotrypsin appeared to bind at the same site on the inhibitor as trypsin.

2.6.2.3 Serum proteinase inhibitors

Yang et al. (1976) studied the effect of serum proteinase inhibitors on the fertilizing capacity of rabbit spermatozoa and reported that these interfere fertilization and the relative effectiveness of acrosin inhibitors is inversely related to their molecular weight.

Human plasma has also been found to constitute various proteinase inhibitors like $\alpha_1$-proteinase inhibitor (initially called $\alpha_1$-antitrypsin), antithrombin III, $\alpha_2$-antiplasmin, $\alpha_1$-antichymotrypsin, $C_1$-inhibitor ($C_1$-esterase inhibitor), $\alpha_2$-macroglobulin, inter- $\alpha$-trypsin inhibitor, beta-anticollagenase and $\alpha$-cysteine proteinase inhibitor (Travis and Salvesen, 1982).

2.6.2.4 Mucus proteinase inhibitor

In other pathological conditions where elastase is responsible for tissue destruction as in case of chronic bronchitis and emphysema, due to leucocyte elastase, the mucus proteinase inhibitor has been found to be a fast-acting inhibitor of leucocyte elastase (Boudier and Bieth, 1989). It has also been suggested that the net balance of neutrophil elastase and its inhibitor, $\alpha_1$-proteinase inhibitor is a critical determinant in the development of destructive lung disease (Perimutter et al., 1988).