

Split ejaculation study: semen parameters and calcium and magnesium in seminal plasma

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KEY WORDS

split ejaculate ► semen parameters ► calcium ► magnesium

ABSTRACT

Objectives. Male infertility is on the rise. Artificial insemination is an option in many cases like oligozoospermia or oligoasthenozoospermia. Homologous insemination is helpful for some couples in whom sperm count is low. This study was aimed to understand the most suitable portion of split ejaculate for insemination and also the level of calcium and magnesium in each split.

Materials and methods. A total number of 31 normal and clinically healthy adults participated in this study. They were instructed to maintain abstinence for 2-5 days prior to sample collection in three splits. Each split was evaluated as if it were a whole sample, following WHO criteria. Seminal plasma was separated. Calcium and magnesium levels were measured in seminal plasma and spermatozoa of each split.

Results. The split ejaculate study revealed that the first portion contained a higher number of spermatozoa with better motility than the 2nd and 3rd splits. Similarly, the level of calcium and magnesium in seminal plasma and spermatozoa was greater in the first split, followed by 2nd and 3rd splits.

Conclusion. The presented split ejaculate study has shown the first ejaculate as superior in quality in terms of total sperm count and percentage of active motility. The first split of ejaculation is recommended for homologous artificial insemination in case of oligozoospermia and oligoasthenozoospermia. This study has also shown that a major portion of two important elements, calcium and magnesium, is contributed by the prostate gland.

INTRODUCTION

Artificial insemination is one of the methods widely used in the treatment of male infertility [1-4]. Many childless couples prefer homologous artificial insemination in which a portion of split ejaculate containing rich and active spermatozoa was shown to give good results in such cases [5-8]. Furthermore, in cases of oligospermia it was the preferred choice. These outcomes prompted split ejaculation studies in order to understand semen details and the origin of each component in semen [9-12]

The different features of two elements, calcium and magnesium, in the human reproductive system and in semen, indicate their prominent role in fertilization. The aim of the current study is to investigate the quality of semen in each split ejaculate in order to recommend a suitable split for artificial insemination as well as to identify the origin of calcium and magnesium in semen.

MATERIALS AND METHODS

All glassware and plasticware were cleaned thoroughly [13, 14] (Valsa et al. 2012a; 2012b). Thirty-one healthy adults belonging to the Province of Gujarat, aged of 20 to 35 years, volunteered for the study. Prior to sample collection, their consent was obtained and they were instructed to maintain abstinence for three to five days prior to semen sample collection [15]. They were further instructed to collect complete semen samples following masturbation in three splits. Each split was to be collected in a clean, numbered wide mouthed sterile bottle [16].

Samples were evaluated as per WHO [8] criteria. Seminal plasma was separated by centrifugation (10x2000 rpm) [17]. The pellet of spermatozoa and seminal plasma were kept under -20°C until the estimation of calcium and magnesium.

Calcium and magnesium was estimated by a colorimetric method [18]. A comparative study of colorimetry and atomic absorption spectrophotometry produced similar results in case of calcium and magnesium [14].

RESULTS

The study showed that the 1st split of ejaculate was superior in semen quality and the level of calcium and magnesium in spermatozoa and seminal plasma was more in 1st split than in 2nd and 3rd splits (Table 1).

DISCUSSION

Understanding the source of the different components of semen was made possible by the split ejaculation study. Hunter [19] was the first to observe and report the partitional characteristic nature of human semen in regards to its color and consistency of each split. Lundquist [20] introduced the study of split ejaculation. Split ejaculate studies have been collected in samples of two splits [21], three splits [22], five splits [23], or six splits [24]. In general, many researchers opted for three-split studies of ejaculate [12, 16, 22, 25, 26]. The first split of ejaculate contains secretions of Cowper's gland, prostate, and epididymis. The second split consists of prostate and seminal vesicles secretions. While the third split is rich in vesicular secretions [22]. Lundquist [20] reported the source of fructose in semen as the seminal vesicles. Others reported the source of various components of semen using the split ejaculate technique [12, 16, 22]. This was established as a technique to under-

Table 1. Showing the results of semen parameters and level of calcium and magnesium in three splits of ejaculate

Splits	Volume (ml)	Total count (million/ml)	Spermatozoa Motility			Seminal Plasma		Spermatozoa	
			Total	Active	Sluggish	Ca (mg/dl)	Mg (mg/dl)	Ca (mg/gm)	Mg (mg/gm)
1	1.16 ±0.07	88.0 ±5.65	77.16 ±1.87	60.35 ±1.82	16.81 ±1.85	130.32 ±13.64	51.50 ±3.94	35.24 ±7.39	9.73 ±4.77
	(0.5-2.1)	46-132	(60-94)	(40-78)	(7-25)	(32-324)	(22.80-80.88)	(12.62-198.18)	(1.59-19.38)
2	0.95 ±0.04	29.42 ±2.61	55.65 ±1.91	31.0 ±2.85	24.0 ±3.23	33.10 ±2.33	13.20 ±1.74	12.54 ±2.12	3.24 ±0.23
	(0.7-1.6)	(8-59)	(33-72)	(0-54)	(5-70)	(12-64)	(1.23-25.74)	(1.58-50.0)	(0.85-4.10)
3	0.92 ±0.03	10.11 ±1.45	9.93 ±1.56	9.04 ±0.85	40.53 ±2.36	21.92 ±1.93	8.46 ±1.36	8.20 ±1.93	2.15 ±0.32
	(0.5-1.5)	(5-40)	(5-80)	(5-40)	(5-100)	(8-44)	(1.23-17.16)	(2.78-16.88)	(0.53-3.35)

stand the origin of the different components present in semen as well as to evaluate the pathological condition of different glands.

The first fraction of ejaculate is more fertile [8, 12, 16, 27]. The present study observed a different quality of each split ejaculate – a decrease in total semen volume from the first to third ejaculate (Table 1). Statistically, the difference was highly significant. Tauber et al. [28] reported a reverse pattern. The first fraction did not coagulate, whereas the second and third fractions required more time due to lack of prostate secretion. Certain enzymes present in vesicular secretion are involved in coagulation and a few are prostate fluids that are involved in liquefaction of the clot [26, 29]. The morphology of spermatozoa cells was normal in three splits. Cohen et al. [30] and Amelar and Hotchkiss [6] reported normal spermatozoa in all splits. In the present study, total spermatozoa count was more in the first fraction of ejaculate and decreased in subsequent ejaculates (Table 1) as shown in earlier reports [6, 7, 16, 28, 30-33]. This is due to the epididymal content present in the first fraction. The spermatozoa rich fraction in the first split ejaculate was widely used for artificial insemination [2, 3, 5, 6, 7, 34].

In this study the percentage of total and active, motile spermatozoa was more in the first split than in the second and third splits (Table 1). An earlier report also showed the first fraction had a greater percentage of sperm with active motility [16]. However, Eliasson and Lindholmer [35] did not find any difference between the whole sample and the first split ejaculate.

Calcium and magnesium content in human semen has been reported previously [10, 11, 13, 14]. Calcium is essential for sperm motility [36-42], metabolic functions [44], acrosome reaction, and fertilization [38, 41, 44, 45]. It activates immotile sperm collected from the epididymis and stimulates respiration in the spermatozoa [46], stimulating the contractile apparatus of the sperm flagellum [47] (Babcock et al. 1978), and binds to the protein on the cell membrane [48]. The calcium inside the cell influences its motility, either by modifying the wave-form of tail movement or by inhibiting the motility completely [27].

We observed that the level of calcium was highest in the first split and decreased in the second (<0.001) and third splits (<0.001). Homonnai et al. [49] (1978) observed the highest level of calcium in prostate fluid. Prostatic secretion was fully seen in the first ejaculate [49]. In spermatozoa, the calcium level was more in the first fraction when compared with the second (<0.001) and third (<0.01). This indicates that calcium enters the spermatozoa cells in the absence of seminal vesicle fluid. The study of Rufo et al. [50] showed that seminal vesicles released a protein that inhibited the entry of calcium into cells. In the third split the content was mostly from the seminal vesicle.

Magnesium is essential for enzymes involved in anaerobic glycolysis and release of energy (ATP) and, thus, for sperm motility [17]. For survival of sperm, an optimal concentration of magnesium is essential [51]. Magnesium is at a greater concentration inside the sperm than outside [52].

Just as calcium in seminal plasma, magnesium was found to be decrease from the first to second (<0.001) to third ejaculate (<0.001) (Table 1). This was supported by studies of Eliasson and Lindholmer [25] and Lindholmer and Eliasson [53]. But in the case of magnesium in spermatozoa, a reverse pattern was observed. This was absent in earlier studies [53]. This study might be indicating that there is an entry of magnesium during ejaculation.

A correlation was not observed between calcium and any other semen parameter. It was the same in the case of magnesium. A significant positive correlation was found between seminal plasma calcium and magnesium in all three splits indicating that the source of both was the same. It was more in the first, less in the second, and even less in the third. The study confirmed earlier reports that the prostate gland is the major supplier of calcium and magnesium [10, 36, 49, 53].

CONCLUSION

We found the present split ejaculate study to show that the first fraction of split ejaculate was superior when the level of calcium and magnesium was high. Since the first fraction of split was superior in quality, it should be used for homologous insemination.

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