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# Evaluation of Hormonal Profile on Women with Secondary Infertility Attending Federal Teaching Hospital, Owerri, Imo State

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## Abstract

Secondary infertility, characterized by the inability to conceive or carry a pregnancy to term following a previous successful pregnancy, is a complex reproductive disorder influenced by various factors, including hormonal imbalances. This study aimed to evaluate serum levels of Estradiol (E2), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Anti-Müllerian Hormone (AMH) in Secondary Infertility subjects attending Federal Teaching Hospital, Owerri. The study population comprised of Twenty (20) women diagnosed of secondary infertility within the ages 20 and 45 years and twenty (20) age-matched apparently healthy women without secondary infertility served as control group. Five (5) mls of venous blood was collected aseptically from each subject by venipuncture using sterile needle and syringes. The supernatant sera were analyzed using enzymatic immunoassay methods for Estradiol, FSH, LH and AMH. The result showed significantly lower mean levels of estradiol and luteinizing hormone (p=0.006 and p=0.024 respectively) in females with secondary infertility (67.93±42.48 pg/ml and 18.11±16.53 mIU/ml respectively) compared to the control subjects (108.59±45.43 pg/ml and 36.76±31.52 mIU/ml respectively). However, there was no significant difference in the mean levels of follicle-stimulating hormone and anti-müllerian hormone (p=0.710 and p=0.173 respectively) between females with secondary infertility (67.93±42.48 pg/ml and 4.72±5.89 ng/ml respectively) and the control subjects (16.28±9.05 mIU/ml and 7.04±4.56 ng/ml respectively). Furthermore, there were no significant correlations observed between anti-müllerian hormone and estradiol (r=0.08, p=0.749), follicle-stimulating hormone (r=0.01, p=0.952), or luteinizing hormone (r=0.16, p=0.509) in females with secondary infertility. In conclusion, the study revealed that while estradiol and LH levels were significantly lower in females with secondary infertility compared to controls, no significant differences were observed in FSH and AMH levels. These findings suggest a potential disruption in the hypothalamic-pituitary-ovarian axis, impacting follicular development, ovulation, and overall reproductive health.

***Keywords***: *Endocrine Disorders, Ovarian Function, Hormonal Imbalance, Reproductive Hormones, Menstrual Irregularities*

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## Introduction

Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 years of age) or 6 months (in a woman above 35 years of age) despite adequate, regular (3-4 times per week), unprotected sexual intercourse.[1-2] Infertility may also be referred to as the inability to carry a pregnancy to the delivery of a live baby. Infertility can be due to the woman, the man, or both; primary or secondary. In primary infertility, the couples have never been able to conceive; while in Secondary Infertility there is difficulty in conceiving after having conceived (either carried the pregnancy to term or had a miscarriage).[3-5]

Infertility has been described as the most important reproductive health concerns of Nigerian women.[6] Estimations from demographic health surveys found an 11 % prevalence rate, while clinical based studies suggested rates between 14.5 % and 30 %.[7] In Nigeria, the prevalence of primary infertility is 5 % and secondary infertility is 8 %.[8] The causes of infertility vary and have been linked to environmental issues, occupational related, genetics, and infectious diseases.[9] Both genders are affected in 40 % of cases, but the most common reasons for reproductive problems are conditions such as ovulatory disorders (25 %), fallopian tubal damage (20 %), and uterine or peritoneal abnormalities (10 %). However, in 30 % of cases, causes are unknown.

Infertility is a condition that attracts stigma in the Nigerian society, where childbearing is considered the hallmark of womanhood, and an inability to bear children makes the society see the woman as incomplete.[10] Infertility has been recognized as a potentially serious, costly, and burdensome issue for families.[11] The discovery that one cannot become pregnant is often unforeseen and results in invasive and demanding medical tests and procedures for both men and women. Furthermore, infertility may put a strain on couples' relationships, causing feelings of shame, anger, low self-esteem, depression, anxiety, grief, guilt, and suicidal ideation.[12]

The study was done to evaluate serum levels of Estradiol (E2), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Anti-Müllerian Hormone (AMH) in Secondary Infertility subjects attending Federal Teaching Hospital, Owerri.

## Materials and Methods

### Study Area

The study was conducted at the Federal Teaching Hospital, Owerri.

### Ethics Advocacy and Pre-Survey Contacts

An introductory letter was acquired from the Head of the Department of Medical Laboratory Science at Imo State University (Appendix I). This letter, along with the proposal, was submitted to the ethical committee of the Federal Teaching Hospital, Owerri. Subsequently, ethical approval was granted for the collection of samples. Informed written and structured questionnaires were provided to the study subjects after a thorough explanation of the extent, nature, and goals of the study. Additionally, sociodemographic data of the study subjects were recorded, including age, marital status, gender, tribe, and profession. A specific day was designated for the collection of samples.

### Study Population

A total of forty (40) subjects within the ages twenty and forty-five years were recruited for this study. Twenty (20) were female subjects who had been attending clinic for not less than three months diagnosed of secondary infertility in Federal Teaching Hospital, Owerri. Twenty (20) were apparently healthy individuals who served as controls subjects of the same age limits and sex who had no record of any other ailment.

**Selection Criteria**

**Inclusion Criteria**

The participants were those recently diagnosed of secondary infertility, the subjects were selected based on the criteria that:

i. Subjects within the ages of twenty (20) and forty-five (45)
ii. Subjects who were diagnosed of secondary infertility based on WHO
iii. Subjects without any other chronic diseases or other health related complications.
iv. Subjects who were apparently healthy and served as control subjects.

**Exclusion Criteria**

This study excluded

i. Subjects who were below age twenty (20) and above forty-five (45).
ii. Subjects with chronic diseases such as HIV/AIDS, diabetes, kidney failure and other carcinomas.
iii. Subjects who could not meet the clinical criteria for the diagnosis of secondary infertility.
iv. Subjects whose informed consents could not be obtained because they are skeptical about the purpose of the research work.

**Study Design**

A cross-sectional study was conducted in the month of February, 2024 and all eligible individuals who filled the questionnaire and gave a written informed consent for the study period were sampled. The research was grouped into two categories, group A represents twenty (20) women with diagnosed of secondary infertility attending Federal Teaching Hospital, Owerri while group B represents twenty (20) apparently healthy women without secondary infertility of the same age limit and they served as control.

**Sample Collection**

Venous blood samples (5ml) were collected aseptically by venipunture from each of the subjects using a 5ml sterile disposable syringe and needle. The whole blood samples were dispensed into a pre -labeled plain dry specimen container and allowed to clot. The clotted samples were centrifuged at 3000rpm for 5minues to separate and obtain the serum. The separated serum was used to assay Estradiol, FSH, LH and AMH.

**Laboratory Procedures**

All reagents used were commercially purchased and the manufacturer's standard operating procedures were strictly followed.

**A. Determination of Estradiol (E2)**

The test was done by enzymatic immunoassay (De Boever *et al.*, 1986) as modified by AccuBind Monobind Inc, Lake Forest. Catalogue number: CA 92630.

**Procedure**

The microplates' wells were formatted for each serum reference, control, or subject specimen to be assayed in duplicate. Then, 0.025 mL (25 µL) of the appropriate serum reference, control, or specimen was pipetted into the assigned well. Subsequently, 0.050 mL (50 µL) of the Estradiol Biotin Reagent was added to all wells, and the microplate was gently swirled for 30 seconds to mix. The microplate was covered and incubated for 30 minutes at room temperature. 0.050 mL (50 µL) of the Estradiol Enzyme Reagent was added to all wells, and the microplate was gently swirled for 30 seconds to mix.

The microplate was covered and further incubated for 90 minutes at room temperature. After incubation, the contents of the microplate were discarded by decantation, and the plate was dried with absorbent paper. Next, 0.350 mL (350 µL) of wash buffer was added to each well, and this step was repeated two (2) additional times for a total of three (3) washes. Following the washing steps, 0.100 mL (100 µL) of the Working Substrate solution was added to all wells. Importantly, the plate was not shaken after substrate addition. The microplate was incubated at room temperature for fifteen (20) minutes. To conclude the procedure, 0.050 mL (50 µL) of stop solution was added to each well, and the plate was gently mixed for 20 seconds. Finally, the absorbance in each well was read at 450 nm using a microplate reader.

**B. Determination of Follicle-Stimulating Hormone (FSH)**

The test was done by enzymatic immunoassay [13] as modified by AccuBind Monobind Inc, Lake Forest. Catalogue number: CA 92630.

**Procedure**

The microplate wells were prepared for each serum reference, control, or subject specimen to be duplicated. Then, 0.050 mL (50 µL) of the appropriate serum reference, control, or specimen was pipetted into their designated wells. Subsequently, 0.100 mL (100 µL) of FSH enzyme reagent solution was added to all wells, followed by gentle swirling and covering. The microplate was incubated for 45 minutes at room temperature. Afterward, the microplate's contents were discarded through decantation, and the plate was dried with absorbent paper. Next, 0.350 mL (350 µL) of wash buffer was added, decanted, and this process was repeated four (4) more times for a total of five (5) washes. Following that, 0.100 mL (100 µL) of Working substrate reagent was added to all wells without shaking the plate. The microplate was then incubated at room temperature in the dark for five (5) minutes. Finally, 0.050 mL (50 µL) of stop solution was gently added to each well, mixed for 15-20 seconds, and the absorbance in each well was read at 450 nm using a microplate reader.

**C. Determination of Luteinizing Hormone (LH)**

The test was done by enzymatic immunoassay by Uotila *et al*.[13] as modified by AccuBind Monobind Inc, Lake Forest. Catalogue number: CA 92630.

**Procedure**

The microplate wells were prepared for each serum reference, control, or subject specimen to be duplicated. Then, 0.050 mL (50 µL) of the appropriate serum reference, control, or specimen was pipetted into their designated wells. Subsequently, 0.100 mL (100 µL) of LH enzyme reagent solution was added to all wells, followed by gentle swirling and covering. The microplate was incubated for 45 minutes at room temperature. Afterward, the microplate's contents were discarded through decantation, and the plate was dried with absorbent paper. Next, 0.350 mL (350 µL) of wash buffer was added, decanted, and this process was repeated four (4) more times for a total of five (5) washes. Following that, 0.100 mL (100 µL) of Working substrate reagent was added to all wells without shaking the plate. The microplate was then incubated at room temperature in the dark for five (5) minutes. Finally, 0.050 mL (50 µL) of stop solution was gently added to each well, mixed for 15-20 seconds, and the absorbance in each well was read at 450 nm using a microplate reader.

**D. Determination of Anti-Müllerian Hormone (AMH)**

The test was done by enzymatic immunoassay Jopling *et al*.[14] as modified by AccuBind Monobind Inc, Lake Forest. Catalogue number: CA 92630.

**Procedure**

The microplate wells were prepared for each serum reference, control, or subject specimen to be duplicated. Then, 0.050 mL (50 µL) of the appropriate serum reference calibrator, control, or specimen was pipetted into their designated wells. Subsequently, 0.050 mL (50 µL) of AMH enzyme reagent solution was added to all wells, followed by gentle swirling for 30 seconds and covering. The microplate was incubated for 60 minutes at room temperature. Afterward, the microplate's contents were discarded through decantation, and the plate was dried with absorbent paper. Next, 0.350 mL (350 µL) of wash buffer was added, decanted, and this process was repeated two (2) more times for a total of three (3) washes. Following that, 0.100 mL (100 µL) of TMB substrate reagent was added to all wells without shaking the plate. The microplate was then incubated at room temperature for twenty (20) minutes. Finally, 0.050 mL (50 µL) of stop solution was gently added to each well, mixed for 20 seconds, and the absorbance in each well was read at 450 nm using a microplate reader.

**Statistical Analysis**

Data obtained in this study was analysed using SPSS (Statistical Package for Social Science) version 21. The student independent T-test was used to determine the mean differences. Results were expressed as Mean ± standard deviation. Test with probability of $p<0.05$ was statistically significant.

**Results**

**Table 1: Mean ± SD Values of E2, FSH, LH and AMH in Females with Secondary Fertility**

**of the Study Population**

| Parameters | Females with Secondary Fertility (n=20) | Control Subjects (n=20) | t-value | p-value (0.05) |
|---|---|---|---|---|
| **E2 (pg/ml)** | 67.93±42.48* | 108.59±45.43 | 2.92 | 0.006 |
| **FSH (mIU/ml)** | 14.52±18.94 | 16.28±9.05 | 0.38 | 0.710 |
| **LH (mIU/ml)** | 18.11±16.53* | 36.76±31.52 | 2.34 | 0.024 |
| **AMH (ng/ml)** | 4.72±5.89 | 7.04±4.56 | 1.39 | 0.173 |

**KEY:**
**n: population size**
***: Statistically significant (P<0.05)**
**E2: Estradiol**
**FSH: Follicle Stimulating Hormone**
**LH: Luteinizing Hormone**
**AMH: Anti-müllerian Hormone**

Table 1 indicates the test parameters of the females with secondary infertility and control group. It shows the mean ± SD values of E2, FSH, LH and AMH. The mean ± SD values of estradiol was lower in female with secondary fertility (67.93±42.48) pg/ml which was statistically significant (p=0.006) when compared to mean ± SD values of the control subjects (108.59±45.43) pg/ml. The mean ± SD values of luteinizing hormone was lower in female with secondary fertility (18.11±16.53) mIU/ml which was statistically significant (p=0.024) when compared to mean ± SD values of the control subjects (36.76±31.52) mIU/ml.
The mean ± SD values of follicle stimulating hormone was lower in female with secondary fertility (67.93±42.48) pg/ml which was not statistically significant (p=0.710) when compared to mean ± SD values of the control subjects (16.28±9.05) mIU/ml. The mean ± SD values of anti-müllerian hormone was lower in female with secondary fertility (4.72±5.89) ng/ml which was not statistically significant (p=0.173) when compared to mean ± SD values of the control subjects (7.04±4.56) ng/ml.

**Table 2: Pearson Correlation of AMH with E2, FSH and LH in Females with Secondary Fertility**

| Variable | N | R | p-value |
|---|---|---|---|
| **E2** | 20 | 0.08 | 0.749 |
| **FSH** | 20 | 0.01 | 0.952 |
| **LH** | 20 | 0.16 | 0.509 |

**KEY:**
**n: population size**

**r: Pearson correlation**
**E2: Estradiol**
**FSH: Follicle Stimulating Hormone**
**LH: Luteinizing Hormone**
**AMH: Anti-müllerian Hormone**
Table 2 indicates the test parameters of the females with secondary infertility. It shows the Pearson correlation of AMH with E2, FSH and LH in females with secondary fertility. There was no significant correlation between anti-müllerian hormone and estradiol (r=0.08, p=0.749), follicle stimulating hormone (r=0.01, p=0.952) and luteinizing hormone (r=0.16, p=0.509) in females with secondary fertility.

**Table 3: Pearson Correlation of FSH with E2, LH and AMH in Females with Secondary Fertility**

| Variable | N | R | p-value |
|---|---|---|---|
| **E2** | 20 | -0.33 | 0.144 |
| **LH** | 20 | 0.73** | 0.000 |
| **AMH** | 20 | 0.01 | 0.952 |

**KEY:**
**n: population size**
**r: Pearson correlation**
****: Statistically significant (P<0.05)**
**E2: Estradiol**
**FSH: Follicle Stimulating Hormone**
**LH: Luteinizing Hormone**
**AMH: Anti-mullerian Hormone**
Table 2 indicates the test parameters of the females with secondary infertility. It shows the Pearson correlation of FSH with E2, LH and AMH in females with secondary fertility. There was a positive significant correlation between follicle stimulating hormone and luteinizing hormone (r=0.73, p=0.000) in females with secondary fertility. There was no significant correlation between follicle stimulating hormone and estradiol (r=-0.33, p=0.144) and anti-müllerian hormone (r=0.01, p=0.952) in females with secondary fertility.

## Discussion

Secondary infertility, characterized by the inability to conceive or carry a pregnancy to term following a previous successful pregnancy, is a complex reproductive disorder influenced by various factors, including hormonal imbalances.[15] In this study, we evaluated the levels of estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and anti-Müllerian hormone (AMH) in females with secondary infertility, aiming to elucidate their role in the pathophysiology of this condition. The study revealed significantly lower mean estradiol levels in females with secondary infertility compared to controls, indicating a potential hormonal imbalance impacting reproductive health.[16] Lower estradiol levels may signify impaired ovarian function or

reduced follicular development critical for ovulation and conception, suggesting disruptions in the feedback loop between the hypothalamus, pituitary gland, and ovaries, leading to diminished estradiol production, underscoring the importance of assessing estradiol levels in secondary infertility.

This finding is consistent with prior research linking decreased estradiol levels to infertility, highlighting its significance in female reproductive health. However, variations in study populations, methodologies, and locations may contribute to observed differences, despite some studies reporting contrasting results, indicating the complexity of hormonal regulation in infertility.[17] Moreover, the study found significantly lower mean LH levels in females with secondary infertility compared to controls, suggesting potential disruptions in the hypothalamic-pituitary-ovarian axis regulating ovulation and menstrual cycles.[18] Lower LH levels may indicate impaired ovarian function or reduced gonadotropin secretion, impacting pregnancy achievement, aligning with similar studies suggesting LH as a biomarker for ovarian function and fertility potential, despite some reporting conflicting results due to variations in populations and methodologies.[18]

Notably, mean FSH levels did not significantly differ between females with secondary infertility and controls, implying FSH may not reliably indicate secondary infertility.[18] Although lower FSH levels were observed, compensatory mechanisms within the hypothalamic-pituitary-ovarian axis may balance altered FSH secretion with changes in other hormones like LH or estradiol. Similarly, mean AMH levels did not significantly differ between the two groups, suggesting it may not be a distinguishing factor in secondary infertility within this population. Although some studies reported significantly lower AMH levels in women with secondary infertility[19-20], discrepancies exist, likely due to variations in populations and methodologies. Additionally, no significant correlation was found between AMH levels and estradiol, FSH, or LH in females with secondary infertility, suggesting AMH may be regulated independently or by other factors[21], despite mixed findings in the literature, indicating potential interactions between AMH and other reproductive hormones. However, a significant positive correlation was observed between FSH and LH levels, emphasizing their association within the hypothalamic-pituitary-ovarian axis and their roles in regulating ovarian function and menstrual cycles. This underscores the importance of maintaining hormonal balance in women with secondary infertility to address fertility challenges effectively.[22]

## Conclusion

In conclusion, our findings shed light on the intricate hormonal dynamics among women with secondary infertility. While estradiol and LH levels were significantly lower in females with secondary infertility compared to controls, no significant differences were observed in FSH and AMH levels. These findings suggest a potential disruption in the hypothalamic-pituitary-ovarian axis, impacting follicular development, ovulation, and overall reproductive health. Further research is needed to elucidate the underlying mechanisms driving these hormonal imbalances and to explore their implications for fertility treatment strategies.

## References

1. Edward U, Ogbonna GN, Obeagu EI. Evaluation of Fertility Hormonal Profile on Women with Intramural Fibroid in Owerri. Elite Journal of Health Science. 2023;2(5):10-7.

2. Obeagu EI, Njar VE, Obeagu GU. Infertility: Prevalence and consequences. Int. J. Curr. Res. Chem. Pharm. Sci. 2023;10(7):43-50.
3. Obeagu EI, Obeagu GU. The Vital Role of Antioxidants in Enhancing Fertility and Pregnancy Success: A Review. Elite Journal of Nursing and Health Science. 2023;1(1):1-2.
4. Obeagu EI, Obeagu GU. Harnessing the Power of Antioxidant-Rich Diet for Preconception Health: A Review. Elite Journal of Health Science. 2023;1(1):1-3.
5. Obeagu EI, Obeagu GU. Harnessing the Power of Antioxidants: Enhancing Gamete Quality and Fostering Successful Pregnancy. Elite Journal of Nursing and Health Science. 2024;2(3):73-83.
6. Uadia PO, Emokpae AM. Male infertility in Nigeria: A neglected reproductive health issue requiring attention. Journal of Basic and Clinical Reproductive Sciences. 2015;4(2):45-53.
7. Hailemariam S, Tessema F, Asefa M, Tadesse H, Tenkolu G. The prevalence of depression and associated factors in Ethiopia: findings from the National Health Survey. International journal of mental health systems. 2012; 6:1-1.
8. Jabeen F, Khadija S, Daud S. Prevalence of primary and secondary infertility. Saudi J Med. 2022;7(1):22-28.
9. Gimenes F, Souza RP, Bento JC, Teixeira JJ, Maria-Engler SS, Bonini MG, Consolaro ME. Male infertility: a public health issue caused by sexually transmitted pathogens. Nature Reviews Urology. 2014;11(12):672-687.
10. Esan DT, Nnamani KQ, Ogunkorode A, Muhammad F, Oluwagbemi OO, Ramos CG. Infertility affects the quality of life of Southwestern Nigerian women and their partners. International Journal of Africa Nursing Sciences. 2022; 17:100506.
11. Ombelet W, Cooke I, Dyer S, Serour G, Devroey P. Infertility and the provision of infertility medical services in developing countries. Human reproduction update. 2008;14(6):605-621.
12. Dube L, Nkosi-Mafutha N, Balsom AA, Gordon JL. Infertility-related distress and clinical targets for psychotherapy: a qualitative study. BMJ open. 2021;11(11):e050373.
13. Uotila M, Ruoslahti E, Engvall EJ. Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. *Journal of Immunological Methods.* 1981; 42(1):11-15.
14. Jopling H, Yates A, Burgoyne N, Hayden K, Chaloner C, Tetlow L. Paediatric Anti-Müllerian Hormone measurement: Male and female reference intervals established using the automated Beckman Coulter Access AMH assay. *Endocrinology, Diabetes and Metabolism*, 2018; 1(4):e00021.
15. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, Petraglia F. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Human reproduction update. 2016;22(1):104-115.
16. Bendarska-Czerwińska A, Zmarzły N, Morawiec E, Panfil A, Bryś K, Czarniecka J, Ostenda A, Dziobek K, Sagan D, Boroń D, Michalski P. Endocrine disorders and fertility and pregnancy: An update. Frontiers in Endocrinology. 2023; 13:970439.
17. White HA, Garcia CD, Roberts AB. Variations in estradiol levels among women with secondary infertility: a comparative analysis. *Fertility and Sterility*, 2019; 48(2):156-165.

18. Chen X, Xiao Z, Cai Y, Huang L, Chen C. Hypothalamic mechanisms of obesity-associated disturbance of hypothalamic–pituitary–ovarian axis. Trends in endocrinology & metabolism. 2022;33(3):206-217.
19. Meczekalski B, Czyzyk A, Kunicki M, Podfigurna-Stopa A, Plociennik L, Jakiel G, Maciejewska-Jeske M, Lukaszuk K. Fertility in women of late reproductive age: the role of serum anti-Müllerian hormone (AMH) levels in its assessment. Journal of endocrinological investigation. 2016; 39:1259-1265.
20. Gleicher N, Weghofer A, Barad DH. Discordances between follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in female infertility. Reproductive Biology and Endocrinology. 2010; 8:1-7.
21. Bedenk J, Vrtačnik-Bokal E, Virant-Klun I. The role of anti-Müllerian hormone (AMH) in ovarian disease and infertility. Journal of assisted reproduction and genetics. 2020;37(1):89-100.
22. Squatrito M, Vervier J, Bindels J, Bernet L, Blacher S, Nisolle M, Munaut C. Impaired fertility in adenomyosis: a murine model reveals endometrial receptivity and progesterone resistance imbalances. Reproduction. 2024;167(5).