Effect of Depo-Provera on some hormones in child bearing women attending a Tertiary Hospital in Southern Nigeria

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Abstract
Depoprovera is a popular hormonal medication of progestin type popularly used in the southern part of Nigeria. It is used as a method of birth control and also a part of menopausal hormone therapy. It is an injectable known as depot medroxyprogesterone acetate. It stops pregnancy by preventing ovulation and thickening the cervical mucus. Depo is usually administered as a shot of 150mg/ml injection which last for 12weeks. Advantageously, it is very easy to administer and it helps women against the monotony and forgetfulness in taking daily pills. One hundred and fifty (150) women attending a tertiary hospital in southern part of Nigeria, who has not been on any previous contraceptive, had 5millilitre of blood collected at baseline, 3rd, 6th, 9th and 12th month after quarterly administration of depoprovera. LH, FSH, Prolactin, Progesterone and estradiol were determined using Enzyme Linked Immuno Assay method kit. The data obtained were analysed using statistical package for social science (SPSS) version 21.There is a significant decrease in LH concentration, while there is a significant increase in prolactin and progesterone from baseline to the 12th month. There was no significant difference in FSH and Estradiol concentrations. Also, there was significant difference (P<0.05) in LH concentration at different age groups, while no significant difference (P>0.05) in FSH, prolactin, progesterone and estradiol at different age groups. The result of the study showed that Depoprovera usage caused a decreased in LH with increase in prolactin and progesterone; an endocrinology condition which supports anovulatory cycle.
Keywords: *Depoprovera, progestin, medroxy progesterone acetate, Hormone.*

Introduction

Oral contraceptive agents can induce substantial metabolic changes that resemble those seen in persons at increased risk for premature coronary heart disease (Crook *et al*., 1988). These changes include raised serum triglyceride and low-density lipoprotein (LDL) cholesterol levels, reduced high-density lipoprotein (HDL) cholesterol levels, impairment of glucose tolerance, and elevated insulin levels (Wynn *et al* 1966, Erratum 1966). Although the contribution of these changes to the increased risk of coronary heart disease in users of oral contraceptives is uncertain (Wynn *et al* 1979).

Oral administration of estrogen and progestin provides an effective, reversible means of contraception. There has been controversy, however, about the possible risks, including coronary heart disease and myocardial infarction (Wynn *et al* 1979, Albano *et al* 1972, Stadel 1981, Realini and Goldzieher 1985) of this treatment. The progestin component of oral contraceptives combining progestin and estrogen is primarily responsible for the contraceptive action, but it has been implicated as a risk factor for coronary heart disease (Albano *et al* 1972,

Sturtevant 1989) perhaps through the promotion of potentially adverse changes in lipid and carbohydrate metabolism (Kay, 1982).Low levels of HDL increase the risk of coronary heart disease (Lipson et al 1986) especially in women (Gordon and Rifkind 1989). Ethinyl estradiol raises HDL cholesterol levels (Jacobs *et al* 1990). Conversely, progestins can lower HDL cholesterol levels (Kay, 1982, Godsland *et al* 1987, Wahl *et al* 1983, Bradley *et al* 1978) by increasing hepatic lipase activity (Krauss *et al* 1983).

The aim of this study was to determine the effects of depoproveraa commonly used oral contraceptive on some female hormones concentration of females attending University of Port Harcourt Teaching hospital based in South south Nigeria

Materials and Methods

Study Area: University of Port Harcourt Teaching hospital (UPTH)

Subject Selection: Ethical clearance was obtained from the Ethics and Protocol Review Committee of the University of Port Harcourt Teaching hospital (UPTH). Written informed consent was obtained from each subjects. This was a study of adult females from the Rivers state attending a Reproductive Healthcare Clinic in University of Port Harcourt Teaching hospital. One Hundred and fifty (150) subjects were recruited for this study. Purposive random sampling technique was used. The inclusion criteria were as follows:

females between the ages of 20 and 50 years, females who gave their written consent and females without predisposing factors or conditions to CV disease prior to contraceptive use.

Data and blood sample collection

A questionnaire was administered to obtain basic information on age, duration of drug use, contraceptive type, etc. Blood samples were collected by venepuncture from each subjects at baseline (0 Months), 3 months, 6 months, 9 months and 12 months respectively. After clotting, blood samples were centrifuged at 3,000 rpm for 10 minutes. Serum was aliquoted into Eppendorf tubes and stored at −20°C until use.

Hormonal Studies

Determination of Luteinizing Hormone LH was done by Immunoenzymometric assay. The Principle is that the essential reagents required for an immunoenzymometric assay includes high affinity and specificity antibodies (enzyme and immobilized) with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogeneously added biotinylated monoclonal anti-LH antibody. Upon mixing the monoclonal biotinylated

Antibody, the enzyme labeled antibody and a serum containing the native antigen, reaction result between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_p + Ag_{LH} + {}^{Btn}Ab_{(m)} \underset{ka}{\overset{Ka}{\leftrightarrow}} {}^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}{}^{Btn}Ab_{(m)}$$
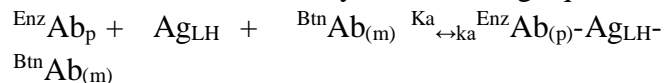
$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

Ag_{LH} = Native Antigen(Variable Quantity)

$^{Enz}Ab(p)$ = Enzyme labeled Antibody (Excess Quantity)

$^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex

$_{ka}$ =Rate Constant of Association

$_{Ka}$= Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below.

$^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}^{Btn}Ab_{(m)}$ +Streptavidin$_{CW}$ → Immobilized complex

Streptavidin$_{CW}$ = Streptavidin immobilized on well

Immobilized complex = Antibody-Antigen sandwich bound

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing values, a dose respond curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure: The microplates wells for each serum reference, control and patient specimen were formatted to be assayed in duplicate .0.050ml (50µl) of the appropriate serum reference, control and specimen was pipetted into the assigned well.0.100ml(100µl) of LH-Enzyme reagent was pipetted to all wells. The microplate was gently swirled for 20 seconds to mix. This was covered and incubated for 60minutes at room temperature. The contents of the microplate were discarded by decantation or aspiration (the plate was blotted dry with absorbent paper) 350µl wash buffer was added and decanted. This was repeated two additional times for a total of three washes.0.100ml (100µl) of substrate solution was added to all wells (added in the same order). It was incubated at room temperature for fifteen minutes. 0.050ml (50µl) of stop solution was added to each well and was mixed gently for 15 seconds. Absorbance was read in each well at 450nm using a reference wavelength of 620 nm. It was covered and incubated for 90minutes at room temperature.

Follicle Stimulating Hormone was done by the method of FSH Accubind ELISA Test System

The principle is thatUpon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation.
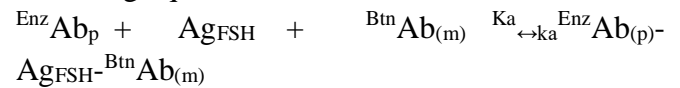
$^{Enz}Ab_p$ + Ag_{FSH} + $^{Btn}Ab_{(m)}$ $^{Ka}_{\leftrightarrow ka}$$^{Enz}Ab_{(p)}\text{-}Ag_{FSH}\text{-}^{Btn}Ab_{(m)}$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

Ag_{LFSH} = Native Antigen(Variable Quantity)

$^{Enz}Ab(p)$ = Enzyme labeled Antibody (Excess Quantity)

$^{Enz}Ab_{(p)}$ − Ag_{FSH} −$^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex

$_{ka}$ =Rate Constant of Association

$_{Ka}$= Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below.

$^{Enz}Ab_{(p)}\text{-}Ag_{FSH}\text{-}^{Btn}Ab_{(m)}$ +Streptavidin$_{CW}$ → Immobilized complex

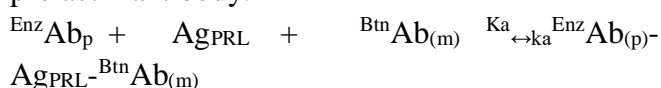Streptavidin$_{CW}$ = Streptavidin immobilized on well

Immobilized complex = Antibody-Antigen ssandwich bound

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing values, a dose respond curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure: The microplates wells for each serum reference, control and patient specimen were formatted to be assayed in duplicated. 0.050ml (50µl) of the appropriate serum reference, control and specimen was pipetted into the assigned well. 0.100ml(100µl) of FSH-Enzyme reagent solution was pipetted to all the wells. The microplate was

gently swirled for 20seconds to mix. This was covered and incubated for 60minutes at room temperature. The contents of the microplate were discarded by decantation (the plate was blotted dry with absorbent paper). 350µl wash buffer was added and decanted. This was repeated two additional times for a total of three washes. 0.100ml(100µl) of substrate solution was added to all wells (added in the same order). It was incubated at room temperature for fifteen minutes, 0.050ml(50µl) of stop solution was added to each well and was mixed gently for 15 seconds. Absorbance was read in each well at 450nm using a reference wavelength of 620 nm.

Determination of Prolactin- PRL was done by Immunozymometric assay. The Principle of this method is based on assay method including high affinity and specificity antibodies (enzyme labeled and immobilized), with different and distinct epitope recognition in excess, and native antigen. The immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-prolactin antibody.

$^{Enz}Ab_p$ + Ag_{PRL} + $^{Btn}Ab_{(m)}$ $\overset{Ka}{\underset{ka}{\leftrightarrow}}$ $^{Enz}Ab_{(p)}$-Ag_{PRL}-$^{Btn}Ab_{(m)}$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
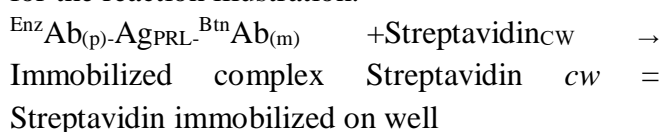
Ag_{LPRL} = Native Antigen(Variable Quantity)

$^{Enz}Ab(p)$ = Enzyme labeled Antibody (Excess Quantity)

$^{Enz}Ab_{(p)}$ – Ag_{PRL} – $^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex

$_{ka}$ =Rate Constant of Association

$_{Ka}$= Rate Constant of Dissociation

Simulteneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. See below for the reaction illustration.

$^{Enz}Ab_{(p)}$-Ag_{PRL}-$^{Btn}Ab_{(m)}$ +Streptavidin$_{CW}$ → Immobilized complex Streptavidin cw = Streptavidin immobilized on well

Immobilised complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound *antigen* by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values.

Procedure: The microplates wells for each serum reference, control and patient specimen were formatted to be assayed in duplicate. 0.025ml (25µl) of the appropriate serum reference, control and specimen was pipetted into the assigned well. 0.100ml (100µl) of prolactin-Enzyme reagent solution was pipetted to all wells. The microplate was gently swirled for 20seconds to mix, covered and incubated for 60minutes at room temperature. The contents of the microplates were discarded by decantation (the plate was blotted dry with absorbent paper). 350µl wash buffer was added and decanted. This was repeated two additional times for a total of three washes. 0.100ml (100µl) of substrate solution was added to all wells (added in the same order) and incubated at room temperature for fifteen minutes 0.050ml(50µl) of stop solution was added to each well and was mixed gently for 15 seconds. Absorbance was read in each well at 450nm using a reference wavelength of 620 nm.

Estrogen Determination was done by method of Estradiol Accubind ELISA Test System which principle i**s** based on delayed competitive enzyme immunoassay (type 9).upon mixing the biotinylated with a serum containing the antigen, a reaction results between the antigen and the antibody.

Ag + Ab_{Btn} ↔ $AgAb_{Btn}$

Ab_{Btn} = Biotinylated antibody Ag = antigen

$AgAb_{Btn}$= immune complex

After short incubation, the enzyme conjugate is added (this delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition

reaction results between the enzyme analogue and the antigen in the sample for a limited number antibody binding site (not consumed in the first incubation).

$$^{Enz}Ag \quad + \quad Ag \quad + \quad rAb_{BtnK-a} \leftrightarrow^{K_a}AgAb_{Btn}+ \quad ^{Enz}AgAb_{Btn}$$

^{Enz}Ag = Enzyme-antigen Conjugate (constant Quality)

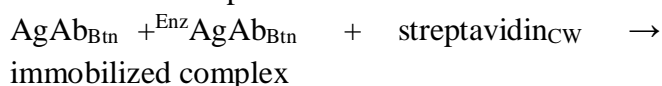$^{Enz}AgAb_{Btn}$= Enzyme-antigen conjugate- antibody complex

rAb_{Btn} = Biotinylated antibody not reacted in first incubation

K_a = Rate constant of association

K_{-a} = Rate constant of disassociation

$K= K_a/ K_{-a}$ = Equilibrium constant

A simultaneous reaction between the biotin attached to the antibody and the streptavin immobilized on the microwell occurs. This affects the separation of the antibody bound fraction after decantation or aspiration.

$$AgAb_{Btn} +^{Enz}AgAb_{Btn} \quad + \quad streptavidin_{CW} \rightarrow immobilized\ complex$$

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Procedure: The microplates wells for each serum reference, control and patient specimen were formatted to be assayed in duplicated. 0.025ml (25µl) of the appropriate serum reference, control and specimen was pipetted into the assigned well. 0.050ml (50µl) of estradiol biotin reagent was pipetted to all wells. The microplate was gently swirled for 30seconds to mix. This was covered and incubated for 30minutes at room temperature. 0.050ml (50µl) of estradiol enzyme reagent was added to all wells. The microplate was swirled gently for 30seconds to mix. It was covered and incubated for 90minutes at room temperature. The content of the microplate was discarded by decantation. (The plate was blotted dry with absorbent paper). 350µl of wash buffer was added

and decanted. This was repeated two additional times for a total of three washes. 0.100ml (100µl) of substrate solution was added to all wells (added in the same order). It was incubated at room temperature for twenty minutes 0.050ml (50µl) of stop solution was added to each well and was mixed gently for 15 seconds. Absorbance was read in each well at 450nm using a reference wavelength of 620 nm.

Determination of Progesterone was done using the method of Progesterone Accubind ELISA Test System whose principle is based on competitive enzyme immunoassay (type 7). Upon mixing biotinylated antibody, enzyme- antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antigen-binding sites. The interaction is illustrated by the following equation:

$$^{Enz}Ag \quad + \quad Ag \quad + \quad Ab_{BtnK-a} \leftrightarrow^{K_a}Agab_{Btn} \quad + \quad ^{Enz}AgAb_{Btn}$$
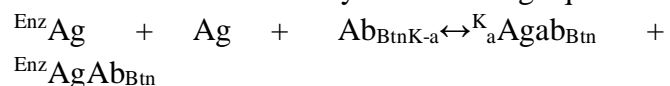
^{Enz}Ag = Enzyme-antigen Conjugate (constant Quality)

Ag = Native Antigen (variable quality)

$AgAb_{Btn}$ = Antigen-antibody complex

$^{Enz}AgAb_{Btn}$= Enzyme-antigen conjugate- antibody complex

Ab_{Btn} = Biotinylated antibody (constant Quality)

K_a = Rate constant of association

K_{-a} = Rate constant of disassociation

$K= K_a/ K_{-a}$ = Equilibrium constant

Procedure: The microplates wells for each serum reference, control and patient specimen were formatted to be assayed in duplicated.0.025ml (25µl) of the appropriate serum reference, control and specimen was pipetted into the assigned well.0.050ml (50µl) of progesterone biotin reagent was pipetted to all wells. The microplate was gently swirled for 30seconds to mix. 0.050ml (50µl) of progesterone enzyme reagent was added to all wells. The microplate was swirled gently for 10seconds to mix. It was covered and incubated for 60minutes at room temperature. The contents

of the microplate were discarded by decantation. (The plate was blotted dry with absorbent paper). 350µl of wash buffer was added and decanted. This was repeated two additional times for a total of three washes. 0.100ml (100µl) of substrate solution was added to all wells (added in the same order) It was incubated at room temperature for 20 minutes 0.050ml (50µl) of stop solution was added to each well and was mixed gently for 30 seconds. Absorbance was read in each well at 450nm using a reference wavelength of 620 nm.

Statistical Analysis: Statistical analyses were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA). P < 0.05 was considered significant. Data are presented as mean (standard deviation, SD). Differences in continuous data were compared using Student's *t*-test (two groups) and one-way analysis of variance (ANOVA; three or more groups) followed by the post-hoc test.

Result

As shown in table 1, The result of the study showed that LH (iU/L) was 8.30 ± 1.69, 8.17 ± 1.45, 7.72 ± 1.27, 7.08 ± 1.18 and 6.49 ± 1.04 while the FSH (IU/L) was 6.73 ± 1.20, 6.63 ± 1.82, 6.78 ± 1.55, 6.75 ± 1.41 and 6.99 ± 1.12 at 0, 3rd, 6th, 9th and 12th month respectively for subjects on Depoprovera. The Prolactin (ng/ml) was 15.94 ± 6.54, 16.88 ± 6.87, 17.62 ± 6.62, 19.14 ± 6.50 and 20.82 ± 6.65 at 0, 3rd,6th,9th and 12th month respectively while the Progesterone (ng/ml) was 3.47 ± 0.76, 3.60 ± 0.72, 3.72 ± 0.59, 3.86 ± 0.49 and 4.22 ± 0.87 at 0,3rd,6th,9th and 12th month respectively. Estrogen (pg/ml) concentration was 69.47 ± 27.41, 70.50 ± 27.63, 70.43 ± 26.82, 74.30 ± 31.85 and 71.10 ± 26.32 at 0, 3rd, 6th, 9th and 12th month respectively.

Table 1 Effect of Depoproveral on some Hormones at different month

Month	LH(iu/L)	FSH(iu/L)	PROL(ng/ml)	PROG(ng/ml)	E2(pg/ml)
Baseline	8.30 ± 1.69	6.73 ± 1.20	15.94 ± 6.54	3.47 ± 0.76	69.47 ± 27.41
3rd month	8.17 ± 1.45	6.63 ± 1.82	16.88 ± 6.87	3.60 ± 0.72	70.50 ± 27.63
6th month	7.72 ± 1.27	6.78 ± 1.55	17.62 ± 6.62	3.72 ± 0.59	70.43 ± 26.82
9th month	7.08 ± 1.18	6.75 ± 1.41	19.14 ± 6.50	3.86 ± 0.49	74.30 ± 31.85
12th month	6.49 ± 1.04	6.99 ± 1.12	20.82 ± 6.65	4.22 ± 0.87	71.10 ± 26.32
F	9.566	0.198	2.534	5.136	0.130
P	0.000	0.939	0.043	0.001	0.971

As shown in Table 2, There was significant difference in LH (IU/L) of 9.63 ± 1.62, 8.11 ± 1.66, 8.42 ± 1.89, 9.13 ± 1.64, 8.03 ± 1.40, 8.25 ± 1.72, 8.37 ± 1.00, 7.58 ± 1.32, 7.97 ± 1.19, 7.80 ± 0.88, 6.88 ± 1.21, 7.67 ± 1.05, 7.10 ± 0.85, 6.34 ± 1.11 and 6.95 ± 0.31 at age groups(years) of 21-30 baseline, 31-40baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively while the respective FSH (IU/L) was 7.70 ± 2.69, 6.37 ± 1.88, 8.10 ± 1.80, 7.10 ± 1.85, 6.31 ± 1.81, 8.07 ± 1.51, 6.83 ± 1.27, 6.56 ± 1.55, 8.02 ± 1.38, 6.57 ± 0.81, 6.57 ± 1.48, 7.87 ± 0.92, 6.83 ± 0.85, 6.87 ± 1.16 and 7.72 ± 0.99. There was no significant difference in Prolactin (ng/ml) concentrations of 16.87 ± 7.60, 16.66 ± 6.68, 11.05 ± 3.18, 16.67 ± 7.64, 17.81 ± 7.06, 11.67 ± 3.03, 17.13 ± 6.98, 18.51 ± 6.86, 12.85 ± 3.02, 18.47 ± 5.35, 19.86 ± 6.94, 15.47 ± 3.74, 20.37 ± 5.30, 21.43 ± 7.09 and 17.65 ± 4.97 at age groups(years) of 21-30 baseline, 31-40baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively while there was no significant difference in Progesterone (ng/ml) concentrations of 3.27 ± 0.20, 3.45 ± 0.84, 3.75 ± 0.53, 3.40 ± 0.40, 3.59 ± 0.78, 3.77 ± 0.66, 3.83 ± 0.60, 3.67 ± 0.61, 3.92 ± 0.55, 4.17 ± 0.71, 3.81 ± 0.45, 3.92 ± 0.59, 4.23 ± 0.60, 4.14 ± 0.77 and 4.67 ± 1.51 at age groups(years) of 21-30 baseline, 31-40baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th,

31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively. The Estradiol(pg/ml) was 83.67$\pm$27.42, 65.21$\pm$28.15, 83.25$\pm$18.96, 83.33$\pm$30.28, 66.30$\pm$28.03, 85.00$\pm$20.70, 83.67$\pm$28.98, 66.87$\pm$27.91, 81.00$\pm$16.12, 84.33$\pm$29.50, 71.61$\pm$34.14, 82.25$\pm$20.86,

84.67$\pm$31.66, 67.78$\pm$26.32 and 80.00$\pm$23.93 at age groups(years) of 21-30 baseline, 31-40baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively.

Table 2 Effect of Depoproveral on Some Hormones at different age groups

Age Group	LH(iu/L)	FSH (iu/L)	PROL(ng/ml)	PROG(ng/ml)	E2 (pg/ml)
21-30 baseline	9.63$\pm$1.62	7.70$\pm$2.69	16.87$\pm$7.60	3.27$\pm$0.20	83.67$\pm$27.42
31-40baseline	8.11$\pm$1.66	6.37$\pm$1.88	16.66$\pm$6.68	3.45$\pm$0.84	65.21$\pm$28.15
41-50baseline	8.42$\pm$1.89	8.10$\pm$1.80	11.05$\pm$3.18	3.75$\pm$0.53	83.25$\pm$18.96
21-30 3rd	9.13$\pm$1.64	7.10$\pm$1.85	16.67$\pm$7.64	3.40$\pm$0.40	83.33$\pm$30.28
31-40 rd	8.03$\pm$1.40	6.31$\pm$1.81	17.81$\pm$7.06	3.59$\pm$0.78	66.30$\pm$28.03
41-503rd	8.25$\pm$1.72	8.07$\pm$1.51	11.67$\pm$3.03	3.77$\pm$0.66	85.00$\pm$20.70
21-306th	8.37$\pm$1.00	6.83$\pm$1.27	17.13$\pm$6.98	3.83$\pm$0.60	83.67$\pm$28.98
31-40 6th	7.58$\pm$1.32	6.56$\pm$1.55	18.51$\pm$6.86	3.67$\pm$0.61	66.87$\pm$27.91
41-50 6th	7.97$\pm$1.19	8.02$\pm$1.38	12.85$\pm$3.02	3.92$\pm$0.55	81.00$\pm$16.12
21-30 9th	7.80$\pm$0.88	6.57$\pm$0.81	18.47$\pm$5.35	4.17$\pm$0.71	84.33$\pm$29.50
31-40 9th	6.88$\pm$1.21	6.57$\pm$1.48	19.86$\pm$6.94	3.81$\pm$0.45	71.61$\pm$34.14
41-50 9th	7.67$\pm$1.05	7.87$\pm$0.92	15.47$\pm$3.74	3.92$\pm$0.59	82.25$\pm$20.86
21-3012th	7.10$\pm$0.85	6.83$\pm$0.85	20.37$\pm$5.30	4.23$\pm$0.60	84.67$\pm$31.66
31-40 12th	6.34$\pm$1.11	6.87$\pm$1.16	21.43$\pm$7.09	4.14$\pm$0.77	67.78$\pm$26.32
41-50 12th	6.95$\pm$0.31	7.72$\pm$0.99	17.65$\pm$4.97	4.67$\pm$1.51	80.00$\pm$23.93
F	3.427	1.214	1.484	1.729	0.630
P	0.000	0.272	0.125	0.057	0.836

Discussion

Hormones are chemical messengers that are secreted directly into the blood, which carries them to organs and tissues of the body to exert their functions. Depo-provera is a hormone used for contraception. It is given by injection, which lasts for 3 months at a time. It is similar to progesterone, which is one of the two hormones produced by the woman's ovaries during her normal cycle. (Royal women hospital).

The present study shows a significance decrease (P<0.05) in the secretion of LH, significant increase (P<0.05) in prolactin and progesterone (P<0.001). However, FSH and Estradiol concentration at the end of the study (12months) after Depo-provera administration did not show any significant difference (P>0.05). This is likely in agreement with the information from the Royal women's hospital in Victoria, Australia on their web site (www.thewomen's.org.au). Their study revealed that hormone levels are low and stable throughout the cycle, so the lining of the uterus does not thicken as it normally would be. The present study showed reduced LH, practically stable FSH and unnoticeable increase in estradiol. The slight increased progesterone concentration is likely due to the synthetic external input progesterone, which is the steady introduction of medroxy progesterone which is given steadily every three months.

The result of the study further showed that LH was affected by the age of the subjects as there was lowering of the LH concentration based on age difference. The other hormonal parameters were not affected by the age. The HPG axis is subject to both positive feed forward and negative feedback regulation at several levels (Bliss *et al*, 2010). Estrogen and progesterone with short and long negative feedback loops regulate the secretion of FSH and LH by the pituitary and GnRH by the hypothalamus (Herbison, 2009).An increase in oxidative activity and increased oxLDL was observed in women treated with MPA which leads to initiation of atherosclerosis (Topcuoglu *et. al.*, 2005).

Conclusion

The result of the study showed that Depoprovera usage caused a decreased in LH with increase in prolactin and progesterone; anendocrinology condition which supports anovulatory cycle

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