THE PREVALENCE OF LUTEAL PHASE DEFECT AMONG INFERTILE WOMEN

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Anatomy, School of Medical Sciences and the Department of Theoretical and Applied Biology, Faculty of Biosciences, KNUST. This work has not been submitted for any other degree.

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ABSTRACT

Normal endometrial receptivity is essential for the establishment of any pregnancy and its evaluation is thus considered a basic goal in the assessment of female infertility. This study was designed to investigate the prevalence of luteal phase defect (LPD) among infertile women, and LPD's association with age and type of infertility. Mid-luteal endometrial biopsies were taken from eighty (80) infertile women attending clinics at the gynaecology units of Komfo Anokye Teaching Hospital, Magazine Clinic and the Bomso Specialist Hospital all in Kumasi and ten fertile women as control using dilatation and curettage and then processed the samples for light microscopy. The results show that 65.0% of the biopsies of the infertile women were normal in development hence their infertility could be due to other factors. In 35.0% of the biopsies the endometrial development was out-of-phase and therefore suggestive of a defective luteal phase which may lead to a non-receptive endometrium during the implantation window.

There was no significant difference when LPD was analyzed according to age ($p=0.472$) as well as type of infertility ($p = 0.157$) suggesting that ageing and type of infertility was not associated with LPD.

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INTRODUCTION

1.1 INFERTILITY

The reproductive processes in humans require both excellent integrity of and excellent interactions between the female and male reproductive systems. Thus the origin of infertility is equally due to inefficient male or female factors. In approximately 40% of infertility cases, where the aetiology has been determined, they are due to female factors, another 40% to male factors, and the remaining 20% are due to both male and female factors (Hatasaka *et al.*, 1997). Infertility is defined as the inability of a non-contraceptive user but sexually active woman to have a live birth after 12 months of unprotected and regularly and properly timed intercourse at least three times a week (Pressat and Wilson, 1985). Millions of women and men worldwide are confronted with infertility. It has been estimated that about 20-30% of marriages in Sub Saharan Africa and 10-15% in the Western world are childless (Cates *et al.*, 1985; Okonofua, 1999). The average infertility in Africa is 10.1% of couples, with a high of 32% in some countries such as Cameroon, Central African Republic and Gabon (Gerais and Rushwan, 1992). This childlessness can be caused by primary infertility in which case the couple has never had a live birth or secondary infertility where the couple has had at least one live birth. However, the incidence of primary and secondary infertility varies enormously in each region of the world (Ericksen and Brunette, 1996). According to data from Larsen (2000) and also from a World Health Organization-sponsored (WHO) collaborative study of (1987), the incidence of primary infertility is relatively low in Africa as compared to secondary infertility which is the major cause of childlessness in Africa.

1.2 INFERTILITY AND SOCIETY

Parenthood is undeniably one of the most universally desired goals in adulthood, and most people have life plans that include children. However, not all such couples who desire a pregnancy will achieve one spontaneously and a proportion of couples will need medical help to resolve underlying fertility problems. Globally, WHO (1997) estimates that sixty to eighty million couples experience unwanted infertility. Several studies show that in resource-poor countries, where children are highly valued for cultural and economic reasons, childlessness often creates enormous problems for couples; especially for the women who are generally blamed for the infertility (Okonofua, 1997). Infertility is of particular concern in Africa because of the extent of the problem and the social stigma attached to it. Motherhood is often the only way for women to enhance their status within their family and community. In some places, the stigma of childlessness is so great that infertile women are socially isolated and neglected (Gerais and Rushwan, 1992). Studies from communities in Egypt (Inhorn, 1994b), Nigeria (Okonofua, 1997), Mozambique (Gerrits, 1997) and the Gambia (Sundby, 1997) showed that infertile women are often excluded from societal events and ceremonies or may even be despised and perceived as inauspicious. In some cases, they are feared as casting the "evil eye" on pregnant women. The psychological consequences of infertility have been well described in literature (Rowe, 2006; Seshagiri, 2001; Singh, 1996).

Available evidence suggests that the social consequences of infertility are particularly profound for African women as compared to men (Inhorn, 1994a; Inhorn, 1994b). Regardless of the medical cause of infertility, women receive the major blame for the reproductive setback and they suffer personal grief and frustration, social stigma, ostracism

and serious economic deprivations (Okonofua, 1997). In Cameroon, Feldman-Savelsberg (1994) reports that infertility is a ground for divorce among the Bangangte tribe causing a woman to lose her access to land distributed by her husband. Where she is able to avoid divorce, an infertile woman receives fewer gifts from her husband and is abandoned in old age with no child to till the land for her. In Egypt, women go through a complicated ritual known as *kabsa* (a form of fertility-producing, polluting boundary violation) in efforts to overcome infertility (Inhorn, 1994b; Inhorn and Buss, 1994).

Among the Ekiti of southwestern Nigeria, Ademola (1982) reports that infertile women are treated as outcasts and their bodies are buried on the outskirts of the town with those of demented persons. Infertile women often feel guilt and worthlessness leading to low self-esteem, depression and anxiety. In terms of the economic impact of childlessness, childless women and their families may feel that they have a lack of social security and support in their old age. Conjugal relations often become stressful due to infertility. Infertile women often receive disrespectful treatment by husbands, and the husbands' families may encourage them to divorce or take a second wife. At the family and lineage level, childless women receive disrespectful treatment and maltreatment by in-laws due to the concern for their family lineage dying out. In more extreme cases, acts of violence are committed against them (Okonofua, 1997; Sayeed, 1999; Sungree, 1987).

1.3 CAUSES OF INFERTILITY

Although data on the etiology of infertility vary in their accuracy from region to region, it is estimated that the most common causes of infertility in Western countries are pelvic endometriosis (30.8%), ovulatory dysfunction (25-40%), tubal disease (11-15%),

completely unexplained infertility (5-15%) and luteal phase deficiency (LPD) 5-10% (Engman and Luciano, 2005). In developing countries such as Ghana, reproductive tract infections, particularly sexually transmitted diseases (STD's), septic abortions and postpartum infections are the leading preventable causes of infertility constituting about 64-85% of the infertile cases in Africa (Abaidoo *et al.*, 2000; Cates *et al.*, 1985; Okonofua *et al.*, 1994). Cultural and social factors also play an important role in causing infertility. Marital and sexual customs such as age at marriage, the number of sexual partners and female genital mutilation can influence the risk of genital infection which if left untreated can lead to infertility (Ericksen and Brunette, 1996).

Studies have shown that there is a section of infertile women who cannot bear children despite regular ovulation with patent uterine tubes and without male factor infertility. In these women there is a latent factor which is attributed to a defect in the correlation between the cellular events occurring in the ovaries and the failure of optimal growth of uterine endometrium during the menstrual cycle. Such a mismatch leads to a non-receptive endometrium which is not conducive for implantation of an embryo (Wentz, 1980). The defect is attributed to the most common endocrine disorder associated with infertility and spontaneous abortion, luteal phase defect.

1.4 LUTEAL PHASE DEFECT

Luteal phase defect (LPD), (also known as luteal phase dysfunction, luteal phase deficiency or luteal phase insufficiency) is defined as an endometrial histology inconsistent with the chronologic date of the menstrual cycle (Engman and Luciano, 2005) and becomes histologically diagnostic when the histological date (observed endometrial development) of

a an endometrial biopsy differs from the chronologic date (expected endometrial development) by at least 2 days with reference to the last menstrual cycle (Engman and Luciano, 2005; Fadare and Zheng, 2005; Noyes *et al.*, 1975). The luteal phase of a menstrual cycle begins after ovulation of a tertiary ovarian follicle and end with the onset of menstruation. During this period, the corpus luteum secretes multiple steroids and peptides including progesterone that stimulate the endometrium into maturation in preparation for implantation. In a cycle with luteal phase defect, these changes fail to take place leading to a non-receptive endometrium with the subsequent failure of implantation and hence infertility (Lenton *et al.*, 1984b; Seibel, 1997).

Normal endometrial milieu is very essential for implantation and evaluation of endometrial receptivity has been considered a basic goal in the evaluation of the infertile woman (Wentz, 1980). Investigation of endometrial function has traditionally been made by dating secretory phase endometrial biopsy according to the morphologic criteria reported by Noyes and others (1975).

1.5 JUSTIFICATION FOR THE STUDY

The existence of a) conflicting data on the prevalence of LPD, b) lack of sufficient data in Sub-Saharan Africa and in Ghana in particular on LPD, c) conflicting view on the effect of age on the occurrence of LPD, and d) the interplay of several factors in causing infertility provoked this work with the hope that the results acruing thereof will be a contribution to the knowledge base on LPD.

1.5.1 VARIATION IN WORLD–WIDE DATA ON LPD

Although LPD may occur in fertile women, its incidence is higher among infertile women (Li and Cooke, 1991; Li et al., 1991); and the incidence of LPD is said to vary widely among infertile women world wide (Garcia, 2004). In two separate studies conducted by Tulppala et al. (1991) in Finland and (Li et al., 1991) in the United Kingdom, the incidence of LPD were 17.4% and 28.0% respectively. Studies in the United States of America, suggest that LPD incidence ranges from 3.7% to 20.0% among infertile women (Mosher and Pratt, 1987).

1.5.2 LACK OF SUFFICIENT DATA IN AFRICA ON LPD

In Africa even though there is some evidence of LPD, published work done on finding the incidence of LPD has been insufficient. In Nigeria, it is estimated that about 10-15% of infertility among women is due to LPD (Jimoh, 2004) but Ilesanmi (1995) put the estimates of LPD in Nigeria as high as 32.7% among infertile women. In Ghana, there is virtually no published work on LPD though some considerable research has been conducted on other causes of infertility.

1.5.3 EFFECT OF AGE ON LPD

It has been well established that female fertility declines with age (Fox and Buckley, 1991; Tietze, 1957). This phenomenon has been attributed to ageing of the ovaries resulting in poor oocyte quality (Abdalla *et al.,* 1991; Navot *et al.,* 1991). Moreover, ovarian follicles from older women contain gametes that have a higher rate of chromosomal abnormality (Richardson and Nelson, 1990; Wramsby *et al.,* 1987). In some animal species, mainly rat and mouse, marked age-related endometrial changes have been described. In older animals

an increase in collagen has been observed (Craig and Jollie, 1985) along with a reduction in stromal cells (Wilcox *et al.*, 1988) and in oestrogen receptors (Han *et al.*, 1989). Furthermore, reductions in oestral periods (Rahima and Soderwall, 1978) and in the endometrial cells' ability to express a decidual reaction in vitro have also been documented (Otha 1987). These data suggest a decline in endometrial receptivity and might contribute to an explanation of the age-dependent decrease in fertility in females of these species.

However other studies have also suggested that age does not appear to have a significant effect on the morphology or histological responses of the endometrium to steroid stimulation (Lenton *et al.*, 1984a; Menken *et al.*, 1986; Noci *et al.*, 1995; van Noord-Zaadstra *et al.*, 1991). In view of the above there is the need to investigate LPD occurrence in relation with the age of the woman in our setting.

1.5.4 INTERPLAY OF SEVERAL FACTORS IN CAUSING INFERTILITY

Some causes of infertility like endometriosis, infections and tubal problems including endometrial abnormalities have been shown to vary among infertile women depending on the type of infertility (Gautray *et al.*, 1981; Mahmood and Templeton, 1991; Wentz, 1980). However, the general infertile population is rather heterogeneous and is composed of various subgroups with different aetiology. It is therefore of clinical interest to investigate the occurrence of LPD among the different infertile subgroups. Based on these facts the present study was designed to throw more light on the endometrial factor of infertility, present study was dat the present study was conducted.

1.6 GENERAL OBJECTIVE

To investigate luteal phase defect among out patient infertile women in selected gynaecologic clinics in Kumasi, Ghana

1.6.1 Specific objectives

- Provide information on the prevalence of LPD among the infertile women
- Study the distribution of LPD with respect to the age of the women
- Study the distribution of LPD in relation to type of infertility

Chapter 2

LITERATURE REVIEW

2.1 THE MORPHOLOGY OF THE UTERUS AND CHANGES IN THE ENDOMETRIUM DURING THE NORMAL MENSTRUAL CYCLE

The uterus of a non-pregnant woman is a hollow thick-walled, inverted pear-shaped muscular organ located between the urinary bladder and the rectum. The uterus measures about 7 cm long, 4 cm wide and 2.5 cm thick in nulliparous women. The uterus is divided into an upper two thirds (2/3) called the body and a distal 1/3, the cervix. The two parts are joined together by the isthmus. The upper rounded part of the body of the uterus known as the fundus, is the usual site of implantation. The function of the uterus is to harbour the embryo, provide a source of nutrition and expel the foetus at the end of its development (Barbara, 1980; Edwards, 1995; Saladin, 1998). The wall of the uterus consists of three layers: an outer perimetrium (serosa), a middle myometrium (muscularis) and an inner endometrium (mucosa). It is the endometrium of the uterus which directly supports and provides nutrition for the embryo (Edward and Froehlich., 1995; Haines and Taylor, 1987). The endometrium itself has two components: an epithelial component which consist of luminal and glandular parts, and a stromal component consisting of connective tissue. The endometrium is divided into two portions; a superficial part known as the functionalis which undergoes changes throughout the menstrual cycle and is shed during menstruation and a basal part called the basalis which remains constant during the menstrual cycle and regenerates the functionalis at the end of the cycle (Edwards, 1995).

The menstrual cycle is simultaneously influenced by changes in the ovaries called the ovarian cycle and corresponding changes in the uterus called the uterine /endometrial cycle. The menstrual cycle may be divided into three as phase's follows; a) menstrual/bleeding phase, b) preovulatory/follicular/proliferative or oestrogenic phase and c) postovulatory /luteal/secretory/progestational phase. At the beginning of the

cycle, the hypothalamus stimulates the pituitary to release follicle-stimulating hormone (FSH) and a small amount of luteinizing hormone (LH). The FSH stimulates the growth of several follicles in the ovaries but only one develops into the mature Graffian follicle. In the uterus, increasing levels of oestrogen from the developing follicles of the ovary induce proliferation of the endometrial glands and stromal connective tissues from the stem cells of the basalis to form the functional layer of the endometrium. During this process the endometrium becomes highly vascularised (Edwards, 1995). The follicular phase, that is the period during which the ovarian follicle develops is the most variable part of the menstrual cycle; varying from 10.3 days to 16.3 days with an average of 12.9 days (Lenton *et al.*, 1984a). In the early stages, the glands in the endometrium have low columnar epithelial lining and small narrow glands with simple tubular appearance with evidence of mitotic activity in both the glands and in the stroma (Seibel, 1997). Mitotic activity is at its peak during the late proliferative phase coinciding with the pre-ovulatory peak of oestrogen. At this point there is some secretory activity pushing some of the nuclei of the columnar cells up to give a pseudostratified appearance (Haines and Taylor, 1987; Noyes *et al.*, 1950). There is a sharp rise in the oestrogen concentration that causes a great surge in the gonadotropin output. The result is a marked increase in both FSH and LH. The increase in LH is greater and this is responsible for the release of the ovum from the Graffian follicle (Haines and Taylor, 1987; Jones, 1949). About 16 hours after the LH surge ovulation occurs and this is assumed to be day 14 of the menstrual cycle. Using the LH dating criteria this is said to be day LH+0 (Lenton *et al.*, 1984b). The ruptured follicle is converted into a corpus luteum, which soon begins to secrete small amount of oestrogen and significant amount of progesterone. The luteal phase begins at ovulation and lasts until the menstrual phase of the next cycle. This is the most constant part of a normal cycle which is about 14.13 ±1.41 (Lenton *et al.*, 1984a). At

the beginning of the secretory phase, progesterone induces the endometrial glands to secrete glycogen, mucus, and other lipid substances. One of the earliest signs of ovulation in the endometrium is the presence of pseudostratification and vacuolation in the gland cells, which occurs 36 to 48 hours after ovulation. The subnuclear vacuoles give a positive staining reaction for glycogen and mucin with Periodic Acid Schiff (PAS) (Haines and Taylor, 1987).

From day 16 (LH+2) to day 19 (LH+5) the secretory materials of the subnuclear vacuoles gradually moves to the apical pole of the cell. This is in response to the increase in progesterone secretion by the corpus luteum, thus increasing its level in the blood. Mitotic activity is still apparent in the glands around day 16 and continuous for about 2-3 days in the stroma (Noyes *et al.*, 1950). At about day 20-21 (LH+ 6- LH + 7) the glands become tortuous. Stromal oedema becomes evident around day 22. At about day 23- 24, pseudeducidual changes occur under the epithelium and around the blood vessels. These cyclical changes in the endometrium prepare it for implantation in the event that ovulation and fertilization have occurred and necessitate menstruation in the absence of fertilization. When fertilization and therefore implantation fail to occur, the corpus luteum begins to regress and ceases to produce the oestrogen and progesterone needed to maintain the endometrial development. The glands begin to show involutional changes and there is gradual disappearance of the luminal secretion. The glands progressively collapse and papillary turf of epithelial cells projects into the lumina giving the characteristics saw-tooth pattern (Fox and Buckley, 1991). During the last few days of the cycle prior to menstruation the stroma shows infiltration by endometrial granulocytes (Edwards, 1995). The menstrual phase begins with the rupture of the spiral arteries secondary to ischemia, releasing blood and apoptosed endometrial tissues. This comes out as menstrual flow which usually lasts for 4-5 days.

During this period, the functionalis layer of the endometrium is completely shed (Edward and Froehlich., 1995; Haines and Taylor, 1987).

2.2 IMPLANTATION

In humans fertilization occurs in the ampulla of the uterine tube, where it resides for some 72 hours (Taylor and Gomel, 2008). Under the influence of ovarian steroids, the autonomic nervous system, and the developing embryo itself, the morula is transported through the ampulla to the isthmus and intramural/interstitial portion of the uterus to the uterine cavity. During this period cell division and compaction occur to form a morula. Following entry of the morula into the uterine cavity, cell polarity is established and lineage differentiation occurs, forming a blastocyst (Tabibzadeh, 1998; Taylor and Gomel, 2008).

Embryo implantation represents the most critical step of the reproductive process in many species including humans (Bentin-Ley *et al.*, 1999). A blastocyst must successfully attach itself to maternal endometrial tissue in order to survive. Implantation requires the synchronous development of competence of the blastocyst to implant and receptivivity of the endometrium by being able to respond to embryonic signals. Implantation begins 6 to 7 days after fertilization in humans (Vigano *et al.*, 2003). Implantation is the process by which the free floating blastocyst attaches to the endometrium, invades into the stroma and establishes the placenta. The process involves a complex dialogue between the endometrium and the conceptus that is mediated by soluble growth factors, hormones, adhesion molecules, the extracellular matrix (ECM) and prostaglandins (Bentin-Ley *et al.*, 1999; Lindenberg *et al.*, 1989; Sharkey and Smith, 2003). Wilco*x et al.*(1988) reported that about 65% of conceptions end in unrecognized loss. These authurs further explained that if this

unrecognized loss is projected into a 100%, mere apposition of embryo to endometrium without invasion of the former into the latter accounts for 45% of the total losses, Aborted implantation accounts for 30% and developmental failure after successful implantation is responsible for the remaining 25% (Wilcox *et al.*, 1988).

Although specific mechanisms of implantation vary widely among species, three fundamental processes have been identified and these are: (a) apposition of the blastocyst to the uterine wall, (b) adhesion of the trophoblast to the uterine epithelial lining, and (c) penetration of the epithelial basement membrane and the underlying stroma by the blastocyst (Campbell *et al.*, 1995; Kimber, 2000b). These three processes occur during the period known as "period of endometrial receptivity" or implantation window. It is during this critical period that a proper dialogue can be established between an intrusive blastocyst and a receptive endometrium (Tabibzadeh, 1998). The "window of endometrial receptivity" (for embryo implantation) is a relatively short period which is confined to day 19-21 (LH+5 to LH+7) of the menstrual cycle (Psychoyos, 1993). Rogers *et al*., (1989), however, believed that the duration of the window must be at least 3.5 days. Others also believed that it is as long as 7 days (Formigli *et al.*, 1987). Beyond this period of receptivity, the endometrium becomes non-receptive and therefore refractory thus any embryo that arrives after this period cannot be implanted and will subsequently be aborted (Tabibzadeh *et al.*, 1999).

The period of uterine receptivity is characterised by certain molecules that are important for the implantation of the embryo. These include pinopodes, glycodelins, intergrins (adhesion molecules), tumour necrosis factor-α (TNF-α), leukaemia inhibition factor (LIF), Interleukin-1 and colony stimulating factor-1 (Lessey, 2000; Ordi *et al.*, 2003b; Tabibzadeh *et al.*, 2000; Tabibzadeh *et al.*, 1999). Lack of these factors has been found to coincide with endometrial non receptivity which is not

conducive for embryo implantation and could lead to infertility (Ordi *et al.*, 2003a; Tabibzadeh *et al.*, 2000).

2.3 EVALUATION OF THE LUTEAL PHASE

The luteal phase of the menstrual cycle begins after ovulation of a Graafian ovarian follicle and ends with the onset of menstrual bleeding. This is the period during which the endometrium prepares itself to receive the developing blastocyst. During this time, the corpus luteum secretes multiple steroids and peptides, but it is progesterone that primarily leads to endometrial gland maturation and decidualization of the endometrial stroma (Coutifaris *et al.*, 2004).

After cyclic menstrual functions have been verified, multiple methods have been proposed to evaluate the luteal phase. Some investigators have used methods such as monitoring cervical mucus texture or recording the basal body temperature (BBT) to identify the time of ovulation (Gautray *et al.*, 1981), and others have combined the BBT with the onset of the next menstrual period (NMP) as a means of timing ovulation (Noyes and Haman, 1953). Other methods for the determination of ovulation include; hormonal assays and ultrasonography (Li and Cooke, 1991).

2.3.1 Determination of ovulation by Basal body temperature

Basal body temperature is the body's temperature at rest in the morning before getting out of bed to do any physical activity. The patient's temperature is recorded every morning before leaving bed throughout the monthly cycle. The basal body temperature before ovulation ranges from 36.1°C to 36.3 °C. This is due to the presence of oestrogen, which keep temperatures down. Temperatures will vary from person to person, but should stay below its range. Once ovulation has occurred, the temperatures

go up to a new higher level, usually ranging from around 36.4°C to 36.6 °C. The day after ovulation, the temperature generally jumps up by at least 0.11°C, and then continues to rise. It is the observation of the dip and subsequent rise in temperature that suggests ovulation. The increase in temperature is caused by the progesterone released from the Graafian follicle after ovulation. The recording of (BBT) over time provides a simple and inexpensive method for evaluating ovulatory function. Whereas biphasic patterns in temperature recording are characteristic in normal ovulatory cycles, monophasic recordings or a grossly shorter interval of luteal phase temperature elevation often less than 11 days,(instead of the projected 14 days) may identify patients with absent or poor quality ovulatory function. However, some normally ovulating women may exhibit monophasic BBT patterns and therefore the test cannot be used exclusively to reliably define the time of ovulation (De Souza *et al.*, 1998). It has been reported that the BBT chart cannot show whether the luteal phase is normal or defective (Li and Cooke, 1991).

Furthermore, the recording of BBT has limitations as an indicator of the day of ovulation as temperature increase occurs after ovulation. In addition, France *et al*. (1992) using the combination of monitored cervical symptoms, records of BBT and pre-ovulatory luteinizing hormone (LH) surge as ovulatory indices, showed that in only 9.2% of cycles did these three markers identify the same day as the day of ovulation. France *et al*., (1992) further reports that it is inconvenient for women to use a thermometer (oral or rectal) in the morning before getting out of bed; and that the wide variation in the graduation of the thermometers also means it is not a reliable method of obtaining an accurate temperature chart.

2.3.2 Evaluation of luteal phase by Serum progesterone measurement

Serum progesterone determinations during the luteal phase may also be used to evaluate ovulatory function. Three to four blood samples are normally taken usually in the morning starting on day 17 of the menstrual cycle (LH+3) for serum progesterone determination. Average values greater than 3.0 ng/ml provide presumptive evidence of ovulation (Wathen *et al.*, 1984). Midluteal phase determinations may offer additional information regarding the quality of luteal function**,** although concentrations may fluctuate widely even in normal women. It is important to note that the pulsatile progesterone secretion could disguise results and this can only be avoided by estimation of the total amount of progesterone by daily determination of serum progesterone throughout the luteal phase. However this daily blood sampling is impracticable given the invasive nature of the procedure produces a severe inconvenience for the patient. The procedure would involve taking at least three to four blood samples starting on day 17 (LH +3) (Garcia, 2004). However, in spontaneous cycles as occurs in humans**,** mid-luteal progesterone levels greater than 10.0 ng/ml correlate well with normal, "in phase" endometrial histology (Jordan *et al.*, 1994).

2.3.3 Urinary Luteinizing Hormone Estimation as a determinant of ovulation

Ovulation predictor tests are performed for four or five days around the periovulatory period around day 11 of the menstrual cycle to detect LH surge before ovulation. The surge in LH is determined by a change in colour as a result of a concentration above a certain threshold in the urine. Ovulation occurs within 24-26 hours following a positive test. Urinary luteinizing hormone (LH) determinations can identify the midcycle LH surge which provides reliable indirect evidence of ovulatory function**,**

and help to define the interval in which conception is most likely (Wilcox *et al.*, 1988). Results generally correlate well with the peak in serum LH, particularly in evening urine specimens (Luciano *et al.*, 1990). Accuracy, reliability, and ease of use vary among products and the level of understanding of the patient since this test is normally done by the patient at home.

2.3.4 The ultrasonography for the determination of ovulation

Serial transvaginal/abdominal ultrasound scan can reveal the size and number of developing follicles and provide presumptive evidence of ovulation and luteinization. This is demonstrated by progressive follicular growth, sudden collapse of the preovulatory follicle, a loss of clearly defined follicular margins, the appearance of internal echoes, and an increase in cul-de-sac fluid volume (O'Herlihy *et al.*, 1982). To monitor the developing ovarian folilicles, transvaginal ultrasonogaphy is normally done on day 8 of the cycle for the first time, then every 2 days, and daily from the day on which follicular diameter is about 14 mm until there is evidence of follicular rupture or disappearance. Serial measurement of endometrial thickness by transvaginal/abdominal ultrasonography can also be used to assess the adequacy of the luteal phase (Abaidoo *et al.*, 2000). Li *et al.*, (1992) however reports that measurement of endometrial thickness is unlikely to replace histological dating of the endometrium in the evaluation of the luteal phase. Although non-invasive, in developing countries such as Ghana the costs involved are enormous. Therefore this method is generally reserved for patients in whom less complicated methods fail to provide the necessary information.

2.3.5 Determination of ovulation /luteal phase by Endometrial Dating

The endometrium is the site of implantation for the developing embryo and there is therefore an excellent rationale for the evaluation of the endometrium of the female

partner in a couple presenting with infertility using endometrial dating. First it is a method to document ovulation and second to evaluate the organ participating in the process of implantation (Coutifaris *et al.*, 2004). During the menstrual cycle, sequential action of oestrogen derived from the developing ovarian follicle followed by the combined action of oestrogen and progesterone on the endometrium after ovulation causes morphological and molecular changes in endometrial cells rendering them receptive to the adhesive and migratory properties of the trophoblast cells of the implanting embryo. Thus, it has long been advocated that the clinical evaluation of the infertile couple should include an evaluation of the quality of the site of implantation (Balasch *et al.*, 1986; Noyes *et al.*, 1950; Wentz, 1980).

During the luteal phase, the morphological changes observed in the endometrium occur in a predictable pattern (Noyes *et al.*, 1950). Traditionally, the endometrium is considered out-of-phase if the standardized menstrual cycle date based on morphological criteria assigned by a pathologist lags by 2 or more days or is advanced in development by 2 or more days than the actual standardized cycle date determined by a physiological marker such as the urinary or serum LH surge, ovulation, or the date of the onset of the next menstrual cycle. The endometrial biopsy has been used for decades in the screening and evaluation of the infertile couple to confirm ovulation and to also evaluate the histological maturation of the endometrium (Li *et al.*, 1987). The endometrial biopsy, with evaluation of morphological changes, has been considered superior to alternatives such as serum progesterone measurements, because of the pulsatile nature of progesterone and a belief that these morphological changes in the endometrium better represent the cumulative effect of cycle-specific pattern of ovarian hormone secretion and it is thus considered the gold standard in the diagnosis of luteal phase defect (Wentz, 1980).

Chapter 3

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Endometrial biopsies were obtained by clinical staff from 80 selected infertile women attending the gynaecological clinics at the Komfo Anokye Teaching hospital, Magazine clinic and the Bomso Specialist Hospital all in Kumasi. At the time of sampling these were the only gynaecological centers in Kumasi where biopsies were being taken. Endometrial biopsies were similarly obtained from ten normal fertile women and used as a control group. These were women who had regular menstrual cycles of between 25 and 29 days with no evidence of menstrual disorders, had not used hormonal contraceptives or intrauterine contraceptive device for at least four months and had had at least one successful pregnancy and had no evidence of pathology associated with their reproductive tract. The subjects were age 23-40 years with a mean of 31.4 yrs and the control were aged 22-35 years with a mean of 29 years.

All biopsies were timed with reference to the last menstrual cycle. Using a Sharman's curette (Down's Surgical Limited, Sheffield, UK), a single biopsy tissue sample was taken from the fundus of the body of the uterus of each subject between days 18-22 of the menstrual cycle. The samples were then fixed immediately in 10% formalin (Sigma Chemicals Company, UK) and sent to the laboratory for processing. The background of these women including age, type of infertility, clinical diagnosis, last menstrual period and hormonal therapeutic histories were retrieved from their medical records for subsequent correlative analysiswith a written informed patient consent. The

entire protocol for the work was approved by the ethics committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi.

3.2 TISSUE PROCESSING FOR LIGHT MICROSCOPY

Each endometrial biopsy which was immediately put in 10% formaldehyde after removal was kept in it for at least for six hours for fixation. The tissues were then washed in phosphate buffer and dehydrated through a graded series (70%, 80%, 90% and 95%) of ethanol (Sigma Chemicals Limited, UK) for one and half hours each. It was then passed through two changes of absolute ethanol (99.7%) for an hour each. The tissues were then cleared in xylene (BDH Chemical Limited, Poole, England) and impregnated with molten wax overnight. The tissues were then trimmed and blocked out using the Leukard embedding blocks (Bright Instrument Company limited, Huntingdon, England) and cooled in water.

3.3 SECTIONING AND STAINING

The wax blocks were sectioned using the Rotary type microtome (Bright Instrument Company limited, Huntingdon, England) at approximately 5µm thick in order to study the tissues using light microscope. Short Ribbons of the sections were floated out in a water bath (Bright Instrument Company Limited, Huntingdon, England) and individual sections were then picked onto a grease free slides with the aid of a camel hair brush (Bright Instrument Company Limited, Huntingdon, England). In order to get the sections to stick to the slides and to get excess wax off, there were first kept in an oven with temperature around 60°c for about 45 minutes. The sections were passed through two changes of xylene for 5 minutes in order to deparaffinize the sections. They were then hydrated by passing them through decreasing concentrations of ethanol (99.5%, 95%, 90%and 70%) for two minutes each and stained with Haematoxylin (BDH Chemical Limited, Poole, England) for 15 minutes, briefly

differentiated in acid-alcohol for 5 seconds (Sigma Chemicals Company, UK) and washed in running tap water for five minutes. The sections were then stained in 1% aqueous eosin (Sigma Chemicals Company, UK) for 5 minutes, washed in tap water and dehydrated through decreasing concentration of ethanol (75%, 90%, 95%, 99.7%), cleared in xylene and mounted with DPX (BDH Chemical Limited, Poole, England).

3.4 ENDOMETRIAL DATING

All the biopsies were chronologically dated in relation to the last menstrual period (LMP). Detailed examination was performed on each of the biopsies using Carl Ziess standard research microscope (Carl Ziess Inc) so as to date it histologically using the combined traditional dating criteria (Noyes *et al.*, 1950) and Li's appraisal (1988). The two day dating was done and expressed as day X ± 1. It is a 2 day reading system for dating the luteal phase of the menstrual cycle based on the cyclic variation in the sequential development of human endometrium in response to the changing levels of oestrogen and progesterone. The following features were used for the dating; a) shape of glands, b) pseudostratification of epithelial cell nuclei and c) the presence and position of vacuoles which were either subnuclear vacuolations or supranuclear vacuolation. d) The presence of luminal secretions and gland mitosis were also considered. e) In the stroma the presence and amount of stromal oedema, stromal mitosis, pseudodecidual reactions and infiltration of the stroma by leucocytes were also considered. An out of phase biopsy was defined as 2-day lag between the chronological date and the histological date. It is asserted that examination of the endometrium during the secretory phase yields more information about the time of ovulation, degree of progestational change, and normality of the endometrium than any other test used in sterility studies. Attention to qualitative changes in 8 morphological factors is most useful in dating the

endometrial biopsy. During the 1st week of luteal activity, attention should be focused on changes occurring in gland epithelium: gland mitosis, pseudostratification of nuclei, basal vacuolation, and secretion. During the 2nd week, stromal changes (including edema), predecidual reaction, stromal mitosis, and leukocytic infiltration are the key criteria(Noyes *et al.*, 1950). This is shown in the figure below.

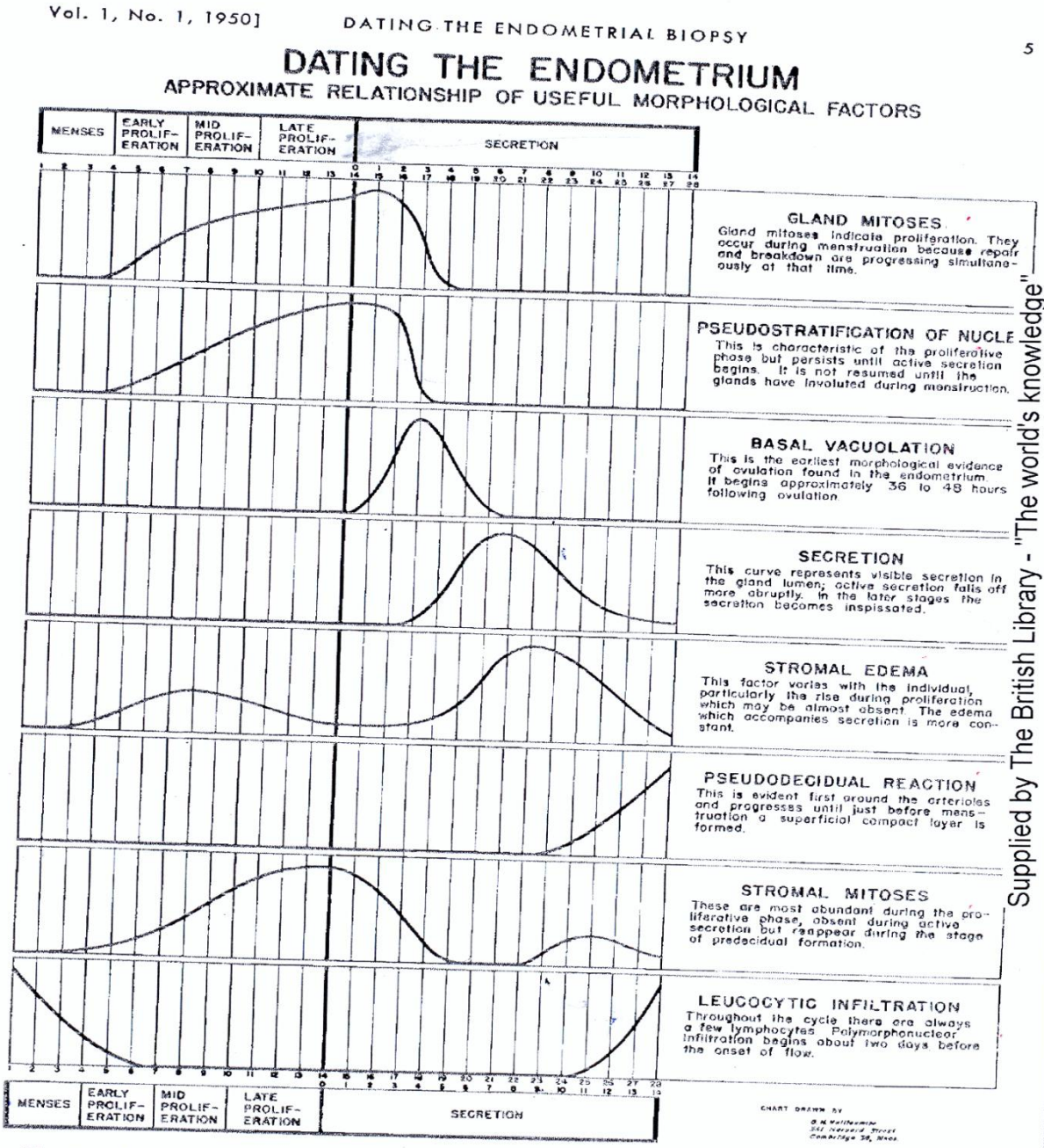


FIGURE 1. This chart has been slightly modified from an original by P. F. Latour. The curves represent approximately estimated quantitative changes in each of eight factors we consider most helpful in dating endometrium.

Chapter 4

RESULTS

4.1 TYPE OF INFERTILITY

Out of the 80 women studied, 16 (20%) of them presented with primary infertility. The remaining 64 (80%) women presented with secondary infertility (fig 4.1).

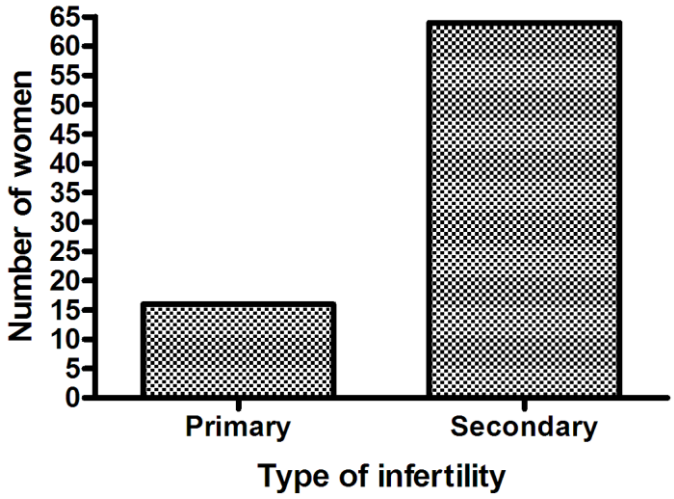


Figure 4.1 Bar chart showing the occurrence of primary and secondary infertility among study subjects

4.2 AGE, INFERTILITY AND FECUNDITY

The age range of the control was 22-35 with a mean of 29 years. The age range of the subjets in this study was 23-40 years with a mean age of 31.4 years ± 0.51 (Mean ± SEM). The number of women presenting with infertility was generally on the increase with ageing. From 13 (16.25%) women at age 20-25 years, the number rose to 24 (30.0%) women at 26-30 and then peaked at 31-35 years with 35 (43.75%) women. The number decreased to 8 women within 36-40 years brackets as shown in figure 4.2.

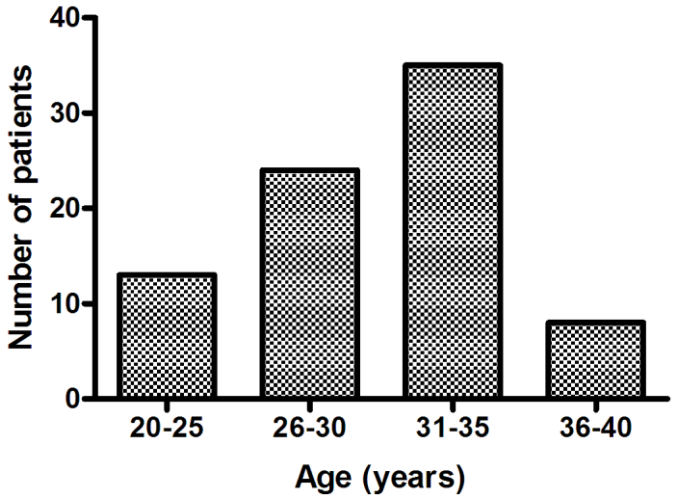


Figure 4.2 Bar chart of age grouping among infertilie women studied

4.2.1 Type of infertility and Age

Figure 4.2.1 represents age distribution of primary and secondary infertility among the study group. At the age brackets of 20-25 years, 7 (8.75%) presented with primary infertility compared with 4 (5.00%) who presented with secondary infertility. At the 26-30 year group, the number of secondary infertile women rose to 21 (26.25%) while primary infertility decreased to 3 (3.75%). Whereas there is decrease in the number of women with primary infertility with age, there appear to be a normal distribution pattern in the women with secondary infertility, rising to peak at the 30-35 age group before declining.

Figure 4.2.1 Bar chart showing occurrence of primary and secondary infertility in the different age groups

4.3 DETERMINATION OF OVULATION USING ENDOMETRIAL BIOPSY

The main objective of endometrial studies in infertility investigations is to determine whether ovulation has occurred and to assess the suitability of the endometrium for implantation in the event of fertilization. Of the 80 biopsies dated histologically, 68 (85%) of them showed secretory activities like tortuoucity of glands (plate 4.3A) and luminal secretion (plate 4.2A) indicating that ovulation had occurred. The remaining 12 biopsies (15%) showed arrested proliferative activities (and therefore with no concomitant secretory activities) such as small round narrow glands (Plate 4.3 B) indicating anovulation even though all the biopsies were taken during the mid luteal phase (Fig 4.3).

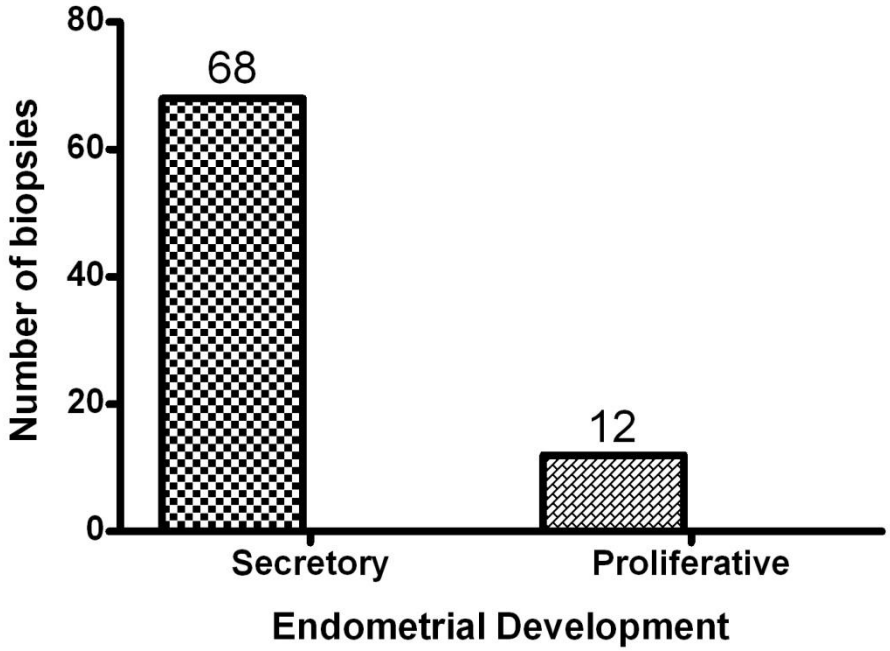


Figure 4.3 Bar chart showing the developmental phase of the endometrium among the infertile women

4.3.1 Observed endometrial histology

Of the 80 biopsies dated, 52 (65.0%) were assessed as being in-phase (there was correlation between the histological development and the expected endometrial development) and 28 (35.0%) assigned a diagnosis of luteal phase defect (LPD). In this LPD group there were discrepancies between the histological date and the chronological date. A biopsy is said to be out-of-phase if there is a lag of 2 or more days between the expected endometrial development and the observed endometrial morphology. Thus analyses of the out of phase biopsies in the LPD group indicated that two of them were advanced in development than expected as these showed features such as saw-toothed glands (Plate 4.1B) around day 20-21 (LH + 6/7) as compared to the control biopsies which showed luminal secretion (Plate 4.1A). Fourteen (14) of the ovulatory biopsies were retarded (lagging by 2 or more days).

The biopsies which were retarded in development exhibited early secretory features such as basal vacuolation of glandular cells and absence of luminal secretion around days 20-21 of the menstrual cycle (Plate 4.2B) compared to the control biopsy which showed luminal secretion (Plate 4.2 A). Twelve (15.0%) of the biopsies showed proliferative features such as small round narrow gland without luminal secretion (Plate 4.3B) compared to the control biopsy which showed tortuous glands (Plate 4.3A) even though all the biopsies were taken during the secretory phase of the menstrual cycle.

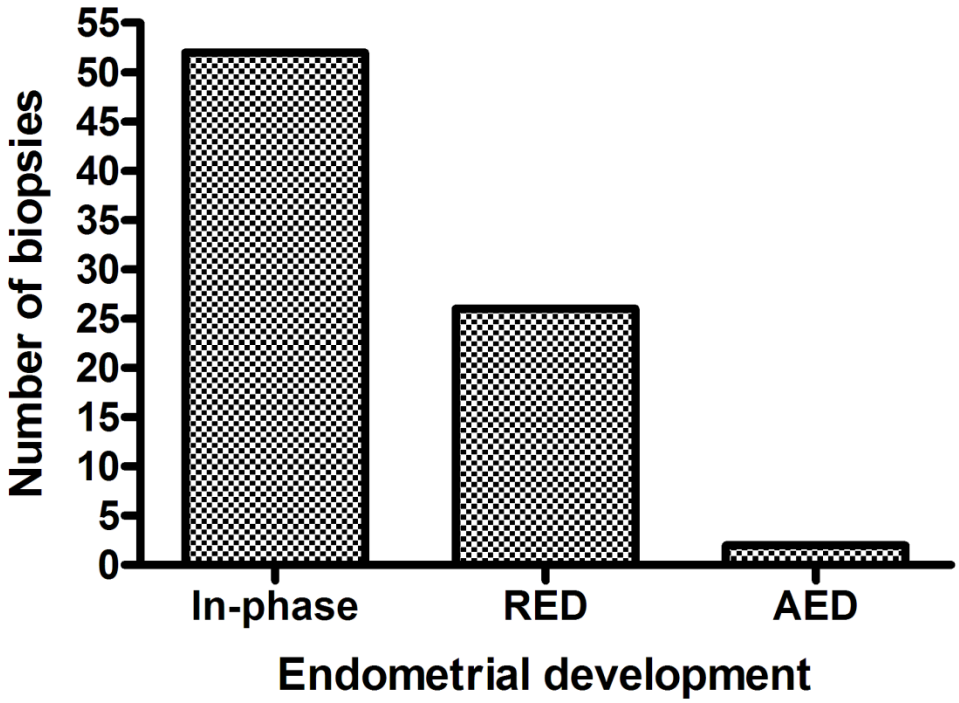


Figure 4.4 Bar chart showing the development of the Endometrium in the infertie women

RED = Retarded Endometrial Development, AED = Advance Endometrial Development

4.4 LUTEAL PHASE DEFECT AND TYPE OF INFERTILITY

Further analysis of the endometrial biopsies using the combined Noyes *et al* (1950) and Li's (1988) criteria gave 50.0% (8) of the 16 women who presented with primary infertility as being in phase and the other 50.0% as out-of phase (LPD). Among the 64 secondary infertile women, 20(31.25%) had out of phase biopsies and 44(68.75%) had in-phase biopsies (Table4.1). The frequency of LPD in these two groups was evaluated by Chi Square. The difference between the two groups was however not statistical significant (P= 0.1597, χ^2 = 1.978, df = 1).

Table 4.1 Endometrial development and type of infertility

Type of infertility	In-phase	Out-of-phase
Primary	8 (50%)	8[a] (50%)
Secondary	44 (68.75%)	20[a] (31.25%)

Figures bearing the same superscripts are not statistically significant (P= 0.1597)

4.5 AGE AND LUTEAL PHASE DEFECT

Among the 27 women of the study group who were in the 20-29 age group, 19 (70.40%) of them showed in-phase endometrial development and 8 (29.60%) out-of phase endometrial development. Similarly, those in the 30-40 year group range of the same group, 33(62.30%) were in phase while 20 (37.70%) had their biopsies showing out-of phase. (Table4.2). When the difference between the two age groups was analyzed using Chi-Square, it was not statistically significant (P=0.4723, χ^2 = 0.5167, df = 1).

Table 4.2 Incidences of LPD and Age

Age	In-phase	Out-of-phase
20-29	19 (70.40%)	8[b] (29.60%)
30-40	33 (62.30%)	20[b] (37.70%)

Figures bearing the same superscripts are not statistically significant (*P*=0.4723).

Plate 4.1A: A light micrograph of a secretory endometrial biopsy from a fertile control around day 20-21 (LH+6/7) showing a gland (**g)** with luminal secretion (**S**).
X400
Stain: Haematoxylin and Eosin

Plate 4.1B: A light micrograph of a secretory endometrial biopsy from an infertile woman taken around day 20-21 (LH +6/7) showing a gland **G** with saw-tooth appearance (**arrow head**) without luminal secretion, an indication of advance endometrial development.
X400
Stain: Haematoxylin and Eosin

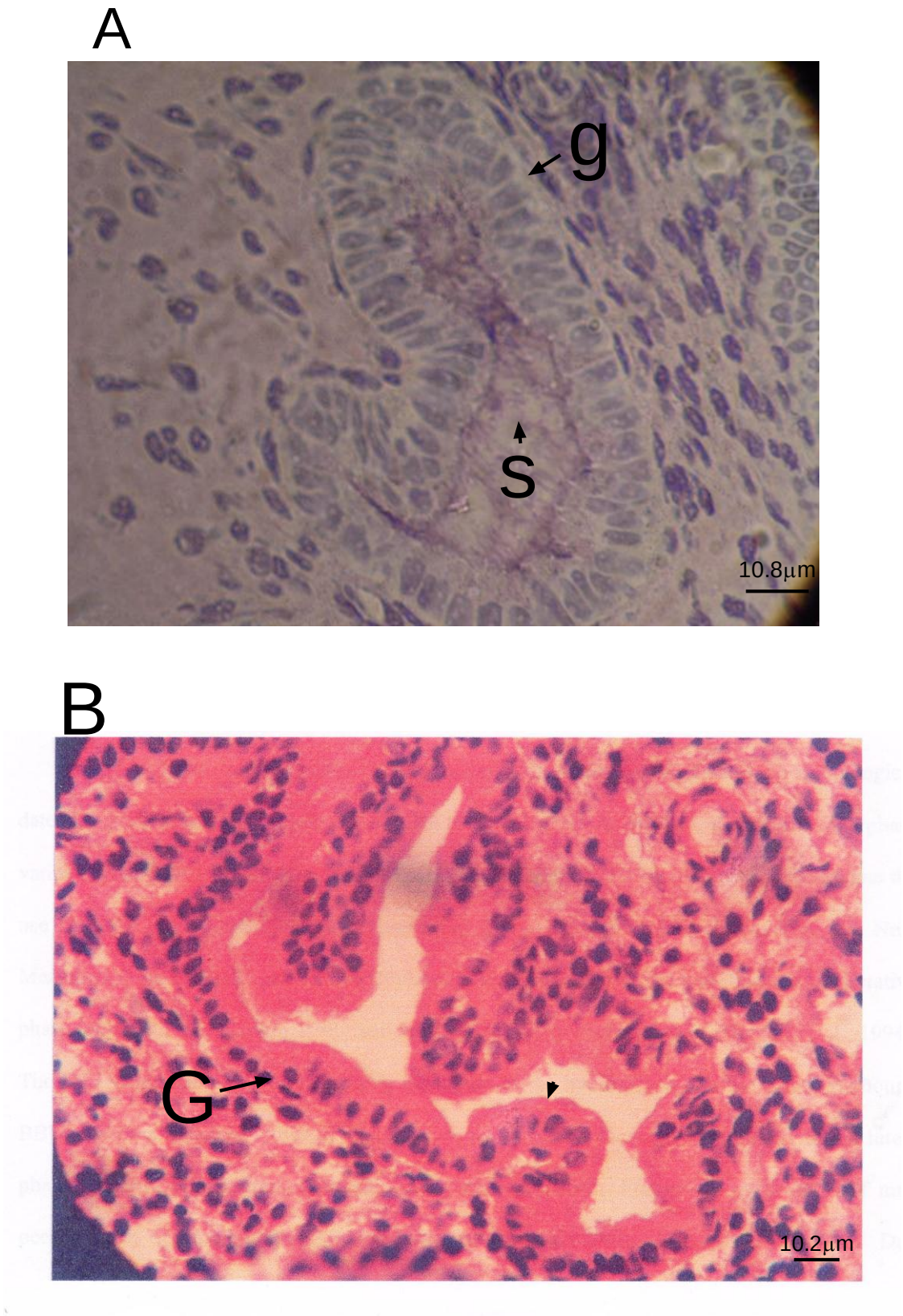


PLATE 4.1

Plate 4.2A: A light micrograph of a secretory endometrial biopsy from a fertile woman taken around day 20-21 (LH+6/7) of the menstrual cycle showing a gland (G) with luminal secretion (**S**)
X400
Stain: Haematoxylin and Eosin

Plate 4.2B: A light micrograph of an endometrial biopsy from an infertile woman taken around day 20-21 (**LH+6/7**) of the menstrual cycle showing a gland (**G**) with basal vacuolation (**BV**) without luminal secretion (**L**). This is a case of retarded endometrial development.
X400
Stain: Haematoxylin and Eosin

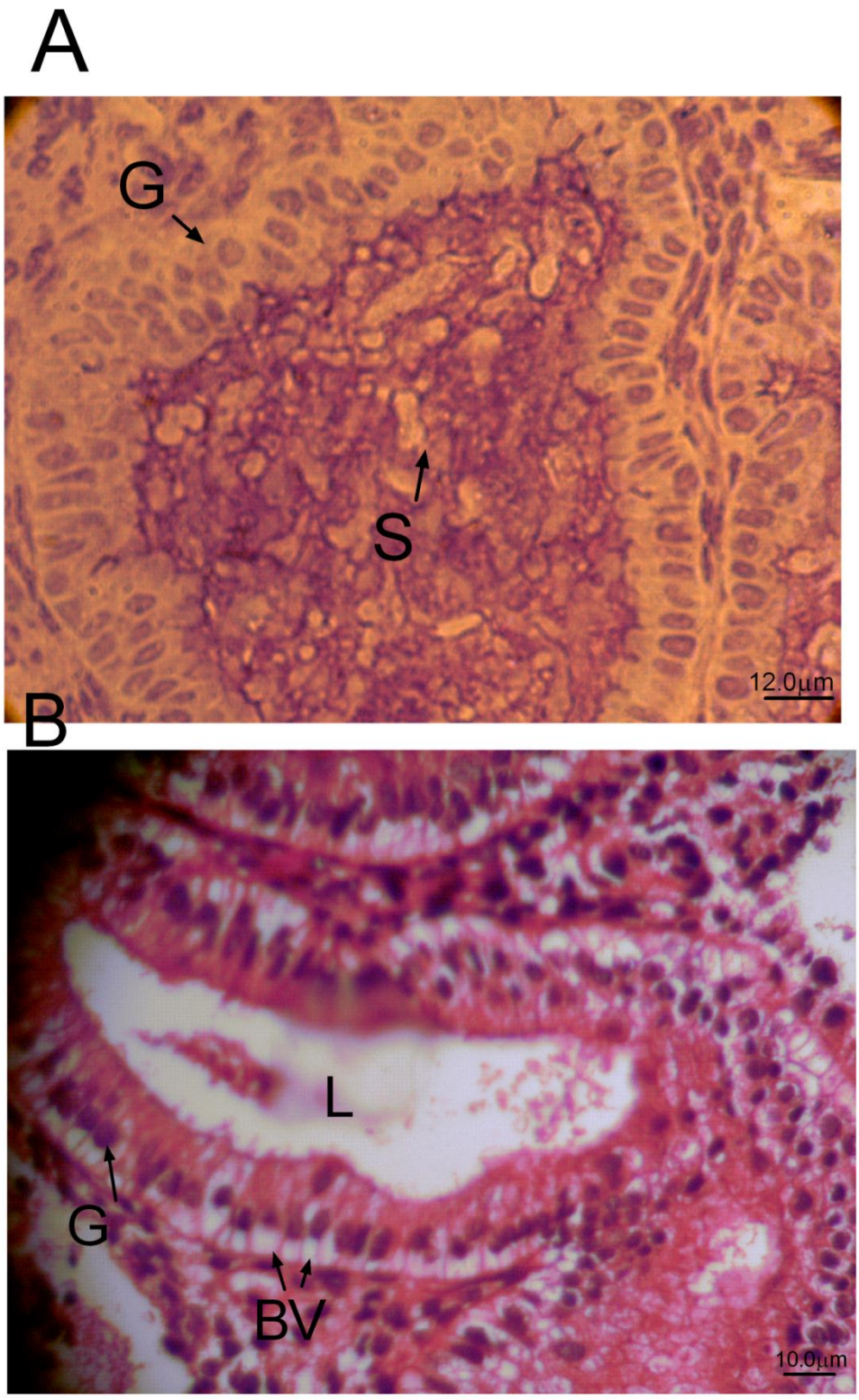
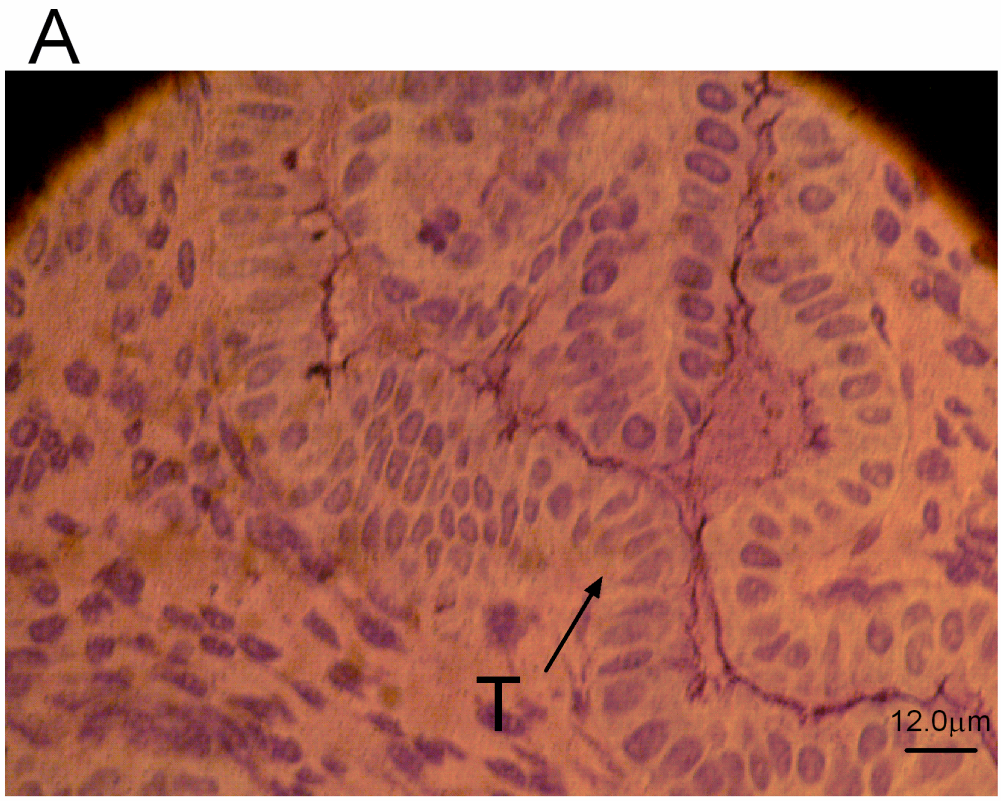


PLATE 4.2

Plate 4.3A: A light micrograph of a secretory endometrial biopsy from a fertile woman showing a tortuous gland (**T**) indicating secretory activity.
X400
Stain: Haematoxylin and Eosin

Plate 4.3B: A light micrograph of an endometrial biopsy from an infertile woman showing small round narrow regenerative gland (**G**) without luminal secretion indicating proliferative activity.
N = Gland nuclei
ST = Stroma
X400
Stain: Haematoxylin and Eosin



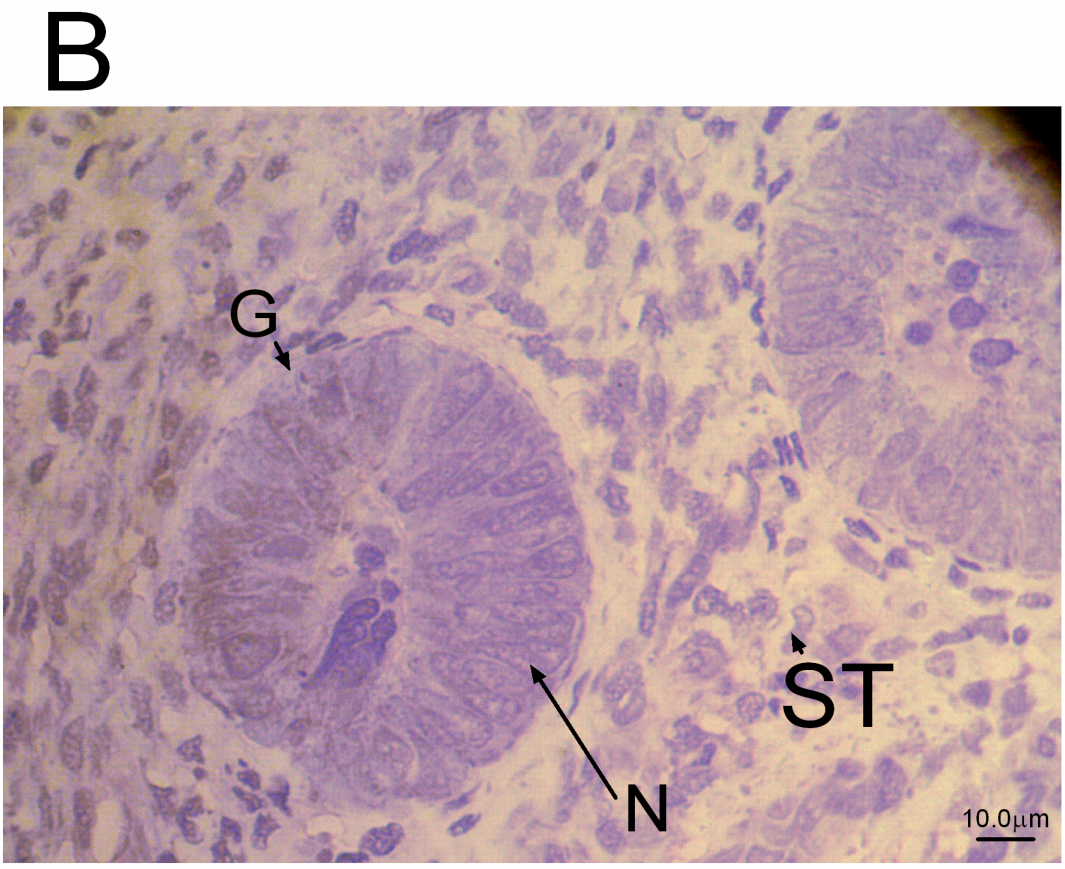


PLATE 4.3

Chapter 5

DISCUSSION

5.1 INFERTILITY

In this work about 20.0% of subjects in the study group presented with primary infertility whilst 80.0% presented with secondary infertility. Thus the finding of this work suggest that given any random sample of infertile women in Ghana, the prevalence in the country in terms of the prevalence of primary and secondary infertility may not be different from the levels in other developing countries; as this work corroborates the work of (Larsen, 2000; Okonofua *et al.*, 1994) who suggested that secondary infertility is the predominant form of infertility in developing countries. The work of (Larsen, 2000) further showed that, primary infertility in developing countries is low affecting less than a third of the infertile population; an observation confirmed in this study as only one quarter of the 80 women in the study presented with primary infertility. In contrast, both Okonofua *et al.*, (1994) and Larsen, (2000) observed that elevated levels of secondary infertility prevailing in most countries in the developing world particularly in Sub Saharan Africa are due to a high prevalence of classical STDS, complications of unsafe abortions and postpartum infections (Larsen, 2000; Okonofua *et al.*, 1994). Similar assertions were alluded to by Abaidoo *et al.*, (2000), who stated that many women in Ghana resort to abortion as a means of contraception leading to secondary infertility at an early age. Even though in this work no direct interviews were carried out with the infertile women it may be reasonable to assume that of 80% of the women in the study group who suffer from secondary infertility, a significant number of the infertility may have been caused by one or combination of the factors above. An important intervention in the prevention of infertility in Ghana and in Africa as a whole would be that directed towards reducing

its incidence through the prevention of infections that lead to infertility. It is therefore imperative for reproductive health programmes to integrate the prevention of infertility with the prevention of sexually transmitted infections, post-abortion and postpartum sepsis so as to engender greater participation of women in those programmes.

5.2 AGEING AND INFERTILITY IN WOMEN

When the infertile women were divided into primary and secondary infertility and then grouped according to age, it was realized that about 70.0% of the secondary infertility cases where within the age brackets of 26-35 years. In the present study 30.0% and 43.7% of the infertile women were within the age brackets of 26-30 and 31-35 respectively These figures are relatively higher than an earlier report by Menken *et al*., (1986) in which they indicated a percentage of 9.0% at age 25–29 and 15.0% at age 30–34. The difference could be attributed to the smaller sample size of 80 in the present study.

Furthermore, a sharp decline in pregnancy rate with advancing female age is well documented. (Schwartz and Mayaux, 1982), the decline was observed in 14.0% for patients under 35 years, 19.0% at age 35 to 37, 25.0% at age 38 to 40, and 40.0% after age 40. It has also been scientifically documented that female fecundity is at its peak during this age bracket of 26-35 years and infertility becomes more pronounced after the age of 35 (Gindoff and Jewelewicz, 1986; van Noord-Zaadstra *et al.*, 1991). Thus for the bulk of the women in this study seeking to resolve their secondary infertility to fall in this optimum fecund age bracket and voluntarily seek medical attention suggests that this knowledge may not only be scientifically known but it may also be a common knowledge in society as to when women are best suited to have children. Hence the anxieties of these women in the study group to voluntarily seek help more

than the others in the other age brackets; for the reason that the expected is not happening and their biological clocks are 'ticking away past their prime'. In other words the relatively lower numbers for those below the age of 25 may suggest they think they have time on their side whereas those after the age of 35 may feel it may be getting too late for them; hence those in the age range 25-34 years may feel time is ripe for them to seek help.

This thought is corroborated by the findings of the Ghana demographic survey, (2003) which indicates that the percentage of age at first birth occurring at age 18 or less had fallen from 25% among the oldest cohort of women age 45-49 to 15% among the youngest cohort aged 20-24. The reports states that this reduction in the percentage of women giving birth early implies that more young women are postponing marriage and using contraceptives at an earlier age to pursue education or careers . It is thus reasonable to assume that when these life ambitions are fulfilled in the young women and they have settled down expecting to have babies readily they may rush to fertility clinics after a few years of trying. As a result of the above evidence it is important for infertility care givers to carefully counsel patients regarding family planning issues, especially with regards to advancing age and diminishing pregnancy rates. Patients who are in their early to mid thirties or beyond who are considering pregnancy or have been trying for any length of time without success warrant an early referral for infertility evaluation.

5.3 DATING THE ENDOMETRIAL BIOPSIES

In the present work, previously reported structural changes in the endometrium, which were described using LH peak were used in combination with information on the last menstrual period (LMP) and light microscopic analysis of the endometrial biopsies

using the combined Noyes *et al.,* (1950) and (Li *et al.,* 1988) dating criteria. The LMP was used to in the present study to determine the beginning of the menstrual cycle. Multiple methods have been proposed to evaluate the luteal phase. Some investigators have used self methods such as monitoring cervical mucus symptoms or recording the basal body temperature (BBT) to identify the time of ovulation (Gautray *et al*., 1981), and others have combined the BBT with the onset of the next menstrual period (NMP) as a means of timing ovulation (Noyes and Haman, 1975; Shoupe *et al.*, 1989). However, the lack of precision of these particular methods in timing ovulation has been reported (Li and Cooke, 1991). The method of choice in the evaluation is the combination of the luteal phase measurement of serum LH and dating endometrial biopsy (Garcia, 2004; Li *et al.*, 1989; Wentz, 1980).

In the evaluation of the luteal phase, measurement of serum LH peak is normally used to identify the day of ovulation (Coutifaris *et al.*, 2004). In the present study, however, only endometrial dating was done. The endometrium is the target organ of steroid hormones and the site of implantation of the blastocyst, it is therefore used in a bioassay in the assessment of the infertile woman (Engman and Luciano, 2005; Fadare and Zheng, 2005; Garcia, 2004). Endometrial biopsy alone, which utilizes evaluation of morphological changes, has been considered superior to alternatives such as serum progesterone measurements. This is because the pulsatile nature of progesterone secretion makes it unreliable to measure in the cycle period. Furthermore, there is the belief that these morphological changes better represent the cumulative effect of cycle-specific patterns of ovarian hormone secretion (Li *et al.*, 1989; Ordi *et al.*, 2003b; Wentz, 1980).

Majority of the endometrial biopsies (EB) in this study were obtained in the mid-luteal phase. The argument for taking the EBs in the mid-luteal phase is that it is the time

during which implantation of the blastocyst takes place and the morphology of the endometrium at this time is more likely to be most receptive and therefore would display the most difference between the normal and defective endometrial structure than one would find at the beginning when the rebuilding of the endometrial lining has just begun or at the end of the luteal phase, when degenerative changes have already commenced. Moreover, Li *et al*, (1989), showed that the precision of histological dating by the traditional criteria in the first half of the luteal phase is better than that obtained in the second half. Also, Castelbaum *et al*., (1994) found that there was a greater detection rate for luteal phase defect (LPD) using the mid-luteal biopsy (12.1%-18.2%) compared with the late-luteal biopsy (6.1%-9.1%). In view of the above considerations the use of mid-luteal phase biopsies in the present study is consistent with current trends in the study of endometrial morphology for evaluation of infertility.

In one peculiar case, a woman who had a chronological date of 36 days had a histological date of 20-21 days. This is a case of a long menstrual cycle; as the normal human menstrual cycle ranges from 25 – 36 days with an average of 28 days. The variation in the length of the menstrual cycle is as a result of variation in the length of the proliferative phase which varies from 10.3 days to 16.3 days with an average of 12.9 days whilst the secretory phase of the menstrual cycle is relatively constant at 14 days (Lenton *et al.*, 1984a). Therefore the biopsy in this case should have been taken around chronological day 29 of the cycle since ovulation would have occurred on day 22 instead of day 14 as in the average 28 day cycle. But the biopsy was taken around day 21-22 like a normal 28 day cycle. The biopsy was therefore showing peri-ovulatory changes instead of peri-implantation changes. This particular case, though rare, calls for a more precise dating of endometrial biopsies in infertility investigations

as against the broad phase dating that is currently been use in most hospitals in Kumasi.

5.4 ENDOMETRIAL DEVELOPMENT

In the present study 85.0% (68/80) of the endometrial biopsies showed secretory activity indicating ovulation had occurred and 15.0 % (12/80) of the biopsies showed proliferative activity even though these were taken during the secretory phase of the menstrual cycle (Fig 4.3). In these women ovulation had not occurred and their infertility could be due to anovulation. Further analysis of the 68 ovulatory biopsies revealed that 76.5% (52/68) of them were in-phase; as there were no discrepancies between the chronological date as determined by the LMP and - observed endometrial development (histological date). Furthermore, when these biopsies were compared with those in the control group they were found to be similar in development in both the epithelial and the stromal components. Therefore their infertility could have been due to other factors such as tubal blockage, improper timing of coitus, low sperm count, malformed sperm cells or coital problems as indicated by Abaidoo *et al*., (2000).

In 35.0% (28/80) of the biopsies studied, the endometrial development was out-of-phase. That is there were discrepancies between the expected endometrial development (Chronologic date) and the observed endometrial development (Histological date). This discrepancy is an out-of-phase infertile biopsy, in which micrographs show proliferative features. The infertility status of these women might be due to luteal phase defect (LPD). This high percentage may suggest that even though LPD is not considered among the major causes of infertility in this part of the world where infections such as sexually transmitted infections, postpartum infections

and infection from septic abortions are considered frontline, this study suggests that LPD, perhaps, deserves equal attention as a frontline cause of infertility. It is generally known that once LPD is diagnosed in a patient, it can be corrected by the use of gonadotropins or clomiphine-citrate to improve folliculogenesis and corpus luteum function; and also to induce multiple ovulations which will in turn increase serum progesterone levels and therefore improve the chance of maintaining a pregnancy (Engman and Luciano, 2005). Some studies among women with recurrent spontaneous abortions and also had LPD have shown that after the correction of the LPD there was an increase in the chances of a term pregnancy (Balasch *et al.*, 1986).

The phenomenon of retarded endometrial development (LPD) as observed in this study, may be caused by several conditions including a) Deficient progesterone secretion from the corpus luteum and b) failure of the endometrium to respond appropriately to ovarian stimulation (Seibel, 1997). Some experts, however, believe that LPD is a combination of a defective of corpus luteum coupled with progesterone out put both in amount and in duration resulting in inadequate stimulation of the endometrium (Kimber, 2000a; Olive, 1991). Others believe c) Poor follicular development as a result of the secretion of low levels of Follicle Stimulating Hormone (FSH) originating from a defect in the hypothalamus or the pituitary. In poor follicular production the body may not produce a normal level of follicle stimulating hormone (FSH) and Luteinizing hormone (LH) or the ovaries may not respond strongly to these hormones leading to inadequate follicle development. Granting that after ovulation, the remnants of a well formed Graafian follicle ultimately becomes the corpus luteum, poor follicle formation leads to poor corpus luteum quality. In turn, a poor corpus luteum will produce inadequate progesterone, causing the endometrium to be inadequately prepared for the implantation of a fertilized embryo (Kimber, 2000b). d)

Failure of the endometrium to respond to progesterone stimulation even in the presence of adequate follicle development and a corpus luteum that persist for the appropriate length of time can cause LPD. In such situations the receptors for progesterone are either not developed or the concentration is less than required for the proper functioning of the endometrium. Thus even though enough hormones would be produced, the endometrium will not respond appropriately and thus making it insensitive and non-receptive (Perez *et al.*, 1981). Since embryo implantation is highly dependent on the state of the endometrium, LPD can consistently interfere with a woman's ability to get pregnant and carry it successfully to term.

Further analysis of the biopsies that were out of phase showed that 7.1% (2/28) of them were advanced in development. Advanced endometrial development (AED) has also been hypothesized to cause infertility since embryo implantation is highly dependent on the state of the endometrium. In these women the development of the endometrium was ahead of time which could be due to hypersecretion of progesterone, or excessive response by the endometrium. The infertility status of these women could be due to AED. This is because before an embryo can implant in the endometrium there must be synchrony in the development of both the embryo and the endometrium (Balasch *et al.*, 1986). The embryo must arrive during the "window of implantation" which is restricted to day 18-22 of an average 28 day cycle. The window is said to close after this date and the endometrium then becomes hostile thus preventing any embryo which arrives from implanting (Coutifaris *et al.*, 2004; Tabibzadeh, 1998). Therefore in those women who had retarded endometrial development or AED, it is possible that fertilization could have occurred but implantation could not have taken place due to dysynchrony between the development of the endometrium and the arrival

of the blastocyst and this could be responsible to some extent for their inability to have children.

5.5 AGE AND LUTEAL PHASE DEFECT

The subjects in the present study were divided into two according to their ages and the incidence of LPD analyzed accordingly. The results show that about 66.2% (53/80) of the total study subjects where within the 30-40 age bracket whilst only 33.8% (27/80) were aged 20-29 year (Fig 4.5). The incidence of LPD among the two age groups did not show any significant difference, giving an indication that age may play a less significant role on the incidence of endometrial retardation in pre- perimenopausal women. Research has shown that the process of aging in humans especially females affects all biological systems and that these changes become apparent at different ages in different systems of the human body and become more obvious when the system is required to function to its maximum potential (Seibel, 1997). The reproductive capacity of the human couple is limited in time by progressive, age-dependent subfertility and eventually by menopause, which imposes absolute sterility (Gindoff and Jewelewicz, 1986). It was therefore hypothesized that as the person is ageing there would be a gradual decrease in the response of the endometrium to steroid hormones but this was not the case as the results of the present study has shown. The result of the present study as it stands, however, is in conformity with others (Lenton *et al.*, 1984b; Noci *et al.*, 1995; van Noord-Zaadstra *et al.*, 1991). These previous studies also suggested that age does not appear to have a significant effect on the morphology or histological responses of the endometrium to steroid stimulation.

The endometrial secretory function and endometrial development appear unaffected significantly among the subjects in the present study and thus arguing on the surface against an increased rate of luteal phase defect in cycling older women. The results of

the present study therefore gives hope to those above 35 years since majority of them have normal endometrial development and may therefore be able to conceive and probably carry pregnancy to term should fertilization occur naturally or in cases of IVF. However, given the small sample size of this study only a repeat study with a larger cohort may confirm this observation or otherwise.

On the other hand, the lack of significant age-related difference in the present study therefore stands in contrast to the work of Meldrum, (1993) and Sterzik *et al.,* (1988) which suggested that the development of the endometrium is frequently abnormal in older women implying that the endometrial receptivity to implantation may also deteriorate with advancing age. They attributed this reduced receptivity to deficient progesterone secretion by the corpus luteum or the inability of the endometrium to respond to progesterone stimulation. However, in protocols used for oocyte retrieval, the endometrium is exposed to very high and perhaps deleterious hormonal levels, which may explain the difference in results found in stimulated as in Sterzik *et al.,* (1988) and spontaneous cycles such as the one evaluated in the present study. Also Sterzik *et al.,* (1988) obtained the endometrial biopsies on day 2 after HCG induced ovulation in infertile women and not at the time of implantation, as was done in the present study. Conflicting results seen in the present study and other studies of reproductive changes in women 35 years and above may be due to sampling difference in the population recruited by the various studies, including fertility status (Lee *et al.*, 1988; Lenton *et al.*, 1991; Sherman *et al.*, 1976).

5.6 INCIDENCE OF LPD AND TYPE OF INFERTILITY

The results of the present study show that out of the 16 women presenting with primary infertility, 50.0% (8/16) had their endometrial biopsies showing out-of-phase

development and 50.0% (8) had in-phase endometrial development. Of the 64 women with secondary infertility 31.25%, (20/64) had out of phase biopsies and 68.75% (44/64) had in-phase biopsies. The findings of the present study is similar to a study by Li *et al*., (1991) who also found no significant relation between women with tubal or male factor infertility and fertile subjects. The finding of the present study gives an indication that endometrial development does not depend on the type of infertility presented. Further research into the development of the endometrium in women presenting with infertility should be directed towards specific causes of the infertility.

The incidence of some causes of infertility like endometriosis, infections and tubal problems has been shown to vary among infertile women depending on the type of infertility. The incidences of endometriosis in primary and secondary infertility are 26.0% and 13.0% respectively (Mahmood and Templeton, 1991). Infections are more associated with secondary infertility than primary infertility (Larsen, 2000).

Chapter 6

SUMMARY OF MAIN FINDINGS, CONCLUSION AND FUTURE WORK

6.1 SUMMARY OF MAIN FINDINGS

- High secondary infertility compare to primary infertility which is consistent with the trend in Sub Saharan Africa
- Majority of the patients were aged 35 and above
- The prevalence of LPD among the 80 study subjects was 35%
- Age did not appear to affect endometrial development
- Prevalence of LPD did not show any significant association with type of infertility

6.2 CONCLUSIONS

In the 65.0% of the women whose endometrial biopsies showed normal endometrial development, their infertility could be due to other factors rather than LPD. Further investigation will therefore have to be carried out to ascertain the cause of their infertility.

In the 35.0% of the infertile women in whom the development of the endometrium appeared retarded indicates a non-receptive and non-adhesive endometrium around the ‘’implantation window’’, which may not be conducive for implantation. Therefore their infertility could be due to LPD.

There was no significant difference when LPD was analyzed according to the age (p=0.472) as well as type of infertility (p = 0.157) suggesting that aging and type of infertility has no effect on endometrial retardation.

6.3 RECOMMENDATION AND FUTURE WORK

1. Further studies with a higher sample size involving hospitals from other parts of the country should be carried out.
2. Biochemical and hormonal assays should be performed with endometrial biopsy in women with LPD in future studies.
3. Because of the existence of RED and AED among normal ovulating women presenting with infertility, it is recommended that specific 2-day histological dating should be used instead of the broad phase endometrial dating.

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APPENDIX