ORIGINAL

Biotin levels in blood and follicular fluid and their associations with pregnancy outcomes in IVF/ICSI patients

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Abstract : It has been shown that biotin, a water-soluble vitamin (B_7), plays roles in reproductive functions, such as oocyte maturation and embryo development, in experimental animals. On the other hand, little is known about the clinical effects of biotin on human reproduction. In this study, serum and follicular fluid biotin levels were measured in patients who underwent in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), and their associations with reproductive outcomes were evaluated. As a result, biotin was detected in follicular fluid, as well as serum, and the biotin levels of follicular fluid were found to be positively correlated with those of serum. The biotin levels of serum were higher than those of follicular fluid, suggesting that biotin may be taken up into the follicular fluid from the blood. Although serum and follicular fluid biotin levels tended to be higher in pregnant patients than in non-pregnant patients, these data did not show the significant statistical difference. These findings indicate that biotin does not contribute to the maintenance of oocyte quality, and hence, it does not increase fertilization and pregnancy rates. J. Med. Invest. 69 : 65-69, February, 2022

Keywords **:** *biotin, IVF/ICSI, follicular fluid, pregnancy*

INTRODUCTION

Biotin, a water-soluble vitamin (B_7), was discovered about 50 years ago (1), and its physiological and biochemical roles have been well elucidated. Biotin is involved in in vivo carbon dioxide fixation reactions as a coenzyme of four types of carboxylase : methylcrotonyl CoA carboxylase, acetyl CoA carboxylase, pyruvate carboxylase, and propionyl CoA carboxylase (2). It is well known that biotin deficiency causes dysfunction of these metabolic pathways, and that these physiological impairments induce skin disorders, such as dermatitis and hair loss ; neuritis ; and an increased risk of infections (3, 4). In addition, biotin is also involved in glucose and lipid homeostasis (5-7) and the immune system (8). It has been shown that biotin-deficient conditions may disturb oocyte growth and quality, embryonic development, and fetal development (9-15), and it has been suggested that biotin functions as a growth factor in oocyte maturation and the development and differentiation of embryos (16-19). However, most of these results were obtained from basic research using experimental animals, and there is little data on the clinical effects of biotin on human reproduction. Recently, some clinical studies focusing on in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) patients revealed that specific vitamins play pivotal roles in oocyte maturation and embryonic development (20, 21), whereas the roles of biotin were not evaluated in these studies. Thus, this study aimed to measure the biotin levels in blood and follicular fluid in IVF/ICSI patients and to evaluate the associations between them and reproductive outcomes.

Received for publication October 5, 2021 ; accepted December 2, 2021.

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MATERIALS AND METHODS

Study design

This prospective cohort study was approved by the clinical research review board of Tokushima University (Approved No. 3745). Written informed consent was obtained from all subjects after they had received an explanation about the study. Infertile patients (30 to 44 years old) who were scheduled for IVF/ICSI treatment between July 2020 to January 2021 were enrolled. The primary aim of this study was to evaluate the biotin levels in serum and follicular fluid during treatment and the relationship between serum biotin levels and those in follicular fluid. The secondary aim was to compare the biotin levels in serum and follicular fluid between pregnant and non-pregnant groups.

Ovarian stimulation protocols and embryo transfer policy

The patients were scheduled for controlled ovarian hyperstimulation, involving either a gonadotrophin-releasing hormone (GnRH) agonist- or antagonist-based protocol. In the GnRH agonist-based protocol, the patients were started on buserelin acetate in the midluteal phase of the preceding cycle. The administration of follicle-stimulating hormone (FSH) or human menopausal gonadotrophin (hMG) was initiated within seven days of withdrawal bleeding, and the initial dose was adjusted based on the patient's antral follicular count, age, and anti-Müllerian hormone level and was maintained for about five days. Subsequent FSH or hMG doses were determined according to follicular maturation, as assessed by ultrasound sonography. In the GnRH antagonist-based protocol, ganirelix treatment was started when the dominant follicle reached ≥14 mm in diameter. When the follicle size reached about 18 mm, 5,000 IU human chorionic gonadotrophin (hCG) was administered. Around 36 h after the administration of hCG, transvaginal ultrasound-guided oocyte pick-up (OPU) was performed. Semen from the participant's partner was prepared using the swim-up technique, and IVF,

ICSI, or both was carried out. Single embryo transfer was conducted at the cleavage stage (day 2 or day 3) or blastocyst stage (day 5 or day 6) based on the fertilized ovum count. As some patients were at high risk of ovarian hyperstimulation syndrome, a freeze-all strategy was employed, and frozen-thawed embryo transfer was performed. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac, according to transvaginal sonography.

Serum and follicular fluid collection

Serum samples were obtained on menstrual days 3-5 in the previous cycle, on the first day of stimulation, and on the day of hCG trigger ; i.e., 2 days before the OPU. The serum was separated by centrifugation at 3500 rpm for 15 min, and the samples were frozen at -40℃. To ensure that follicular fluid was obtained from a single follicle and avoid contamination by blood or the flushing medium or the mixing of follicular fluid during oocyte retrieval, the follicular fluid from the first retrieved follicle was collected. Thereafter, follicular fluid samples with matched mature metaphase Ⅱ oocytes were centrifuged at 1500 rpm for 15 min, and the supernatants were stored at -40℃.

Detection of serum and follicular fluid biotin levels

The biotin levels in serum and follicular fluid were analyzed using a commercially available biotin enzyme-linked immunosorbent assay kit (Immundiagnostik, Bensheim, Germany), according to the manufacturer's instructions. This assay kit was used in a previous study, and reliable results were obtained (22, 23). Briefly, each sample (150 μL) was added to individual wells. The samples and standard biotin solutions were pre-incubated with 50 μL of conjugate (enzyme-labeled biotin) solution for 30 min at 25℃. One hundred μL of the enzyme substrate, 3,3',5,5'-tetramethylbenzidine (TMB), was added to each well, before the mixture was incubated for 15 mins at 25℃ in the dark. After the reaction had been stopped by adding 100 μL of hydrochloric acid to each well, absorbance was measured at 450 nm and 620 nm using a microplate reader (SpectraMax i3 ; Molecular Devices). Absorbance was calculated by subtracting the reference absorbance seen at 620 nm from that observed at 450 nm.

Statistical analyses

The Mann-Whitney U test or Kruskal-Wallis test followed by the Steel-Dwass test were used for comparisons between two groups or among multiple groups, respectively. Pearson's correlation coefficient was used as a statistical measure of the strength of a linear relationship between data pairs. *P*-values of <0.05 were regarded as significant.

RESULTS

A total of 31 females were enrolled in this study. Twenty-eight females were treated with the GnRH agonist-based protocol, and 3 females were treated with the GnRH antagonist-based protocol. The patients' clinical background data are summarized in Table 1. All patients underwent OPU and single embryo transfer, and clinical pregnancy was achieved in 11 cases. The background data ; i.e., age, body mass index, hormonal levels, and total FSH/hMG doses, of the patients who did (the pregnant group) and did not (the non-pregnant group) become pregnant did not differ (Table 1).

The serum biotin levels on menstrual days 3-5 in the previous cycle (baseline level) and on the first day of stimulation were significantly higher than the follicular fluid biotin level (Fig. 1). On the other hand, the serum biotin level on the day of hCG trigger did not differ from the serum biotin levels seen at other timepoints or the follicular fluid biotin level. The serum biotin levels seen on menstrual days 3-5 (baseline level) were positively correlated with follicular fluid biotin levels (Fig. 2). Similarly, the serum biotin level on the day of hCG trigger was positively correlated with the follicular fluid biotin level. On the other hand, serum biotin level at baseline and that in hCG trigger was not correlated (data not shown).

The serum biotin levels on menstrual days 3-5 in the previous cycle tended to be higher in the pregnant group than in the non-pregnant group ($P = 0.06$) ; however, this trend did not reach significance (Table 2). Similarly, the follicular fluid biotin level tended to be higher in the pregnant group than in the non-pregnant group ($P = 0.05$). On the other hand, the serum biotin level at the hCG trigger did not differ between the pregnant and non-pregnant groups.

DISCUSSION

In this study, we demonstrated that biotin is detectable in follicular fluid, as well as in serum, and that the biotin level in follicular fluid is positively correlated with that in serum. Although serum and follicular fluid biotin levels tended to be higher in pregnant patients than in non-pregnant patients, these data did not show the significant statistical difference. On the other hand, serum biotin levels may not change during the IVF/ICSI cycle. As far as we know, this is the first study to evaluate the clinical effects of biotin on human reproduction.

As noted above, it has been reported that biotin deficiency may disturb oocyte growth and quality, embryonic development, and fetal development in some species (9-13, 23), and it has been

Table 1. Patient characteristics

	Pregnancy	Non-pregnancy	Total
Number of patients	11	20	31
Age (years)	35.5 ± 2.9	38.3 ± 3.5	37.6 ± 3.6
BMI (kg/m^2)	21.0 ± 2.4	21.9 ± 3.1	21.6 ± 2.9
Baseline E2 level (pg/mL)	56.0 ± 23.5	54.6 ± 34.4	55.1 ± 31.0
Baseline LH level (IU/L)	6.3 ± 2.8	5.5 ± 1.6	5.8 ± 2.1
Baseline FSH level (IU/L)	8.4 ± 3.3	7.2 ± 1.9	7.7 ± 2.6
E2 (pg/mL) level at hCG trigger	4270 ± 2559	3778 ± 2708	3952 ± 2667
Total hMG dose (IU)	3259 ± 1222	3090 ± 1118	3150 ± 1159

Baseline LH, FSH, and E2 levels were measured on menstrual day 3-5 in prior cycle. E2 level was measured on the day when hCG trigger was determined.

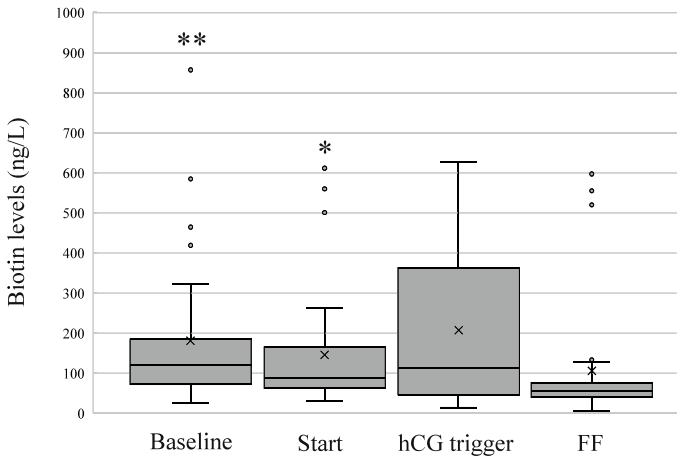

Figure 1. Biotin levels in serum and follicular fluid. Serum biotin levels were measured on menstrual days 3-5 (Baseline), on the first day of stimulation (Start), and on the day of hCG trigger (hCG trigger). The biotin level of follicular fluid (FF) was also measured in representative oocytes. The boxes extend from the 25th to 75th percentiles. The whiskers indicate the 5th and 95th percentiles. The lines in the middle of the boxes indicate median values. *$P < 0.05$, **$P < 0.01$ vs. FF.

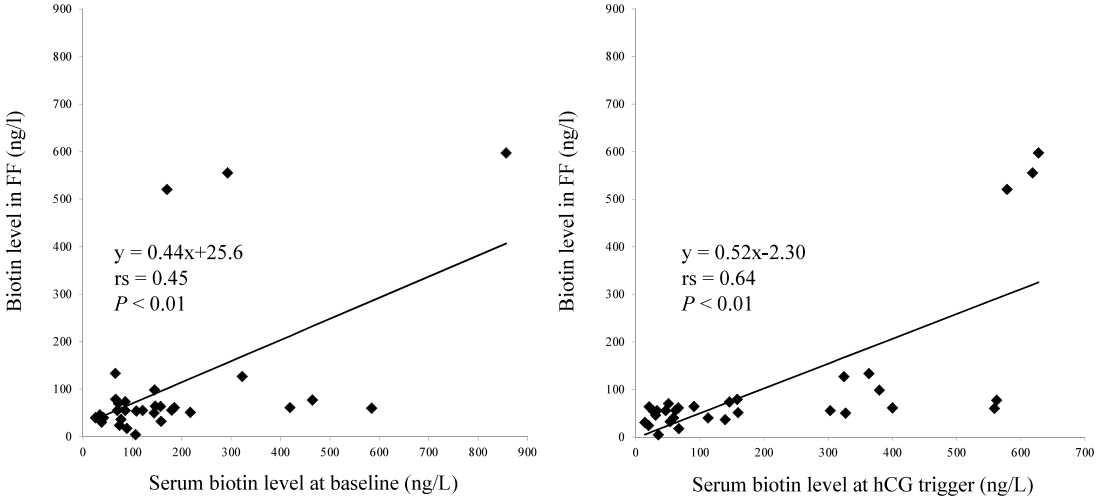

Figure 2. Correlation between the biotin levels of follicular fluid and serum. The baseline serum biotin level was measured on menstrual days 3-5 in the previous cycle. The serum biotin level at the hCG trigger was measured on the day of hCG trigger.

Table 2. Biotin concentrations in pregnancy and non-pregnancy groups

		Pregnancy group	Non-pregnancy group	*P* value
Number of patients		11	20	-
Biotin level (ng/L)	in serum at baseline	292 ± 253	117 ± 64.6	0.06
	in serum at hCG trigger	259 ± 227	127 ± 117	0.15
	in follicular fluid	147 ± 178	83.6 ± 123	0.05

Serum biotin level at baseline was measured on menstrual day 3-5 in prior cycle. Serum biotin level at hCG trigger was measured on the day when hCG trigger was determined. Follicular fluid biotin level was measured in representative oocyte.

suggested that biotin functions as a growth factor in oocyte maturation and development and embryo differentiation (16-19). In a study of domestic fowl, Taniguchi *et al*. demonstrated that biotin is an essential nutrient and suggested that it may play a major role in the normal morphogenesis of embryos (19). Similarly, several rodent studies have indicated that biotin deficiency during pregnancy may increase the rates of abnormal fetal development and growth and affect the rates of absorption and embryonic death (9, 11, 13). In addition, some studies have suggested that biotin deficiency disturbs oocyte growth in the ovaries and that sufficient biotin intake is required for the production of high-quality oocytes in mice (14, 15). Furthermore, maternal biotin deficiency during pregnancy might be the risk of preterm labor or fetal growth restriction (23). As shown in the results section, the serum biotin levels of the pregnant group tended to be higher than those of the non-pregnant group in the present study. However, these data did not show the significant statistical difference. Therefore, it should have been concluded that the serum and follicular fluid biotin levels did not have significant difference between pregnant patients and non-pregnant patients. These findings indicate that biotin does not contribute to the maintenance of oocyte quality, and hence, increase fertilization and pregnancy rates. On the contrary, the serum biotin levels seen before the administration of hCG varied widely, but did not differ between the pregnant and non-pregnant groups. As serum hormone levels, such as the levels of estradiol, FSH, and luteinizing hormone, are highly variable at this point, it is possible that these factors may have affected serum biotin levels and obscured any underlying differences between the pregnant and non-pregnant groups.

It has been reported that the composition of follicular fluid strongly influences oocyte quality, developmental competence, and the quality of embryos (24-26). For this reason, many studies have highlighted follicular fluid as an important source of potential non-invasive biomarkers of oocyte and embryo quality, as well as biomarkers for predicting clinical outcomes (25-27). However, follicular fluid biotin levels and their associations with reproductive outcomes have not been evaluated in previous studies. In this study, we showed that biotin can be detected in follicular fluid and that its levels in follicular fluid and serum were positively correlated. In addition, the levels of biotin in serum were higher than those seen in follicular fluid, suggesting that biotin may be taken up into follicular fluid from the blood. As noted above, in this study the mean follicular fluid biotin level of the pregnant group was higher than that of the non-pregnant group.

Some studies have shown that sufficient nutritional conditions are very important for suppressing oocyte abnormalities (28-30) and that oocytes consume pyruvate as their main energy source during meiosis (31-33). Pyruvate is metabolized to acetyl-CoA and oxaloacetate in oocytes, and biotin plays pivotal roles in the formation of oxaloacetate from pyruvate. In addition, biotin is known to be related to the mRNA expression of histones H2A, H3, and H4, further supporting the idea that it contributes to maintaining oocyte quality (34-36). As fertilization and pregnancy rates are strongly influenced by oocyte quality, it is possible that the trend towards high biotin levels in follicular fluid may have increased the pregnancy rate in this study.

In summary, we showed that biotin could be detected in follicular fluid and that serum biotin levels and follicular fluid biotin levels were positively correlated. On the other hand, although serum and follicular fluid biotin levels tended to be higher in pregnant patients than in non-pregnant patients, these data did not show the significant statistical difference. Recently, the number of patients receiving IVF/ICSI treatment has increased year by year in Japan (37-39). The present and previous studies provide useful information for improving the clinical results of these treatments.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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