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Study of The Effect of Vit D On Sex Hormones in Females in Basrah City

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Serum vitamin D; female Infertility; Sex hormone; Lipid profile.

ABSTRACT

The present study evaluates the effect of Vitamin D on infertility in females by measuring sex-related hormones (testosterone(TESTO), estradiol(E2), luteinizing hormone(LH,) and follicle-stimulating hormone(FSH)) and some biochemical markers (prolactin (PRL) and lipid profile). The study included 56femalese divided between 28 infertile patients and 28 healthy individuals (control group), all the participated individuals were aged between 18-45 years. Infertile patients with other acute or chronic diseases were excluded from the study. Serum levels of Vitamin D, TESTO, FSH, LH, E2, PRL, and total lipid were measured by standard methods. Statistical analysis has performed the results indicated statistically significant differences between the two studied groups ($p \leq 0.05$). The results showed that infertile female patients have significantly lower levels of vitamin D ($p \leq 0.05$) when compared to the healthy control groups. The results also indicated a significant increase in the level of LH and FSH ($P<0.05$), compared to the control group. Our study indicated a significant positive correlation between vitamin D and each of; TESTO ($r=0.426$, $P<0.05$), LH ($r=0.009$, $P<0.01$), HDL ($r=0.039$, $P<0.05$) and LH/FSH ($r=0.044$, $P<0.05$) in the serum samples of the studied infertile females, respectively.

1. INTRODUCTION

Female infertility may be caused by low vitamin D levels [1]. It has been proposed that vitamin D insufficiency reduces sex hormone-binding globulin levels. As a result, androgen levels are rising, laying the groundwork for the polycystic ovarian syndrome (PCOS) [2]. Vitamin D levels were linked to lower insulin resistance. With the addition of vitamin D to therapy, patients with PCOS had improved insulin resistance, androgen levels, folliculogenesis, and monthly abnormalities [3]. However, 25(OH)D levels in follicular fluid were shown to be inversely linked to body mass index. As a consequence, 25(OH)D vitamin levels in follicular fluid were expected to be an independent predictor of IVF success. It was discovered that a 1 ng/mL increase in vitamin D in follicular fluid resulted in a 6% increase in live pregnancy [4]. Infertility is an illness with psychological, cultural, and physiological ramifications that cause trauma and stress, especially in a social setting like ours that places a heavy premium on reproduction. According to the World Health Organization's International Committee for Monitoring Assisted Reproductive Information, infertility is a

reproductive system illness identified as the inability to produce clinical pregnancy after 12 months or more of frequent sexual intercourse [5]. Infertility is a public health issue throughout the reproductive age, affecting around 10-15% of couples seeking to conceive in the world [6]. Primary infertility and secondary infertility are two types of infertility [7]. The Americans with Disabilities Act [8] of the United States has defined infertility as a condition. Infertility in women was identified as the sixth most significant worldwide handicap (among rural populations under 60 years old) [9].

Types of infertility:

1-Primary infertility

It is described as a female who has never been diagnosed with pregnancy and fits all of the criteria for infertility [10]. According to the World Health Organization, primary infertility occurs when a woman is unable to give birth to a child because she was unable to become pregnant or was unable to bring a pregnancy to term [11].

2-Secondary infertility

It is characterized as a female who is unable to become pregnant yet has previously been diagnosed with pregnancy [10]. Infertility is a woman's inability to bear a child due to a failure to become pregnant or difficulty carrying a pregnancy to a live birth following either a prior pregnancy or a previous pregnancy to a live delivery that the WHO describes [11].

Ovulation problems, tubal disorders, uterine abnormalities, endometriosis, and peritoneal factors are the most common causes of female infertility [12]. Cervical factors are also thought to play a minor effect, even though they are rarely the only cause [13].

In females, gonadotropin-releasing hormone (GnRH) stimulates and estrogen inhibits luteinizing hormone (LH) secretion. LH has a variety of roles that differ between men and women. LH stimulates the production of steroid hormones from the ovaries in both sexes [14]. Similarly, LH regulates the duration and sequence of the female menstrual cycle by acting in both ovulation and egg implantation in the uterus [15].

GnRH decreases the release of follicle-stimulating hormone (FSH) and LH from the anterior pituitary, inhibiting ovulation and estrogen production in women. GnRH agonists, such as leuprolide, function clinically through this pathway [16]. In women, FSH controls the menstrual cycle and ovarian follicular development. FSH works by binding to the granulosa cell surface on the granulosa cell surface in the ovaries. [17].

Both males and females require estrogen, a sex hormone generated from cholesterol. Women generate three forms of estrogen from adolescence to menopause: 17-lifespan estradiol (E2), estriol (E3) during pregnancy, and andestrone (E1) after menopause. Other estrogens include estetrol, or E4, which the fetal liver creates, and estrone sulfate, or E1S, which primarily acts as a reservoir for excess estrogen (produced by the enzyme estrogen sulfotransferase on E1) [18]. Estrogen's major function in women is to mature and maintain the reproductive system; nevertheless, estrogen has additional effects. It also affects other physiological systems and psychological responses. Its broad effects are most visible after menopause when the ovaries' production of E2 decreased significantly [19].

Testosterone is in charge of the initial stages of sexual development. Sex hormone-binding globulin (SHBG) and albumin are two plasma proteins that are connected to testosterone. This excessive protein-bound testosterone acts as an overabundance of testosterone in the body. In the zona reticularis of the adrenal glands,

both men and women manufacture weak acting androgens. Dehydroepiandrosterone and androstenedione are examples of weak-acting androgens. They have a low affinity for testosterone receptors, but if enough of them are created, they can be converted to testosterone in the peripheral tissues [20].

Vitamin D was initially thought to be a vitamin required for bone metabolism, but in recent years, it has been "rediscovered" as not just a "bone vitamin," but also a "multitasking vitamin." This is because vitamin D has been linked to a variety of extra-skeletal functions as well as the homeostasis of several systems. Because of vitamin D's numerous methods of action and the rising prevalence of vitamin D deficiency in the general population, the scientific community has shown a growing interest in vitamin D research. There is evidence that vitamin D deficiency is a major risk factor for a variety of acute and chronic illnesses., including musculoskeletal discomfort., Types of diabetes one and two, cardiovascular disease, autism, and PCOS [21]. PCOS is a complicated hormonal and metabolic disorder with an unknown cause. With a potential rate of up to 15%. Is also the most prevalent endocrinopathy among reproductive-age women [22]. Several studies have discovered that a deficiency of vitamin D affects both insulin secretion and insulin resistance. Insulin resistance is one of the diagnoses of PCOS, which is one of the most common endocrine diseases Associated with women of reproductive age [23]. It has been proposed that vitamin D insufficiency reduces sex hormone-binding globulin levels. As a result, androgen levels are rising, laying the foundations for PCOS [24]. Vitamin D levels were linked to lower insulin resistance. With the addition of vitamin D to therapies, patients with PCOS had improved insulin resistance, androgen levels, folliculogenesis, and menstrual irregularities [25].

2. Materials and methods

2.1 Subject and samples

The study samples of patients were collected from Infertility patients. Patients' forearm veins were sampled for 5ml of blood. Friends and family members were asked to provide healthy controls. Venous blood samples were collected and placed in vacationer gel tubes. After clotting, it was centrifuged for ten minutes at 5000 rpm. The serum was kept at -20 0C for later analysis. The study samples included 56 females infertile (patients 28 and healthy individuals 28) between the ages of (18-45) years.

Exclusion Criteria

- Cases and controls aged under 18 and above 45 years old.
- Patients with chronic diabetes mellitus, and thyroid disorders.
- Cases who take supplements replacement.
- pregnant women (control cases).
- Obese cases (controls and patients).

2.2 Methods

2.2.1 Measurement of Vitamin D concentration & Hormones

Serum vitamin D level & hormones were measured by using a fully automated COBAS e411 analyzer which was using the electrochemiluminescence Immunoassay “ECLIA” method in analysis. The normal value of total vitamin D according to the manufacturer of the kit was more than 30 to 50 ng/ml which is considered the optimal value, while the values below 20 ng/ml are considered a severe deficiency, and the values below 30 ng/ml considered as insufficiency.

While the reference value for testosterone was (0.048-0.8 ng/ml) for females and males (2.49-8.36 ng/ml) according to the manufacturer of the kit. The reference value of luteinizing hormone (LH) according to the manufacture of kit for females was (2.4-12.6 mIu/mL) in a follicular while (14.0-95.6 mIu/mL) in midcycle

and male (1.7-8.6 mIu/ml). The reference value of follicle-stimulating hormone (FSH) according to the manufacture of kit for females was (3.5-12.5 mIu/ml) in a follicular while (4.7- 21.5 mIu/ml) in midcycle and male (1.5-12.4 mlu/ml). The reference value of estradiol (E2) was (85.8-498 pg/ml) according to the manufacture of kit for females in midcycle and luteal (43.8-211 pg/ml) and male (7.63-42.6 pg/ml). The reference value of Prolactin was (4.79-23.3 ng/ml) according to the manufacture of kit for females and males (4.04-15.2 ng/ml).

2.2.2 Estimation Lipid Profile Level.

The levels of all lipid molecules (cholesterol, triglycerides, high-density lipoprotein(HDL), Low-Density Lipoprotein(LDL), and Very Low-Density Lipoprotein(VLDL)) were measured by using the deferent principle method and Enzymatic reagent [26- 30].

2.2.3 Glucose Estimation Level

Glucose was measured after enzymatic oxidation in the presence of glucose oxidase. Peroxidase catalyzes the reaction of hydrogen peroxide with phenol and 4-aminophenazone (paraminophenazone "PAP") to generate a red-violent quinoneimine dye as an indicator [31].

2.3 Statistical Analysis

Statistical analyses were achieved with the help of a statistical package for social sciences (SPSS) version 26. Under the p-value <0.05, the results were considered statistically significant. The data was represented using the mean and standard deviation (SD). T-tests were used to investigate differences between parametric quantitative data in two groups, the Mann-Whitney test was used to investigate differences between non-parametric quantitative data in two groups, and Spearman's correlation test to investigate the correlation coefficient (r) between every two parameters.

3. Results

This study is a case-control design, that included 56 females, equally divided between infertile patients and healthy control. Table (1) shows the levels of all parameters that were investigated in this study such as; VIT.D, TESTO, LH, FSH, PRO, E2, RSB, CHOL, TG, HDL, VLDL, LDL. Approximately, 25% of the infertile patients have irregular ovulation. The monthly period of these patients was frequently delayed or disappeared. Changes in the hypothalamus (which generates gonadotropin-releasing hormone [GnRH]) and the pituitary gland's production of specific hormones might cause ovulation to be disrupted. LH and FSH hormones can also be used as indicators for the ovary's development and the release of an egg [32].

Table (1): The Differences in the Levels of Vit.D, Hormones, and Biochemical Biomarker between Patient and Control Female

Variables	Patients (No=28)			Control (No=28)			*P* value
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	
VIT. D (ng/ml)	9.53±6.622	6.160	3.0-27.7	17.99±12.05	15.30	3.00-52.1	0.001
TESTO (ng/ml)	0.16±0.14	0.027	0.025-0.48	0.140±0.188	0.03	0.025-0.72	0.193
LH (m.IU/ml)	13.81±10.65	9.60	4.0-49.2	7.23±6.84	6.15	0.50-33.2	0.002
FSH (m.IU/ml)	5.03±2.42	4.70	4.0-49.20	4.90±8.61	2.85	0.40-47.6	0.011

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PRO (ng/ml)	**15.85±8.47**	**14.25**	**2.0-40.5**	**13.38±8.84**	**11.35**	**5.4-52.8**	**0.130**
E2 Pg/ml	**90.05±197.00**	**37.40**	**5.0-1075.0**	**79.20±64.12**	**65.75**	**5.0-194.0**	**0.412**
CHOL (mg/dl)	**165.50±33.69**	**161.00**	**114-242**	**178.04±52.23**	**164.0**	**124-388**	**0.446**
TG (mg/dl)	**126.96±72.43**	**108.0**	**44-340**	**173.96±113.84**	**127.0**	**34-499**	**0.161**
HDL (mg/dl)	**38.43±11.77**	**39.0**	**21-69**	**37.29±10.32**	**36.0**	**19-56**	**0.793**
VLDL (mg/dl)	**25.39±14.48**	**21.0**	**8.8-68.0**	**39.48±30.63**	**26.3**	**6.80-146.0**	**0.075**
LDL (mg/dl)	**99.60±32.67**	**98.0**	**32-168.6**	**105.92±47.55**	**97.0**	**45.2-304.2**	**0.922**
RBS (mg/dl)	**107.93±40.17**	**101.0**	**70-280**	**87.68±15.35**	**85.50**	**68-140**	**0.002**

Mann-Whitney test, significant ($p < 0.05$) high significant ($p \leq 0.001$) very high significant ($p \leq 0.0001$).

The study showed a very high significant decrease in the level of Vit D ($p \leq 0.001$) in patients with infertility (6.622±9.53), compared to healthy controls (12.05±17.99), Table (2) and Figure (1).

Table (2): Vit. D Level in Patients and Control Female

Variables	**Patients NO=28**			**Control NO=28**			***P* value**
	Mean±SD	**Median**	**Min-Max**	**Mean±SD**	**Median**	**Min-Max**	
VIT D ng/ml	**9.53±6.622**	**6.160**	**3.0-27.7**	**17.99±12.05**	**15.30**	**3.00-52.1**	**0.001**

Mann-Whitney test

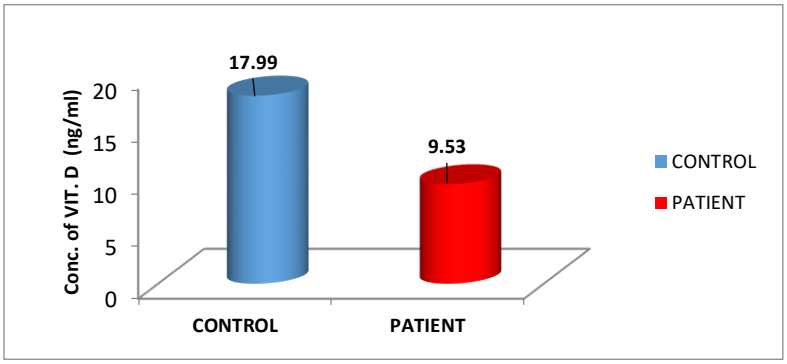

Figure (1): Mean Level of Vit D in Serum of Patients and Healthy Control Female

The results of this study showed a highly significant increase in the level of LH (p> 0.01) in patients with infertility (10.65±13.81) compared to healthy controls (6.84±7.23), Table (3) and Figure (2).

Table (3): LH Level in Patients and Control female

Variables	Patients NO=28			Control NO=28			*P* value
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	
LH m.IU/ml	13.81±10.65	9.60	4.0-49.2	7.23±6.84	6.15	0.50-33.2	0.002

Mann-Whitney test

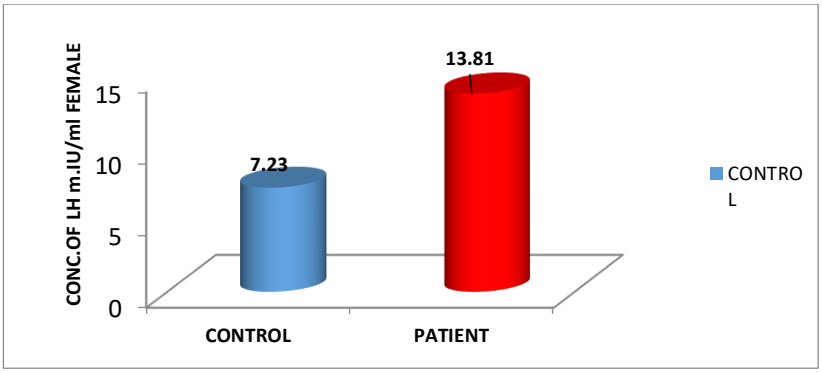

Figure (2): Mean Level of LH in Serum of Patients and Healthy Control female.

Level of FSH in patients and control females, the study indicated a significant FSH (p< 0.05) in patients with infertility (2.42±5.03) compared to healthy controls (8.61±4.90), Table (4) and Figure (3).

Table (4): FSH Level in Patients and Control female

Variables	Patients NO=28			Control NO=28			*P* value
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	
FSH m.IU/ml	5.03±2.42	4.70	4.0-49.20	4.90±8.61	2.85	0.40-47.6	0.011

Mann-Whitney test

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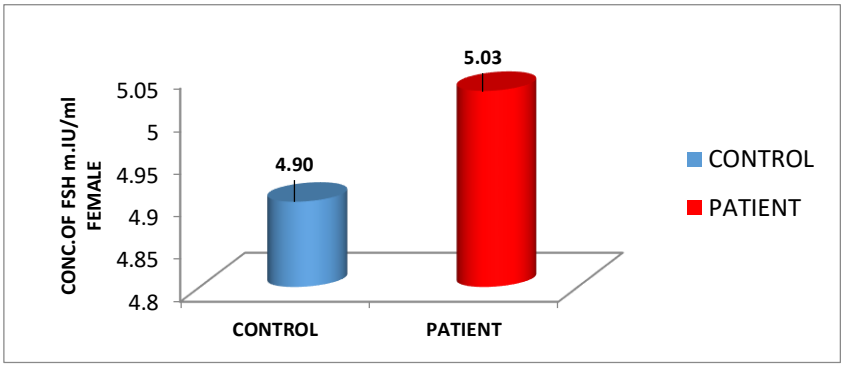

Figure (3): Mean Level of FSH in Serum of Patients and Healthy Control female.

The statistical analysis of other variables (hormone and lipid profile), listed in Table (1) showed no significant difference in the mean concentrations of TESTO ($P > 0.05$), Prolactin level ($P > 0.05$), E2 level ($P > 0.05$), CHOL level ($P > 0.05$), TG level ($P > 0.05$), HDL level ($P > 0.05$), VLDL level ($P > 0.05$) and LDL level ($P > 0.05$).

The correlations between Vit D and other biochemical variables in female patients were also investigated. Table (5) shows the results of the Spearman correlation coefficient (r), between the Vit.D and other studied biomarkers in female patients. The results in Table 5 indicated a significant positive correlation between the Vit.D and the TESTO (r=0.426, P<0.05), LH (r=0.009, P<0.01), HDL (r=0.039, P<0.05) and LH/FSH (r=0.044, P<0.05), respectively. In contrast, the results showed no significant correlation between Vit.D with FSH (r=- 0.123, p= 0.534), PRL (r=0.143, p=0.467), E2 (r=0.289, p= 0.135) and no significant with CHOL, TG, LDL and VLDL (Table 5).

Table (5): The Correlations Between Vit D, Hormones, and Other Biochemical Variables in Patients Female

Correlations	r	*P* value
Vit D *vs*. AGE	**-0.049**	**0.806**
Vit D *vs*. TESTO	***0.426**	**0.024**
Vit D *vs*.LH	****0.482**	**0.009**
Vit D *vs*.FSH	**-0.123**	**0.534**
Vit D *vs*.PRO	**0.143**	**0.467**
Vit D *vs*.E2	**0.289**	**0.135**
Vit D *vs*.CHOL	**0.036**	**0.856**
Vit D *vs*.TG	**-0.082**	**0.678**
Vit D *vs*.HDL	**0.392***	**0.039**
Vit D *vs*.VLDL	**-0.082**	**0.678**
Vit D *vs*.LDL	**-0.141**	**0.473**
Vit D *vs*.RBS	**-0.173**	**0.378**
Vit D *vs*.LH/FSH	***0.384**	**0.044**

Spearman's correlations test, R = strength of correlation or correlation coefficient, (-) inversely correlation, (+) proportional correlation., significant ($*p < 0.05$), high significant ($**p \leq 0.001$), very high significant ($***p \leq 0.0001$)

4. Discussion

infertility Because unexplained infertility affects more than 25% of patients who consult reproductive and

infertility care clinics, it may be characterized as an exclusion condition [33]. The study found a significant decrease in vitamin D levels in female infertility patients compared to healthy controls, Table (2) and Figure (1). Vitamin D's Effect on Endometriosis, PCOS, the most prevalent female endocrine illness, as well as raising progesterone and estrogen levels, regulating menstrual cycles, and improving the chances of a successful pregnancy [34]. Results of this study were matched with the results reported by other authors [35], that Serum 25(OH)D levels are severely low in women seeking medical treatment for infertility in couples and [36], who believed that vitamin D was heavily involved in female reproduction. The study showed a highly significant increase in the level of LH in patients with female infertility compared to healthy controls, Table (3), and Figure (2). LH controls only higher levels during the duration and timing of a female menstrual cycle, which includes ovulation, uterine preparation for implantation of a fertilized egg, and ovarian estrogen and progesterone production in response to LH stimulation, theca cells in the ovary produce TESTO. In women, the latter is transformed into estrogen via the granulosa cells. LH flow causes mature follicles on the ovary to ovulate. Residual cells grow within ovulated follicles to create corpora lutea, which secretes the steroid hormones progesterone and estradiol. In most mammals, progesterone is necessary for pregnancy maintenance, whereas LH is required for the continual development and function of the corpora lutea [37]. Our study agrees with the study, which found LH to be one of the most important causes of infertility in women and was statistically significant ($p=0.0025$). also by [39], Who discovers that high or lowered FSH and LH levels indicate the existence of a mechanism. at the anterior pituitary gland that indicates an irregular distribution of FSH and LH, which may further explain it to be a probable cause of infertility owing to improper or delayed ovum maturation., and agrees with [40]. The study showed an increasingly significant value of FSH in patients with female infertility compared to healthy controls, Table (4), and Figure (3). FSH is important in both male and female sexual development and reproduction. During the follicular phase of the menstrual cycle, FSH promotes the development of ovarian follicles. As a dominant follicle takes over, estrogen and inhibin are secreted. FSH causes granulosa cells in ovarian follicles to generate aromatase, which converts androgens produced by thecal cells to estradiol [41]. Our study matched with [38], who finds that FSH serum level was a statistically significant difference between fertile and infertile females ($P=0.0022$), and [39]. The correlation coefficient (r) between the Vit.D and other biochemical variables in the patient's female, there was a significant positive correlation between the Vit.D and the Testo in the infertility patient (Table 5). The findings of the current study are consistent with [42], the last study show serum vitamin D level positively correlated with total TESTO ($P <0.001$) and free androgen index ($P < 0.001$). Vitamin D's effect on Total TESTO (TT) levels may be related to increased ovarian/adrenal TESTO synthesis and/or greater transformation of the precursor hormones de-hydroepiandrosterone (DHEA) sulfate and DHEA in peripheral tissues [43].

There was a significant positive correlation between the Vit.D and the LH in the infertility patient (Table 5). That agrees with [44], which correlation test showed a significant positive correlation between mean LH and vitamin D/25(OH)D ($P<0.05$). Increasing data suggest that Vitamin D might be important in female reproductive regulation [45]. There was a significant positive correlation between the Vit D & the HDL in the infertility patient (Table 5). Similar results were obtained by [46], who finds a positive correlation between Vit.D and HDL ($r=0.199$, $p\leq0.01$). Hyperandrogenemia and hyperinsulinemia may be the major causes of dyslipidemia in PCOS. This results in increased adipose cell access and consumption of fatty acid and lipolysis composition released into the circulatory system. Excess fatty acids in the liver enhance VLDL production, which finally results in dyslipidemia. A high TG blood concentration causes a decrease in HDL and an increase in LDL levels by altering the opposite direction of cholesterol. Hyperandrogenism also likely impacts lipid metabolism by increasing hepatic lipase activity, which plays a role in HDL molecule degradation [47]. There was a significant positive correlation between the Vit D & the LH/FSH in the infertility patient (table 5). Our study matched with [48], Who demonstrates a link between vitamin D and

LH/FSH ratio, vitamin D deficiency correction leads to a significantly higher rate of LH/FSH ratio correction (less than two) than women with uncorrected low vitamin D.

5. Conclusions

The findings further demonstrated that low levels of Vit.D may cause infertility in the female. It has been shown that changes in Vit.D levels affect infertility through several mechanisms. For this reason, it is necessary to consider examining the blood levels of Vit.D, when routinely exanimating patients with infertility.

6. Conflicts of interest

The authors declare no conflicts of interest.

7. Acknowledgments

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