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SERUM PROTEIN PROFILE IN LUTEAL PHASE OF CYCLING WOMEN

ABDUL MAJEED CHEEMA, NABILA ROOHI, ASMA ABDUL MALIK AND ABDUL QAYYUM NAYYER

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

Abstract: Serum samples of unmarried young females (N=34) of ages ranging 19-25 years, were drawn on mid luteal phase (at 7th or 8th day before the next menstruation). Prior to sampling, it was made certain that the subjects were definitely passing through the required phase. In case of irregular cycle, the sampling was made on 21st day of the menstrual cycle. Different samples were categorized into normal regular (27-29 days), short regular (24-26 days), long regular (30-31 days) and irregular cycle. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) based on the method of Laemmli (1970), was employed for studying the low molecular weight protein fractions in all cycling patterns. The gel photography, image storing and quantification of various protein fractions were carried out by Gene Genius Bioimaging Gel Documentation System that provided the data of molecular weights and percent areas covered by each of the fractions. The data was analysed statistically using Student 't' test and employed in finding the enhancement/reduction and/or appearance/disappearance of particular protein fractions for comparisons among normal, short, long and irregular cycles. Low molecular weight serum protein fractions were ranging between 26-11 kDa in all cycling patterns. Significant protein fractions of 14 and 13 kDa were found to be declined in short compared to normal regular cycle. Protein fractions of 17 , 15 and 13 kDa indicated a pronounced decline in irregular compared to short regular cycle. The fractions of 17 and 11 kDa indicated a significant elevation in long compared to short regular cycle. No significant protein was found in irregular compared to long regular cycle.

Key words: Gel electrophoresis, luteal phase, protein fractions

INTRODUCTION

Reproductive function, in women and female primates, follows a cyclic pattern between menarche and menopause that is termed as menstrual cycle (Randall *et al.*, 2000). The average age of menstrual bleeding in girls is 12 years although it starts as early as 10 or may be as late as 16. At menopause, when periods stop, the average age is 50, however, it can vary (Marshall, 2001).

The time course of gonadotropin secretion throughout the menstrual cycle is regulated by negative and positive feedback actions of estradiol on gonadotropin secretions. The secretions of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland is dependent on interplay of two major regulatory components,

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the ovary and the hypothalamus (Cone *et al.*, 2003). The length of the cycle is counted from the beginning of the menstrual period until the day before the next period commences. The average cycle lasts about 28 days but cycle can vary from 23 to 31 days (Cutler *et al.*, 1998).

Menstrual cycle is generally divided into different phases, the menstrual phase, follicular phase, ovulation phase and the luteal phase. Following ovulation, progestational or luteal phase is dominated. Normal length of the luteal phase is 10-16 days and average is 14 days. The estrogen level is elevated at mid luteal phase and decreased at the end of the menstrual cycle. The secretion of progesterone during the luteal phase is episodic and pulses are correlated with pulses of LH. LH acts as a luteotropic agent. Formation of oxytocin and vasopressin within the corpus luteum promotes luteolysis by modulating autocrine or paracrine mechanisms. Finally, LH down regulation of its own receptors may play a role in the termination of luteal phase (Marshall, 2001).

Severe reducing diets cause low levels of progesterone, slowing follicular growth, inhibiting the surge of LH and preventing ovulation (Wynn and Wynn, 1994). Exercise of sufficient rigor, particularly, when coupled by weight loss and dietary restriction is capable of producing reversible disturbances of many otherwise healthy young women (Campbell *et al.*, 2001). However, it is clear that there are many health benefits of moderate and regular exercise (Green, 1993). Menstrual cycle may be shorter regular (less than 28 days), longer regular (more than 28 days) and irregular. In physiological set up, particularly, related to reproduction, the hormonal patterns are indicative of the regulation of cycle and determine the duration of different phases. Polyacrylamide gel electrophoresis (PAGE) of serum proteins has an important role as diagnostic investigation and variations in menstrual cycle duration may be evaluated on the basis of appearance or disappearance of certain regulatory proteins in the blood. Von Wolf *et al.* (2001) analysed endometrial mRNA and protein expression of osteopentin and its receptor beta (3)-integrin throughout the menstrual cycle. Beier and Beier-Hellwig (1998) studied the proteins during menstrual cycle and identified and isolated the molecular structure of several proteins like histones, cyclophilins, transthyretin, haptoglobin and uteroglobin. Beier-Hellwig and Beier (1994) had also studied the endometrial proteins by SDS-PAGE, during luteal phase, and observed that numerous individual protein bands mainly resolved between 68 and 65 kDa. They identified several of these proteins as histones; H_2A, H_2B, H_3 and H_4. Itoh and Manaka (2001) analyzed the vaginal secretions by SDS-PAGE and reported a relatively low concentration of 67 kDa and high concentration of 56 kDa in pre and post menstrual phases. A relatively high concentration of 52 kDa protein characterized the mid cycle. By keeping in view the importance of proteins in so many physiological phenomena, it is very likely that in the present investigation, various regulatory proteins may be synchronously associated with the behaviour of the cycle.

MATERIALS AND METHODS

Serum samples of unmarried young females (N=34) age range 19-25 years were drawn at mid luteal phase (mostly on 7th and sometimes on 8th day before the onset of next menses). In case of irregular cycles the sampling was made on 21st day of menstrual cycle. Menstrual cycle was categorized into 4 types. A cycle with duration of 24-26 days was termed as short regular; of 30-31 days categorized as long regular and 27-29 days was considered as normal regular. The subject experiencing varying duration in subsequent cycles are placed in irregular category.

Polyacrylamide gels (15%) for low molecular weight protein fractions was prepared by using the method of Laemmli (1970). Protein size markers and each of the samples were loaded in separate wells and gels were electrophoresed at a current supply of 30 mA and voltage of 200 V in a cooling chamber maintained at 4°C until the tracking dye reached the lower end of the gel. Following Electrophoresing run, the gels were stained with coomassie brilliant blue up to 30 minutes and destained afterwards until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background.

Stained gels were photographed afterwards and their images were saved on a floppy disk with Gene Genius Bio-imaging Gel Documentation system. The quantification of separated protein fractions was carried out by gene tools software that provided the data of molecular weights and percent areas covered by each of the fractions.

For the assessment of the variations in the protein profiles among different individuals in normal, short, long and irregular cycle during luteal phase, values of individual protein fractions were averaged and expressed as mean±SEM. The data was analyzed statistically using Student t-test and employed in finding the enhancement/reduction and appearance/disappearance of particular protein fractions for comparisons among different individuals.

RESULTS

An overall view of protein profile resulted in the detection of seven low molecular weight protein fractions ranging between 26-11 kDa during luteal phase of all cycling patterns in the unmarried young females (Fig. 1).

Among these, protein fractions of 26 kDa and 20 kDa were found to be elevated by 16 and 17% covering the average areas of 29.88±2.17 and 8.94±3.72, respectively, in short compared to normal regular cycle. Protein fractions of 17, 15, 14, 13 and 11 kDa were found to be declined by 60, 66, 42, 82 and 46%, respectively, with average values of 1.87±0.31, 1.71±0.46, 1.89±0.40, 0.50±0.12 and 3.41±0.83%, in short compared to normal regular cycle, respectively.

In the comparison of normal and long regular cycle, protein fractions of 26, 17 and 11 kDa did not exhibit significant variations. Protein fractions of 20, 15 and 14 were, however, declined by 17, 25 and 14% with average areas of 7.63±0.44, 3.76±1.03 and

2.79±0.32%, respectively, in long compared to normal regular cycle.

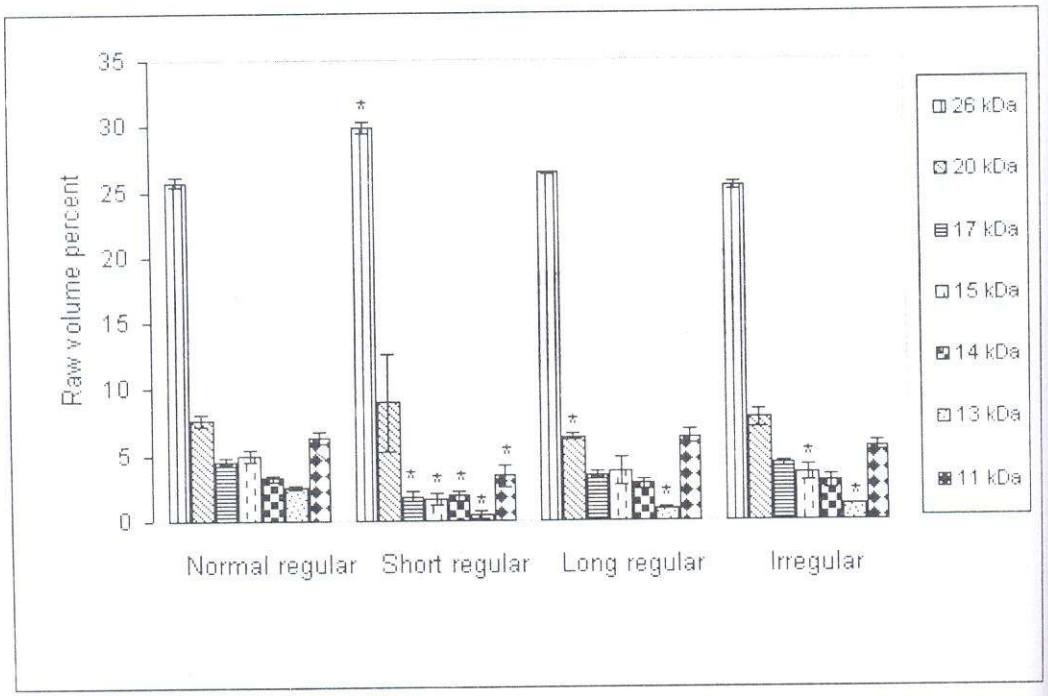

Fig. 1. Average raw volumes percent exhibited by various protein fractions in different subjects during luteal phase in normal, short, long and irregular cycles.
Values are mean±SEM * Significance at P<0.05, as compared to normal regular

Among the comparison of normal and irregular cycle, protein fractions of 17, 15, 14 and 13 kDa were declined by 23, 26, 63 and 14% with average areas of 4.33±0.24, 3.60±0.62, 297±0.54 and 19.00±0.20% in irregular compared to normal regular cycle. Protein fractions of 20, 17 and 11 kDa were almost in the same range in both cycling patterns.

In the comparison of short and long regular cycle, protein fraction of 20 kDa, exhibited a significant decline of 29%, whereas, 17, 15, 14, 13 and 11 kDa fractions were significantly elevated by 88, 119, 47, 84 and 87%, respectively, in short compared to long regular cycle.

Among the comparisons of short and irregular menstrual cycle, protein fractions of 26 and 20 kDa indicated a decline of 16 and 15% in irregular compared to short regular cycle. Other protein fractions of 17, 15, 14, 13 and 11 kDa were found to be elevated by 131, 110, 57, 133 and 63, respectively, in irregular compared to short regular cycle.

Among the comparison of long regular and irregular cycle the protein fractions of 20 and 17 kDa were elevated, respectively, by 22 and 23%, whereas, 13 kDa protein

fraction was declined by 22%, in irregular compared to long regular cycle. The rest of the protein fractions did not vary considerably, in this group comparison.

DISCUSSION

The term menstrual cycle technically refers to a series of changes that occurs in sexually mature non-pregnant females (Labb *et al.*, 2000). Complex series of hormonal interactions between thyroid, adrenal, pituitary gland, hypothalamus and ovary control menstruation (Hundscheid *et al.*, 2000). Therefore, changes in the amount or timing of hormone released by the thyroid, pituitary gland or hypothalamus may cause menstrual irregularities (Weiss, 2001). Membrane cofactor protein is found throughout the menstrual cycle and plays a role in normal reproductive function. Many insulin like growth factors binding proteins are involved in human folliculogenesis during normal and abnormal menstrual cycle (Rabahi *et al.*, 1991).

In the regulation of menstrual cycle in terms of duration of its various phases, it is evident that directly or indirectly through hormonal mediation various regulatory proteins eventually determine the behaviour of the cycle. The regulatory proteins in serum through their varying concentrations reflect their role in the function. No doubt, such responses are the main feature to know variations in luteal phase of human females. In the present study serum samples of young unmarried females were collected at luteal phase of normal, short regular, long regular and irregular cycle. The study of the protein fractions is an approach to locate these in different cycling patterns and to understand the regulatory nature of these serum proteins in four cycling patterns. The various protein fractions observed included trypsin I (26 kDa), trypsin inhibitor (20 kDa), apolipoprotein A II (17 kDa), Lysozyme (15kDa), α-lactalbumin (14 kDa), β2-microglobulin (11kDa) and an unknown fraction (13 kDa). Trypsin I (26 kDa) varied non significantly in short, long and irregular subjects compared to normal regular cycle. Trypsin inhibitor was found to be declined in long compared to normal regular cycle. It strongly indicates that this fraction's appearance in the circulation is associated with the normal behaviour of the cycle as its decrease changes cycle's duration (Bouckart *et al.*, 1986). Apolipoprotein A-II (17 kDa) was found to be declined by 66% in short compared to normal regular cycle. The same fraction was enhanced by 88% in long and 131% in irregular compared to short regular cycle. Solerte *et al.* (1996) have reported that apolipoprotein A-II remain unchanged throughout the menstrual cycle. Hence, variations in its appearance, in the present investigation, may be related to irregularity. There is variation in the level of lysozyme and albumin during the luteal phase of menstrual cycle (Schumancher *et al.*, 1977). Alpha lactalbumin (14 kDa) and many other proteins showed no significant differences in luteal phase of cycling women (Vizoro *et al.*, 1989) as, in the present study, the decrease in alpha lactalbumin in short compared to normal regular cycle suggests that decrease in duration of cycle length may be linked with the reduction of alpha lactalbumin. Lysozyme (15 kDa) was significantly enhanced by 110% in irregular compared to short regular cycle. Highly significantly expressed fraction of 13 kDa was declined by 63% in irregular

compared to normal regular cycle, whereas, an average elevation of 133% was observed in irregular subjects compared to short regular cycle indicating the role of the fraction in varying cycle duration. Beta-2 microglobulin was declined by 46% in short compared to normal regular cycle while this fraction was elevated in long compared to short regular cycle. On the other hand, Paaby *et al.* (1980) demonstrated that beta-2 microglobulin was appeared significantly during the luteal phase but not during the following phase in the serum.

Conclusively, analysis of the results of present study, analysing the pattern of serum protein fractions, indicated that some fractions alterations in luteal phase are strong indicators of their role in alteration of cycle and phase duration. The results of the present study are in agreement and also in disagreement with other reports. However, the differences, in the pattern of present study may be related to genetic and environmental differences of the populations.

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