THE ROLE OF SEMINAL CALCIUM IN MALE INFERTILITY

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SUMMARY: Concentrations of total calcium were analyzed in the seminal fluid of 20 fertile and 35 infertile men by the use of automated analyzer and commercial kit. Normozoospermic infertile men exhibited a significantly lower calcium level, compared to the fertile group. The lower calcium concentration determined in the oligo-azoospermic infertile men, was not significantly different when compared to the fertile men. In the subject and control groups, no significant correlations existed between seminal calcium and sperm density or percent motility. The data from our study strongly suggest that seminal calcium level has to be determined in normozoospermic infertile men, who also possess the asthenozoospermia problem.

Key Words: Seminal calcium, infertility, male.

INTRODUCTION

Conflicting data have been presented concerning the effect of seminal calcium on male infertility. Umeyama *et al.* observed that seminal calcium concentrations were almost the same between fertile and infertile men (1). Prien et al. demonstrated no significant difference in the seminal concentration of total calcium, regardless of spermatozoa motility; however, semen of men with hypomotility exhibited a significantly lower Ca^{++} concentration when compared with that of men with normal motility (2). In another study, seminal calcium levels were determined in four groups of infertile men (normo-, oligo-, severely oligo-, and azoospermic), and no statistically significant variations were found in relation to infertility classification (3). Some studies (1,3) have demonstrated that a relationship exists between high calcium level and fertility in men. The main objectives of this study are: (1) to check the variations of seminal calcium levels among fertile and two groups of infertile men; and (2) to determine the effect of seminal calcium on spermatozoa motility.

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MATERIALS AND METHODS

Infertile men (n=35) in the age range of 24 to 40 (mean ± SE, 30.4±0.57) years, with no apparent chronic or acute disease, were selected for the study. These subjects were chosen from men who consulted the Çukurova University Medical School Clinic for infertility tests after being unable to achieve pregnancy for at least 2 years and from whom the female partners have shown no diagnosed cause of infertility or any disorder of ovulation (hormone test, laparoscopy). Infertile men were divided into two groups; normo-zoospermic (n=17), a sperm density of not $<20X10^6$ /mL; and oligo or azoospermic (n=18). In the second infertile group, two of the subjects were azoospermic.

Selection of fertile control subjects (in the age range of 20 to 40; means ± SE, 29.2±2.33) was restricted to 20 men who had fathered a child in the last 2 years.

Before sample collection, the subject had to abstain from any form of sexual activity for at least 3 days. Semen samples were collected by masturbation, and the entire ejaculate was

passed into a wide neck sterile container. Since the specimen has to be collected in as pure form as possible, subjects were advised not to use any kind of lubricants or contraceptive creams and especially not to collect the sample in a condom "sheath". The samples were delivered to the laboratory within 30 minutes after collection, ensuring that they were kept warm (at nearly body temperature) in order not to affect the sperm motility. The number, motility, and morphology of spermatozoa were assessed in a fraction of each sample (200μL), by using computer assisted semen analysis system (Hamilton Thorn Research, Inc. HTM-C 2030 Motility Analyzer, Danvers, USA); the remainder of the sample was centrifuged, and the seminal plasma was stored frozen at -70°C until analysis for total calcium. Measurements of seminal calcium levels were carried out by the use of automated analyzer (Technicon, RA-XT model, England) and commercial kit (Cromatest).

Data were analysed using Student's t-test, and Pearson correlation coefficient and correlation coefficient significance tests (SPSS Release 4.1 for IBM VM/CMS). All values were expressed as the mean ± SE of the mean.

Table 1: Classification of infertile semen samples, according to WHO criteria (4).

	n
Normozoospermic*	5
Oligozoospermic	1
Asthenozoospermic*	7
Asthenoteratozoospermic*	5
Oligoasthenozoospermic	10
Oligoasthenoteratozoospermic	5
Azoospermic	2
Total	35

(*) Normozoospermic samples.

RESULTS

Characteristics of infertile semen samples, are indicated in Table 1. Seminal fluid of normozoospermic infertile men exhibited a significantly lower calcium concentration ($p<0.05$), when compared with that of fertile men (mean ± SE values, respectively, 113±15.2 and 152±9.9 μg/mL). The lower calcium concentration determined in the oligo-azoospermic infertile men (mean±SE, 137±14.5 μg/mL), was not significantly different when compared to the fertile group (Table 2). Seminal calcium concentration did not differ significantly between two infertile groups.

Table 2: Seminal calcium levels in the fertile and infertile men (mean ± SE).

Groups	Seminal calcium (μg/dL)
Fertile (n=20)	152 ± 9.9
Normozoospermic infertile (n=17)	113 ± 15.2*
Oligo-/azoospermic infertile (n=18)	137 ± 14.5

(*) $p<0.05$, when compared to the fertile group.

In fertile and infertile groups, there were no significant relationships between seminal calcium and sperm density or percent motility (Table 3).

Table 3: Relationship between seminal calcium and sperm density or percent motility (values express coefficient of correlation).

	Fertile (n=20)	Infertile (n=33)
Spem density	-0.16	-0.10
% Motility	-0.30	-0.13

DISCUSSION

It has long been known that extracellular calcium is required for successful fertilization. In all systems examined, an influx of Ca^{+2}, is required to initiate the acrosomal reaction, with its attendant release of enzymes and membrane alterations necessary for sperm-egg interaction (5). There is also evidence that this ion may be involved in sperm motility (6-8).

In the present study, the mean seminal calcium value in fertile men was found to be higher than those of both infertile groups. The lowest value detected in the normozoospermic infertile groups was significant when compared to the fertile men (Table 2). These findings may suggest that significantly decreased seminal calcium may be related to infertility seen in normozoospermic subjects.

In a study performed by Umeyama *et al.* (1), seminal calcium concentrations were almost the same between fertile and infertile men, and the highest level was determined in the normozoospermic infertile sub-

jects. Our findings do not confirm these results, but are similar with those of Abou-Shakra (3), who determined the lowest concentration in the normozoospermic infertile group, being nonsignificant in relation to infertility classification.

A study by Prien *et al.* (2) examined the relationship between sperm motility and seminal Ca^{++}. They determined that seminal fluid of men with hypomotility exhibited a significantly lower Ca^{++} concentration when compared with that of men with normal motility. Although we have assessed total calcium level, our results do not support this finding, since we have found weak and negative, but nonsignificant correlations between seminal calcium and sperm motility both in the fertile and infertile groups (Table 3). However, another study indicating that a high concentration of calcium suppresses sperm motility, confirms our findings (9). Of the 33 infertile subjects included in our study, 27 were asthenozoospermic (Table 1); in order to draw a more definite conclusion about seminal calcium and sperm motility, we plan to determine ionized calcium (Ca^{++}) level in the seminal fluid in a group including only asthenozoospermic infertile men, and make comparison with samples exhibiting normal sperm motility.

When our results are evaluated with those of other reports, it may be concluded that seminal calcium may be involved in sperm motility, and this effect can be stimulatory or inhibitory, depending on its concentration. As reported by Fraser (10), an optimal seminal calcium concentration is required to promote sperm motility, and all steps leading to successful fertilization. The most important result of our study reveal that seminal calcium concentration has to be determined in normozoospermic infertile men, who also have the asthenozoospermia problem. Further study is necessary for an understanding of the male infertility and seminal calcium relationships.

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