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ORIGINAL ARTICLE

Effect of maternal age on endometrial morphology among Ghanaian infertile women

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As more women choose to delay childbearing, increasing numbers of them face age-related fertility problems. 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. It is unclear as to whether women who present to infertility clinics at older age have age-related endometrial retardation or luteal phase defect. This study was designed to investigate the prevalence of luteal phase defect (LPD) among infertile women and its relationship with age. Mid-luteal endometrial biopsies were taken from eighty (80) infertile women attending fertility clinics of Komfo Anokye Teaching Hospital, Magazine Clinic and the Bomso Specialist Hospital in Kumasi metropolis and ten fertile women as control using dilatation and curettage and then processed 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 suggesting that ageing has no significant effect on endometrial retardation from this study.
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Keywords*:* Luteal phase defect, Infertility, Ageing, Non-receptivity

INTRODUCTION

Female age is the single most important determinant of spontaneous as well as treatment related conception, with a gradual decline in fertility especially after the age of 35 years (Menken *et al.*, 1986). Demographic studies have shown that more women are delaying childbearing at the present time than previously (Abaidoo *et al.*, 2000; Botting *et al.*, 2000). This trend is expected to cause a corresponding rise in the mean age at which women first present with infertility (Botting *et al.*, 2000). It is unclear as to whether Ghanaian women who present to fertility clinics at an older age have a different diagnostic profile from that in younger women. It has been well established that female fertility declines with age (Fox *et al.*, 1991; Tietze, 1957). This phenomenon has been attributed in-part to the 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 *et al.*, 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 *et al.*, 1985) along with a reduction in stromal cells (Wilcox *et al.*, 1988) and oestrogen receptors (Han *et al.*, 1989). Furthermore, reductions in oestral periods (Rahima *et al.*, 1978) and the endometrial cells' ability to express a

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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). Although there have been some studies on female infertility in Ghana, there has not been any study to evaluate the effect on maternal age on endometrial development in Ghana. The present study was therefore designed to study endometrial development among infertile with respect to maternal age in Ghanaian setting.

MATERIALS AND METHODS

Sample collection

Endometrial biopsies were obtained by qualify clinical staff from 80 selected infertile women attending the fertility clinics at the Komfo Anokye Teaching hospital, Magazine clinic and the Bomso Specialist Hospital in Kumasi metropolis between January 2005 to December 2008. Endometrial biopsies were similarly obtained from ten 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 have had at least one successful pregnancy and had no evidence of pathology associated with their reproductive tract.

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. Socio-demographic characteristic of the participant including age, type of infertility, clinical diagnosis, last menstrual period and hormonal therapeutic histories were retrieved from their medical records for subsequent correlative analysis with 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.

Endometrial dating

All the biopsies were chronologically dated in relation to the last menstrual period (LMP). All the biopsies were processed using the wax embedding techniques and stained with haematoxylin and eosin (H and E) for light microscopic examination. 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 sub-nuclear vacuolations or supra-nuclear 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.

Staistical analysis

All categorical variables were analyzed using Chi- Square analysis. In all statistical tests, a value of $P<0.05$ was considered significant. All analysis was performed using Sigma Plot for Windows, Version 11.0, (Systat Software, Erkrath, Germany; www.systat.com).

RESULTS

Generally, the number of women presenting with infertility increases with age as shown in figure 1. 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 before decreasing to 8 women among the 36-40 years women as shown in figure 1.

Type of infertility and Age

Figure 2 represents age distribution of primary and secondary infertility among the study group. Among the 20-25 years group, 7 (53.8%) presented with primary infertility compared with 6 (46.2%) who presented with secondary infertility. Among the 26-30 year group, the number of secondary infertile women rose to 21 (87.5%) while primary infertility decreased to 3 (12.5%). Whereas there is general decrease in the number of women with primary infertility

with age, there appear to be a normal distribution among the women with secondary infertility.

Maternal age and endometrial development

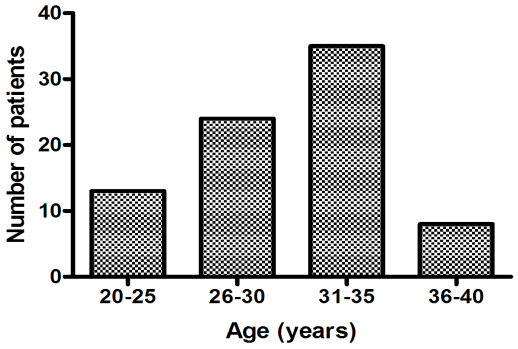

Figure 1: Age distribution of the infertile subjects

Among the 27 women 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, 33 (62.30%) were in-phase while 20 (37.70%) had their biopsies showing out-of phase (Table 1). When the difference between the two age groups was analyzed, there were no significant difference between the groups (P=0.4723, χ^2 = 0.5167, OR = 1.439, 95% CI = 0.53 to 3.90).

DISCUSSION

From this study, when the studied population was stratified based on infertility and age, about 70.0% of those who had secondary infertility cases where within the 26-35 years age group. Also, 30.0% and 43.7% of the infertile women were within the 26-30 and 31-35 age group respectively These figures are relatively higher than the 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 fact that whilst we use infertile subjects, their study was a population base study.

It has been documented that female fecundity is at its peak within 26-35 years and infertility becomes more pronounced after the age of 35 (Gindoff *et al.*, 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 group 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 group because they think their biological clocks are 'ticking away past their prime'. 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

Table 1: Incidences of LPD and Maternal Age

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

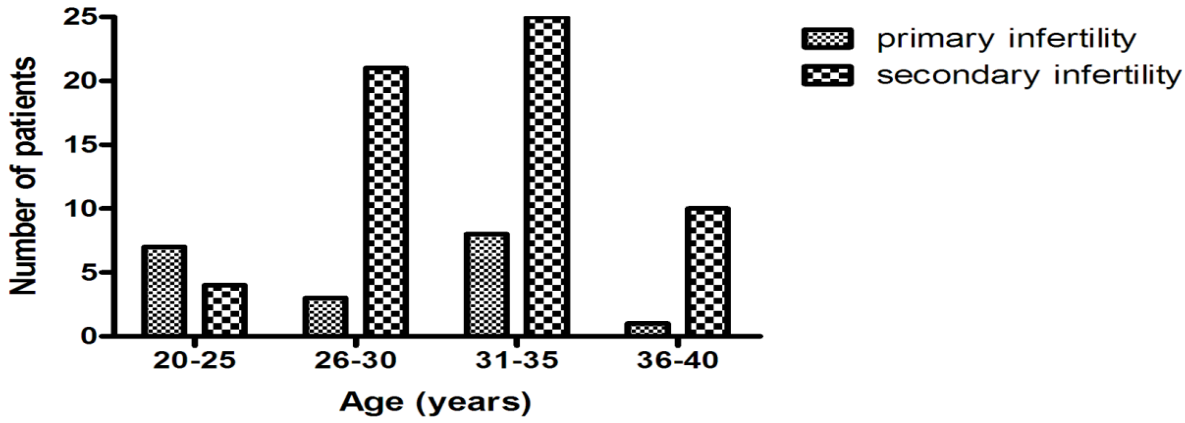

Figure 2: Distribution of primary and secondary infertility in the different age groups

women age 45-49 to 15% among the youngest cohort aged 20-24. The reports associate the reduction in the percentage of women giving birth early to the fact 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.

Stratification of the studied population based on age and LPD indicates that about 66.2% (53/80) of the study population where within the 30-40 age group whilst only 33.8% (27/80) were aged 20-29 year (Fig 2). 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 *et al*., 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 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.

On the other hand, the lack of significant age-related difference is 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.

CONCLUSION

Infertility was generally on the increased with advancing age peaking at the age of 30-35 years with secondary infertility being higher among the study population. This study thus confirm the earlier findings which suggest that age and infertility among Ghanaian women is an increasing problem due to general societal trends for women to delay childbearing until later ages. Though prevalence of luteal phase defect among the study subjects was relatively high, the findings show that age was not a predictive factor for luteal phase defect indicating that maternal age may not be associated with increase endometrial retardation.

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