GENERAL INTRODUCTION
INTRODUCTION

Investigations on reproductive biology in humans are almost entirely limited to women who spend the majority of their reproductive years in menstrual cycling (Strassmann, 1997). During the last two decades there has been a marked increase in the assisted reproductive technology (ART) patient population in all infertility clinics all over the world (Rao et al., 2004). The potential fertile period of the ovulatory menstrual cycle, sometimes termed the window of fertility, is defined as that period during which a woman can conceive from an act of intercourse. Its accurate definition has many implications in the management of human fertility and infertility (Blackwell et al., 1997). Identification of the period of ovulation in woman is critical in the treatment of infertility. Success in invitro fertilization and embryo transfer has been associated with the exact time of ovulation. Ovulation, a complex process, initiated by the surge of luteinizing hormone (LH) which is characterized by resumption of meiosis and restructuring of the follicular wall, resulting in follicular split and the discharge of a ruptured mature fertilizable ovum. The sequence of events occurring during the normal ovulatory process has been the subject of considerable attention since 1950s (Guraya and Dhanju, 1992).

Invitro fertilization/embryo transfer (IVF/ET) is an effective method of treating various causes of infertility. The first successful pregnancy in this method employed laparoscopy to aspirate a single follicle in a natural menstrual cycle (Steptoe, 1978). Since then, ovarian stimulation has been widely used in assisted reproduction programs all over the world (Ng, 2001). A normal menstrual cycle is divided into two phases, the follicular and the luteal phase, with ovulation generally occurring between the two. The follicular phase is characterized by low but steady levels of
luteinizing hormone (LH) and follicle stimulating hormone (FSH) released from the pituitary. The gonadotropins act in the form of gonads to stimulate growth of the follicle (developing egg cell) and eventual stimulation of estrogen production by the follicle. Estrogen released by the follicle produces a negative feedback keeping LH/FSH levels steady. A few days before ovulation (around mid cycle), estrogen production, followed shortly after by LH and FSH, increases dramatically and rapidly, signaling the ovulation. The levels of LH and FSH then decline rapidly to their pre-peak levels, usually in about 48 hours (Yen et al, 1999). The average length of the menstrual cycle in woman in the India and the United States is 28 days (Wood, 1994; Yen et al., 1999) but there is much variation within individuals and between populations (Wood, 1994; Vizthum, 2001; Creinin et al., 2004).

An important step in the evaluation and management of female infertility is the detection and timing of ovulation. More importantly, the development of a simple, self administered method of ovulation detection or prediction would increase the efficacy of the rhythm method of contraception and provide a more reliable technique of family planning for millions of couples who depend exclusively on periodic abstinence for conceptional control. Definite proof of ovulation is the establishment of pregnancy or the recovery of an ovum from the oviduct. Direct observations of a corpus luteum with the presence of a stigma by pelvic endoscopy or laparotomy may be considered strong evidence of ovulation.

Presumptive evidence of ovulation may be obtained by steroid or gonadotropic hormone assays in the blood or urine by observance of peripheral changes in the reproductive tract and other sites has also associated with ovulation. An understanding of the hormonal events which control the ovulatory process is essential to appreciate the physiologic basis of many tests which
have been devised for documentation and timing of ovulation. Maturation of ovarian follicles is effected by the tropic action of follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior lobe of the pituitary gland. Release of FSH and LH, is in turn, controlled by the release of gonadotropin hormone (GnRH). The pattern of FSH and LH secretion during the menstrual cycle has been established (Abraham et al., 1972; Moghissi et al., 1972; Speroff and Vandewiele, 1971; Yen et al., 1970).

ASSESSING OVULATION

Ovulation is a phase of the female menstrual cycle that involves the release of an egg (ovum) from one of the ovaries. New life begins if the ovum meets with a sperm during its journey down the fallopian tube. Ovulation depends on a complex interplay of glands and their hormones, and generally occurs about two weeks before the onset of the menstrual period. Typical ovulation symptoms and signs include changes in cervical mucus and a small rise in basal temperature. In general, ovulation occurs almost once in every month until menopause, apart from episodes of pregnancy and breastfeeding. However, some women experience irregular ovulation or no ovulation at all. The women generally show several signs of ovulation such as mucus changes, abdominal pain, premenstrual symptoms and temperature rise.

i) Regular menstrual cycles - menstrual periods that arrive every 24-35 days are more likely to be ovulatory than periods that occur more or less often.

ii) Mucus changes - about two weeks before menstruation, an ovulating woman may notice slick and slippery mucus.

iii) Abdominal pain - some women experience pain during ovulation. The pain may be general or localized to one side of the abdomen.
iv) Premenstrual symptoms - ovulation may accompany premenstrual symptoms such as breast enlargement and tenderness, abdominal bloating and moodiness and symptoms of facial allergy.

v) Temperature rise - women who use a natural family planning method of contraception will notice a small rise in their basal temperature after ovulation. The temperature rise is about half a degree Celsius. This temperature rise does not predict ovulation - it suggests that ovulation has already taken place.

Common causes of ovulatory problem include:

- **Hypothalamus** - events that can alter the functioning of the hypothalamus include polycystic ovary syndrome, over exercising, poor nutrition and chronic stress.
- **Pituitary** - events that can prevent the pituitary gland from producing enough hormones include benign pituitary tumours or direct injury to the pituitary itself.
- **Ovary** - events that can prevent the ovaries from releasing of ova include early menopause (also known as ovarian failure), or damage or removal of the ovaries.

Ovulation disorders are believed to be responsible for infertility in approximately 20% to 25% of fertile women. These disturbances include anovulation, luteal phase defect, and subtle anomalies of the ovulatory process.

Medical tests can check whether ovulation has taken place or not. These tests include; blood test, pregnancy ultrasound, postcoital test, basal body temperature, cervical mucus and ultrasonography.
• **Blood test** - to check for the presence of progesterone. A level greater than 20 nmol/L indicates that ovulation has taken place. This test must be taken about three to 10 days before the first day of the next expected period.

• **Pregnancy ultrasound** - the presence of a fetus is the only 100 per cent proof of the occurred ovulation. Medical tests such as ovulation predictor kits and blood tests can only ascertain that ovulation would occur definitely or not.

Commonly performed techniques for documentation of ovulation include recording the basal body temperature, identifying cervical changes, measuring levels of urinary luteinizing hormone (LH), serum progesterone, endometrial biopsy, and ultrasonography.

**POSTCOITAL TEST**

Ferning represents crystallization of the mucus, which depends on the concentration of electrolytes—principally sodium chloride. Ferning is assessed by spreading cervical mucus onto a glass slide and allowing it to dry. It is customarily graded from 0 (no ferning) to 4+ (intense crystallization) depending on the extent of crystal formation. Preovulatory mucus has 4+ crystallization (ferning) and is readily penetrated by normal motile sperm.

**THE BASAL BODY TEMPERATURE CHART (BBT)**

The BBT chart is a simple, noninvasive, and inexpensive because the required information can be acquired from the ovulation and the duration of the luteal phase. Patients are instructed to take their temperature on awaking early morning daily, before any physical activity. A temperature increase of 0.4°F (0.22°C) for two consecutive days indicates ovulation, because the expected temperature increase is in the order of degree decimals. The temperature increase is a function of the thermogenic effect of
progesterone on the hypothalamus. The initial rise in serum progesterone may occur anytime between 48 hours before ovulation and 24 hours after. Therefore, an increase in temperature is useful for establishing that ovulation has occurred, but it should not be used to predict the onset of ovulation in a given cycle. In most women, a biphasic basal body temperature chart indicates ovulation, whereas a monophasic basal body temperature chart suggests anovulation. However, ovulation has been reported to occur in 3% to 12% of women with monophasic basal body temperature charts (Johansson et al., 1972). Furthermore, ovulation may not occur in some patients with biphasic basal body temperature charts. Once the temperature change has occurred, it usually persists for 12 to 14 days. Temperature elevations that are not sustained for at least 11 days suggest the presence of luteal phase defects (e.g., a short luteal phase). However, the diagnosis of luteal phase deficiency should rely on histologic dating of the endometrium and not on basal body temperature records. Patients should also indicate on the basal body temperature chart the days when they experience bleeding that of the intercourse. The chart is useful for assessing the frequency of intercourse and its relationship to the cycle, and it can be used to schedule other diagnostic tests, such as the Hysterosalphinogram or Hysteroscopy (HSG), the post coital test (PCT), serum progesterone measurement, and endometrial biopsy.

**CERVICAL CHANGES**

Changes in the appearance of the cervix and in the physical properties of the cervical mucus form the basis of many tests commonly used to determine the time of ovulation. These include appearance of the cervix, mid-cycle mucorrhea, ferning, spinnbarkeit, and viscosity or consistency of cervical mucus. The appearance of the cervix varies during the menstrual cycle. In mid-cycle, the cervix softens progressively, and the orifice space dilates
and exudates a clear, profuse mucus. Within a few days after ovulation, the cervix becomes firm, and the orifice space closes and covered by scanty, turbid, tenacious mucus. With instruction, women are able to predict and identify the approximate time of ovulation by recognizing the increased mid-cycle mucus discharge, which occurs around the time of ovulation (Moghissi, 1987)

**ULTRASONOGRAPHY**

A rapid and reliable method for monitoring follicular growth, rupture, and regression. Ultrasonography provides good presumptive—but not definitive—evidence of ovulation. Ovulation is deemed to have occurred if the follicle has reached a mean diameter of 13 to 28 mm. Accuracy of determining ovulation timing with ultrasonography is approximately 85%. However, serial ultrasonographic determination, which may be expensive, is required (Moghissi, 1988).

**OTHER LABORATORY TESTS**

Women who experience irregular menstrual cycle or who have galactorrhea. Measuring levels of serum thyroid-stimulating hormone and prolactin should be tested. Hypothyroidism and hyperprolactinemia can cause ovulatory dysfunction and are readily treatable. Screening for the human immunodeficiency virus, hepatitis, rubella, and syphilis also should be tested among the infertility patients. Women who are suspected of having polycystic ovarian syndrome or hyperandrogenism, measurements of serum LH, dehydroepiandrosterone sulfate, 17α-hydroxyprogesterone, and testosterone are of diagnostic value. During the initial visit, cervical cultures can be obtained for *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*, all of which have been associated with infertility. Both *Neisseria gonorrhoeae* and *Chlamydia trachomatis* can cause salpingitis and fallopian tube
damage, which can lead infertility (WHO, 1983). However, the roles of *Ureaplasma urealyticum* and *Mycoplasma hominis* in infertility are not clear. *U. urealyticum* has been associated more frequently with infertile women compared with fertile women. In addition, *U. urealyticum* is associated with an increased risk of preterm delivery. The idea of the eradication of *U. urealyticum* with antibiotic therapy enhances fertility and improves pregnancy outcome is doubtful; however, previous report shows the improvement with treatment (Friberg, 1980). The relationship between *M. hominis* and infertility in pregnancy outcome is even more doubtful. *M. hominis* has been recovered from infertile and fertile women with similar frequency (Friberg, 1980). Women who harbor *M. hominis*, however, more often have a history of pelvic inflammatory disease and are more likely to be currently infected with *U. urealyticum*. They are also more likely to have bacterial vaginosis, which has been associated with an increased risk of salpingitis and endometritis resulting in infertility and, in pregnant women, with premature rupture of membranes (Hillier et al., 1996).

**LAPAROSCOPY AND HYSTEROSCOPY**

An important element of the infertility evaluation is laparoscopy and hysteroscopy and need not be performed until after the basic refine has been completed. Laparoscopy is necessary to evaluate the peritoneal cavity for endometriosis and pelvic adhesions, as well as to verify the presence of tubal disease when it is suggested by HSG. Hysteroscopy is necessary for confirmation and treatment of intrauterine abnormalities, such as fibroids, intrauterine synechia, or congenital abnormalities, which may have been noted on previous imaging studies like HSG. A laparoscopy with chromopertubation and hysteroscopy can be performed simultaneously for the assessment of ovulation.
Increase the chances of ovulation as follows:

i) Women who are seriously obese or underweight may have problems with ovulation.

ii) Excessive exercise can prevent ovulation. Ease back on physical activity levels - this may require expert help if the exercise is actually a form of bulimia.

iii) Repeated crash dieting, fasting, skipping meals and other disordered eating habits can hamper the body's ability to regularly ovulate. Chronic emotional stress can play havoc with menstrual cycle.

In recent years, attention has been paid on the biochemical importance of saliva. Saliva is a complex fluid produced by a number of specialized glands which discharge into the oral cavity of the glands of mammalian vertebrates. Mostly, saliva is produced by the major salivary glands (parotid, submandibular, and sublingual), but a small contribution is made by the numerous small labial, buccal, and palatal glands which line the mouth (Van Dam and Van Loenen, 1978; Vining and McGinley, 1985). The easy noninvasive, stress-free nature of saliva collection makes it one of the most accessible body fluids to obtain.

The major disadvantage of saliva is that many biochemical assays are retained for a shorter period of time than they are in urine. New collecting devices should make physicians more comfortable with using saliva as an alternative to blood or urine. So far, no device could be found to serve for all salivary analyses. Highly sensitive methods of detection are required, as most of the biochemical profiles can be detected in salivary secretions (Mandel, 1974). Salivary secretion is generally accepted to be a two-stage process, with initial secretion of an aqueous plasma-like primary fluid by the acinar cells and its subsequent modification during passage through the water-impermeable ductal cell system.
Secretion is controlled by the autonomic nervous via signal transduction systems that couple receptor stimulation to ion transport and protein secretory mechanisms. The volumes of saliva produced vary depending on the type and intensity of stimulation, the largest volumes occurring with cholinergic stimulation. Neurotransmitters released in response to secretory stimuli bind to specific protein receptors on the acinar cell membrane. This causes alterations in membrane-bound G-proteins and a subsequent series of intracellular second messenger events is initiated. In the case of muscarinic cholinergic stimulation, the signal transduction system involves release of calcium from intracellular stores by inositol triphosphate (IP3) and the subsequent activation of a variety of ion channels and transport systems, ultimately leading to the trans-epithelial movement of water.

Although the primary secretion is a plasma ultra-filtrate (i.e., isotonic), energy-dependent reabsorption of sodium and chloride ions in the ductal system results in a markedly hypotonic secretion, which facilitates taste. Bicarbonate allows buffering, while calcium and phosphate allow for maintenance of tooth mineral integrity. Early work defined the effects of flow on electrolyte composition. Interestingly, the pH and ionic composition may also influence the activity of organic components in the saliva (Davenport, 1977; Jacobson, 1981; Vining and McGinley, 1982). For example, lysozyme activity is influenced by electrolytes and salivary anions of low-charge density (Burgen, 1956). Thiocyanate, the anionic product of the salivary peroxidase system, also potentiates lysozyme activity. Saliva also contains a starch-digesting enzyme called amylase (ptyalin), which initiates the process of enzymatic hydrolysis; it split starch into molecules of the double sugar maltose. Many carnivores, such as dogs and cats, have no amylase in their saliva; therefore, their natural diet
contains very little starch. Substances must be in solution for the
taste buds to be stimulated; saliva provides the solvent for food
materials.

The composition of saliva varies, but its principal
components are water, inorganic ions similar to those commonly
found in blood plasma, and a number of organic constituents,
including salivary proteins, free amino acids, and the enzymes
lysozyme and amylase. These enzymes and other proteins,
including saliva-specific glycoproteins are synthesized by the
acinar cells. The transport of proteins into saliva has been reviewed
by Young (1979). Almost all of the organic compounds of plasma,
such as hormones, immunoglobulins, enzymes, DNA and viruses
may be detected in saliva in trace amounts (Vining and McGinley,
1985). The total protein concentration in saliva is negligible
because this concentration is less than 1% of that in plasma
(Breimier and Danhof, 1980). It seems likely that a major source of
these trace amounts originates from the gingival crevicular fluid
(from the tooth/gum margin) (Cimasoni, 1974). Although saliva is
slightly acidic, the bicarbonates and phosphates contained within
it serve as buffers and maintain the pH and saliva relatively
constant under ordinary conditions.

Hormones used to measure in blood have been now
estimated in saliva, though the quantities are comparatively less
(Braat et al., 1998). Hence, saliva is considered as the best non-
invasive source for chemical and biochemical study (Freundl et al.,
1996). Report shows that saliva is a very good source of both
hormones and enzymes and their levels changed in accordance
with the phases of menstrual cycle (Flynn and Lynch, 1976).
Appearance of "fern pattern" is established in bovine vaginal mucus
to predict the time of ovulation (Etchepareborda et al., 1983;
Affandi et al., 1985; Wagrowska-Danilewicz and Danilewicz, 1986;
Rajanarayanan, 2004), and the technique was then extended in human vaginal mucus (Moghissi, 1986). Eventhough studies concerning salivary ferning have been conducted comparing with other fertility markers; the results are not convincing (Guida et al., 1993; Fehring and Gaska, 1996). Saliva test works on the basis that estrogen levels rise in a woman’s body during the days leading up to her releasing an egg (or ovulation). This 'fertile phase' may be detected by looking at changes in a small sample of a woman’s saliva each day. The increased estrogen hormone in the body causes a woman's saliva to contain more salt. This 'salty' saliva crystallizes on the glass slide to form ‘fern-like' patterns that can be seen under the microscope. These fern-like patterns can usually be seen in the saliva about 2 to 4 days before ovulation and are thought to be about 90% accurate.

The numerous retrospective methods of hormonal assays described to predict ovulation (Strott et al., 1969; Yussman and Taymor, 1970; Sanyal et al., 1974). At present there is no single completely reliable parameter for ovulation prediction. Variations among hormonal and clinical parameters during a specific menstrual cycle are troublesome, and there is considerable variation exists even in the same patient (Garcia et al., 1981). Advances in ultrasound technology have allowed better delineation of ovarian follicular growth before ovulation. There is, however, still a gap between observations from follicular measurements and the understanding of biophysical relationships that provide the patterns of follicular growth. Even the later study showed a wide variation of follicular size before ovulation, and it was concluded that ultrasound examinations alone would not provide sufficient information for the prediction of ovulation. However, ultrasound experience has shown that not only does follicular volume at ovulation vary, but there is also wide variability in the time of ovulation, which indicates that there are different length of the
folicular growth periods and thus, the quality of the ova may be reflected by the size of the follicles, the duration of folicular growth or by combination of those factors (Rossavik and Gibbons, 1985).

Earlier reports have been devised to monitor ovarian function and predict variations of fecundity (potential fertility) in the women (Albertson and Zinaman, 1987). In particular, results from studies of ovarian function and steroid metabolism have suggested that changes in the concentration of urinary estrogen and progesterone metabolites might be used as practical index of reproductive status. Recent data suggest that the onset of puberty, rather than chronological age, is linked to the increase in rates of depression in women (Worthman et al., 1998). Thus, changes in the reproductive hormonal milieu may precipitate or alleviate depression in women. This seems particularly in the case of rapid-cycling affective illness. Fluctuations in reproductive hormone levels in women can have a significant impact on mood. Thyroid function also plays an important role in the regulation of mood in women, and it should be monitored during times of reproductive hormonal change, when there may be an increased risk of developing hypothyroidism.

Reproductive ageing is associated with reduced fertility and endocrine changes that become more pronounced during the premenopausal period (Ebbiary et al., 1994). An early sign of the aging of the reproductive mechanism can be detected in women who are having normal ovulatory cycles. The regulation of FSH and LH secretion appears to be sufficiently independent to permit the observed differences in the age of onset of these premenopausal increases (Lenton et al., 1998). The mid cycle LH surge triggers a series of biochemical reactions that lead to follicular rupture and expulsion of the ovum (Yen, 1978). Other pituitary hormones, prolactin (PRL) and follicle stimulating hormone (FSH) are also released at this time (Vekemans et al., 1977) but their role in
ovulatory events is uncertain. A noninvasive method for evaluation of luteal function is the need of the hour. A single assay of serum progesterone is not sufficient to characterize the duration or adequacy of luteal activity and cannot detect the onset of progesterone secretion at the time of ovulation. Progesterone determined the adequacy of measuring the concentration of pregnanediol, in early morning saliva specimen as a means of detecting the occurrence of ovulation (Judge et al., 1978) and as a method for describing luteal function during normal menstrual cycle. The importance of predicting human ovulation for either optimizing or avoiding conception has been considered from an endocrine, morphological and clinical viewpoint. Of the biochemical markers in peripheral blood, a knowledge of the LH peak is the most clearly defined, with a two to four fold increase above baseline levels for a relatively short 24-30 hour preovulatory period. Daily assessment of the rise in preovulatory estrogen reflects graafian follicle development but the rise is less distinct and spread over 3-4 days with marked day to day fluctuations. LH induces a marked reduction in estrogen production some 12 hours prior to ovulation and at the same time induces a two to three fold increase in progesterone production above baseline levels. While these changes in themselves are not great enough for day to day discrimination, a knowledge of their reciprocal relationship may be. The preovulatory period shows a rise in FSH which is relatively small compared to LH. Yet the radioimmunoassay technique has not generally been refined to be as rapid and reliable.

During menstrual cycle the level of hormones like estrogen, progesterone and thyroid hormones will vary during different phases. These hormones are nothing but they are the metabolic products of cholesterol. Cholesterol breakdown leads to higher concentration of fatty acids into the blood stream which will be released in the body fluids (i.e. saliva is a dilute aqueous fluid
originating from salivary glands which serves various functions like digestion, lubrication and protection of the oral mucosa (Vining et al., 1983) during reproductive phases. The identification of these fatty acids will help for detection of the ovulation. The most important way to enhance the fertility is to identify the days which are more conducive for conception in order to ascertain the days to perform the act in intercourse. The most fertile time of a woman is the 24 hours period around ovulation.

Many constituents of saliva have been studied and their relationship with menstrual cycle and ovulation has been established. These constituents include proteins, urea, amino acids, electrolytes, mucin, lipids derived compounds, sugars (glycosaminoglycans), and enzymes like alkaline phosphatase and lactate dehydrogenase. Most of these constituents bear no precise relationship to the time of ovulation. N-acetyl-β-D-glucosamine concentration in saliva apparently shows cyclic changes during the menstrual cycle, an increase at mid cycle and a decrease at the end of the menstrual cycle (Rosado et al., 1977).

The cyclic nature of circulating levels of sex hormones in premenopausal women and its possible impact on the levels of lipids and lipoproteins, emphasize the need to determine how these levels vary between the follicular and luteal phases of the menstrual cycle. This is especially important because even small changes may be seen between menstrual cycle phases (Gordon et al., 1989). The biosynthesis of steroid hormones is dependent on a source of cholesterol, which may be derived from both de novo synthesis and uptake of plasma lipoproteins (Brown et al., 1979; Carr and Simpson, 1981). Evidence that favors an important physiological role for these receptors and plasma LDL in steroid hormone synthesis have been provided from several sources. This study provides important data on the levels of lipids and
lipoproteins as well as sex hormones during the follicular and luteal phases of the menstrual cycle under very controlled metabolic conditions.

Specific high affinity receptors for low density lipoprotein (LDL) have been demonstrated in steroid hormone-producing tissues from bovine (Faust et al., 1977), mouse (Kovanen et al., 1979; Savion et al., 1982), and human (Simpson et al., 1978; Carr et al., 1980; Winkel et al., 1980a; Winkel et al., 1980b; Brown et al., 1981) sources and appear to be identical to those that are genetically absent in patients with receptor-negative homozygous familial hypercholesterolemia (Brown et al., 1981). On account of the cyclic nature of circulating levels of sex hormones in premenopausal women and their possible impact on the levels of lipids and lipoproteins, and hence CHD appears risk, it is important to determine how these levels vary between the follicular and luteal phases of the menstrual cycle. Fluctuations in the levels of T-C, Triglycerides, phospholipids, free fatty acids and its derivatives and lipid protein metabolites like HDL-C, LDL-C and VLDL-C are compared between menstrual cycle phases, although short term and small, need to be considered in the screening and medical monitoring of premenopausal women, especially those with borderline levels. Although it is small, such fluctuations may prove to be clinically significant in the long run.

A variety of endogenous biochemical substances such as salivary glucose, alkaline phosphatase, arylsulfatase, β-glucuronidase, N-acetyl-β-D-glucosaminidase, as well as phosphorus have been found to fluctuate during the ovulatory period of the menstrual cycle. A little work has been done on electrolytes. Reports of serum levels of phosphorus and/or phosphorus-containing substances in the blood are inconclusive to suggest either a significant or nonsignificant pattern of
periovulatory fluctuation. Simpson and Dale (1972) found no significant variation in serum phosphorus, calcium, or magnesium during the menstrual cycle in normally cyclic women, or women who took oral contraceptives. MacDonald and MacDonald (1977) measured erythrocyte 2, 3-diphosphoglycerate (2, 3 DPG) levels in women during the menstrual cycle and reported no significant change in concentration. Punnonen (1978) reported low levels of phospholipids at various phases of the menstrual cycle. Pitkin et al., (1978) found levels also vary throughout the menstrual cycle, but no significant pattern was present even though parathyroid hormone levels rose progressively through the follicular phase to peak at or slightly before the LH surge, then fell progressively through the luteal phase.

The fact that phosphorus in sputum was found to peak during the periovulatory period while no significant fluctuations was found in serum phosphorus and suggests that there may be changes in phosphorus metabolism around ovulation. It is possible that hormone-induced changes in phosphorus may be compensated by the active secretion of phosphorus into saliva. This hypothesis has been advanced (Ben-Aryeh et al., 1976) and seems tenable since phosphorus in its association with intracellular messenger c-AMP is involved in regulatory aspects of the menstrual cycle in a variety of ways. In addition to the above regulatory function of phosphorus in the menstrual cycle, the ion appears to be itself regulated by a spectrum of feedback mechanisms and hormone systems. Pitkin et al., (1978) found an increased in the associated progressive parathyroid hormone (PTH) during the follicular phase which is at peak or slightly before LH surge and then fell progressively throughout the luteal phase. Calcium, however, appeared to fall three to four days before ovulation and then increased due to the fluctuation of estrogen.
The concentration of salivary glucose, sialic acid and glycosaminoglycans was considered as indicators of ovulation (Davis and Balin, 1972; Oster and Yang, 1972). During that part of the cycle between ovulation and the onset of menstruation (the luteal phase), the concentration of progesterone is high. Progesterone reportedly has a natriuretic effect (Landau and Lugibihil, 1958) and the increase in progesterone after ovulation is thought to be followed by a compensatory rise in aldosterone concentration (Korda and Horvath, 1979). After the menopause the concentration of uric acid in plasma increases (Forsling et al., 1981). This is believed to result from the decrease in sex-steroid concentration, similar to that which occurs at the time of onset of menstruation. The fact that the change in plasma sodium is not associated with changes in weight or in concentrations of creatinine, urea, or albumin suggests that total body water and intravascular volume remain constant. Thus it appears that sodium is lost in excess of water during the period after ovulation (Mira et al., 1984). Estrogens leads to a marked acceleration of calcium uptake and decrease of its elimination through pigeon's gut (Silberberg, 1956). In non pregnant women estrogen administration produces increased parathyroid activity (Wernly et al., 1965). It is known that the calcium homeostasis is maintained by parathyroid glands; however, the effect of menstrual cycle on serum calcium remains controversial (Southam and Gonzaga, 1965). Magnesium is involved in basal metabolism that changes over the course of menstrual cycle (Solomon et al., 1982). These evidences suggest that ovarian hormone possibly influence calcium, magnesium and inorganic phosphate metabolism during different phases of menstrual cycle.

The glycolytic enzymes, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase, and hexokinase in cervical mucus of ovulatory women were serially measured daily during the
menstrual cycle. Among all the enzymes, the cyclic changes in LDH activity were most significant, being high during the proliferative phase, gradually decreasing to the lowest level around ovulation, and then increasing markedly. Estrogen inhibits cervical mucus LDH activity while progesterone accelerated it (Takehisa, 1980). The cellular content of the saliva has shown to be a major source of the three enzymes. The enzymes were found to have maximum levels of activity during the periovulatory phase of the cycle. This observation can be attributed to the presence of increased numbers of exfoliated cells from the oral mucosa resulting from the pre-ovulatory estrogen stimulus to cell proliferation. The activity of alkaline phosphatase decreased significantly at midcycle just prior to the LH surge and begins to rise after ovulation. Earlier reports pointed out that the activity of cervical mucus alkaline phosphatase decreased significantly at midcycle just prior to the LH surge and began to rise after ovulation. Self-detection of cervical mucus alkaline phosphatase may provide a practical method of ovulation prediction (Moghissi et al., 1976).

Reactive oxygen species (ROS) play a physiological role during ovulation that is similar in some respects to inflammation (Bjersing and Cajander, 1974; Espey, 1980). Ovary is a metabolically active organ and, hence, it is under a variety of stresses continuously. Ovulation is suppressed by agents that inhibit acute inflammatory reactions (Espey et al., 1982). Since ROS is generated during inflammatory process, it is reasonably hypothesized that ROS is released in connection with follicle rupture and is involved in the process (fig.1). The source of ROS appears to be inflammatory cells, such as macrophages and neutrophils, as they are present in ovary at ovulation (Nakamura et al., 1987; Adashi, 1990; Brannstrom et al., 1993) and produce tremendous amount of free radical. This notion is supported by the findings that the suppression of ROS by SOD and/or catalase in
in-vitro perfused rabbit ovary preparations hinders ovulation (Miyazaki et al., 1991). Monoxygenase reaction, mediated by P450, is required for the steroidogenic process that inevitably produces ROS as byproducts. ROS levels in the corpus luteum actually rise during the regression phase (Riley and Behrman, 1991; Sawada and Carlson, 1991; Sugino et al., 1993; Sawada and Carlson, 1994). The NADPH-dependent generation of superoxide in the mouse ovary increases during the early pre-ovulatory phase in cyclic females and during the luteal phase in pregnant animals (Jain et al., 2000). Ovarian as well as uterine NADPH-dependent superoxide production appears to be LH-inducible. ROS and related compounds may function as intracellular regulators of steroidogenesis and progesterone release in the corpus luteum (Carlson et al., 1993; Sawada and Carlson, 1994; Carlson et al., 1995; Sawada and Carlson, 1996).

(fig.1)
Excess oxidation, however, causes oxidative stress, resulting in the dysfunction of the reproductive process. Antioxidation that reduce the levels of reactive oxygen species are of prime importance in reproductive systems in maintaining the quality of gametes and support reproduction. While anti-oxidative enzymes, such as superoxide dismutase and peroxidase, play a central role in eliminating oxidative stress, reduction-oxidation (redox) systems, comprised of mainly glutathione and thioredoxin, function to reduce the levels of oxidized molecules (Fujii et al., 2000). Since the production of ROS is high in reproductive tissue due to active metabolism and steroidogenesis, the tissue is under continuous oxidative stress. ROS modifies susceptible molecules including DNA, lipids, and proteins. It damage the oocytes and increases the risk of hereditable disease, and, hence, living organisms must eliminate such gametes to preserve the species. On the other hand, the reproductive system utilizes ROS in some processes that are essential for reproduction. Rises in some polymorphonuclear neutrophil leucocyte enzymes were also seen around ovulation. The mid-cycle peak peroxidase activity is affected by a number of factors, such as immunization, allergies, infection, and even some foodstuffs and time of day, therefore it is, at least at this stage of investigations, difficult to say whether it would be possible to use it to detect ovulation and to control fertility (Cockle and Harkness, 1978).

Cyclic changes in various physical properties and biochemical constituents of saliva are known to reflect accurately the hormonal changes associated with an menstrual cycle and may be utilized clinically to determine the time of ovulation. For example, the biochemical substances like salivary amino acids as well as hormones like estrogen and progesterone have been found to fluctuate during the ovulatory period of the menstrual cycle (Landau and Lugibihil, 1967: Lyons et al., 1989). In blood plasma,
the large amount of neutral amino acids exhibited maximum reduction and remained low during postovulatory period (Wall and Truswell, 1991). Yet so far, attempt has not been made to correlate the changes in the salivary amino acids with the detection of ovulation. Hence, the aim of the present study was to determine the concentration of amino acids in saliva obtained serially during menstrual cycle and to relate it's quantification to the time of ovulation in the view of reverse phase-HPLC.

Humans emit a complex array of volatile and nonvolatile molecules that are influenced by an individual's genetics, health, diet, and stress. Olfaction is the most ancient of our distal senses and this may be used to evaluate food and environmental toxins as well as to recognize kin and potential predators (Preti et al., 2007). However, saliva and properly collected alveolar air samples must pass over or come in contact with the posterior dorsal surface of the tongue, a site of bacterial plaque development and source of halitosis-related volatiles. The application of gas chromatography/mass spectrometry (GC/MS) in a comparative experiment is to identify volatile compounds from women saliva that differ in concentration between reproductive phases (Willse et al., 2005).

A complex mixture might comprise several hundred or even thousands of volatile compounds. The statistical problems offer significant challenges beyond traditional two-group screening procedures, because their number and location in a chromatogram are generally unknown, and also detected the components overlap in populous chromatograms. The detection and attribution of these salivary volatile markers provide the possibility for new, non-invasive diagnostic methods.
It is widely assumed that, after ovulation, the human endometrium undergoes specific changes and becomes receptive to the implantation of embryo during the mid-secretory phase. When implantation does not take place, further changes occur which eventually result in the shedding of human endometrium (Von wollf et al., 2000). Human endometrium is a steroid-sensitive tissue and there is evidence that supports the belief that heat shock proteins (HSP) are implicated in the regulation of steroid function. Therefore, it was examined the expression of various members of the heat shock family of proteins which were evaluated in the steroid-responsive human endometrium (Tabibzadeh et al., 1996). There are increasing evidences that chaperones (Proteins) are also present outside the cell, exerting cytokine-like effects and influencing immune recognition. Hsp70 has been found to be present in human blood sera. Chaperones act only upon an unfolded or denatured (partial/complete) protein, and thus help in post translational modification. Many chaperones are heat shock proteins, i.e. proteins expressed in response to the elevated temperatures or other cellular stresses. Chaperonins Cpn10 and Cpn60 are present in pancreatic juice, but Hsp70 is not. These observations raise the possibility that molecular chaperones may be present in other secretory fluids, like human saliva (Fabian et al., 2003). Saliva protein concentration is dependent on gland production, time of the day, diet, age, gender and status of disease (Ferreiro et al., 2002). In term of protein composition, the main component is α-amylase, which represents 60% of total saliva protein content. Other saliva proteins are heat shock protein, lactoferrin, immunoglobulins, carbonic anhydrase, albumin and a wide range of peptides which include cystatins, statherin, lyzosyme, histatins and a broad class of typical peptides mainly contributed by prolines labelled as proline rich proteins (PRPs). It is also possible to find small peptides, due to the salivary proteolytic activity (Perinpanayagan et al., 1995). Components of saliva are
secreted from a number of glands including the parotid gland, which in humans, secrete a protein-rich fluid of low viscosity and are thus referred to as a serous gland, and the submandibular and sublingual glands, which in humans secrete a carbohydrate-rich fluid of higher viscosity and are referred to as mucus glands. Additional minor salivary glands include the von Ebner's gland. The proteins in saliva perform many of its functions (Levine et al., 1987; Beeley, 1993). The protein composition of saliva varies considerably among species (Young et al., 1981), reflecting diverse diets and modes of digestion. The function of some of the salivary proteins is known; for instance the glycosidase, amylase, protease, kallikrein, histatin family of fungistatic proteins and the cystatin family of protease inhibitors (Karn et al., 1978; Oppenheim et al., 1988; Freije et al., 1991). They have revealed a wide diversity in primary sequence and extensive posttranslational processing for salivary proteins (Minaguchi and Bennick, 1989). Despite these advances, the primary structure and function of many of the salivary proteins still remain to be determined. A promising approach to the study of saliva is the identification of its protein components in reproductive periods using proteomic techniques. Moreover, a comparison between samples from reproductive phases such as preovulatory (6-12 days) ovulatory phase (13-14 days) and postovulatory phase (15-26 days) subjects may reveal unique or increased levels of specific proteins that may be used as biomarkers (Amado et al., 2004). The present study is to gain a better knowledge on saliva protein composition. To achieve that goal, it is used relatively new technique of MALDI-TOF MS/MS analysis in the present study. This mass spectrometer is specifically designed for use in proteomic studies with the usual sensitivity and accuracy of a standard MALDI-TOF combined with automated MS and MS/MS spectra acquisition of tryptic digest peptides increasing the sensitivity and reliability of protein identification in biological samples (Bienvenut et al., 2002).
The present study mainly focuses on the analysis of chemical profiles in human saliva (quantitative test) from different phases of normal menstrual cycles with the help of physical, biochemical and molecular tools in order to detect the timing of ovulation. The reason behind the investigation is to obtain the biochemical profiles, which may provide the evidence for the detection of fertility status (i.e. ovulation) in women.

**THE FOLLOWING ARE THE MAIN OBJECTIVES OF THE PRESENT STUDY**

1. To check the fern pattern, ultrasonology, hormone level in saliva during different phases of menstrual cycle.

2. To determine the concentration of minerals, sialic acid and glycosaminoglycans in the saliva of human female during various phases of menstrual cycle.

3. To quantify salivary lipids and their constituents like cholesterol, triglycerides, phospholipids, HDL-C, free fatty acids and GC-Fatty acids during menstrual cycle.

4. To evaluate the specific salivary volatile compounds using GC-MS in relation to predict fertile time during menstrual cycle.

5. To quantify and compare the antioxidant enzymes viz, peroxidase, glutathione peroxidase, catalase, superoxide dismutase, lactate dehydrogenase and alkaline phosphatase concentration in the saliva during various phases of menstrual cycle.
6. To identify salivary protein using SDS-PAGE and MALDI-TOF/MS during the menstrual cycle.

Reasons to select the saliva as the source for the present investigation are

ii) Sample **collection is non-invasive and painless**
iii) Obtaining the sample is easy.
iv) Saliva offers an attractive alternative to blood or tissue for the isolation of protein samples for use in diagnostics.