REVIEW OF LITERATURE
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Scientific study relating to ovulation and fertility is still in its infancy in recognizing the complexity of the ecology and it knows even less about how alteration of individual components of the system that would affect the human physique. The understanding of these interactions may make it possible to manipulate the system in favor of promotion and maintenance of oral health. The type and quantity of oral microbes harbored in the oral cavity may contribute to elevate risk levels for a number of systemic diseases.

Infertility can be associated with polycystic ovarian syndrome (PCOS) and endometriosis. Also, an imbalance of the female hormones can disrupt ovulation: further the hormonal imbalance can also interfere with the ability of a fertilized egg to implant and develop in the uterus leading to difficulty in achieving pregnant or an inability to sustain pregnancy. The cause of PCOS is unknown, and it is a condition in which cysts form in the ovaries, disrupting ovulation, leading to infertility and other menstrual problems. It is marked by several hormonal imbalances. Estrogen and progesterone levels must be balanced in order to work together efficiently. Estrogen dominance is mainly due to the too much of estrogen in relationship to the amount of progesterone produced in the body. The causes of the excess of estrogen are:

i) Estrogens in foods and the environment
ii) synthetic hormone use (HRT)
iii) obesity and
iv) low progesterone levels due to lack of ovulation

In women, a common cause of infertility is an ovulation problem—that is, the ovaries do not release even an eggs each month. Ovulation problems result in the malfunctioning of the female reproductive system. This system includes the
hypothalamus (an area of the brain), pituitary gland, adrenal glands, thyroid gland, and genital organs. For example, the ovaries may not produce enough progesterone, the female hormone that causes the lining of the uterus to thicken in the preparation for a potential fetus. Ovulation may not occur because the hypothalamus does not secrete gonadotropin-releasing hormone, which stimulates the pituitary gland to produce the hormones that trigger ovulation (luteinizing hormone and follicle-stimulating hormone). High levels of prolactin (hyperprolactinemia), a hormone that stimulates milk production, may result in the low levels of the hormones that trigger ovulation. Prolactin levels may be high because of the pituitary gland tumor (prolactinoma), which is almost always noncancerous. Ovulation defects may be due to polycystic ovary syndrome, thyroid gland disorders, adrenal gland disorders, excessive exercise, diabetes, weight loss, obesity, or psychologic stress. Sometimes the cause is early menopause—when the supply of eggs has run out early. The dysfunction of the ovulation causes the irregular periods or no periods in women (amenorrhea). Sometimes women, who have regular menstrual periods, do not have premenstrual symptoms, such as breast tenderness, lower abdominal swelling, and changing of moods.

To determine if or when ovulation is occurring, clinicians may ask a woman to take her temperature at rest (basal body temperature) daily. Usually, the best time is immediately after awakening. A low point in basal body temperature suggests that ovulation is about to occur. An increase of more than 0.9° F (0.5°C) in temperature usually indicates that ovulation has occurred. However, basal body temperature does not reliably or precisely indicate when ovulation occurs. At best, it predicts ovulation only within two days. More accurate techniques include ultrasonography and ovulation predictor kits (which detect an increase in luteinizing hormone in the urine 24 to 36 hours before
ovulation). These kits are used at home to test urine on several consecutive days. Also, the level of progesterone in the blood or saliva or the level of one of its by-products in the urine may be measured. A marked increase in these levels indicates that ovulation has occurred. To determine whether ovulation occurs regularly, doctors may perform an endometrial biopsy. Administering the tissue typing technique in the removed tissue, the occurrence of ovulation can be ascertained. If changes occur, it is the proof of the occurred ovulation. If the normal changes appear delayed, the problem may be inadequate production or inactivity of progesterone.

Despite major achievements in elucidating many details of the physiologic processes of human reproduction during the past 2 decades, a simple, reliable, and inexpensive method for the prediction and detection of the time of ovulation is still not available. For clinical purposes, studies of BBT and biophysical and biochemical constituents of cervical mucus appear to be the most practical methods of ovulation detection. The development of rapid and easy-to-use urinary LH assays and steroid assay kits for measurement of urinary and salivary estrogen and progesterone or their metabolites may further improve the precision of ovulation timing (Moghissi, 1992).

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 2-4 fold increase above baseline levels for a relatively short 24-30 hour preovulatory period. Ovulation is considered to occur 28-36 hours after the beginning of the LH rise or 8-20 hours after the LH peak. Daily assessment of the preovulatory stage, rise in 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 2-3 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 due to endocrine axis. The preovulatory phase reflects rise in FSH is relatively small compared to LH, and the radioimmunoassay technique has not generally been refined to be as rapid and reliable. Monitoring the day to day growth of the preovulatory follicle ultrasonically is both linear and potentially predictable but there is a wide range of its final diameter (17-25 mm) prior to making ovulation prediction inaccurate (Kerin, 1982).

Increasing evidence derived from experimental and clinical studies suggests that the hypothalamic-pituitary-thyroid axis (HPT) and the hypothalamic-pituitary-ovarian axis (HPO) are physiologically related and act together as a unified system in a number of pathological conditions. The suggestion that specific thyroid hormone receptors at the ovarian level might regulate reproductive function, as well as the suggested influence of estrogens at the higher levels of the HPT axis, seems to integrate the reciprocal relationship of these two major endocrine axes. Both hyper- and hypothyroidism may result in menstrual disturbances (Doufas and Mastorakos, 2000). It has long been recognized that the deficiency of maternal thyroid hormone or excess can influence the outcome for mother and fetus at all stages of pregnancy, as well as interfere with ovulation and fertility (Glinoer, 1997; Casey and Leveno, 2006). Thyroid dysfunction is extremely common in women and has unique consequences related to menstrual cyclicity and reproduction (Redmond, 2004). Thyroid hormones are essential for normal growth, sexual development and reproductive function.
During puberty, changes in thyroid functions and an increase in thyroid volume occur as an adaptation to body and sexual development (Weber et al., 2004). The effect of hyperthyroidism on pubertal development is not well known, but a short period of hyperthyroidism seems not to have major negative effects. In adolescence or young adulthood, menstrual dysfunction, infertility, and stillbirth or premature birth are associated with thyroid dysfunction. Neurocognitive functioning may be impaired in the luteal phase of the menstrual cycle due to associated changes in hypothalamic-pituitary adrenal (HPA) axis function. This study examines the relationship between changes in neurocognition and HPA axis function in different phases of the menstrual cycle. Reliable methods to expect ovulation are still lacking, therefore predicting the fertile period is most likely to occur, but these guidelines were outdated (Wilcox et al., 2000).

Saliva testing is the most reliable way to measure free, "bioavailable" hormone activity, that is, the hormones actually doing their jobs at the cell level. Furthermore, saliva collection is painless and easy, and can be done conveniently in one's own home. Saliva testing shows patterns over a period of time, for example throughout the entire menstrual cycle. Saliva testing shows multiple hormone levels and their functional interaction. Saliva tests are cheaper than similar blood serum tests. More importantly, it is a noninvasive fluid and easy to handle for biochemical and molecular studies in order to detect directly or indirectly for the prediction of ovulation.

Since the discovery of amylase in saliva, early in the nineteenth century, the excess of 30 other enzymes have been identified in saliva. These include esterases, maltase, phosphatases, hyaluronidase, catalase, peroxidase, ribonucleases, dehydrogenases, carbonic anhydrase, kallikrein, lysozyme,
glutamine oxaloacetate transaminase and glutamine pyruvate transaminase (Burgen and Emmelin, 1961; Chauncey, 1961). Most of the early studies were done on the saliva collected from the buccal space of humans and thus the origin of the enzymes is equivocal, often being from corpuscular or oral bacterial sources (Dreizen et al., 1959; Chauncey, 1961; Eichel et al., 1965; Ferguson, 1968) rather than glandular sources. Chauncey et al. (1963) assayed separately collected parotid and mandibular salivas of man, sheep, pig, dog and rabbit for amylase, acid phosphatase, non-specific esterase, pseudocholinesterase and galactosidase. Common to all of these salivary secretions was the presence of acid phosphatase and esterase activity whereas the other enzymes, whilst present in most of the salivas, were low or absent in one or more of these species. Sheep saliva lacked both amylase and β-galactosidase, which arguably may be related to this species being a foregut fermenter as these two enzymes directly influence carbohydrate breakdown (Beal, 1998).

Plasma estrogen levels may be twofold or threefold higher in hyperthyroid than in normal women during all phases of the menstrual cycle (Akande and Hockaday, 1972). However, hyperthyroidism results in increased levels of sex hormone–binding globulin (SHBG) (Ruder et al., 1971; Tulchinsky and Chopra, 1973). Whether the increase in plasma estrogens is entirely attributable to the elevated sex hormone–binding globulin or whether there is an actual increase in unbound estrogen, as in the case of hyperthyroid men, remains to be determined. The metabolic clearance rate of 17-β-estradiol is decreased in hyperthyroidism and is thought to be largely due to increased binding of 17-β-estradiol to sex hormone–binding globulin (Ridgway et al., 1975). Menstrual disturbances have also been described in patients with hyperthyroidism. Biochemical and hormonal abnormalities, nutritional disturbances, and emotional upheavals associated with
hyperthyroidism may, individually or in combination, influence menstrual function.

Human odour (pheromones) has been reported to excite the residual human vomeronasal organ (Monti-Bloch and Grosser, 1991; Berliner et al., 1996; Grosser et al., 2000), to activate specific regions within the brain (Sobel et al., 1999), to affect social behavior (Grosser et al., 2000, Cowley and Brooksbank, 1991), to regulate ovulation (Stern and McClintock, 1998), and to modulate, in a gender specific way, physiological parameters such as serum levels of testosterone, luteinizing hormone, and follicle stimulating hormone as well as respiration and cardiac frequency (Monti-Bloch et al., 1998; Shinohara et al., 2000). Chemically, the currently known human pheromones are volatile steroid molecules such as, e.g., the 16-androstenes, 5a-androst-16-en-3b-ol or 5a-androst-16-en-3-one. Accordingly, the biosynthesis of pheromones should be strongly associated with the biosynthesis of cholesterol, the essential substrate for steroid hormone synthesis. Recent evidence suggests that many of the enzymes of the cholesterol synthesis pathway exist mainly in the peroxisomes (Appelkvist et al., 1990; Krisans, 1996; Biardi and Krisans, 1996; Olivier et al., 2000). In particular, the enzymes mevalonate kinase (MVK) and farnesyl diphosphate synthase (FPPS) seem to be localized predominantly, if not exclusively, in this organelle (Aboushadi et al., 1999; Krisans et al., 1994; Biardi et al., 1994). Additional evidence for the essential role of peroxisomes in cholesterol biosynthesis is provided by the deficiency of enzymes of cholesterol synthesis in patients with defects in peroxisome biogenesis (Hodge et al., 1991; Wanders and Romeijn, 1998).

Saliva is a mixture of ions, small organic molecules, enzymes and proteins, some in multiprotein complexes and other biochemical's (Kaufman and Lamster, 2000). Add to this the oral microorganisms and their byproducts, and an ecological system is
created that either maintains good oral health or conversely, contributes to its decline (Lenander-Lumikari and Loimaranta, 2000). Whole saliva is a complex mixture of parotid, submandibular, sublingual and minor gland secretions, together with bacteria, leukocytes, sloughed epithelial cells and crevicular fluid. The uses of different stimulants of saliva secretion produce samples where the secretions from the major salivary glands occur in different proportions. The concentration of most salivary constituents depends on the flow rate of saliva. Metabolite measurements in saliva are complicated by the presence of bacteria, epithelial cells and leukocytes. An additional complication is that a result of bacteria action, the composition of saliva changes on standing. In traditional methods of analysis, saliva is therefore collected on ice to arrest the bacterial metabolism. Centrifugation stops the bacterial action and removes both the cells and the turbidity, which can interfere with many analytical techniques. Such clarification however may decrease the levels of certain salivary parameters. Furthermore, saliva can be obtained by non-invasive techniques; this is helpful in cases where multiple serial samples are needed and in monitoring of adults and children.

Puskulian (1972) found a decrease in the sodium and calcium concentrations and increase in potassium concentration in submandibular, but not parotid saliva, at the time of ovulation. Although she discusses possible hormonal causes the most probable explanation is the fact that flow rate of submandibular saliva was also lower at the time of ovulation. (Chesley and Hellman, 1957; Katrampass and Mylona-Hatziotis (1963) reports indicate that it was unable to detect a menstrual rhythm in the sodium and potassium concentrations in unstimulated whole saliva. Foster and Lorinez (1971) studied the variation in alkaline phosphatase concentration in paraffin-stimulated whole saliva collected each day before breakfast by women over one or more
menstrual cycles. They reported a significant increase in enzyme activity in the immediate preovulatory period. Speirs (1961) reported that the hyaluronidases activity of wax-stimulated whole saliva was higher during menstruation that at other phases of the menstrual cycle. However, Prout and Hopps (1973) found that the bacterial contribution of the hyaluronidase activity of unstimulated whole saliva was independent of the phase of the cycle but the hyaluronidase activity of non-bacterial origin in saliva actually decreased at the time of menstruation.

Quantitative changes in excretion of certain metabolites can be used to characterize several normal endocrine-mediated physiological changes as well as changes caused by pathological disorders (Chen et al., 1970; Hutterer et al., 1971; Dubowski, 1974; Thompson and Markey, 1975; Jellum, 1977; Preti and Huggins, 1978; Tonzetich, 1978; Zelson et al., 1980; Janak et al., 1980;). Exhaled mouth air has received attention as a diagnostic medium and has been shown to contain a complex mixture of organic compounds of both systemic and oral-cavity origin (Tonzetich, 1971; Dubowski, 1974; O'Neill et al., 1977). Many volatiles are produced in the oral cavity through putrefactive action of microorganisms on proteaceous substrates in whole saliva (Spouge, 1964; Tonzetich and Kestenbaum, 1969; Lee et al., 1972), which consists of primarily secretions from glands, but also contains gingival exudates, food debris and skin lipids (Miles, 1958; Sicher and Blaskor, 1972; MacFarland and Mason, 1975). Increased numbers of leukocytes, exfoliated cells, and bacteria as well as increased gingival exudates are thought to be responsible in part for the accelerated putrefaction seen in the saliva of patients with degenerative periodontal disease (Berg et al, 1947; Tonzetich, 1978, 1977). Volatile sulfur compounds have been extensively studied because they are responsible for oral malodor (halitosis) and their concentration have been shown to increase with the
severity of periodontal involvement. Hydrogen sulfide, ammonia and urea are found in gingival crevicular fluid and have been implicated in the etiology of periodontal degradation (Rizzo, 1967; Golub et al., 1971; Golub and Kleinberg, 1976).

Saliva is produced in three major gland as well as minor salivary glands, which together produce an impressive amount of 1000-2000 ml/day (Castle and Castle, 1998; Bardow et al., 2001; Turner and Sugiya, 2002; Gorr et al., 2005). Salivary secretion is mainly controlled by the autonomic nervous system. Studies in animals show that secretion of protein in saliva is dependent on both parasympathetic stimulation (Edwards and Titchen, 1992; Calvert et al., 1998; Proctor et al., 2000; Carpenter et al., 2005) and sympathetic stimulation, as has been shown to occur in the parotid gland (Edwards and Titchen, 1992) and also in the submandibular gland (Turner and Sugiya, 2002). All the normal daily imbalances on the cholinergic and adrenergic systems, or exogenous conditions that could induce different stimulation such as medication, radiation and food ingestion, among others will produce changes in salivary flow or/and saliva composition (Jensen and Barkvoll, 1998; Bardow et al., 2001). The proteome is defined as the complete set of proteins in a cell at a certain period of time and, thus this definition does not apply to salivary analysis. In fact, the salivary proteome should be understood as the complete set of proteins existing in a given moment on whole saliva present on the oral activity independently of it's origin. In saliva, the major sources of proteins are salivary glands, but microorganism (in particular bacteria) (Macarthur and Jacques, 2003), blood (Huang, 2004), oral tissues (Kojima et al., 2000; Madden et al., 2002) could also be important protein contributors and should be included as a part of the salivary proteome.
The menstrual cycle-dependent changes in the amounts of heat shock protein suggest the regulation is by steroid hormones (Tabibzadeh et al., 1995). Human endometrium, in response to steroid hormones, undergoes characteristic cycles of proliferation, secretory changes, and tissue shedding. Human endometrium expresses a molecular repertoire which includes the heat shock proteins (Hsps) Hsp27, Hsp60, Hsp70, Hsp90, and alpha crystallin B chain. The expression of Hsp27, Hsp60, and the constitutive form of Hsp70 (Hsc70) shows a sharp increase in human endometrium after ovulation. The maximal expression of the molecular chaperone, alpha crystallin B chain, occurs during the secretory phase (Tabibzadeh and Broome, 1999). In view of known functions of the Hsps, it is likely that these proteins are involved in protection of the endometrial proteins against factors with the potential to lead to protein denaturation. The function of the Hsps may be to protect cells against the cytotoxic damage of TNF-alpha, particularly during the critical period of "implantation window."

Most of the work carried out in this field has been performed on human saliva but other mammals, such as rats and swine have been as models when invasive techniques are needed (Proctor and Mansson, 1990; Etzel et al., 1997; Proctor et al, 1997; Malberti et al., 1998; Marshall et al., 1999a and b; Amasaki et al., 2001 and Nishita et al., 2001; Matsushita et al., 2002). More than 300 proteins have been identified in saliva and it is possible that this number will quickly increase in the near future, particularly when the actual major limiting problems of protein identification in saliva such as high contents of mucins, debris and bacteria, as well as its high proteolytic activity and the drawbacks of protein separation and identification methodologies are overcome (Ghafouri et al., 2003; Yao et al., 2003; Huang, 2004; Vitorino et al., 2004; Wilmarth et al., 2004; Ramachandran et al., 2005; Dixon et al., 2005).
Saliva protein concentration is dependent on gland production, time of the day, diet, age, gender, and disease status (Ferreiro et al., 2002). In terms of protein composition, the main component is α-amylase, which represents 60% of total saliva protein content. Other saliva proteins are lactoferrin, immunoglobulins, carbonic anhydrase, albumin and a wide range of peptides which include cystatins, statherin, lysozyme, histatins and a broad class of typical peptides mainly constituted by prolines labelled as proline-rich proteins (PRPs). It is also possible to find small peptides, due to salivary proteolytic activity (Perinpanayagam et al., 1995). Peptides in saliva have important biological function namely associated with calcium binding to enamel, maintenance of ionic calcium concentrations (PRPs and statherin) or associated with antimicrobial action (histatins and cystatins; Amerongen and Veerman, 2002). Otherwise, these peptides are pellicle precursors (Lendnemann et al., 2000; Amerongen and Veerman, 2002).

Saliva is constituted by glycoproteins, mucins being the most representative class. Mucins are high molecular weight glycoproteins, mainly with O-linked glycoslations. It is considered to exist in two operational kinds, MG2 and MG1, based on their separation profile by gel exclusion chromatography. MG2 is the low molecular weight mucin fraction and it’s supposed to be involved in modulation of flora and oral cleanliness (Tabak, 1995). MG1 has a high affinity to tooth material, participating in pellicle formation (Tabak, 1995; Iontcheva et al., 1997; Offner and Troxler, 2000). Interactions of these glycoproteins with other salivary components affect the saliva viscoelastic properties and increase the complexity of this fluid (Offner and Troxler, 2000). Moreover, the scavenging properties of mucins, namely salivary proteins and peptides, make saliva difficult fluid to analyze.
Human saliva is a complex biological fluid, which contains a large array of proteins and peptides that have important biological functions including the maintenance of oral health. In addition, blood concentrations of many identifiable components are reflected in saliva, since saliva contains an ultrafiltrate of the blood. The relatively easy non-invasive nature of collection and the relationship of saliva with plasma levels make saliva diagnosis of diseases (Streckfus and Bigler, 2002; Veerman, et al., 2004).

In whole saliva, the major sources of proteins are the contralateral major (parotid, submandibular, sublingual) and minor (von ebner) salivary glands but also blood, oral tissues and microorganisms can be contributors to the salivary proteome. The protein composition of whole saliva also depends on circadian rhythm, diet, age, gender and physiological status (Battino et al., 2002). With respect to protein content, saliva is mainly constituted by glycoproteins (e.g., mucins, proline-rich glycoproteins), enzymes (e.g., α-amylase, carbonic anhydrase) and a wide range of peptides (cystatins, statherin, histatins, proline-rich proteins). The biological functions of most of saliva proteins are still poorly understood, although protein components in saliva have been partially revealed by conventional biochemical strategies focused on individual molecules or specific group of salivary proteins. A promising new approach to study saliva is the global analysis of salivary proteins using proteomic techniques. Such exploration of the salivary proteome will not only improve our knowledge of oral physiology, but can also allow the identification of novel proteins and the examination of changes in protein levels under different physiological or pathologic conditions. Further, nowadays as in the present study the state-of-the art proteomic methods are applied to the analysis of salivary peptides/proteins (Amado et al., 2005).
It is analysed an assortment of conditions (chip surface type, matrix composition, sample dilution, the use of chaotropics/detergents) obligatory for generation of optimized and reproducible spectra. In addition, we have studied several pre-analytical steps that can alter the analysis of saliva including sample type, centrifugation speed, proteolytic degradation and stability during storage. The hyphenation of liquid chromatography with mass spectrometry (HPLC-MS) allows a fast separation and, at the same time, protein identification based on the theoretical molecular weight values obtained on available data bases. Another advantage of this association is the visualization and identification of possible post translational modification on complex mixtures (Li et al., 1999).