ORIGINAL ARTICLE

Interrelationship between surging reproductive hormones and blood viscosity indices in apparently healthy females

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Abstract

Background: In active female reproductive life, a cascade of physiological phenomena occurs during each menstrual cycle day. *Aim and Objectives*: The study was designed to determine the relationship between surging reproductive hormones and blood viscosity indices. *Material and Methods*: One hundred apparently healthy females between the ages of 18-25 years, with regular cycle length of 28 days were recruited for the study. Reproductive hormones [Luteinizing Hormone (LH) and progesterone], blood viscosity indices [Relative Plasma Viscosity (RPV), fibrinogen and hematocrit] and other blood related parameters were analyzed during days 12, 13, 14 and 21 of menstrual cycle. *Results*: Progesterone secretion reached nadir and zenith on days 12 and 21 respectively while peak LH level occurred on day 12. On days 12 and 21, RPV correlated with progesterone negatively while on days 12 and 13, fibrinogen was inversely related to progesterone. Hematocrit correlated positively with progesterone throughout the menstrual cycle days. Although fibrinogen and mean corpuscular volume were unchanged throughout the menstrual cycle days, RPV was high on day 13. Significant decreases in red and white blood cell counts and hematocrit were observed on day 21. *Conclusion*: The findings of the study indicated that blood viscosity indices were not influenced by surges in progesterone and luteinizing hormone.

Keywords: Relative Plasma Viscosity, Reproductive Hormones, Blood Viscosity Indices, Menstrual Cycle Days, Blood Cells

Introduction

The menstrual cycle consists of a series of cyclic events that occur in the ovary and other reproductive organs in physiologically normal and non-pregnant women [1]. For instance, during follicular phase, under the influence of follicle stimulating hormone, dominant follicle in the ovary increases in size and matures with attendant increase in estradiol secretion. The rising estradiol replenishes the endometrium that was sloughed at the end of the preceding cycle. Surging level of Luteinizing Hormone (LH) towards ovulation converts the mature ovarian follicle into progesterone- secreting

cells with attendant rupturing of dominant follicle and release of secondary oocyte into the fallopian tube where fertilization and final oocyte maturation may occur [2]. During luteal phase, corpus luteum secretes large amount of progesterone in order to prepare the uterus for implantation. The cycle comes to an end once steroidogenic capacity of corpus luteum declines sequel to failure of fertilization to occur.

Besides reproductive structures, changes occur extra-gonadally during menstrual cycle. For example, increase in leucocytes has been reported during menstrual phase by Agoreyo and Asowata [3]. Calcium, a mineral which plays major role in bone formation and signal transduction was reported by Dullo *et al.* [4] to peak during follicular phase. Higher magnesium level was also reported during menstruation by Dullo *et al.* [4]. In rats, catalase level was low during proestrus and estrus with the zenith and nadir levels at metestrus and diestrus respectively [5]. Moreover, lower hemoglobin concentration has been observed in the follicular phase relative to the luteal phase of menstrual cycle [6], low hematocrit was documented by Lugos *et al.* [7] during menstruation compared to pre-menstruation and post-menstruation phases.

Estradiol is known to act on the liver and induces synthesis of proteins including fibrinogen [8]. Hence peak increase in estrogen level should lead to zenith fluctuation in blood viscosity. A study by Larsson *et al.* [9] indicated a reduction in blood viscosity at the beginning of menstruation with a peak level occurring only at day 7. A study by Agoreyo and Okorie [10] showed that relative blood viscosity was high during premenstrual phase when compared to menstrual phase. However, none of these studies conducted hormonal profile thus making it difficult to determine when the hormones peaked and the related rheological consequences. The aim of the study was to determine the relationship between surging reproductive hormones and blood viscosity indices.

Material and Methods

Subjects

One hundred apparently healthy females who satisfied inclusion criteria were randomly selected from the population of female students of University of Benin, Nigeria. Before the commencement of the experiment, three simultaneous cycles were studied for each of the subjects to ascertain the menstrual cycle regularity. Subjects who were with irregular menstrual cycles were excluded.

Written consent was obtained from each subject and a well-structured questionnaire was administered to exclude subjects who were below 18 and above 25 years of age, having medical history of musculoskeletal, respiratory, cardiovascular, kidney, hepatic and metabolic diseases or anatomical deformities. Subjects with history of smoking, alcoholism and caffeine, and any form of medications including contraceptives were also excluded. Only those female students who had regular menstrual cycles and were between 18 and 25 years of age were selected. The study conformed to the provisions of the declaration of Helsinki 1995 (as reviewed in Tokyo in 2004) and lasted for 4 months. Physical and medical examinations were also done and those that were not medically fit were disqualified. Specifically those whose systolic and diastolic blood pressures were not within the ranges of 90-120mmHg and 60-80mmHg respectively were excluded. Those whose heart rates and respiratory rates did not fall within the range of 60-100 beats per minutes and 12-20 cycles/per minutes respectively were excluded.

Samples collection

Venous blood was obtained from each subject between 8 am and 11 am on days 12, 13, 14 and 21 of their menstrual cycles. The blood samples were put in anti-coagulant bottles for various analyses.

Hormonal assay

Blood samples were collected into lithium heparin anticoagulant bottles and mixed thoroughly. Tubes were placed properly in the centrifuge and allowed to spin for 15 minutes at 3500 rpm. Clear plasma was collected into clean tubes by suction using the Pasteur pipette. Each sample was tested for concentrations of LH and progesterone using Enzyme Linked Immune Sorbent Assay Technique (ELISA), as indicated in the manufacturer's instruction. The ELISA kits used for the study were manufactured by Calbiotech Incorporation, United States.

Blood cell counts and mean corpuscular volume

As far as blood cell counts and mean corpuscular volume were concerned, blood from each subject was collected into Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tubes. Blood cell count and mean corpuscular volume were done using Diatron Automated A38-1 Abacus Hematology Analyzer.

Fibrinogen concentration

This was measured using gravimetric assay method as described by Ajayi *et al.* [11]. Blood was collected into clean tubes containing sodium citrate anticoagulant and centrifuged for about 15 minutes at 3500 rpm. One milliliter (ml) of clear plasma was obtained and introduced into test tubes containing 1ml of pre-warmed calcium chloride and placed in a water bath for 30 minutes. The clot formed was collected and compressed to express fluids and non-clotted proteins, washed in distilled water, dried and weighed with an accurate microbalance.

Relative plasma viscosity (RPV)

The modified needle and syringe method of Reid and Ugwu, as described by Ajayi *et al.* [11] was used. Plasma viscosity was measured using 1.0ml graduated syringe to which a vertical needle was fitted. The composite syringe with its plunger and needle was held vertically in a retort stand. The plasma to be tested was drawn up carefully, excluding air bubbles into the vertical syringe until the end of the plunger passed the 1.0 ml mark. The plunger was completely withdrawn and immediately the lower meniscus of the plasma fell to the 1.0 ml mark, a stopwatch was started. The time required by 1.0 ml of plasma to flow down the syringe was noted. The plasma viscosity was expressed as RPV which is the ratio of the flow-time for 1.0 ml of plasma to the flow-time of 1.0 ml of distilled water at the same temperature.

Statistical analysis

GraphPad Prism (version 8.0.1) was used to analyze the data generated. Results are presented as Mean ± SEM. Bar charts were plotted to show the results. Analysis of Variance (ANOVA) and *post hoc* test (Bonferroni) were used to compare the results in different groups. Correlation analysis was conducted using Pearson. Value of p less than 0.05 ($p<0.05$) was taken to be statistically significant.

Results

Reproductive hormones

Figure 1 shows significant reduction ($p <0.05$) in the serum LH concentration on days 13, 14 and 21 of ovarian cycle when compared with day 12. Figure 2 shows that the serum progesterone concentration was significantly higher ($p <0.05$) on day 21 of ovarian cycle when compared with days 12 and 13.

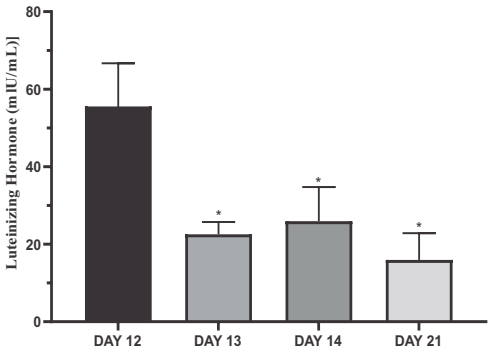

Figure 1: Serum luteinizing hormone concentrations during day 12, 13, 14 and 21 of the menstrual cycle

**Significant difference (p<0.05)*

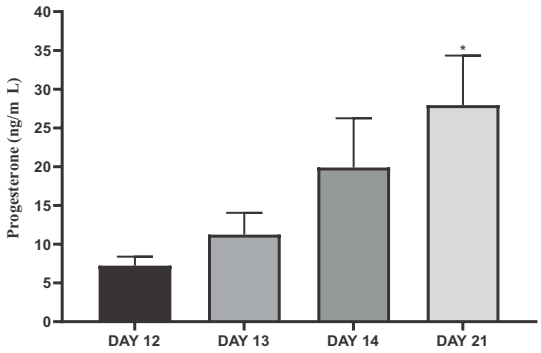

Figure 2: Serum progesterone concentrations during day 12, 13, 14 and 21 of the menstrual cycle

**Significant difference (p<0.05)*

Correlation between reproductive hormones and blood viscosity indices

Progesterone positively correlated with hematocrit and negatively correlated with RPV and fibrinogen on day 12 respectively (Table 1). On day 13, progesterone positively correlated with hematocrit and negatively with fibrinogen. On day 14, LH was inversely correlated with RPV. Progesterone was positively correlated with fibrinogen and hematocrit. On day 21, progesterone was positively correlated with hematocrit and negatively correlated to RPV.

Blood cells counts and mean corpuscular volume

Figure 3 shows significant reduction in White Blood Cell (WBC) count on days 14 and 21 of ovarian cycle compared with day 13. Figure 4 shows that Red Blood Cell (RBC) count was significantly lower on day 21 of the ovarian cycle than day 12 and day 13. Figure 5 shows that there was no significant difference in the mean corpuscular volume throughout the days. On day 21, RBC and HCT were least but progesterone was highest. Also, there was a positive correlation between progesterone and hematocrit.

Blood viscosity indices

Figure 6 shows that fibrinogen concentration was relatively unchanged all through the study. Figure 7 shows that RPV was significantly higher on day 13 than day 12. Figure 8 indicates that the hematocrit was significantly reduced on day 21 than days 12 and 13.

Table 1: Correlation between reproductive hormones and blood viscosity indices

R	Day 12		Day 13		Day 14		Day 21	
	LH	Prog	LH	Prog	LH	Prog	LH	Prog
RPV	-0.4	-0.9*	0.41	0.5	-0.92*	-0.44	0.46	-0.92*
Fibrinogen	0.43	-0.89*	0.45	-0.85*	0.39	0.87*	0.39	0.48
Hematocrit	-0.42	0.87	-0.35	0.89*	0.38	0.97*	-0.48	0.96*

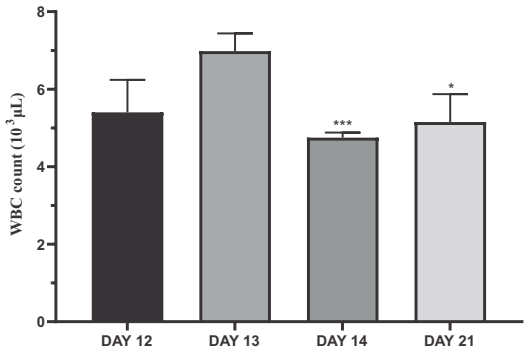

Figure 3: White blood cells count during day 12, 13, 14 and 21 of the menstrual cycle

****Significant difference (p<0.05)*

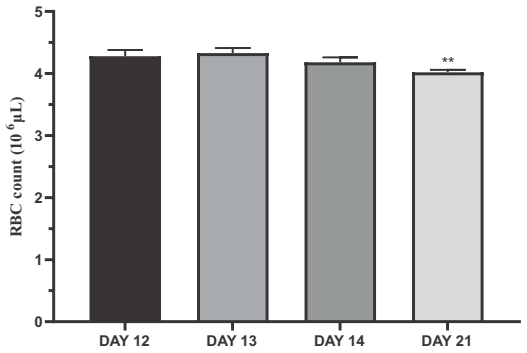

Figure 4: Red blood cells count during day 12, 13, 14 and 21 of the menstrual cycle

***Significant difference (p<0.05)*

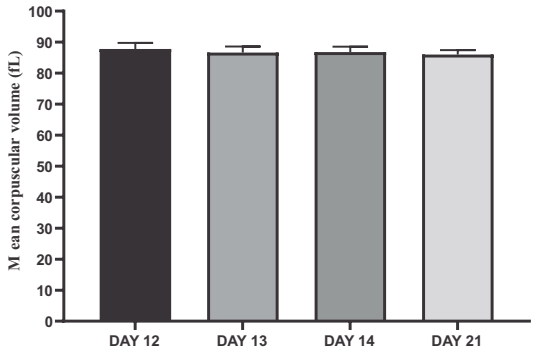

Figure 5: Comparison of mean corpuscular volume during day 12, 13, 14 and 21 of the menstrual cycle

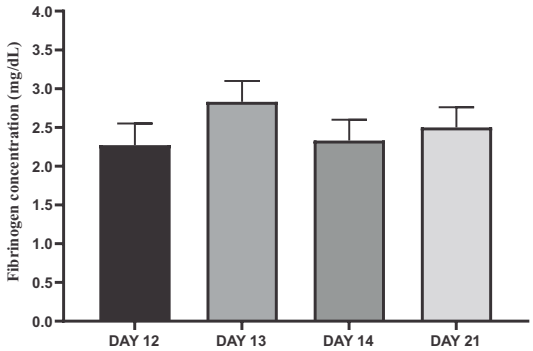

Figure 6: Fibrinogen concentration during day 12, 13, 14 and 21 of the menstrual cycle

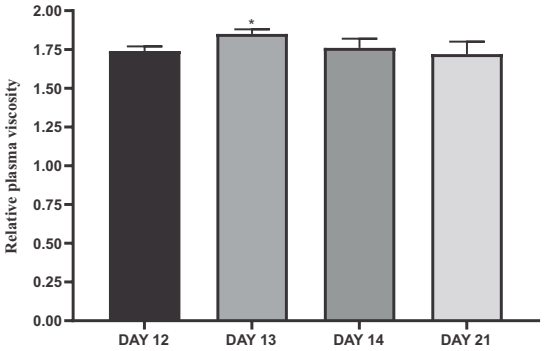

Figure 7: Relative plasma viscosity during day 12, 13, 14 and 21 of the menstrual cycle

**Significant difference (p<0.05)*

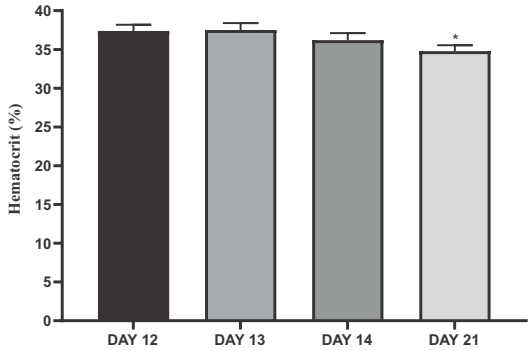

Figure 8: Hematocrit during day 12, 13, 14 and 21 of the menstrual cycle

**Significant difference (p<0.05)*

Discussion

Reproductive hormones exert their effects on virtually all body tissues besides organs of the reproductive system [12-17]. Estradiol, a reproductive hormone induces secretion of plasma proteins including fibrinogen [8, 18], a blood viscosity index. During menstrual cycle, cyclical fluctuation occurs in reproductive hormones culminating in profound variation in body functions [19].

The major finding of the study was that blood viscosity indices were not influenced by surges in progesterone and luteinizing hormone during days 12, 13, 14 and 21 of menstrual cycle. Days 12, 13, 14 and 21 were specifically selected to track fluxing pattern of reproductive hormones before and after mid-cycle. At day 21, progesterone secretion was higher than other days and a negative correlation was found between the hormone and RPV. LH peaked at day 12 but there was no correlation between the hormone and RPV, fibrinogen and hematocrit. At days 12, 13, 14 and 21, positive correlations were found between progesterone and hematocrit. According to the Brun [20], hormones that may influence blood rheology were documented. However, the rheological roles of endogenous reproductive hormones are sketchy.

Acting on evidence from oral contraceptives users, it would be easy to conclude how reproductive hormones affect blood viscosity. Erythrocyte aggregation and high blood viscosity due to increased level of fibrinogen and reduced erythrocyte deformability have been reported in oral contraceptives users [21-22]. However, the results may not indicate the exact pattern that occurs in endogenously controlled rhythms of reproductive hormones in humans during menstrual cycle.

Furthermore, a significantly high concentration of LH was observed on day 12 of menstrual cycle, followed with gradual decrease on days 13, 14 and 21. Although no screening was conducted to ascertain the timing of ovulation, it was likely to have occurred around days 13 or 14 following the reports of Bull *et al.* [23] and Holesh *et al.* [2] that ovulation occurs within 24 hours after LH surge.

Elevated level of progesterone was observed on day 21, predicting ovulatory cycles.

According to the report of Wagner *et al.* [24], serum progesterone concentration of 10 ng/ml or more a week before the next menses indicates normal ovulation. Although, mean corpuscular volume did not change throughout days 12, 13, 14 and 21, there were significant decreases in RBC and WBC counts on day 21. The decrease could have occurred as a result of pooling of RBCs into the spiral arteries supplying the endometrium prior to menstruation. This reduced RBC count accounted for the decrease in hematocrit observed on day 21, since hematocrit is a reflection of RBC count.

Throughout the menstrual cycle days, progesterone positively correlated with RPV, fibrinogen and hematocrit, though there was no significant change in fibrinogen on days 12, 13, 14 and 21. It was also observed that peak RPV occurred on day 13.

Conclusion

Blood viscosity indices were not influenced by surges in progesterone and luteinizing hormone during days 12, 13, 14 and 21 of menstrual cycle.

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References

1. Harlow SD, Paramsothy P. Menstruation and the menopausal transition. *Obstet Gynecol Clin* 2011; 38(3):595-607.
2. Holesh JE, Bass AN, Lord M. Physiology, Ovulation. In: StatPearls. Treasure Island (FL): StatPearls, Publishing 2021.
3. Agoreyo FO, Asowata EO. Assessment of total leukocyte counts during menstruation. *Int J Med Med Sci* 2021; 3(1):19-21.
4. Dullo P, Vedi N. Changes in serum calcium, magnesium and inorganic phosphorus levels during different phases of the menstrual cycle. *J Hum Reprod Sci* 2008; 1(2):77-80.
5. Singh D, Pandey RS. Changes in catalase activity and hydrogen peroxide level in rat ovary during estrous cycle and induction of catalase in rat ovary by estradiol-17 beta. *Indian J Exp Biol* 1998; 36(4):421-423.
6. Javaid A, Hasan R, Naim T. A comparative study of body weight, hemoglobin concentration and hematocrit during follicular and luteal phases of the menstrual cycle. *J Med Sci* 2007; 7(1):146-149.
7. Lugos MD, Vwamdem NI, Polit UY, Ofojekwu MN, Damen JG. Screening for anemia at different phases of the menstrual cycle among female students in a Nigerian University. *J Hematol Blood Transf Disord* 2019; 6:022.
8. Gyawali P, Richards RS. Association of altered hemorheology with oxidative stress and inflammation in metabolic syndrome. *Redox Rep* 2015; 20 (3):139-144.
9. Larsson H, Persson S, Hedner P, Odeberg H, Gustafson A. Studies on blood viscosity during the menstrual cycle and in the postmenopausal period in healthy women. *Acta Obstet Gynecol Scand* 1989; 68(6):483-486.
10. Agoreyo FO, Okorie C.Evaluation of relative blood viscosity during menstruation in reproductive females.*Nig J Basic Appl Sci* 2016; 24(2): 60-65.
11. Ajayi OI, Ekakitie OO, Okpalaugo OC. Differential rheology among ABO blood group system in Nigerians. *J Afr Assoc Physiol Sci* 2015; 3(1):30-35.
12. Onyinye AV, Emeka AG. Citrullus lanatus ethanolic seed extract improved male sexual behavior in rats via enhancement of sexual hormone and hypothalamic-pituitary-gonadal pathway. *J Krishna Inst Med Sci Univ* 2019; 8(3): 96-107.
13. Adeniyi MJ, Agoreyo FO, Ige SF, Fabunmi OA, Ozolua OP, Biliaminu SA. Physiological implications of prolonged selenium administrations in normal and desynchronized adult female rats. *J Krishna Inst Med Sci Univ* 2021; 10(4):21-36.
14. Adeniyi MJ, Agoreyo FO. Estrous cycle ratio as a reproductive index in the rats. *Am J Biomed Sci Res* 2019; 4(2).
15. Agoreyo FO, Adeniyi MJ. Pattern of estrous cycle and ovarian antiperoxidative activity in light deprived Sprague-dawley rats treated with sodium selenate. *J Med Res Biol Stud* 2018; 1(1): 103.
16. Adeniyi MJ, Agoreyo FO. Duration-related modulation of body temperature rhythm and reproductive cycle in rats by photoperiodic perturbation. *Dir Res J Health Pharmacol* 2020; 8 (1): 1-6.
17. Adeniyi MJ, Agoreyo FO, Olorunnisola OL, Olaniyan OT, Seriki SA, Ozolua PO, *et al*. Photo-pollution disrupts reproductive homeostasis in female rats: The duration-dependent role of selenium administrations. *Chin J Physiol* 2020; 63(5): 235-43.
18. Adeniyi MJ and Soladoye AO. Plasma lipid profile and uric acid in high fat fed female rats treated with oral contraceptive. *Biomed J Sci Tech Res* 2017; 1(2): 526-535.
19. Draper CF, Duisters K, Weger B, Chakrabarti A, Harms AC, Brennan L, *et al*. Menstrual cycle rhthmicity: metabolic patterns in healthy women. *Sci Rep* 2018;8: 14568.
20. Brun JF. Hormones, metabolism and body composition as major determinants of blood rheology: Potential pathophysiological meaning. *Clin Hemorheol Microcirc* 2002; 26(2):63-79.

21. Campbell SJ, Mackie IJ, Robinson GE,Machin SJ. Contact activation mediated fibrinolysis is increased by the combined oral contraceptive pill. *Br J Obstet Gynaecol 1993;* 100(1):79-84.
22. Norris LA, Devitt M, Bonnar J. The role of thromboxane A2 in increased whole blood platelet aggregation in oral contraceptive users. *Thromb Res* 1996;81(4):407-417.
23. Bull JR, Rouland SP, Scherwitzl EB, Scherwitzl R, Danielsson KG, Harper J. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. *NPJ Digital Med* 2019; 2(83):1-8.
24. Wagner EC, Prevolsek JS, Wynne-Edwards JE, Williams TD. Hematological changes associated with egg production: estrogen dependence and repeatability. *J Exp Biol* 2008; 211(Pt 3):400-408.

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