

Clinical Application of Sex Hormone in Different Physiological Periods in the Diagnosis of Infertility Patients

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Keywords: Different Periods, Sex Hormone Level Detection, Infertility, Auxiliary Diagnosis

Received: April 26, 2024

Accepted: June 14, 2024

Published: June 17, 2024

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ABSTRACT

Background: Infertility is characterized by the inability to conceive after a year of regular unprotected intercourse. **Aims:** This study aimed to investigate the diagnostic value of sex hormone levels during different physiological periods in the diagnosis of infertility patients. **Methods:** From December 2019 to May 2021, a total of 93 infertility patients were admitted and selected as the observation group. Among them, 31 cases were in the follicular stage, 31 cases in the ovulation stage, and 31 cases in the luteal stage. Ninety-three healthy women for fertility evaluation due to male infertility were selected as the control group. The control group included 31 women in the follicular phase, 31 women in the ovulatory phase, and 31 women in the luteal phase. The levels of sex hormones (prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E₂), testosterone (T), and progesterone (P)) during different physiological phases were compared between the observation and control groups. **Results:** The follicular phase showed no significant difference in LH levels between the observation group and the control group. The observation group showed higher levels of PRL and P compared to the control group, while the levels of FSH, E₂, and T were lower in the observation group compared to the control group. The ovulation phase showed no significant difference in PRL levels between the two groups. The observation group showed lower levels of LH, FSH, E₂, T, and P compared to the control group. The luteal phase showed no statistical difference in E₂ levels between the two groups. The observation group showed higher levels of PRL, LH, and FSH compared to the control group, while

the levels of T and P were lower in the observation group compared to the control group.
Conclusion: Infertile women show variations in hormone levels compared to the normal levels during the follicular phase, ovulatory phase, and luteal phase.

1. INTRODUCTION

The incidence of female infertility has significantly increased due to the obvious changes in people's work pressure and life rhythm. As per the World Health Organization (WHO), infertility is the failure to conceive despite two years of cohabitation and exposure to pregnancy or the inability to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse, epidemiological and clinical definition, respectively [1]. This has important psychological implications for the couple and a negative impact on the quality of their life [2, 3]. Moreover, the incidence is very high and gradually getting younger. According to the Centers for Disease Control (CDC), 1.5 million women in the US (6%) are infertile, and 25% of infertile couples have more than one factor that contributes to their infertility [4]. Therefore, effective treatment should be taken as soon as possible to restore normal fertility. The main factors that cause infertility can be divided into three main categories: male causes, mixed causes, and female causes [5]. There are many factors to consider in terms of pathogenesis, such as endocrine, reproductive organs, immune function, and fallopian tube factors, among which endocrine has the highest probability of causing infertility and becomes the main pathogenic factor. Therefore, it is necessary to combine clinical experience and theoretical knowledge to select appropriate examination methods to diagnose infertility, help improve the patient's pregnancy function, and meet their fertility requirements [6]. At present, the application rate of hormone level detection is high, and the diagnostic accuracy of reproductive endocrine diseases is significantly improved. The monthly normal physiological cycle of the female body can be divided into three periods, which are the follicular stage, ovulation stage, and luteal stage; the level of sex hormones in the female body at different periods has its particularity; the stage of the physiological cycle of the female body can be determined by hormone level, and the infertility factors of the patients can be analyzed by index level. Sex hormones promote the development of follicles and ovulation, which is beneficial to women's normal pregnancies. The abnormal secretion of sex hormones will affect the normal ovulation of the body, resulting in irregular menstruation and infertility.

In this case, the value of testing the level of sex hormones in patients with infertility at different physiological stages is analyzed.

2. MATERIALS AND METHODS

2.1. General Information

From December 2019 to May 2021, 93 patients with infertility admitted to our hospital were selected as the observation group, among which there were 31 cases in the follicular stage, ovulation stage, and luteal stage, respectively, aged 23 - 46 years with an average of (34.23 ± 3.12) years, and the duration of infertility was 1 - 5 years with an average of (3.12 ± 0.35) years; at the same time, 93 healthy women who came to the hospital for physical examination due to male fertility factors were selected as the control group, among which there were 31 cases in follicular stage, ovulation stage, and luteal stage respectively, aged 21 - 45 years with an average of (34.34 ± 3.36) years, and the duration of infertility was 2 - 5 years with an average of (3.25 ± 0.28) years. The data were collated and compared, and the results showed no statistical significance ($P > 0.05$), which was comparable.

The experiments were performed at the Department of Clinical Laboratory and were approved by the Experimental Ethics Committee of the Reproductive Hospital of Guangxi Zhuang Autonomous Region, Nanning 530029, Guangxi, China (No: KY-LW-2024-02). All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with Helsinki guidelines. Before this study, all participants gave informed written consent.

2.1.1. Inclusion Criteria for Infertility

1) Those who meet the diagnostic criteria for infertility in Obstetrics and Gynecology. 2) The physical examination personnel have a normal reproductive function and normal sexual life. 3) All are married women. 4) The spouse has a normal reproductive function.

2.1.2. Exclusion Criteria for Excluded Subjects

1) Patients with hematological diseases. 2) Patients with malignant tumors. 3) Those with abnormal liver and kidney function. 4) The clinical data is incomplete and cannot be consulted at any time.

2.2. Methods

The 4 mL of early morning fasting venous blood was extracted from all the subjects, including the patients at the follicular, ovulation, and luteal stages. The centrifugation speed was 3500 r/min, the centrifugation time was 15 min, and the centrifugation radius was 10 cm. After centrifugation, the upper layer of liquid was obtained and refrigerated in a -20°C refrigerator. The indexes of sex hormones were determined by the Roche Cobas e601 automatic immune analyzer.

2.3. Observation Indicators

The comparison of sex hormone levels in different physiological periods (follicular stage, ovulation stage, and luteal stage) between the two groups mainly included PRL, LH, FSH, E_2 , T, and P.

2.4. Statistical Analysis

SPSS 25.0 was used for processing; the (%) rate was the expression form of all measurement data; X² test was used, ($\bar{x} \pm s$) was the expression form of all counting data; t-test was used; $P < 0.05$ showed that there was a statistically significant difference.

3. RESULTS

3.1. Comparison of Sex Hormone Levels in the Follicular Phase between the Two Groups

Follicular Stage: There was no statistical difference in LH level between the two groups ($P > 0.05$); the levels of PRL (22.67 ± 5.33) ng/ml, P (1.81 ± 0.49) ng/ml in the observation group were higher than those in the control group, and the levels of FSH (4.25 ± 1.12) mIU/ml, E_2 (28.89 ± 6.37) pg/ml, T (0.12 ± 0.02) ng/ml were lower than those in the control group; thus, the results were statistically significant ($P < 0.05$) (Table 1).

3.2. Comparison of Hormone Levels during Ovulation between the Two Groups

Period of Ovulation: There was no statistical difference in PRL level between the two groups ($P >$

Table 1. Comparison of different sex hormone levels in the follicular phase between the two groups ($\bar{x} \pm s$).

Group	PRL (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	E_2 (pg/ml)	T (ng/ml)	P (ng/ml)
Observation group (n = 31)	22.67 ± 5.33	4.87 ± 1.12	4.25 ± 1.12	28.89 ± 6.37	0.12 ± 0.02	1.81 ± 0.49
Control group (n = 31)	14.44 ± 2.67	4.65 ± 1.02	5.94 ± 1.02	52.21 ± 9.82	0.26 ± 0.05	0.52 ± 0.10
<i>T value</i>	6.318	0.665	5.112	11.094	40.846	11.816
<i>P value</i>	<0.001	0.509	<0.001	<0.001	<0.001	<0.001

0.05). The levels of LH (18.24 ± 2.31) U/L, FSH (6.02 ± 1.28) mIU/ml, E₂ (110.50 ± 14.83) pg/ml, T (0.29 ± 0.07) ng/ml, P (1.03 ± 0.20) ng/ml in the observation group were lower than those in the control group, and the results were statistically significant ($P < 0.05$) (Table 2).

3.3. Comparison of Hormone Levels in the Luteal Phase between the Two Groups

Luteal Phase: There was no statistical difference in E₂ level between the two groups ($P > 0.05$). The levels of PRL (28.00 ± 4.67) nmol/L, LH (11.46 ± 1.12) mIU/ml, FSH (12.16 ± 1.82) mIU/ml in the observation group were higher than those in the control group, and the levels of T (0.41 ± 0.10) ng/ml, P (3.21 ± 0.78) ng/ml were lower than those in the control group; the results were statistically significant ($P < 0.05$) (Table 3).

4. DISCUSSION

Infertility has become a reproductive health problem between couples that poses a threat to family harmony and greatly affects social health; therefore, more in-depth clinical research on infertility disease with a view to a definitive diagnosis and treatment. The endocrine imbalance has become the main factor of female infertility; the hypothalamus-pituitary-ovarian axis (HPOA), the female's lifelong gonadal axis, has its main physiological function to control female development, normal menstruation, and sexual function, and this functional regulation is completed through neural regulation and hormone feedback regulation [7]. Therefore, sex hormone levels are used clinically to understand the female endocrine function and diagnose diseases related to endocrine disorders. The six commonly used sex hormones, namely FSH, LH, E₂, P, T, and PRL, basically meet the clinician's screening of endocrine disorders and a general understanding of physiological functions. Folliculogenesis is controlled by a complex interaction among hormones in the hypothalamus, anterior pituitary gland, and ovaries [8]. The increase in gonadotropin-releasing hormone (GnRH) released in the hypothalamus before puberty results in the increased release of LH and FSH in the anterior pituitary; both hormones act on the gonad, stimulating their development, gametogenesis,

Table 2. Comparison of hormone levels during ovulation between the two groups ($\bar{x} \pm s$).

Group	PRL (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	E ₂ (pg/ml)	T (ng/ml)	P (ng/ml)
Observation group (n = 31)	15.11 ± 4.00	18.24 ± 2.31	6.02 ± 1.28	110.50 ± 14.83	0.29 ± 0.07	1.03 ± 0.20
Control group (n = 31)	13.78 ± 2.67	60.35 ± 7.68	15.46 ± 1.54	309.68 ± 35.81	0.42 ± 0.08	1.55 ± 0.23
<i>T value</i>	1.270	24.061	21.602	28.610	5.698	8.006
<i>P value</i>	0.211	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3. Comparison of hormone levels in the luteal phase between the two groups ($\bar{x} \pm s$).

Group	PRL (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	E ₂ (pg/ml)	T (ng/ml)	P (ng/ml)
Observation group (n = 31)	28.00 ± 4.67	11.46 ± 1.12	12.16 ± 1.82	373.29 ± 49.46	0.41 ± 0.10	3.21 ± 0.78
Control group (n = 31)	13.78 ± 4.00	6.23 ± 0.62	5.28 ± 1.21	368.30 ± 48.93	0.54 ± 0.13	7.80 ± 1.49
<i>T value</i>	10.603	18.721	14.425	0.399	10.735	12.518
<i>P value</i>	<0.001	<0.001	<0.001	0.848	<0.001	<0.001

and sexual steroid production [9]. After the onset of puberty, the reproductive organs become mature and begin to perform reproductive activities. The female menstrual cycle is mainly regulated by the HPOA as the main sexual axis, which reflects the process of functional development, maturity, and decline of the HPOA axis [10]. LH and FSH are secreted from the anterior pituitary, and FSH stimulates the development of follicles, while LH is involved in the development and maturation of follicles. The production or insufficient action of LH and FSH will affect gametogenesis and the production of gonadal steroids [8]. The hypothalamus secretes the hypothalamic GnRH, which can effectively regulate the pituitary gonadotropin, thus improving and regulating the ovarian function; the ovarian gonadotropin secretion can effectively feedback and regulate the hypothalamus-pituitary secretion function; obstacles in any link can cause infertility [4, 11]. The level of sex hormones can well reflect the endocrine status of the female body; the occurrence and periodic changes of normal menstruation are regulated by the periodic changes of the ovary, that is, the follicular phase: FSH and LH remain at a low level, E gradually increases with the development and secretion of the follicle; P is only a small amount. Ovulation period: 24 hours before ovulation, FSH is low peak secretion, LH is steep peak secretion, and E is also peak secretion, which appears slightly earlier than FSH and LH; FSH and LH both drop sharply during ovulation. Luteal phase: FSH and LH remain at a low level again; the secretion of E and P gradually increases with the development of corpora luteum, reaching the highest level 7 - 8 days after ovulation and then falling back [12].

According to the results of this study, compared with the control group, the levels of PRL and P in the follicular stage of the observation group were higher, while the levels of FSH, E₂, and T were lower ($P < 0.05$), and there was no statistical difference in LH levels between the two groups ($P > 0.05$); it was confirmed that the sexual axis function of infertility patients was abnormal, and the levels of P and PRL were significantly increased. Hyperprolactinemia could inhibit the activities of FSH and LH, and the levels of FSH, E₂, and T were decreased, which led to ovulation dysfunction [13]. The current study demonstrated ovulation failure as the most common direct factor for infertility in women 24%, followed by ovarian disease 21%, and undetectable in 19% of cases [14]. There was no significant change in LH level, which may be directly related to the positive feedback of the sexual axis. Compared with the control group, the levels of LH, FSH, E₂, T, and P were lower in the observation group during ovulation ($P < 0.05$), and there was no statistical difference in PRL level between the two groups ($P > 0.05$). It was confirmed that the infertile patients suffered from ovulation disorders due to insufficient E₂ secretion by the ovary, which reduced the positive feedback effect of the sexual axis and affected the levels of FSH, LH, and P T but had no significant impact on the level of PRL. If the egg is not fertilized, the level of E₂ and P will drop significantly, the endometrium will fall off, and the corpus luteum will atrophy, FSH levels will rise again, and a new ovarian cycle will begin. Therefore, the redevelopment of follicles will affect the menstrual cycle, and the E₂ level can be used as an ovulation monitoring indicator. If the level of E₂ secretion is insufficient, the positive feedback effect cannot be established, which is not conducive to ovulation, and clinical symptoms, such as amenorrhea, obesity, and hirsutism, will occur. Basic FSH/LH $> 2 - 3.6$ indicates diminished ovarian reserve (DOR), which is the early manifestation of ovarian dysfunction [15] and usually indicates that patients have a poor response to superovulation/controlled ovarian hyperstimulation (COH). The COH plan and gonadotropin (Gn) dose should be adjusted in time to improve ovarian responsiveness and achieve an ideal pregnancy rate [16]. Since the increase of FSH/LH only reflects the DOR rather than the decrease in fertility ability, an ideal pregnancy rate can still be obtained once ovulation is obtained [17, 18]. Compared with the control group, the levels of PRL, LH, and FSH in the luteal phase of the observation group were higher, while the levels of T and P were lower ($P < 0.05$), and there was no statistical difference in E₂ level between the two groups ($P > 0.05$). Under normal conditions, blood P in the follicular stage was always at a low level, averaging 0.6 - 1.9 nmol/L, and generally < 10 nmol/L (3.15 ng/ml); when LH peak appeared before ovulation, P secretion began to increase; after ovulation, the corpus luteum of ovary produced a large amount of P, and the blood P concentration increased rapidly; when the corpus luteum matured the blood P concentration reached the peak, reaching 47.7 - 102.4 nmol/L (15 - 32.2 ng/ml) or higher [19, 20], and then decreased continuously, reaching the lowest level in the premenstrual period. The change of P content in the peripheral blood of the whole corpus luteum was parabolic. Studies have confirmed that

infertility patients are accompanied by luteal insufficiency, and there is normal granulocyte development and a sufficient number in the luteal phase, but it is difficult to generate blood vessels and luteinization, and the PRL level rises, while the T and P levels show a downward trend, which has no obvious impact on the E₂ level. The research results confirmed that both healthy women and infertile women have changes in various sex hormone levels at different periods, so sex hormone levels can be used as an auxiliary diagnostic method for infertility. Under the normal physiological environment of the female body, various hormone indicators will change in varying degrees; FSH and LH can accelerate follicle development, and their combination can form corpus luteum and secrete progesterone while increasing the total amount of E₂ secreted by the ovarian follicle. After follicle maturation, E₂ will be in a peak state, and the endometrium will turn into a proliferative stage. At the same time, the hormone generated by the hypothalamus has a positive feedback effect, which promotes the release of sex hormones in large quantities and significantly increases LH, which is conducive to ovulation. After ovulation, if the egg is not fertilized, the corpus luteum gradually atrophies while other follicles continue to mature until fertilization is successful. Therefore, E₂ can be used as an indicator to detect and evaluate female ovarian ovulation, which directly affects female reproductive health. P belongs to the hormone secreted by the corpus luteum of the ovary, which achieves the promoting effect during the transition from the endometrium to secretion. T is mainly produced by the transformation of peripheral androstenedione, which accounts for a relatively low proportion of ovarian secretion; it can effectively antagonize estrogen and affect the level of its own endocrine metabolism; through the joint detection of the level of sexual hormones, it can play a predictive role in the diagnosis of infertility.

5. LIMITATIONS

The main business of this reproductive health research center is to carry out work around human-assisted reproductive technology. Most of the selected research objects are the infertility population, and the regional scope is narrow. The research results obtained do not fully represent the hormone levels of normal healthy women who have given birth in different physiological periods, and also have certain limitations, so the research scope needs to be expanded, or it is more representative to select healthy women who have had their first child in natural cohabitation as a controlled study.

6. FUTURE PERSPECTIVES

This study aims at the close correlation between sex hormones at different periods and infertility in women of childbearing age. The detection of serum sex hormone levels at different periods is of great significance for the diagnosis of infertility and dynamic observation of follicle development. At the same time, the content of sex hormones in the body is closely related to women's bone mineral density (BMD). Sex hormone levels combined with changes in bone mineral density and bone morphology can guide patients with perimenopausal syndrome to carry out hormone supplement therapy.

7. CONCLUSION

The sex hormone levels of infertile women vary significantly from normal levels during the follicular phase, ovulation phase, and luteal phase. The detection of sex hormone levels at different periods serves as an effective auxiliary diagnostic indicator in the diagnosis of infertility.

AUTHORS' CONTRIBUTIONS

J.N. provided the methodology, performed a formal analysis, supervised the study, and contributed to funding acquisition. D.T. and A.M. professionally edited and proofread this manuscript. T.W., X.L., L.D., and B.W. provided the methodology, resources, and software and performed a formal analysis. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved the submission.

ETHICS APPROVAL

The experiments were performed at the Department of Clinical Laboratory and were approved by the Experimental Ethics Committee of the Reproductive Hospital of Guangxi Zhuang Autonomous Region, Nanning 530029, Guangxi, China. All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with Helsinki guidelines.

CONSENT TO PARTICIPATE

Before this study, all participants gave informed written consent.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

All processed data used in this study can be obtained from the corresponding author upon reasonable request.

FUNDING

This study was supported by the Self-Funded Project of Guangxi Zhuang Autonomous Region Administration of Traditional Chinese Medicine in 2021 (No. GXZYZ20210050).

CONFLICTS OF INTEREST

The authors declare no competing interests.

ACKNOWLEDGEMENTS

In the research process for this subject, we have received the help of many departments and individuals, as well as other people who are not involved in the research. All the members of the project would like to express our heartfelt thanks to the departments and individuals who have provided support and help to the research of this project, and we wish them good health and all the best.

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LIST OF ABBREVIATIONS

PRL = Prolactin
LH = Luteinizing hormone
FSH = Follicle-stimulating hormone
E₂ = Estradiol
T = Testosterone
P = Progesterone
WHO = World Health Organization
CDC = Centers for Disease Control
HPOA = Hypothalamus-pituitary-ovarian axis
GnRH = Gonadotropin-releasing hormone
DOR = Diminished ovarian reserve
COH = Controlled ovarian hyperstimulation
Gn = Gonadotropin
BMD = Bone mineral density

HIGHLIGHTS

- The normal secretion of estrogen, progesterone, and a small amount of androgen is guaranteed through the feedback regulation of the female gonadal axis (hypothalamus-pituitary-ovary).
- The sex hormone levels of the female body in the follicular, ovulatory, and luteal phases each month are special.
- The indexes of sex hormones in different periods of the body of infertile patients are different from the normal level.
- The detection of sex hormone levels in different periods can be used as an auxiliary means for the diagnosis of infertility.

SIGNIFICANCE STATEMENT

Infertility refers to a disease in which couples have not taken contraceptive measures and have a regular sex life within one year but are not pregnant. It is mainly divided into primary infertility and secondary infertility. Female infertility is mainly caused by ovulation disorders, fallopian tube factors, endometrial receptivity abnormalities, and immunologic factors. Male infertility is mainly caused by abnormal spermatogenesis. However, female endocrine disorders lead to ovulation dysfunction and infertility. At the same time, the sex hormone levels of female infertility patients in the follicular, ovulatory, and luteal phases of each month are different from the normal levels. The detection of their sex hormone levels in different periods can provide an accurate scientific basis for the diagnosis and treatment of infertility. Our study provides an endocrinology perspective on the importance of sex hormone level detection on infertility.