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Spectral Analysis of the luteinizing hormone in the blood samples

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INTRODUCTION

In medicine and sciences of the life in general, it is so important the evolution of one measurement as well as its value at a certain moment. Nevertheless the analysis of the temporary series, that is the appropriate methodology for this type of study, and in individual the version of the spectral analysis or analysis of the time series in the dominion of frequencies, is little known and therefore underused. The medicine books are concentrated almost exclusively in explaining methodology that analyzes fixed measures, measures done in a certain moment, Campbell (1996).

In this work we try to analyze the frequency whereupon it happens the pulsating secretion of luteinizing hormone LH, that is to say, we are going to determine as they are the significant frequencies obtained by analysis of Fourier. The main difficulty with that are the professionals of the biomedicine, to detect the frequencies with which the pulsating secretion of the LH takes place is that random errors in the measures and problems in the sampling exist. This does that pulsating secretions of small amplitude do not detect because random errors are considered and that random variations of the secretion are considered like pulsating secretions. In physiology it is accepted that cyclical patterns in the secretion of the LH exist and in this work we are going to confirm this pattern and to determine its frequency. By another part we are going to give diffusion to a statistical methodology little used, without an excessive formalism, but with sufficient rigor and that it is very useful for the investigators, without any excessively deep knowledge of the mathematics. A simplified vision of the subject we can find it in Diggle (1990), and one more developed of the theory on spectral analysis we can consult Brockwell *at al*. (2000) and one more formalized vision in Brockwell *et al.* (1991).

1. MATERIAL AND STATISTICAL METHODOLOGY

In this section we are going to expose of which the physiology of the secretion of the luteinizing hormone consists and are going to make a description of the analysis of temporary series in the dominion of frequencies or spectral analysis.

1.1. Luteinizing hormone

The fertile life of the woman begins in menarquia that is the first menstrual period and finishes with the menopause. This period is divided in cycles of approximately 28 days, separated by the menstruation. Arbitrarily it is considered that the cycle begins the first day of the menstruation and finishes the day previous to the following one. It is characterized by monthly rhythmical variations of the secretion of the hormone LH that helps to regulate the menstrual cycle and the ovum production. The cycle is divided in two periods: the follicular phase that goes from the beginning of the cycle to the ovulation and the luteal phase from the ovulation to the end of the cycle. The follicular phase, in its turn, has two phases: the early phase that embraces the period starting with the beginning of the menstruation until its finishing, and the late phase, from the end of menstruation until the ovulation, that is normally the 14th day of the normal feminine sexual cycle. In this stage a quick increment of the LH secretion occurs that is known as the LH pick in medicine.

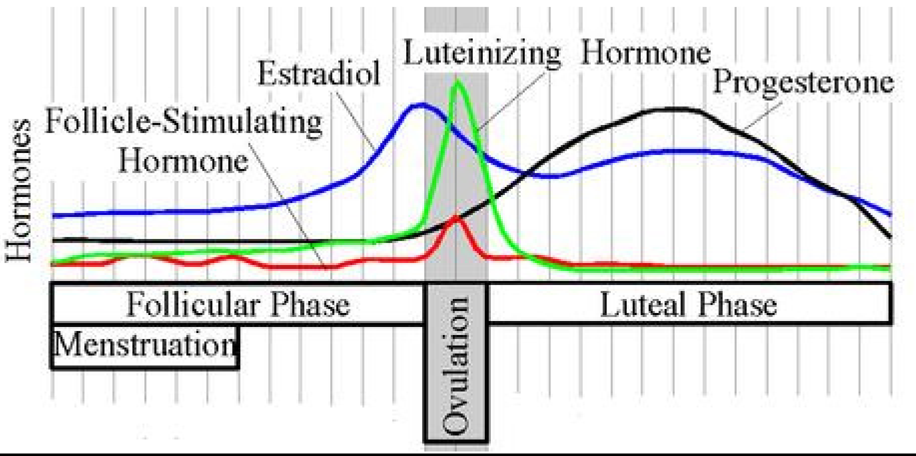

Figure 1. Hormonal behaviour in the follicular and luteal phases.

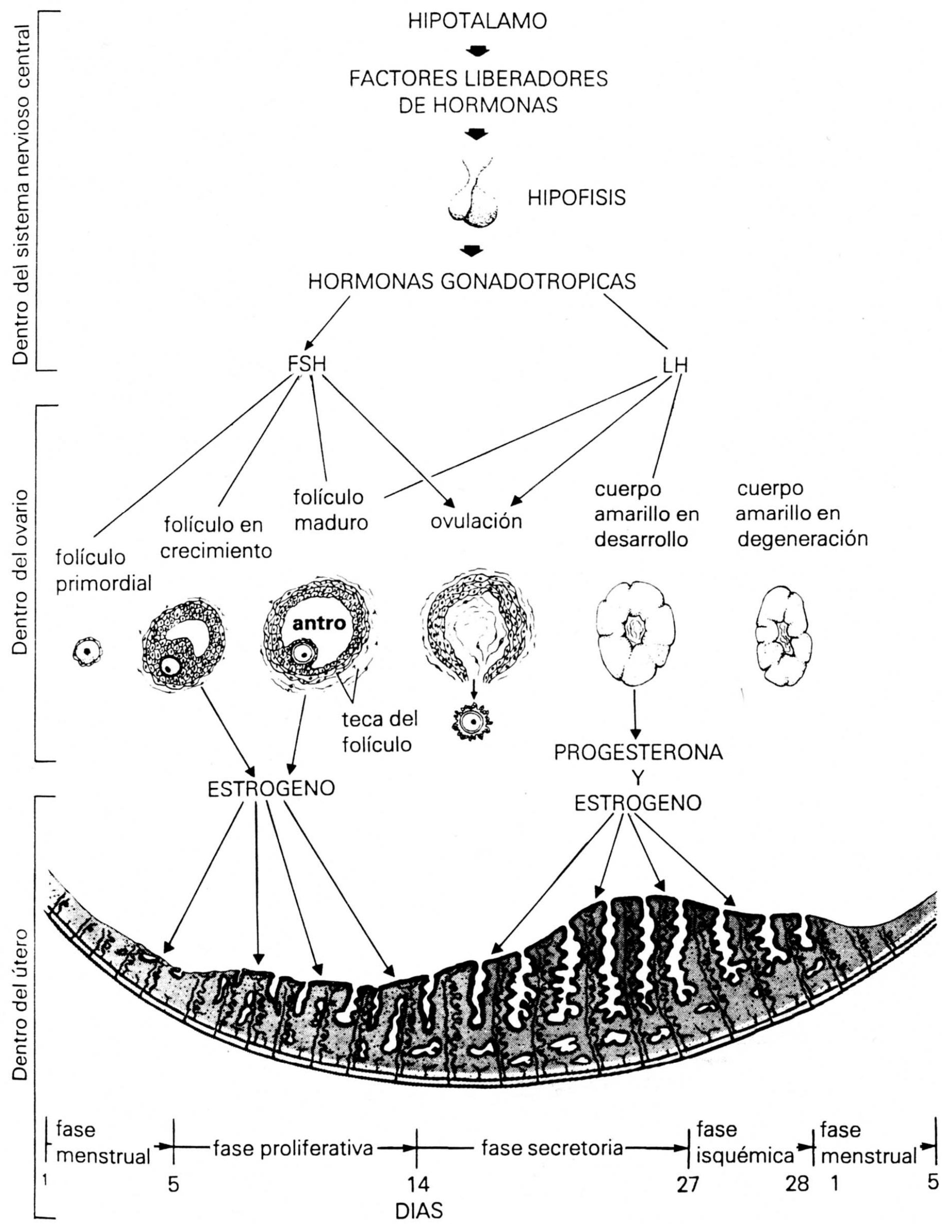

Fig. 2-10. Ciclo reproductivo de la mujer. FSH: hormona folículo estimulante; LH: hormona luteinizante. (Adaptado de Moore KL. Before we are born, 3a. ed. Filadelfia, WB Saunders, Co., 1989.)

Figure 2. The menstrual cycle.

1.2. Spectral Analysis

Isaac Newton published one article in the Royal Society in 1672 where he had used the term “spectrum” for the description of the colours of the rainborn, in which the white light, solar light was discomposed passing via a crystal prism.

We know from the physics that each colour corresponds to the determinate frequency (see Figure 3), thus, light analysis is one of the forms of frequency analysis. A role of the prism will be associated with the Fourier series, as it will be shown later that the decomposition of the observed series values during some time period is a lineal combination of sinus and cosines.

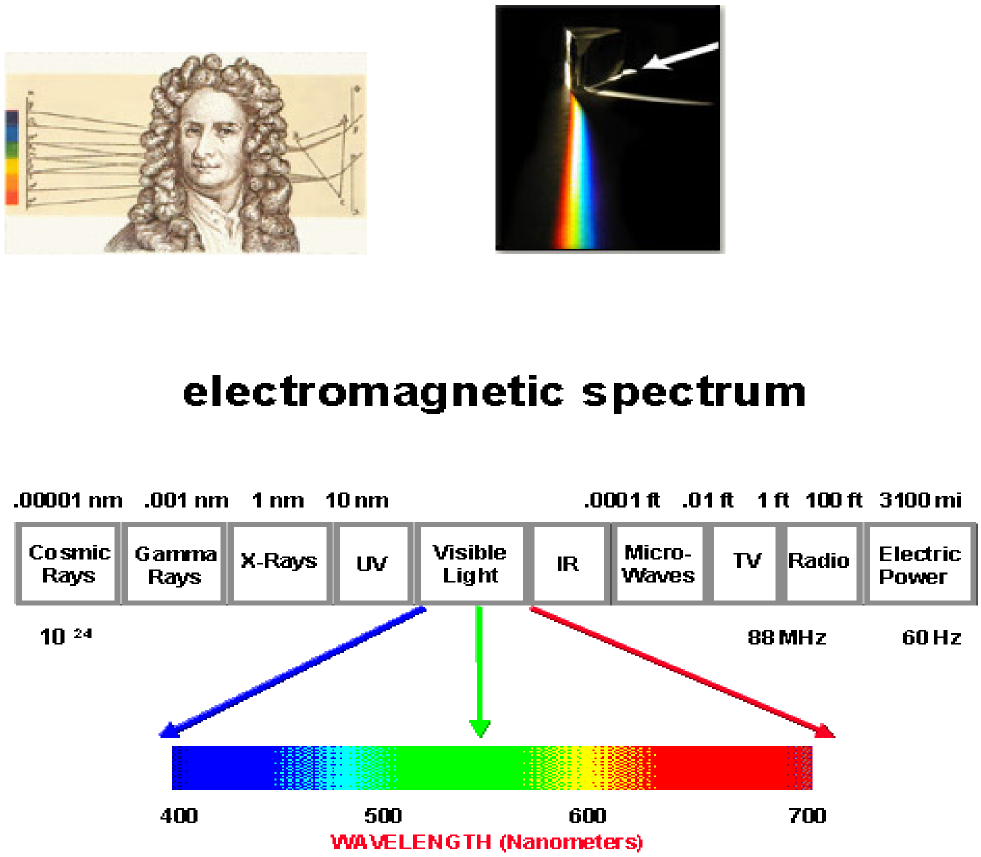

Figure 3. Electromagnetic spectrum and light decomposition discovered by Isaac Newton.

The process to obtain this decomposition is called frequency or spectral analysis.

1.2.1. Temporal Series of the Complex Stationary Values

Although the data we are going to work with in this work are real numbers, we are going to develop the theory for the complex stationary processes that result easier to treat mathematically, and then consider our data as particular cases for the complex processes.

a) Definition. The process $\{X_t\}$ of the complex values is a stationary process if its absolute value has moment of the second order and if $E(X_t)$ and $E(X_{t+h}X_t)$ are independent of t.

b) Definition. The autocovariance function $\gamma(h)$ of one stationary process of complex values $\{X_t\}$ is:

$$\gamma(h) = E(X_{t+h}\overline{X}_t) - E(X_{t+h})E(\overline{X}_t)$$

1.2.2. Spectral Density Function.

At this point we are going to introduce the concept of the spectral density function justifying its formal definition.

If $\{X_t\}$ is one stationary process with zero mean and autocovariance function $\gamma(h)$, accomplishing $\sum_{h=-\infty}^{\infty}|\gamma(h)| < \infty$. The spectral density function of the process $\{X_t\}$ can be defined as

$$f(\lambda) = \sum_{h=-\infty}^{\infty} e^{-ih\lambda}\gamma(h) \qquad \lambda \in R$$

This expression is defined under the condition that the sum of $|\gamma(h)|$ exists and $\left|e^{-ih\lambda}\right| = 1$.

Since sinus and cosines functions are periodical with the 2π period, it is enough to define it in the interval $(-\pi,\pi]$.

From another hand we have

$$\int_{-\pi}^{\pi} e^{ih\lambda} f(\lambda)d\lambda = \int_{-\pi}^{\pi}\sum_{h=-\infty}^{\infty} e^{i(k-h)\lambda}\gamma(h)d\lambda = \sum_{k=-\infty}^{\infty}\gamma(h)\int_{-\pi}^{\pi} e^{i(k-h)}d\lambda = 2\pi\gamma(h).$$

Supposing, as a general case, that the sum of $|\gamma(h)|$ doesn't exist, we could define the spectral density function as:

a) Definition. One function f is a spectral density function of one stationary process $\{X_t\}$ with autocorrelation function $\gamma(h)$ if:

$$\text{i)} \qquad f(\lambda) \geq 0 \quad \forall \lambda \in (0, \pi]$$

$$\text{ii)} \qquad \gamma(h) = \int_{-\pi}^{\pi} e^{ih\lambda} f(\lambda) d\lambda \quad \forall h \in Z$$

Using the Fourier theory it is possible to demonstrate that if $\int_{-\pi}^{\pi} e^{ih\lambda} f(\lambda) d\lambda = \int_{-\pi}^{\pi} e^{ih\lambda} g(\lambda) \quad \forall h \in Z$, then f and g are equals. Consequentially the spectral density function is unique.

b) Suggestion. If $\{X_t\} \sim WN(0, \sigma^2)$ then its spectral density function is:

$$f(\lambda) = \frac{\sigma^2}{2\pi}, \quad -\pi \leq \lambda \leq \pi$$

- It is easy to check it if we consider that.
- This process is called as a white noise since all frequencies influence equally in the variance.

1.2.3. The Periodogram.

If {Xt} is one stationary process with the autocorrelation function γ(h), spectral density function f(λ) and is a realization of this process the periodogram In(λ) is defined from the observations and will play the same role above 2πf(λ) as the sample autocorrelation function γ*(h) above γ(h).

Let us consider the vector of the complex numbers as

$$\vec{x} = (x_1, x_2, ..., x_n)' \in C^n .$$

Let $\omega_k = 2\pi k / n$, where k is an integer number between *–(n-1)/2* and *n/2* (both included), or

$$\omega_k = 2k\pi / n, \quad k = -[(n-1)/2], ..., [n/2].$$

Where $[y]$denote the major integer less o equal to *y.* These values are called Fourier frequencies associated to the sample of the size *n,* and as we can check, they are values belong to the interval of *(-π,π]*.

Let us consider now that the column of vectors *n*

$$\vec{e}_k = \frac{1}{\sqrt{n}} (e^{i\omega_k}, e^{2i\omega_k}, ..., e^{ni\omega_k})', \quad k = -[(n-1)/2], ..., [n/2].$$

It is easy to check that $\vec{e}_j^* \vec{e}_k = \delta_{ij}$ where $\vec{e}_j^*$ denotes the row vector which components are the values conjugated from the components of the vector $\vec{e}_j$ and where δ_{ij} is the delta of Kronecker, $\delta_{jj} = 1, \quad \delta_{kj} = 0 \quad k \neq j$.

Thus the vectors $\{\vec{e}_k\}$ are orthogonal and constitute one base of C^n. That means that any $\vec{x} \in C^n$ is possible to express as a lineal combination of $\{\vec{e}_1, ..., \vec{e}_n\}$

$$\vec{x} = \sum_{k=-[(n-1)/2]}^{[n/2]} a_k \vec{e}_k$$

Each component x_t of the vector $\vec{x}$ is given by

$$x_t = \sum_{k=-[(n-1)/2]}^{[n/2]} a_k e^{it\omega_k} = \sum_{k=-[(n-1)/2]}^{[n/2]} a_k (\cos(\omega_k t) + isen(\omega_k t)) \quad t = 1,...,n$$

The last expression demonstrates that each observation x_t can be expressed as a lineal combination of sinus y cosines with frequencies

$$\omega_k = 2k\pi / n, \quad k = -[(n-1)/2],...,[n/2]$$

a) Proposition. If

$$\omega_k = 2k\pi / n, \quad \text{and} \quad \vec{e}_k = \frac{1}{\sqrt{n}}(e^{i\omega_k}, e^{2i\omega_k} ..., e^{ni\omega_k})', \quad k = -[(n-1)/2],...,[n/2]$$

then $\vec{e}_j^{\,*}\vec{e}_k = \delta_{ij}$ (Delta of Kronecker).

In reality if j=k, $\vec{e}_j^{\,*}\vec{e}_j = \frac{1}{n}(1+...+1) = 1$.

From another side, if $\omega_k \neq 0$, $\sum_{t=1}^{n} e^{-it\omega_k}$ =0. If $j \neq k$, then $\vec{e}_j^{\,*}\vec{e}_k = \sum_{t=1}^{N}$ e^(-it(ωj - ωk))

b) Proposition. The coefficients a_k of the decomposition

$$\vec{x} = \sum_{k=-[(n-1)/2]}^{[n/2]} a_k \vec{e}_k$$

are easy to obtain since

$$a_k = \vec{e} * \vec{x} = \frac{1}{\sqrt{n}} \sum_{t=1}^{n} x_t e^{-it\omega_k} \ .$$

c) Definition. The periodogram of the realization $\{x_1, x_2, ..., x_n\}$ of one stationary process *{X_t}* is the function:

$$I_n(\lambda) = \frac{1}{n}\left|\sum_{t=1}^{n} x_t e^{-it\lambda}\right|^2$$

If $\lambda = \omega_k$ then $I_n(w_k) = |a_k|^2$ and thus the absolute value of the $\vec{x}$ squared is

$$\vec{x} * \vec{x} = \sum_{t=1}^{n} |x_t|^2 = \sum_{k=-[(n-1)/2]}^{[n/2]} |a_k|^2 = \sum_{k=-[(n-1)/2]}^{[n/2]} I_n(\omega_k).$$

If the square of the absolute value of the observations vector $\vec{x}$ represents the variance of the observations, this variance is a result of the sum of the *n* values of the periodogram at the frequencies of ω_k, and the value of the periodigram at the frequency ω_k represents the contribution of the frequency ω_k in the variability of the observations.

d) Proposition. If $\{x_1, x_2, ..., x_n\}$ is the realization of the one real process y, ω_k is a Fourier frequency $2k\pi/n$, in the $(-\pi, \pi]$ then

$$I_n(\omega_k) = \sum_{|h|<n} e^{-ih\lambda} \gamma^*(h)$$

where $\gamma^*(h)$ is the autocovariance simple function of $\{x_1, x_2, ..., x_n\}$.

Since the values of x_k are real ones,

$$I_n(\omega_k) = \frac{1}{n}\left(\sum_{s=1}^{n} x_s e^{-is\omega_k}\right)\left(\sum_{t=1}^{n} x_t e^{it\omega_k}\right)$$

Given that $\sum_{i=1}^{n} e^{-it\omega_k} = 0$ if $\omega_k \neq 0$ we can rest the simple mean $\bar{x}$ a x_k and have:

$$I_n(\omega_k) = \frac{1}{n}(\sum_{s=1}^{n}(x_s - \bar{x})e^{-is\omega_k})(\sum_{t=1}^{n}(x_t - \bar{x})e^{it\omega_k}) = \frac{1}{n}\sum_{s=1}^{n}\sum_{t=1}^{n}(x_s - \bar{x})(x_t - \bar{x})e^{-i(s-t)\omega_k} =$$

$$= \sum_{|h|<n}\gamma^*(h)e^{-ik\omega_k}$$

From another hand:

$$f(\lambda) = \sum_{h=-\infty}^{\infty}\gamma(h)e^{-ih\lambda}$$

Due to the similarity between both expressions we can consider $I_n\ (\lambda)$ as a natural estimator of the $f(\lambda)$.

e) Theorem. If $\{X_t\}$ is a stationary process with a spectral density positive function and $\lambda_1,\ldots,\lambda_m$ are frequencies that $0<\lambda_1<\ldots<\lambda_m<\pi$, the distribution join function $F_n(x_1,\ldots,x_m)$ of the periodigram values $(I_n(\lambda_1),\ldots,I_n(\lambda_m))$ converges in $F(x_1,\ldots,x_m)$ when $n\rightarrow\infty$ where

$$F(x_1,\ldots,\mathrm{x_m}) = \begin{cases} \prod_{i=1}^{m}(1-\exp(\frac{-x_i}{2\pi f(\lambda_i)})), & \textit{if}\ \ x_1,\ldots,x_m > 0 \\ 0 \quad \textit{otherwise} \end{cases}$$

Thus for the values of n sufficiently big the ordinates' values of the periodigram $I_n(\lambda_1),\ldots,I_n(\lambda_m)$ are approximately distributed as freedom exponentially independent values with means $f(\lambda_1),\ldots,f(\lambda_m)$ respectively.

For each value $\lambda\in(0,\pi)$ and $\varepsilon>0$,

$$p\left(\left|I_n(\lambda) - f(\lambda)\right| > \varepsilon\right) \rightarrow k, \quad n\rightarrow\infty$$

This means that the difference between the estimator I_n and the density function f it is impossible to reduce as we would like to even increasing simple size, that means that it is not consistent estimator. To see a simple and practical demonstration of the properties of the estimators, please, refer to Cuadras (2000).

1.2.4. White Noise

It is utile to explore the special case of the white noise, i.e. a process $\{Z_t\}$ where the variables Z_t are mutually independent with a common mean 0 and variance σ^2 and let denote as $WN(0, \sigma^2)$. This process is called as a white noise since each frequency is constant and thus contributes by equal manner in the process variability.

a) Densidad espectral. If $\{Z_t\} \sim WN(0, \sigma^2)$ then $\gamma(0)= \sigma^2$ y $\gamma(h)= 0$ for all $|h|>0$. Thus the spectral density function is

$$f(\lambda) = \sigma^2 \text{ , } -\pi \leq \lambda \leq \pi.$$

b) Periodogram of the white noise. If $\{z_1, z_2, ..., z_n\}$ is a realization of the process $\{Z_t\} \rightarrow WN(0, \sigma^2)$. taking into account

$$I_n(\lambda) = \frac{1}{n}\left|\sum_{t=1}^{n} z_t e^{-it\lambda}\right|^2$$

it can be expressed as

$$I_n(\lambda) = \frac{1}{n}\left|\sum_{t=1}^{n} z_t e^{-it\lambda}\right|^2 = \frac{1}{n}[(A(\lambda))^2 + (B(\lambda))^2]$$

Where $A(\lambda) = \sum_{t=1}^{n} z_t \cos(\lambda t)$ and $B(\lambda) = \sum_{t=1}^{n} z_t sen(\lambda t)$.

Thus $E(A(\lambda)) = E(B(\lambda)) = 0$ and

$$\text{var}(A(\lambda)) = \sigma^2 \sum_{t=1}^{n} \cos^2(\lambda t) = \frac{\sigma^2}{2}\sum_{t=1}^{n}(1+\cos(2\lambda t)) = \frac{\sigma^2}{2}[n + \sum_{t=1}^{n}\cos(2\lambda t)]$$

$$\text{var}(B(\lambda)) = \sigma^2 \sum_{t=1}^{n} sen^2(\lambda t) = \frac{\sigma^2}{2}\sum_{t=1}^{n}(1-\cos(2\lambda t)) = \frac{\sigma^2}{2}[n - \sum_{t=1}^{n}\cos(2\lambda t)]$$

But

$$\sum_{t=1}^{n} e^{i\lambda t} = \frac{e^{i\lambda}(1-e^{i\lambda n})}{1-e^{i\lambda}}$$

Since

$\lambda = k\frac{2\pi}{n},\ k \in Z$; $1-e^{i\lambda n} = 1-(\cos(2k\pi)+isen(2k\pi)) = 0$ and that is why

$$\sum_{t=1}^{n} e^{ik\frac{2\pi}{n}t} = \sum_{t=1}^{n}\cos(k\frac{2\pi}{n}t) + i\sum_{t=1}^{n} sen(k\frac{2\pi}{n}t) = 0+0i\,.$$

Since the parts real and imaginary must be equals:

$$\sum_{t=1}^{n}\cos(k\frac{2\pi}{n}t) = \sum_{t=1}^{n} sen(k\frac{2\pi}{n}t) = 0 \quad k \in Z$$

In particular

$$\sum_{t=1}^{n}\cos(2k\frac{2\pi}{n}t) = \sum_{t=1}^{n} sen(2k\frac{2\pi}{n}t) = 0$$

since $2k \in Z$.

Finally if $\lambda = k\frac{2\pi}{n},\ k \in Z$

$$\mathrm{var}(A(\lambda)) = \mathrm{var}(B(\lambda)) = \frac{n\sigma^2}{2}$$

As well

$$\mathrm{cov}[A(\lambda), B(\lambda)] = E[\sum_{t=1}^{n}\ \sum_{s=1}^{n} z_t z_s \cos(\lambda t) sen(\lambda s)] = \sigma^2 \sum_{t=1}^{n}\cos(\lambda t) sen(\lambda t) = 0$$

since

$$\sum_{t=1}^{n}\cos(\lambda t) sen(\lambda t) = \frac{1}{2}\sum_{t=1}^{n} sen(2\lambda t) = 0; \quad \lambda = k\frac{2\pi}{n},\ k \in Z\,.$$

From all the above we deduct that

$$U(\lambda) = A(\lambda)\sqrt{\frac{2}{n\sigma^2}} \to N(0,1) \qquad V(\lambda) = B(\lambda)\sqrt{\frac{2}{n\sigma^2}} \to N(0,1)$$

Since $U(\lambda)$ y $V(\lambda)$ are independents then

$$\frac{2}{n\sigma^2}[A^2(\lambda) + B^2(\lambda)] = \frac{2}{\sigma^2} I_n(\lambda) \to \chi_2^2 \quad \textit{(chi-square with 2 degrees of freedom.)}$$

2. DATA

The data of this research are obtained from the blood samples taken from one healthy woman without any treatment. The samples were taken each 10 minutes within three periods of 8 hours. One period was correspondent to the late follicular phase, another to the early one of the following menstrual cycle, and the third period of 8 hours corresponded to the late phase of the second cycle. The data originate from Murdoch et al (1985) and given in the Table 1.

Table.1. Levels of the luteinizing hormone in the blood samples (Murdoch et al.)

Time*10 min.	Late phase 1	Early phase 2	Late phase 2	Time*10 min.	Late phase 1	Early phase 2	Late phase 2
1	5,5	2,4	4,3	25	4,8	2,3	4,5
2	4,5	2,4	4,6	26	5,5	2	4,6
3	5,1	2,4	4,7	27	5,1	2	5,8
4	5,5	2,2	4,1	28	5,2	2,9	5
5	5,7	2,1	4,1	29	5	2,9	5,1
6	5,1	1,5	5,2	30	4	2,7	4,5
7	4,3	2,3	5	31	3,7	2,7	4,2
8	4,8	2,3	4,4	32	4,8	2,3	6
9	5,6	2,5	4,2	33	5,9	2,6	5,6
10	5,9	2	5,1	34	5,5	2,4	5,4
11	6	1,9	5,1	35	4,9	1,8	5
12	5,1	1,7	4,7	36	4,4	1,7	4,4
13	5,2	2,2	4,4	37	4,7	1,5	4,6
14	4,4	1,8	3,9	38	4,2	1,4	5,7
15	5,5	3,2	5,4	39	5,5	2,1	5,2
16	5,4	3,2	5,9	40	4,9	3,3	5
17	4,1	2,7	4,2	41	4,8	3,5	4,4
18	4,4	2,2	4,1	42	4,5	3,5	5,7
19	4,7	2,2	4,1	43	4,9	3,1	5,7
20	4,6	1,9	3,6	44	4,9	2,6	4,8
21	6	1,9	3,1	45	4,5	2,1	3,4
22	5,6	1,8	4,8	46	4,2	3,4	5,5
23	5,1	2,7	5,1	47	4,9	3	5,5
24	4,7	3	5,1	48	5,9	2,9	5,6

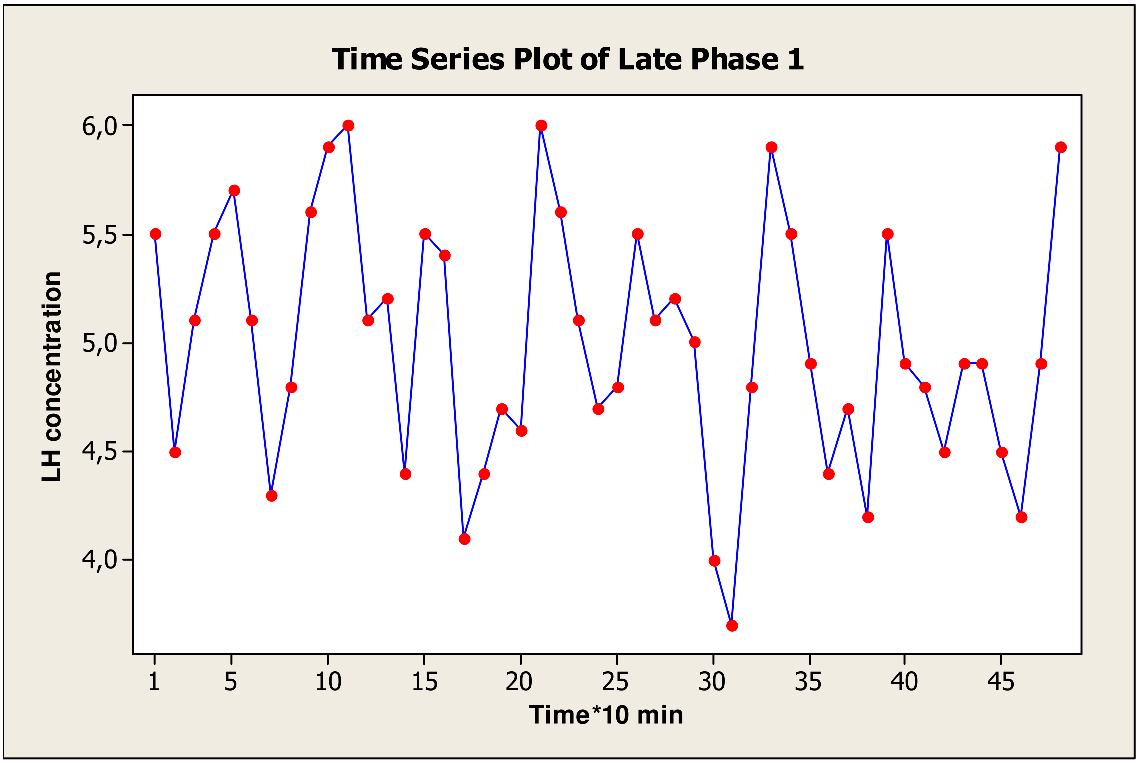

Figure 4. Time Series of Late Follicular Phase. 1st Cycle.

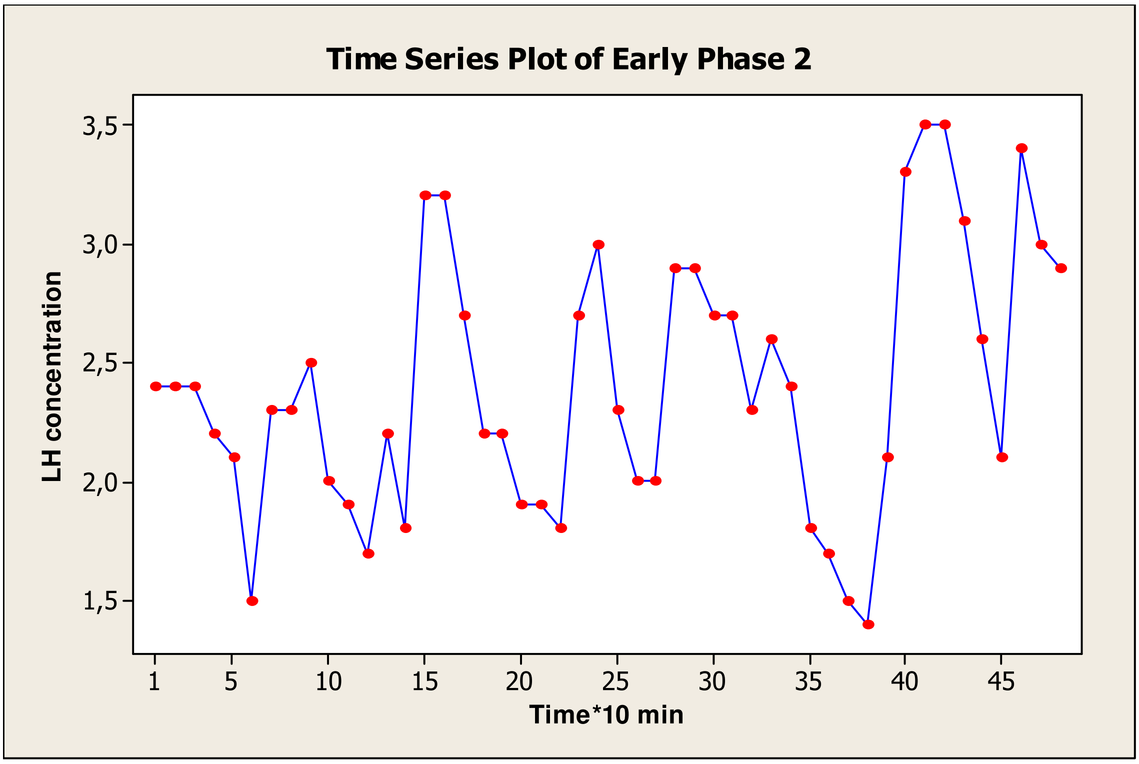

Figure 5. Time Series of Early Follicular Phase. 2^{nd} Cycle.

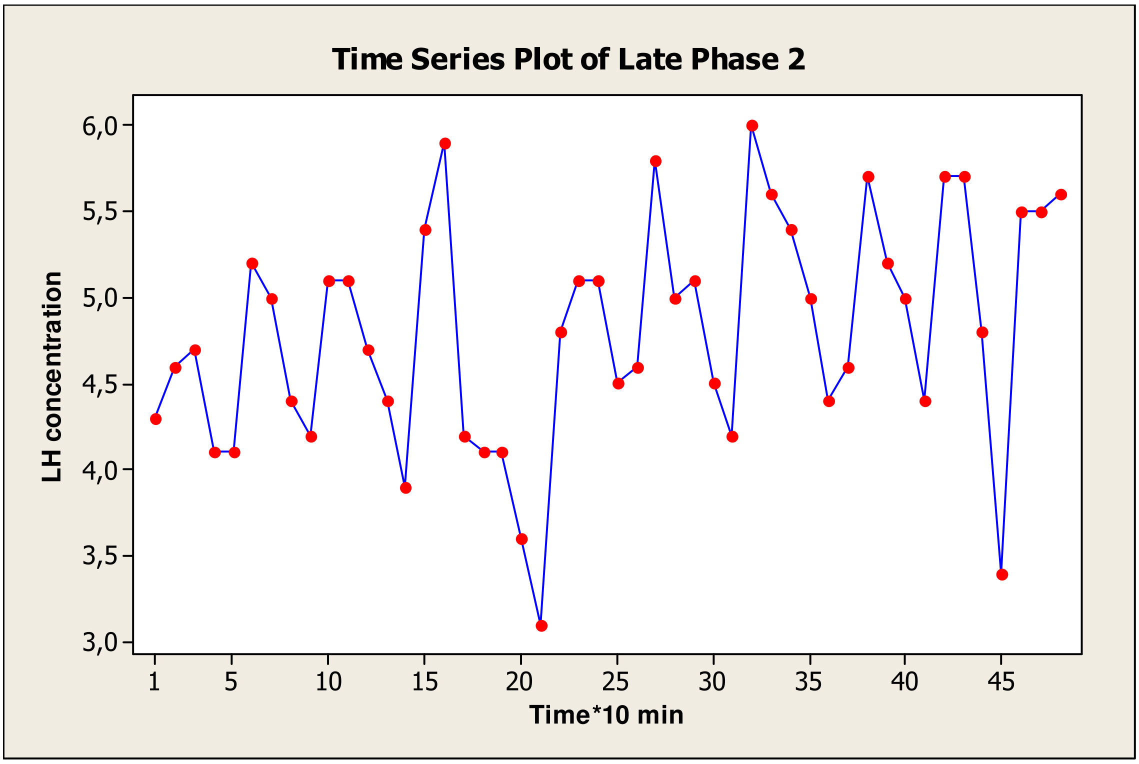

Figure 6. Time Series of Late Follicular Phase. 2nd Cycle.

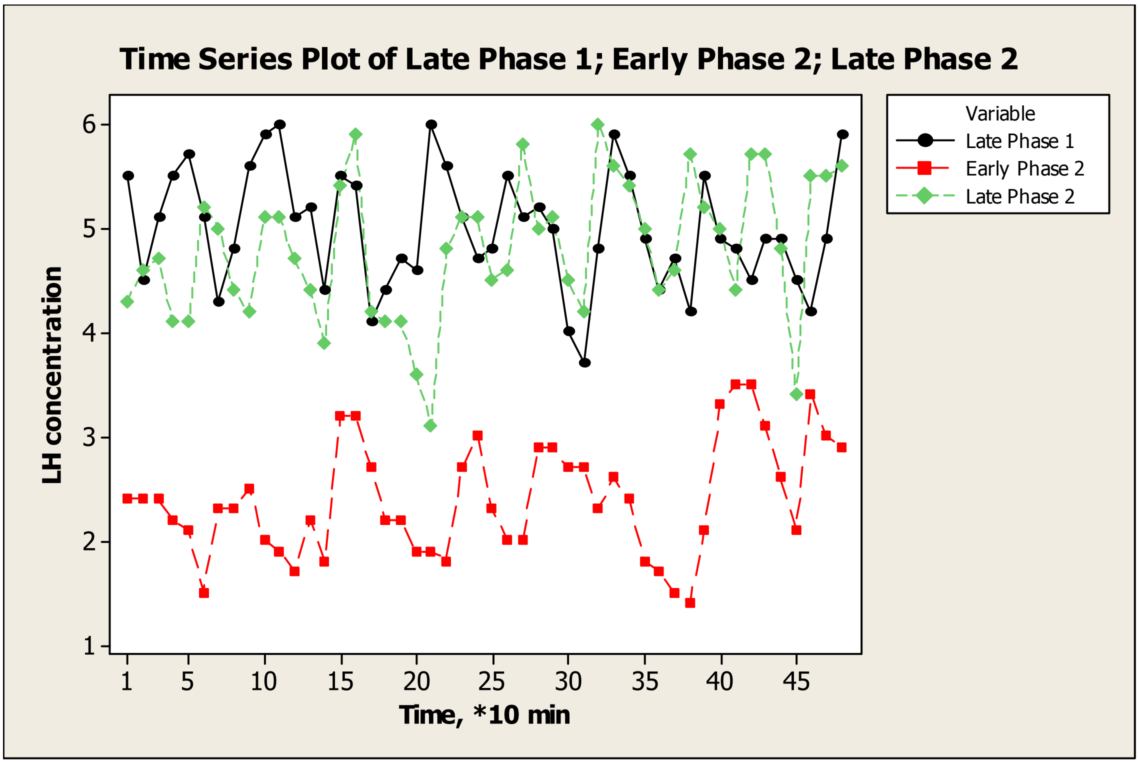

Figure 7. LH concentrations during all three observed phases.

3. RESULTS

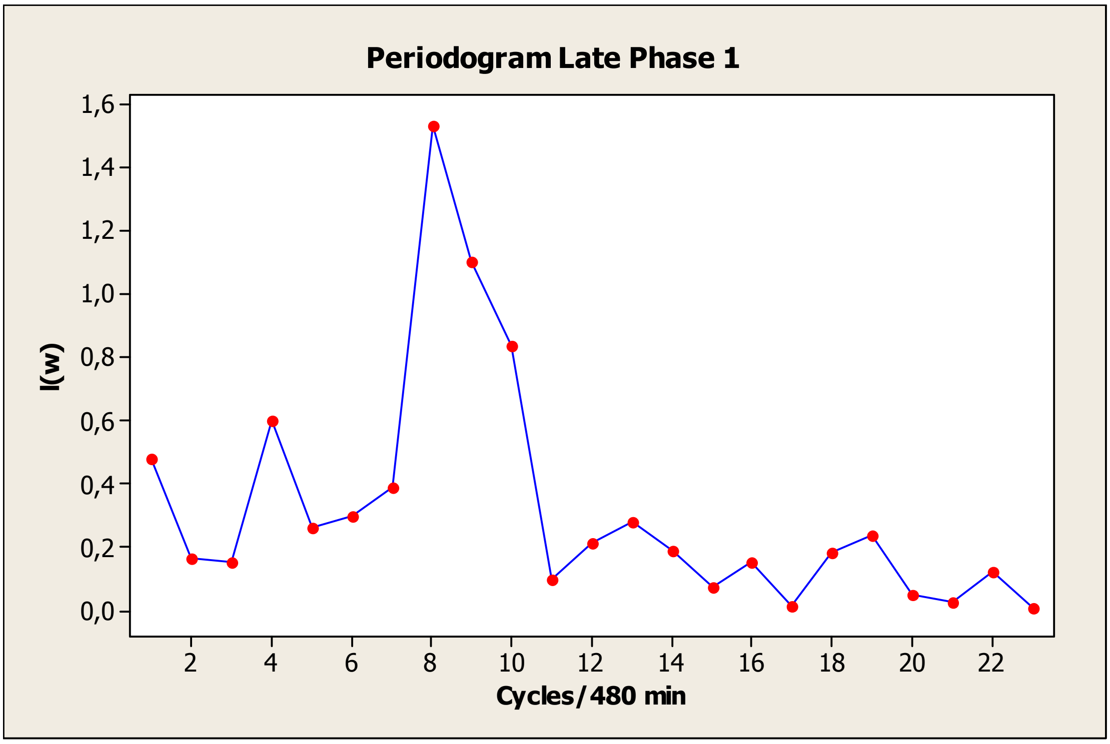

Figure 8. Periodogram of Late Follicular Phase. 1st Cycle.

Figure 8 shows the periodogram of the first of the three LH series corresponding to the late follicular phase of the cycle 1. The dominant peak indicates a cyclic component to the time variation in the data, the location of the peak suggesting a frequency of about 8 cycles in the 8 hours time-span of the data, i.e. one cycle per 1 hour.

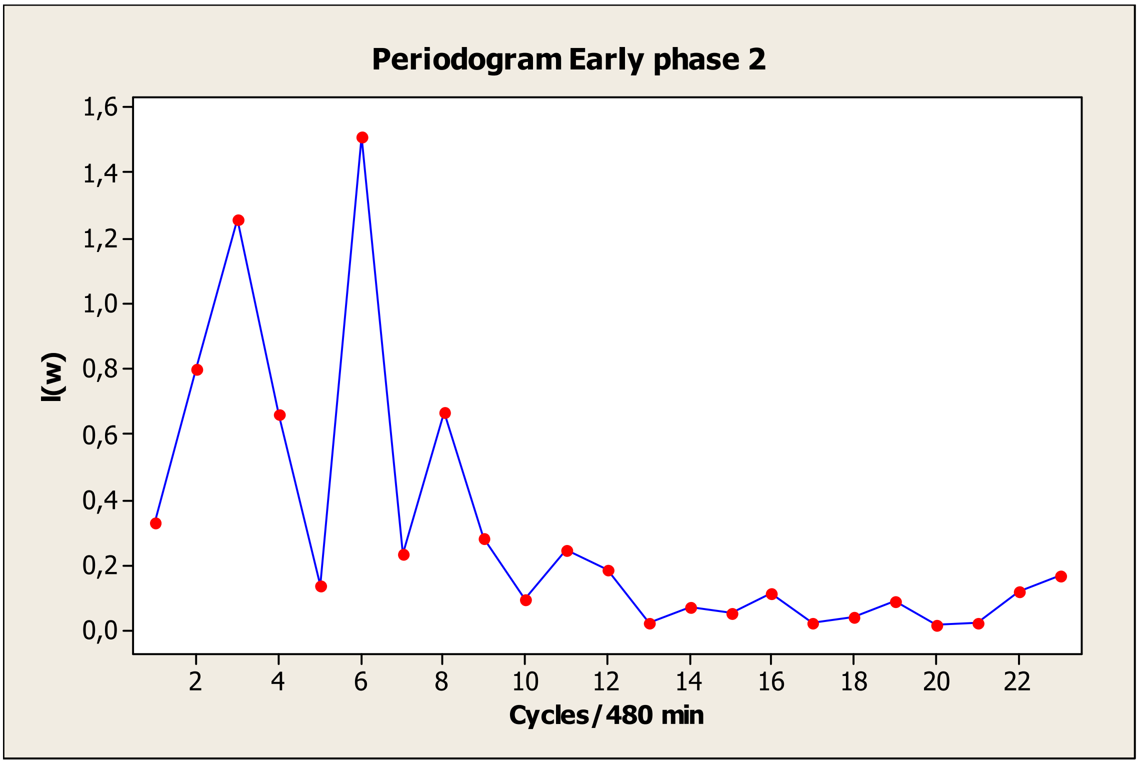

Figure 9. Periodogram of Early Follicular Phase. 2nd Cycle.

Figure 9 shows the periodogram of the second of the three LH series corresponding to the early follicular phase of the cycle 2. The dominant peak indicates a cyclic component to the time variation in the data, the location of the peak suggesting a frequency of about 6 cycles in the 8 hours time-span of the data, i.e. one cycle per one e per 1 hour and 20 minutes.

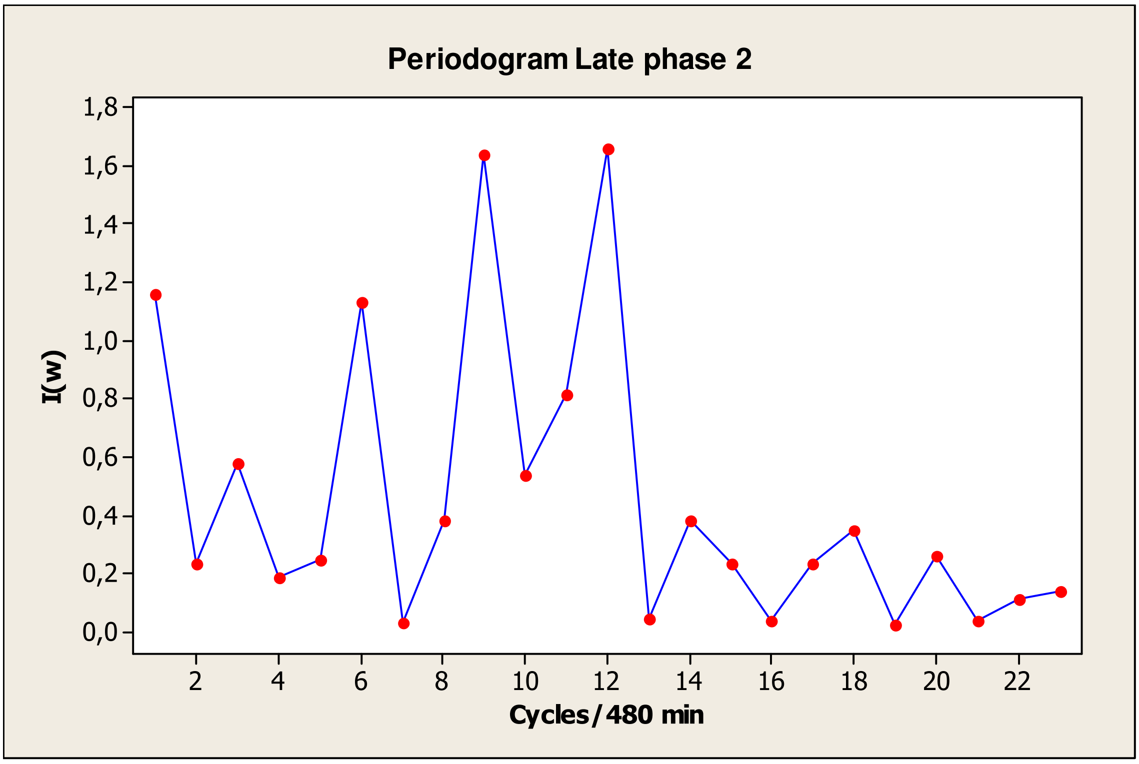

Figure 10. Periodogram of Late Follicular Phase. 2^{nd} Cycle.

Figure 10 shows the periodogram of the third of the three LH series corresponding to the late follicular phase of the cycle 2. The dominant peak indicates a cyclic component to the time variation in the data, the location of the peak suggesting a frequency of about 12 cycles in the 8 hours time-span of the data, i.e. one cycle per 40 minutes; as well there is another significant frequency of about 9 cycles that corresponds to one cycle per 53 minutes approximately.

4. CONCLUSIONS

The methods of spectral analysis and its applications are well developed and explained by different authors such as Chatfield (1980), Priestly (1981), Diggle (1990). From another hand, the spectral analysis was little appreciated by some authors such as Clifton & Steiner (1983) since they consider that it can be applied only in the series of values that follow a regular cycle, however, the spectral analysis is applied to other types of data as it is shown in this research work.

It is known that various hormones, such as the luteinizing hormone or the growth hormone, have a pulsate secretion that makes them effective. That means that its action does not depends on its absolute levels, but on the pulsate ones, as per Lincoln et al. (1985).

As per values of the luteinizing hormone concentrations in the blood samples, taken each 10 minutes, it is demonstrated in the current research that the behavior of these values is cyclic with the frequency of approximately of one cycle per hour in the first of the series that corresponds of the late stage 1 of the follicular cycle (the frequency of the cycle is each 1 hour); in the second series that corresponds to yearly follicular phase 2 (frequency of the cycle is each 1 hour and 20 minutes); and, finally, in the third series that corresponds to the follicular late phase 2 (frequency of each cycle is each 40 minutes). That is meant that the cyclical secretion of LH is more frequent in the late phase then in the early one.

If the samples were taken with more frequency, probably, there would be observed the rhythms with a shorter cycle period.

From this research we can make a conclusion that the spectral analysis is an important tool for improving of the fundaments of the secretion physiology of the luteinizing hormone. Moreover it is indicated as well what should be the frequency in the sampling in order to observe the picks of the hormone secretion.

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