

## ระดับสังกะสีในน้ำอสุจิ (seminal plasma) ในชายที่มีบุตรยาก

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## Zinc Level in Seminal Plasma of Infertile men

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### บทคัดย่อ

สังกะสีเป็นจุลสารอาหารที่สำคัญในระบบสืบพันธุ์ มีหน้าที่เกี่ยวข้องกับการสร้างและการทำงานของเซลล์สืบพันธุ์ในเพศชาย อย่างไรก็ตามความสัมพันธ์ระหว่างระดับสังกะสีในชายที่มีบุตรยาก (infertile) และผลการตรวจวิเคราะห์น้ำอสุจียังไม่มีข้อสรุปที่ชัดเจน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อวัดระดับสังกะสีใน seminal plasma โดยวิธี atomic absorption spectrophotometer ในกลุ่มชายที่มีบุตรยากที่มีผลการวิเคราะห์น้ำอสุจิปกติและผิดปกติ โดยทำการศึกษาในกลุ่มชายที่มีบุตรยาก 135 ราย ในตัวอย่างที่เหลือจากการวิเคราะห์ของหน่วยชีววิทยาการเจริญพันธุ์ ภาควิชาสูติศาสตร์และนรีเวชวิทยา โรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่น โดยแยกตัวอย่างเป็น 2 กลุ่มคือ กลุ่มที่มีผลการตรวจวิเคราะห์น้ำอสุจิปกติ (normozoospermia) จำนวน 61 ราย และกลุ่มที่มีผลการวิเคราะห์น้ำอสุจิผิดปกติ (abnormal group) ซึ่งประกอบด้วย กลุ่มเซลล์สืบพันธุ์มีจำนวนน้อย กลุ่มเซลล์สืบพันธุ์เคลื่อนที่ช้า หรือกลุ่มที่ผิดปกติทั้งสองอย่างรวมกัน และกลุ่มที่ไม่มีเซลล์สืบพันธุ์รวมกันจำนวน 74 ราย ผลการศึกษาพบว่าในกลุ่มที่มีผลการวิเคราะห์น้ำอสุจิปกติและกลุ่มที่มีผลการวิเคราะห์น้ำอสุจิผิดปกติ มีค่าระดับสังกะสีเฉลี่ยเท่ากับ 14.21 มก% และ 14.72 มก% ตามลำดับ และค่าที่ได้แตกต่างอย่างมีนัยสำคัญทางสถิติ ( $P < 0.001$ ) ถึงแม้ว่าสังกะสีจำเป็นสำหรับการทำงานของเซลล์สืบพันธุ์ แต่ระดับสังกะสีที่มากเกินไปอาจเป็นสาเหตุให้เซลล์สืบพันธุ์ทำงานผิดปกติ จากผลการศึกษาจะเห็นได้ว่าระดับสังกะสีในเพศชายที่มีบุตรยากที่มีผลการวิเคราะห์น้ำอสุจิปกติและผิดปกติมีค่าแตกต่างกันแต่ค่าเฉลี่ยของทั้งสองกลุ่มแตกต่างกันน้อยมาก การตรวจวัดระดับสังกะสีในชายกลุ่มที่มีบุตรยากจึงอาจไม่จำเป็นต้องทำ เนื่องจากไม่ได้ช่วยในการแยกกลุ่มปกติออกจากกลุ่มผิดปกติอย่างชัดเจน แต่ควรมีการศึกษาเพิ่มเติมเช่น เปรียบเทียบระดับ

### Abstract

Zinc is an essential micronutrient for spermatogenesis and normal sperm function. However, the correlation of zinc level in seminal plasma and the results of semen analysis is controversial. This study aimed to evaluate zinc levels in seminal plasma of infertile men. One hundred thirty five left-over semen of infertile men collected from the Reproductive Biology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, KGU were analyzed for semen analysis. The seminal plasma was then classified into 2 groups according to its results of semen analysis. Normal group was named normozoospermia ( $n = 61$ ). The samples classified as oligospermia, asthenozoospermia, oligoasthenozoospermia, and azoospermia were included in abnormal group ( $n = 74$ ). They were used for zinc determination by atomic absorption spectrophotometer. Zinc levels of normal and abnormal group were 14.21 mg% and 14.72 mg%, respectively. Mean values of zinc level in two groups were significantly different ( $P < 0.001$ ) with 95 % CI of 11.75-16.68 mg% for normal group, and of 12.64-16.81 mg% for abnormal group. The results implied that determination of zinc level in seminal plasma of infertile men might not be very useful due to a miniscule difference mean value of this ion in both groups. Further study of zinc contents should be investigated in subgroup of semen analysis, or in comparing of fertile and infertile men.

สังกะสีในกลุ่มย่อยแต่ละกลุ่มหรือศึกษาระดับสังกะสีในชายปกติ และชายที่เป็นหมัน เป็นต้น

**คำรหัส:** สังกะสี, น้ำอสุจิ, ผลการวิเคราะห์น้ำอสุจิ

**Key words:** Zinc, Seminal plasma, Semen analysis

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## Introduction

Infertility is defined as a failure to conceive after 1 year of regular unprotected intercourse with the same partner. However, the term "infertility" implies a definitive inability to conceive. Therefore, couples who do not conceive in less than 1 year should be regarded as subfertile.<sup>1</sup> Infertility problem affects about 10-12% of couples. It has been reported that 40-50% of infertility is due to a male factor.<sup>2</sup> One of basic investigations of male infertility is semen analysis. Therefore, the results of semen analysis provide a basic data regarding reproductive male function.

Zinc is one of the most interesting nutritional trace elements in reproductive system. Zinc in man is a cofactor of more than 200 metalloenzymes and plays an important role in normal testicular development, spermatogenesis, and sperm motility. The impairment of zinc in reproductive system causes hypogonadism, and gonadal hypofunction.<sup>3</sup> The clinical symptoms of hair loss, skin lesions, delayed wound healing, dysfunction of the immune system and growth retardation can be found in a patient with zinc deficiency status. It has been reported that zinc in seminal plasma involved in nuclear chromatin decondensation<sup>4</sup> and acrosin activity.<sup>5</sup> Zinc deficiency in nucleus may destabilize quaternary structure of the chromatin, thus reducing the fertilizing capacity of the spermatozoa.<sup>6</sup> The experiment conducted in adult males revealed that leydig cell synthesis of testosterone was depended on adequate dietary zinc.<sup>7</sup> In addition, zinc is required to alter genetic expression from phase to phase of the cell cycle.<sup>8</sup> The depletion of dietary zinc decreases semen volume and serum testosterone.<sup>9</sup> It has been suggested that zinc is necessary for the conversion of testosterone into biologically active form 5 $\alpha$ -dihydrotestosterone (DHT) via the role of 5 $\alpha$ -reductase enzyme.<sup>10</sup>

Zinc content in seminal plasma is predominantly secreted by prostate gland, and may reflect the prostatic function. However, the association of zinc contents in

seminal plasma and other spermatic parameters in both fertile and infertile men is still controversial. Standwell-Smith<sup>11</sup> found the positive correlation of sperm concentration and zinc level in fertile men, but not in infertile men, which is in accordance with the finding of Mahan.<sup>12</sup> However, Wong WY<sup>13</sup> demonstrated that zinc contents in fertile were not differed from those of infertile men. Abou-Shakra<sup>14</sup> reported that zinc content in men grouped by sperm concentration was not differed from each other. However, this finding was not observed by Behne.<sup>15</sup> The relevance of zinc in spermatic function has been focused on sperm motility. The percentage of sperm motility was correlated to zinc level.<sup>16-17</sup> The oral zinc supplementation in men increased proportion of spermatozoa with progressive motility.<sup>18</sup>

Because zinc plays a critical role in sperm function, and the relation of zinc in seminal plasma and spermatic function has not yet been concluded. This study attempts to assess a correlation between zinc contents in seminal plasma and spermatic parameters in infertile men.

## Materials and methods

### Samples preparation

Zinc level in seminal plasma of 135 Thai males who consulted for infertile problem in the year 2003 at Srinakarind Hospital, Khon Kaen University, Khon Kaen Province, Thailand was studied. Semen samples were collected in plastic cups by masturbation after 3-4 days abstinence. Semen analysis of all samples according to WHO criteria<sup>19</sup> were performed including of sperm count (Makler counting chamber), viability (eosine-nigrosine stain) and morphology (hematoxylin-eosine stain) within 30 min after liquefaction. Semen volume was measured in graduated tubes and total sperm counts were calculated. Sperm motility was determined under light microscope. On the basis of these parameters, however, two of sperm concentration and sperm motility were considered as the important parameters. According to

semen analysis results, the patients were assigned into 2 groups of normal and abnormal group. Samples with normal sperm quality (n = 61) were classified as normozoospermia or normal group. Abnormal group (n = 74) included the samples with oligozoospermia (sperm concentration is lower than  $20 \times 10^6$  cell/ml, asthenozoospermia (sperm with forward motility is less than 50% viewed under light microscope), oligoasthenozoospermia (sperm count is lower than  $20 \times 10^6$  cell/ml with forward motility is lower than 50%), and azoospermia (complete absence of spermatozoa in the ejaculate).

After determining semen analysis, seminal plasma was separated from spermatozoa by centrifugation at 1,800 xg for 10 min at room temperature. A drop of well-mixed seminal plasma was viewed under light microscope to assure that seminal plasma was free from contaminated cells. The supernatant was frozen at  $-20^\circ\text{C}$  for further analysis.

Zinc contents in seminal plasma were determined by using flame atomic absorption spectrophotometer<sup>20</sup> by GBC 800 (Australia). The parameters of the instrument were adjusted as followed : lamp current 5.0 mA, optimal wavelength at 213.9 nm, air:acetylene.

The seminal plasma was diluted 200 times with deionized water before measurement, and the results were expressed as zinc content in  $\mu\text{g}\%$ . Standard zinc solutions were prepared using zinc standard solution (Fluka, Switzerland) in deionized water. The standard curve was plotted from 25-250 ppm concentration of zinc. The mean of duplicate testing was used for analysis. Contamination-free preparation was assured by washing out all glasswares with 1% nitric acid and rinsed properly with deionized water used for both samples and standard dilutions.

The mean values of two groups were compared by student t-test. Statistical analysis was performed by using SPSS 10.0.1 program.

## Results

According to semen analysis, normal group revealed a higher mean value of % motility, % viability and sperm concentration than those of abnormal group. However, the mean of semen volume from normal group was slightly lower than those of abnormal group, 2.74 ml and 2.93 ml, respectively. Table 1 showed the mean values obtained in this study for the various parameters measured in the

semen sample and zinc content in seminal plasma. It should be taken into consideration that all spermiatic parameters of normal and abnormal group demonstrated higher mean value than normal range of semen analysis. WHO suggests criteria to normal semen included the semen volume of 2 ml or more, sperm concentration of 20 million/ml or more, sperm motility of 50% or more with forward movement, and sperm morphology of 30% or more with normal form.<sup>19</sup> This finding might be due to the individuals with at least one spermiogram anomaly such as sperm motility or sperm concentration being grouped in abnormal group.

For the determination of zinc by atomic absorption spectrophotometer, it was found that zinc level in seminal plasma from normal and abnormal group was 14.21 mg% and 14.72 mg%, respectively. The mean value of zinc contents in abnormal group was significantly higher than in normal group ( $P < 0.001$ ). 95% confidence interval of the difference of both groups were ranged from 11.75-16.68 mg% and 12.68-16.81 mg% in normal group, and in abnormal group, respectively.

## Discussion

The semen analysis of our study is in accordance with previous report.<sup>21</sup> However, the mean volume of both groups in this present study was slightly higher than previously documented. World Health Organization (WHO) criterion for normal semen includes the volume of 2 ml or more, sperm concentration of 20 million/ml or more, sperm motility of 50% or more with forward movement and sperm morphology of 30% or more of normal forms.<sup>19</sup> We interestingly found that semen analysis of samples divided upon spermiatic parameters into normal and abnormal group showed higher mean value of parameters than this criteria. This might be due to our group method using at least one abnormal parameter to classify the sample. Meanwhile, other parameters of the sample could be normal.

In the present study we also calculated zinc level in seminal plasma of all samples. The results revealed the mean value of zinc content of  $14.5 \pm 9.25$  mg%. This zinc concentration was correspond well with previously published results that showed zinc concentration in infertile men ranging from 13.2 mg% to 15.7 mg%.<sup>22,23</sup> This result was also in comparable with different method measurement. Colorimetric method demonstrated zinc of

**Table 1** Zinc level in seminal plasma of normal and abnormal group and semen analysis  
(Values expressed as the mean  $\pm$  standard deviation)

Groups	Volume (ml)	Motility (%)	Viability (%)	Sperm count (X10 <sup>6</sup> cell/ml)	Zinc Contents (mg%)	95% CI (mg%)
Normal (n = 61)	2.74 $\pm$ 1.19	73.92 $\pm$ 7.62	72.73 $\pm$ 20.25	115.53 $\pm$ 70.65	14.21 $\pm$ 9.61 <sup>δ</sup>	11.75 - 16.68
Abnormal (n = 74)	2.93 $\pm$ 1.8	52.12 $\pm$ 17.73	59.33 $\pm$ 14.74	32.56 $\pm$ 61.64	14.72 $\pm$ 8.99	12.64 - 16.81

<sup>δ</sup>Statistically significant (P < 0.001)

14.77 mg%<sup>24</sup> and neutron activation analysis was found zinc ranged from 2.5-25.7 mg% .<sup>25</sup> In our study, we found the mean value of zinc level in seminal plasma of abnormal group was significantly higher than those of normal group. Although zinc is needed for normal sperm function, high level of this ion may cause spermatozoa functional defect such as sperm motility in asthenozoospermia samples.<sup>27</sup> Moreover, the mean value of both groups was in miniscule difference. In this study, therefore, normal group could not be distinguished from abnormal group using zinc level in seminal plasma.

The role of zinc in relation to male infertility is inconclusive. Some reports have found no significant correlation between zinc concentration in seminal fluid and sperm density or motility<sup>11</sup>, while others concluded that zinc concentration in semen decreased with decreasing number and activity of spermatozoa.<sup>25-26</sup> However, the highest zinc level in seminal plasma was revealed in asthenozoospermic group<sup>16,27</sup>, and this level was significantly differed from other studied. This observation suggested the influence of zinc in sperm motility. Although, relevance of zinc and sperm motility has been reported, high zinc concentration may inhibit progressive motility of spermatozoa.<sup>16,27,28</sup> However, zinc is necessary to stabilize spermatozoa membrane by promotion the oxidation of -SH to form -S-S.<sup>29-30</sup>

Finally, seminal plasma zinc content may not be the most appropriate fluid used as an elemental indicator of male infertility. However, it would be of interest to investigate the relationship between seminal plasma zinc level in fertile and infertile men, or in subgroup of semen quality.

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