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# Cyclical Changes in Prolactin Levels among Infertile Women Attending University of Port Harcourt Teaching Hospital

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**ABSTRACT:** Study was carried out to determine the effect of cyclical changes of prolactin concentrations on infertility in 3 groups of infertile subjects attending university of Port Harcourt teaching hospital for infertility treatments. Prolactin samples of the subjects were determined at follicular (day3), Midcycle (day14) and luteal phase (day21) using enzyme immune assay method (EIA). Prolactin was significantly increased in infertile subjects compared with the controls (Apparently fertile) suggesting an increase in prolactin concentration in infertile subjects. There was also cyclical changes observed in prolactin secretion with midcycle (day14) being the peak. Cyclical changes were also observed in prolactin concentration in both fertile and infertile subjects which peaked at midcycle (day14) suggesting that cycle of subjects should be taken into consideration in estimation, interpretation and investigation of infertility while the elevation of prolactin in the 3 study groups might be responsible for the infertility observed. @JASEM

Keyword: Prolactin, immuno assay, follicular phase, midcycle, luteal phases

Prolactin was first identified as a product of the anterior pituitary in 1933 (Benjaminsen, 1981). It has been found in nearly every vertebrate species. The specific activities of human prolactin have been further defined by the separation of its activity from growth hormone and subsequently by the development of radioimmunoassays (Bervers, et al 1983) and its release in blood are inhibited by dopamine and enhanced by some other hormones. In mammary gland, it induces growth of cells that secrets milk, and also acts with other hormones to produce milk proteins. In some species, lack of prolactin causes infertility through defects in ovulation fertilization and implantation. Prolactin also appears to have a role in the immune response, although this is poorly understood (Ravault *et al* 1982).

In women prolactin secretion by the pituitary gland is rapidly increased during late gestation due to the effect of high levels of placental oestrogen. After labour, maternal plasma prolactin drops at a very slow rate and in women as in other species, the post-partum levels of prolactin are related to the amount of suckling stimulus and then to the length of lactational anestrus. Prolactin plays many biological roles in the post-partum period. It has a significant role in the establishment of maternal behaviour (Bridges *et al* 1987) and increases gene expression of milk proteins (Rosen et al 1980). Hyperprolactinaemia can cause irregular or no ovulation resulting in infertility. Women with this disorder often have irregular periods, and may also experience galactorrhoea.

When the pathologically high prolactin concentration was suppressed by treatment with bromocriptine, normal menstrual cycles resumed (Rolland, *et al* 1975) suggesting that prolactin can directly influence luteinizing hormone secretion and affect the resumption of ovarian activity. However, since the suckling-related increase in Prolactin is not acutely related to any inhibition of basal or pulsatile secretion of luteinizing hormone (Glasier *et al* 1984). Elevated prolactin may impact reproduction through an action on the GnRH neurons of the hypothalamus and/or on the pituitary gland to affect secretion of the gonadotrophins, LH, and FSH (Evans *et al* 1982, McNeilly, 2001). In humans, hyperprolactinemia is associated with a marked reduction in both the frequency and amplitude of LH pulses (Bohnet, *et al* 1976, Matsuzaki, *et al* 1994), indirectly suggesting that both the brain and pituitary might be targets for prolactin while high concentrations of Prolactin inhibit gonadotrophic releasing hormone secretion in humans (Yazigi *et al* 1997). Ravault *et al.,* (1982) found a trend for gilts to have higher prolactin level in summer than in winter.

This study is aimed at determining prolactin fluctuations in infertile women (both Primary and Secondary infertility cases) at different phases of their cycle to (a)ascertain the best time of the woman cycle when sample for prolactin assay is to be collected and (b) subsequent effect of cyclical changes on diagnosis of infertility and infertility in the study groups.

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## METHOD AND MATERIALS

*Subjects:* Three hundred and twenty (320) women with cases of infertility made up of 60 primary infertile subjects, 140 secondary infertile subjects' and120 apparently fertile women as the control were used in this study. They were women within the child bearing age range of 15-44 years with cases of infertility including primary and secondary as well as those with symptomatic galactorrhoea and amenorrhea attending clinics at University of Port Harcourt Teaching Hospital (UPTH), Rivers state.

*Biochemical Assay:* The subjects had their blood collected by venepuncture at days 3 (follicular), 14(ovulatory) and 21( luteal phases)of their menstrual cycle and the serum Prolactin (PROL) was determined by enzyme immune assay (EIA).method using quantitative test kit, produced by Immunometrics (UK) limited, United Kingdom as described below. The assay is of an Immunometric (sandwich) design using two anti-prolactin antibodies (Polyclonal and monoclonal antibodies) which bind to different epitopes of the prolactin molecule.
One hundred microlitres (100ul) working suspension of Prolactin EIA magnetic antibody was added to 100ul of standards, samples or control in labelled plastic tubes. The tubes were vortex mixed, covered and incubated at $37^0$c in a water bath for 15 minutes. Fifty microlitres (50ul) of diluted Prolactin EIA wash buffer was added, mixed and stood on a magnetic base in a rack. The supernatant was decanted while 250ul of diluted Prolactin, EIA labeled antibody was added to the tubes. They were mixed, covered and incubated for 1 hour in a water bath at $37^0$c. The washing step was repeated twice using 500ul diluted Prolactin wash buffer. Five hundred microlitres (500ul) of EIA substrate solution was added to the tubes, mixed, covered and incubated for 1 hour at $37^0$c. One millilitre (1.0ml) of EIA stop solution was added to end the reaction and stabilize the color formed. Absorbance were read at 490nm using a spectrophotometer.

*Statistical Analysis***:** The biochemical data were subjected to some statistical analysis. Values were reported as Mean $\pm$ SEM while student's t-test was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16.A value of $P<0.05$ was accepted as significant

## RESULT AND DISCUSSION

The result revealed that there were cyclical changes in prolactin concentration as shown below. There was significant difference in prolactin concentrations at days 3, 14 and 21 between the controls and infertile subjects ($P<0.0001$) as shown below in table 1.At day 3 prolactin (miu/l) value of 311.45$\pm$ 12.78 in control was significantly lower than 414.72 $\pm$ 13.63 in infertile subjects ($P<0.0001$).Also prolactin (miu/l) of 327.93 $\pm$ 13.59 in the controls at day 14 was significantly lower than 502.75 $\pm$19.00 in infertile subjects($P<0.0001$). At day 21, prolactin (miu/l) 315.23 $\pm$12.86 in control was also significantly lower than 454.88 $\pm$16.92 of infertile subjects ($P<0.0001$).

There was significant difference in prolactin concentrations at days 3, 14 and 21 between the controls and Primary infertile subjects.($P<0.0001$) as shown below in table 1.Prolactin (miu/l) value of 311.45$\pm$12.78 was significantly lower than 424.33$\pm$28.11 in Primary infertile subjects ($P<0.0001$) at day 3. Also prolactin (miu/l) 327.93 $\pm$13.59 in the control at day14 was significantly lower than 489.42 $\pm$ 33.27 in Primary infertile subjects($P<0.0001$)while at day 21, 315.23 $\pm$12.86 in control was significantly lower than 436.53 $\pm$ 29.48 of primary infertile subjects ($P<0.0001$).

Prolactin (miu/l) value of 311.45 $\pm$ 12.78 was significantly lower than 463.74 $\pm$ 21.25 in secondary infertile subjects ($P<0.0001$) at day 3 while 327.93 $\pm$13.59 in the control at day14 was significantly lower than 508.46 $\pm$ 23.16 in secondary infertile subjects ($P<0.0001$). At day 21, prolactin (miu/l) concentration of 315.23 $\pm$12.86 in control was significantly lower than 462.74 $\pm$ 20.63 of secondary infertile subjects ($P<0.0001$) as shown in table 1 below.

The result of this study revealed elevated prolactin concentrations in infertile subjects compared with fertile controls. Also there were cyclical increases in prolactin concentration of infertile subjects (Primary and secondary infertile) subjects compared with their respective controls with the day 14 prolactin as the peak secretion at the three cycles suggesting release of prolactin releasing factor at mid cycle i.e day 14 (midcycle). This study agrees with Liu *et al.,* (1986) whose result showed significantly higher values at mid cycle phase than at the Follicular and luteal phases in normal Chinese women. Prolactin is unique among the anterior pituitary hormones in that its secretion is under tonic inhibitory control. The hypothalamus secretes a prolactin release-inhibiting factor which is secreted into the portal capillaries and thus transported to the anterior pituitary. Prolactin plays a significant role in the establishment of maternal behaviour (Bridges *et al.*, 1987), and increases gene expression of milk proteins (Rosen *et al.*, 1980). Ben – David and Schenker (1983) reported

that transient hyper prolactinaemia at mid cycle might disturb fertilization and embryo implantation.

This study also showed significant difference in the Prolactin values at Follicular phase (Day 3) midcycle (Day 14) and Luteal (Day21) phases of the primary and also secondary infertile women while there was no difference in the mean value of prolactin in control subjects at different phases of cycle. The significant increase observed in prolactin values of both primary and secondary infertile women at the follicular (Day 3), Mid cycle (Day 14) and luteal (Day 21) phases is similar to the study by Azima and Samina (2002) which showed increase in prolactin levels at the follicular, ovulatory and luteal phases in women with primary and secondary infertility. Hyperprolactinaemia is a well-established cause of infertility in both male and female mammals. Elevated prolactin may impact reproduction through an action on the GnRH neurons of the hypothalamus and/or on the pituitary gland to affect secretion of the gonadotrophins, LH, and FSH (Evans et al 1982, McNeilly 2001). In humans, hyperprolactinemia is associated with a marked reduction in both the frequency and amplitude of LH pulses (Bohnet et al 1976, Matsuzaki et al 1994), indirectly suggesting that both the brain and pituitary might be targets for prolactin.

There was significant increase in prolactin levels of secondary infertile women when compared with primary infertile women ($P<0.05$) at the mid cycle and luteal phases while at the follicular phase, there was no significant difference ($P>0.05$). Increase secretion of prolactin at day 14 (mid cycle) compared to other days/phases (i.e days 3 and 21) suggests release of prolactin releasing factor at mid cycle than the luteal and follicular phases. The result is also suggestive that concentration of prolactin is always higher in secondary infertile women than the primary infertile women. Prolactin is unique among the anterior pituitary hormones in that its secretion is under tonic inhibitory control. The hypothalamus secretes a prolactin release-inhibiting factor which is secreted into the portal capillaries and thus transported to the anterior pituitary (Thorner, 1976). As with other pituitary hormones, the release of prolactin is episodic and varies predictably during the day with the lowest levels at mid day and highest values shortly after the onset of deep sleep. Secretion of prolactin is under hypothalamic control and is unique because the main control of its secretion is inhibitory rather than stimulatory (Demers 1999).

**TABLE 1** Cyclical Changes Of Prolactin Concentration In Infertile, Primary And Secondary Infertile Subjects Mean (SEM)

| | infertile subjects | | | primary infertility | | | secondary infertility | | |
|---|---|---|---|---|---|---|---|---|---|
| parameter | control n = 120 | infertile subjects n = 290 | p-value | control n = 120 | primary infertile n = 60 | p-value | control n = 120 | secondary infertile n = 140 | P-value |
| PROL (miu/l)3 | 311.45 (12.78) | 414.72 (13.63) | 0.0001 | 311.45 (12.78) | 424.33 (28.11) | 0.0001 | 311.45 (12.78) | 463.74 (21.25) | 0.0001 |
| PROL (miu/l)14 | 327.93 (13.59) | 502.75 (19.00) | 0.0001 | 327.93 (13.59) | 489.42 (33.27) | 0.0001 | 327.93 (13.59) | 508.46 (23.16) | 0.0001 |
| PROL (miu/l)21 | 315.23 (12.86) | 454.88 (16.92) | 0.0001 | 315.23 (12.86) | 436.53 (29.48) | 0.0001 | 315.23 (12.86) | 462.74 (20.63) | 0.0001 |

The result also revealed variations and cyclic changes in Prolactin concentrations at different age group as shown below. At the 3rd day (follicular phase) the result of the study showed that subjects at 20-24 years of age had the highest secretion of prolactin (miu/l) in the controls with 473.51± 42.10 followed by 25 -29 years at 314.12 ± 21.19 while the others were 292.42 ± 25.60, 283.64 ± 26.03 and 313.38 ± 47.15 at 30-34 years35-39 years and 40-44 years respectively. In primary infertile subjects at day 3 (follicular Phase) subjects at 20-24 years of age also had the highest secretion of prolactin (miu/l) with 501.60± 40.20,followed by 40-44 at 450.00 ± 0.00.Others include 424.26 ± 51.00, 466.32 ± 55.90, 339.00 ± 89.01 and 307.39±38.83 at 25-29 years,30-34 years,35-39years and 15-19years respectively. In the secondary infertility subjects at 25 -29years had the highest prolactin (miu/l) secretion of 508.54 ± 37.23 followed by 20-24 years at 472.51 ± 40.60.Others include 453.65 ± 35.17, 441.00 ±93.84, 409.06 ± 38.12 and 310.23±99.75 at 30-34 years, 40-44 years,35-39years and 15-19years respectively.

In amenorrhoea subjects prolactin secretion (miu/l) was highest at 30-34 years with 349.78 ± 48.97, followed by335.59 ± 30.22 at 25 -29 and 332.75 ± 90.48 at 40-44 years respectively. Others include 282.50 ± 35.39 and 300.90±42.84 at 35-39years and 15-19years respectively as shown below in table 2.

Hyperprolactinaemia is a common endocrine disorder with an incidence of 9–17% (Biller et al 1999, Bayrak et al 2005).

**Table 2**: Prolactin concentrations at day 3 in different age groups

| | control | | primary | | secondary | | amenorrhoea | |
|---|---|---|---|---|---|---|---|---|
| Age (years) | n | prol (miu/l) | n | prol (miu/l) day 3 | n | prol (miu/l) day 3 | n | prol(miu/l) |
| 15-19 | 0 | 0.00 ± 0.00. | 5 | 307.39±38.83 | 3 | 310.23±99.75 | 8 | 300.9±42.84 |
| 20-24 | 13 | 473.51± 42.1 | 8 | 501.60± 40.2 | 10 | 472.51 ± 40.6 | 15 | 0.00 ± 0.00 |
| 25-29 | 41 | 314.12 ± 21.19 | 23 | 424.26 ± 51.00 | 41 | 508.54 ± 37.23 | 27 | 335.59 ± 30.22 |
| 30-34 | 36 | 292.42 ± 25.60 | 19 | 466.32 ± 55.90 | 43 | 453.65 ± 35.17 | 18 | 349.78 ± 48.97 |
| 35-39 | 22 | 283.64 ± 26.03 | 4 | 339.00 ± 89.01 | 31 | 409.06 ± 38.12 | 14 | 282.50 ± 35.39 |
| 40-44 | 8 | 313.38 ± 47.15 | 1 | 450.00 ± 0.00 | 12 | 441.00 ± 93.84 | 8 | 332.75 ± 90.48 |
| Total | 120 | 311.45 ± 12.78 | 60 | 424.33 ± 28.11 | 140 | 463.74 ± 21.25 | 90 | 332.04 ± 19.45 |

In an earlier study, with human granulosa cells, increasing amount of prolactin when added to the culture medium inhibited the secretion of progesterone in a dose dependent manner (Mc Natty *et al.,* 1974). It has also been demonstrated that high levels of prolactin can block FSH induction of aromatase in the estrogen synthetase system (Wang *et al.,* 1980, Dorrington and Gore-Langton 1981) thereby causing infertility. The observation that infertile subjects at age groups 20-24 and 30-34 had the highest production of prolactin at the three phases of cycle in primary infertile suggests that hyperprolactineamia is a common occurrence in these groups of subject and likely be the cause of infertility in women with primary infertility while in secondary infertile subjects 25-29 years had the highest prolactin concentration. In amenorrhea, infertile subjects of 30-34 years had the highest concentration of prolactin.

Hyperprolactinaemia is a common cause of gonadal dysfunction, particularly in women. Its incidence in women with secondary amenorrhoea varies from 13% to over 30% (Franks et al., 1975; Seppala et al., 1975; Bohnet et al., 1976). Most patients with hypogonadism due to pituitary tumours do not in fact suffer from gonadotrophin deficiency, they suffer from hyperprolactinaemia. Only about 30% of patients with hyperprolactinaemia have galactorrhoea. Galactorrhoea often accompanies the amenorrhoea related to the cessation of oral contraceptives. Women with hyperprolactinaemia may present in a variety of ways-with secondary amenorrhoea or any menstrual abnormality, oligomenorrhoea, polymenorrhoea, or even with a normal menstrual cycle. It has been suggested that women with hyperprolactinaemia who present with infertility and have a normal cycle may be suffering from defective luteal function (Del Pozo et al., 1977). As shown below, at the 14$^{th}$ day (MIDCYCLE phase) the result of the study showed that subjects at 20-24 years of age had the highest secretion of prolactin (miu/l) in the controls with 445.00±34.65 followed by 25 -29 years at 329.61 ± 22.86 while the others were 325.38 ± 48.39, 312.69 ± 26.79 and 297.77 ± 27.09 at 40-44 years, 30-34 years and 35-39 years respectively. In primary infertile subjects at day 14 (midcycle Phase) subjects at 20-24 years of age also had the highest secretion of prolactin (miu/l) with 541.60± 46.2,followed by 30-34 years and 40-44 respectively with 524.95 ± 62.69 and 485.00 ± 0.00.Others include 489.74 ± 59.22,383.25 ± 113.48and 309.92±60.45 at 25-29 years, ,35-39years and 15-19years respectively. In the secondary infertility subjects at 25 -29years had the highest prolactin secretion (miu/l) of 541.98 ± 38.11 followed by 30-34 years at 499.44 ± 37.10 and 20-24 years at 492.51 ± 34.6.Others include 489.50 ± 97.77, 446.00 ± 41.01and 329±125.39 at, 40-44 years,35-39years and 15-19years respectively as shown below in table 3. In humans, prolactin secretion has been investigated mainly in relation to induction of puberty and menarche with ovulatory cycle, breast development pregnancy and lactation. Its role in gonadal dysfunction is now widely recognized including amenorrhoea with or without galactorrhea, anovulation and infertility in females (Ratcher and Dokumov 1995).

**Table 3** Prolactin concentrations at day 14 in different age group (Mean ± SEM)

| age(years) | control n | control prol (miu/l) day 21 | primary n | primary prol (miu/l) day 21 | secondary n | secondary prol (miu/l) day21 |
|---|---|---|---|---|---|---|
| 15-19 | 0 | 0.00 ± 0.00 | 5 | 323.77±43.34 | 3 | 295.46±88.94 |
| 20-24 | 13 | 477.80± 32.5 | 8 | 451.98± 32.4 | 10 | 468.90 ± 24.2 |
| 25-29 | 41 | 324.61 ± 21.83 | 23 | 445.00 ± 52.92 | 41 | 512.80 ± 36.91 |
| 30-34 | 36 | 291.53 ± 25.37 | 19 | 472.00 ± 58.63 | 43 | 443.14 ± 34.77 |
| 35-39 | 22 | 283.68 ± 25.75 | 4 | 352.50 ± 96.99 | 31 | 416.16 ± 37.37 |
| 40-44 | 8 | 314.63 ± 44.43 | 1 | 460.00 ± 0.00 | 12 | 446.83 ± 92.45 |
| Total | 120 | 315.23 ± 12.86 | 60 | 436.53 ± 29.48 | 140 | 462.74 ± 20.63 |

**Table 4** Prolactin concentrations at day 21 in different age groups mean ± sem

| age(years) | control n | control prol (miu/l) day 14 | primary n | primary prol (miu/l) day 14 | secondary n | secondary prol (miu/l) day 14 |
|---|---|---|---|---|---|---|
| 15-19 | 0 | 0.00 ± 0.00 | 5 | 309.92±60.45 | 3 | 329±125.39 |
| 20-24 | 13 | 445.00±34.65 | 8 | 541.60± 46.2 | 10 | 492.51 ± 34.6 |
| 25-29 | 41 | 329.61 ± 22.86 | 23 | 489.74 ± 59.22 | 41 | 541.98 ± 38.11 |
| 30-34 | 36 | 312.69 ± 26.79 | 19 | 524.95 ± 62.69 | 43 | 499.44 ± 37.10 |
| 35-39 | 22 | 297.77 ± 27.09 | 4 | 383.25 ± 113.48 | 31 | 446.00 ± 41.01 |
| 40-44 | 8 | 325.38 ± 48.39 | 1 | 485.00 ± 0.00 | 12 | 489.50 ± 97.77 |
| Total | 120 | 327.93 ± 13.59 | 60 | 489.42 ± 33.27 | 140 | 508.46 ± 23.16 |

At the 21$^{St}$ day (Luteal phase) the result of the study showed that subjects at 20-24 years of age had the highest secretion of prolactin (miu/l) in the controls with 477.80± 32.50 followed by 25 -29 years at 324.61 ± 21.83 while the others were314.63 ± 44.43, 291.53 ± 25.37 and 283.68 ± 25.75 at 40-44 Years, 30-34 years and 35-39 years respectively. In primary infertile subjects at day 21 (Luteal Phase) subjects at 30-34 years of age had the highest secretion of prolactin (miu/l) with 472.00 ± 58.63,followed by 40-44 years and 20-24 respectively with 460.00 ± 0.00 and 451.98± 32.40 .Others include 445.00 ± 52.92, 352.50 ± 96.99 and 323.77±43.34 at 25-29 years, ,35-39years and 15-19years respectively. In the secondary infertility, subjects at 25 -29years had the highest prolactin secretion (miu/l) of 512.80 ± 36.91followed by 20-24years at 468.90 ± 24.2 and 40-44 years at 446.83 ± 92.45. Others include 443.14 ± 34.77, 416.16 ± 37.37 and 295.46±88.94 at 35-39 years,35-39years and 15-19years respectively as shown below in table 4. Chandra and Stephen (1998) showed that impaired fertility increased between 1982-1995 by about 25% in all women aged 15-44, the increase was only 6% in women aged 35-44, 12% in women aged 25-34, and 42% in the youngest group 15-24. These data suggest that delayed childbearing does not fully explain the apparent upward trend and that even younger women are experiencing fecundity problems (Chandra and Stephen 1998). Data from historical populations estimated rates of prevalence of infertility to be 5.5%, 9.4% and 19.7% at ages 25-29 years, 30-34 years and 35-39 years, respectively (Bongaarts 1982). Studies in which blood levels of prolactin have been suppressed pharmacologically with bromocriptine, ergocornine or with antiserum to prolactin suggests that only minimal amounts of prolactin are needed for normal follicular growth (McNeilly *et al.,* 1982).

*Conclusion:* In conclusion, present study showed cyclical increase in prolactin concentration of the infertile study groups which peaked at the mid cycle of all the study groups .Release of prolactin by pituitary hypothalamic occurs at the mid cycle causing the surge in prolactin elevation at the mid cycle. The result of this study also bring to fore the need for cycle of subjects to be taken into consideration before prolactin estimation to avoid misdiagnosis as prolactin show cyclic variation.

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