Effect of Prolonged Storage of Seminal Plasma on Levels of Calcium and Magnesium

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Background: In laboratories, the semen or seminal plasma samples are stored before estimation of different elements without knowing the adverse effect of storing. The present study shows the levels of Ca^{++} and Mg^{++} in the seminal plasma before and after the storage. **Methods:** Atomic absorption spectrophotometry (AAS) was used for estimating calcium and magnesium levels in the seminal plasma. Samples were stored for one year in different containers such as polyethylene, plastic, ordinary glass, and borosil glass. Calcium and magnesium levels were estimated after this period by AAS.

Key words: seminal plasma storage; calcium; magnesium; containers; glass; borosil; plastic; polyethylene

The total area in contact with containers was considered in the final calculation. **Results:** After 1-year storage, the levels of calcium and magnesium had changed. The most suitable container for storage of calcium was ordinary glass ($P > 0.05$) whereas ordinary glass ($P > 0.05$), polyethylene ($P > 0.05$), and borosil glass ($P > 0.05$) are the most suitable for magnesium. **Conclusion:** In any elemental study, the most suitable containers are recommended for storage of the semen/seminal plasma samples. J. Clin. Lab. Anal. 26:315–316, 2012. © 2012 Wiley Periodicals, Inc.

Numerous reports highlight the importance of different elements in the seminal plasma for male fertility (1–4). Considering the importance of calcium and magnesium in cell functioning, some of the workers concentrated on the study of these electrolytes. In many elemental studies, samples are preserved for a certain period of time in order to collect new samples. Then the stored and collected samples are estimated together to reduce time and instrument availability making the study economically feasible. The current study was conducted to find out a suitable container used for a prolonged storage of the seminal plasma. The suitability of the container was calculated based on the estimation of levels calcium and magnesium in the seminal plasma before and after the storage.

All the tested containers were thoroughly cleaned to avoid contamination. Semen samples were donated by healthy subjects, who were in 18–35 age group. They gave their consent prior to study. Semen was collected by masturbation after maintaining an abstinence of 3–5 days.

The entire sample of a healthy subject was collected in a glass bottle at 8 am (± 0.5 hr).

Samples were centrifuged at 2,000 rpm for 20 min. The supernatant, seminal plasma, was collected in a clean glass container and stored below -4°C till the estimation. All samples were pooled, thawed, homogenized, and diluted using fresh triple-glass distilled water. The estimation of calcium and magnesium in the seminal plasma was done by atomic absorption spectrophotometry (AAS) (Perkin Elmer A373, Perkin Elmer, Waltham, MA) and values were recorded. Remaining samples were stored in different containers such as polyethylene, plastic, ordinary glass,

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Received 31 March 2012; Accepted 19 April 2012

DOI 10.1002/jcla.21523

Published online in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. Level of Calcium and Magnesium Before and after Storage

Elements (mg/dl)	Before storage	After storage			
		Polyethylene	Plastic	Ordinary glass	Borosil (corning)
Ca++	11.6 ± 0.98	30.0 ± 5.91	53.56 ± 6.09	27.5 ± 6.88	28.61 ± 3.84
Mg++	4.87 ± 0.54	6.4 ± 0.79	30.0 ± 5.69	5.75 ± 1.09	7.1 ± 0.93

and borosil glass. The containers were stored below -4°C for 1 year. After this period, samples were thawed and homogenized and levels of calcium and magnesium were re-estimated.

The levels of calcium and magnesium in the seminal plasma were estimated before and after the prolonged storage in different containers (Table 1). The total surface area of the container in contact with sample was considered while conducting a paired *t*-test. The results show that among different containers used in this study, the most suitable container for the calcium was ordinary glass ($P > 0.05$) whereas ordinary glass ($P > 0.05$), polyethylene ($P > 0.05$), and borosil glass ($P > 0.05$) were the most suitable containers for magnesium.

We found that the level of calcium and magnesium had changed after 1-year storage of seminal plasma in different containers. Plastic containers, which have been regularly used in many laboratories for storing biological fluid, was not suitable for preservation.

We used freshly prepared distilled water for cleaning purpose as well as for dilution of sample as it was known that the level of calcium ions changes (5) significantly in the stored distilled water and leaching of inorganic metals into sample occurs in a prolonged storage (6, 7). Moody and Lindstrom (8) reported that a linear polyethylene container was less contaminating, which did not fully match with our results. Skandhan et al. (9) reported that a polyethylene container was suitable for a prolonged stor-

age of seminal plasma for zinc, copper, and cadmium levels.

In conclusion, the importance of using a suitable container in an elemental study was shown. This study also showed that the concentration of elements changed in the samples stored in different containers.

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