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Time bound changes (in 24 h) in human sperm motility and level of calcium and magnesium in seminal plasma

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Seminal plasma magnesium

Abstract A detailed sperm motility study for 24 h after collection was done. The level of calcium and magnesium in seminal plasma during this period was also seen to understand the role of these electrolytes on sperm motility.

Good care was taken in selection of subjects (young and healthy), collection and pre-physical analysis of sample. Subjects (healthy, 18–25 years) were to maintain abstinence (2–5 days). Collection of sample into wide mouthed container provided was done next to laboratory by masturbation at 8 am ± 30 m. The total study was done in one month period. Samples were studied at ½, 1, 2, 4, 8, 12 and 24 h time for percentage of (1) total sperm motility, (2) active and progressive and (3) non-progressive motility and seminal plasma calcium and magnesium. Total sperm motility deteriorated to 50% in 8 h whereas active and progressive type reduced to 50% in 4 h. At 24 h total sperm motility was 9.8% and active and progressive motility 3.0%.

Level of calcium (27.2 mg/dl) and magnesium (13.54 mg/dl) in seminal plasma did not show any significant changes during study period from that of at ½ h. The study concluded that electrolytes under study were not responsible for the decrease in motility during study period.

1. Introduction

Semen studies took serious thought since sperm cells were shown in it by Antoni Van Leeuwenhoek in 1677.[1] Eliasson and Lindholmer[2] recognized seminal plasma as the environmental medium for sperms, supporting observation of Leeuwenhock why sperms were killed after adding rain water into it.[1] However sperms did not survive long in seminal plasma to name it as a poor medium.[3] Seminal plasma was responsible for initiation and maintenance of progressive motility.[4] Most semen studies were discontinued after 4 h time creating a lacuna in knowledge about motility and survival of sperm after this period.

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Composition of seminal plasma was important for functioning of sperm.[4] Number of organic[5,6] and inorganic[5,7–10] substances were reported in it. Among all two electrolytes, calcium and magnesium secured attention of different groups of workers. Its level in semen, seminal plasma and spermatozoa in normal and infertile patients was measured.[11–15] The origin of these elements in man was located as mainly prostate gland.[1,2] Later on it was identified throughout the reproductive system.[10] Its level differed during different timings of the day.[16] When its level was seen in daily ejaculates a negative correlation was seen between total sperm count and seminal plasma calcium and magnesium. There was negative correlation between sperm count and magnesium. Seminal plasma calcium and sperm calcium were correlated. Correlation was also seen between sperm calcium and sperm magnesium.[14] Considering the importance given to these electrolytes we carried out the present study where the level of these two electrolytes in 24 h after ejaculation was measured and correlated with total sperm count and motility.

2. Materials and methods

The present research proposal was approved by Institutional Ethical Committee of Government Medical College, Surat.

Clinically and mentally normal 24 persons in the age group of 21–26 years belonging to same race of the Province of Gujarat were subjects of this study. Before collection of semen following instructions were given to them, (1) prior to it an abstinence of 2–5 days was to be maintained, (2) its mode was masturbation, (3) to be done in a room provided close to laboratory, (4) to collect on to wide mouthed container supplied by us and (5) to be done at 8.00 (±30 m) a.m. The whole study was conducted in one month period.

Glasswares used in this study were well cleaned by placing it overnight in 6N HNO3, washed in tap water, freshly made glass distilled and triple glass distilled water. Distilled water, if stored it was in plastic carboy. No contact with rubber was made. Except volumetric, all glasswares were dried in hot air oven. Volumetric items were placed on clean filter paper for drying. Calcium, and magnesium in seminal plasma were estimated calorimetrically.[12]

Each sample was studied after liquefaction for total sperm count, details of motility and morphology.[18] Potassium penicillin was added to sample to prevent growth of microorganisms.[19] Illumination of laboratory was maintained constant throughout the study. Samples were evaluated at 1/2, 1, 2, 4, 8, 12 and 24 h time for percentage of total, Active, progressive and forward (PR), non-progressive (NR) and non-motile (NM) sperm. The sample including slide while studying motility remained at 37 °C. At these timings seminal plasma was separated for estimation of calcium and magnesium.

Estimation of calcium was done by using Clark and Collip method.[17] The principle of the biochemical reaction was as follows: calcium present in the sample is precipitated as oxalate by using ammonium oxalate and after washing with ammonia solution, the precipitate is dissolved in acid and titrated with permanganate solution. Reagents used for this techniques are,

1. Ammonium oxalate (AR Grade) (Brittish Drug House, Bombay).
2. Ammonia 2% (V.V. Solution) (AR Grade) (Brittish Drug House, Bombay).
3. Potassium permanganate (AR Grade) (Brittish Drug House, Bombay).
4. Sulfuric acid (AR Grade) (Brittish Drug House, Bombay) and
5. Calcium carbonate (AR Grade) (Brittish Drug House, Bombay), for standard solution of calcium.

Magnesium was measured by colorimetric method of titan yellow. The principle of the biochemical reaction was as follows: titan yellow gives red color with magnesium by using gum ghatti as the color stabilizer and calcium is added in the standard which intensifies the color. Reagents used for this techniques are,

1. Magnesium chloride (GR Grade) (Brittish Drug House, Bombay).
2. Sodium tungstate (AR Grade) (Ranbaxy Laboratories Limited, SAS Nagar, Punjab).
3. Concentrated sulfuric acid (GR Grade) (Brittish Drug House, Bombay).
4. Gum Ghatti (LR Grade) (Modern Chemical Corporation, Bombay).
5. Titan Yellow (GR Grade) (Brittish Drug House, Bombay).
6. Sodium hydroxide (AR Grade) (Ranbaxy laboratories Limited, SAS Nagar, Punjab) and
7. Calcium Chloride (GR Grade) (E. Merck, Germany).

At the end of the procedure the standard and the test against blank was read at 520 mμ.

The statistical study was done at New Jersey Institute of Technology, New Jersey, NJ 071102 USA.

The details of the computer were as follows.

Model	: AT & T 6300
Make	: AT & T Information systems, Inc, Morristown, J.J. 07960, USA
Clock speed	: 4.77 MH 2
Memory	: 640 K
Software used	: SPSS/PC Release 1.1 (SPSS/PC)

The analysis included mean, standard deviation (SD), standard error (SE), student's (unpaired) '*t*' test, paired '*t*' test and correlation of coefficient.

3. Results

All samples were within the normal range considering volume, liquefaction time, PH, presence of fructose, total sperm count, motility and morphology. Percentage of lysperm motility changed from 75.6 ± 3.22 at 30 m to 9.8 ± 2.77 at 24 h. Similarly active, forward and progressive (PR) (50.3 ± 4.92 to 3.0 ± 2.99) and non-progressive (NP) (25.3 ± 3.28 to 6.8 ± 2.47) showed changes (Table 1), whereas seminal plasma calcium in mg% (27.2 ± 1.74 to 28.89 ± 1.38) and magnesium in mg% (13.54 ± 1.67 to 15.09 ± 1.74) did not show any difference (Table 1).

Table 1 Results of 24 h time bound study: changes in sperm motility and seminal plasma calcium and magnesium.

Time of analysis After ejaculation (hour)	Percentage of motility				Seminal Plasma	
	Total	Active forward and progressive (PR)	Non-progressive (NP)	Non-motile (NM)	Calcium (mg/dl)	Magnesium (mg/dl)
½	75.6 ± 3.22 (60–90)	50.3 ± 4.92 (25–70)	25.3 ± 3.28 (10–38)	24.4 ± 3.20 (10–40)	27.2 ± 1.74 (20–36)	13.54 ± 1.67 (6.72–20.73)
1	74.5 ± 3.40 (55–90)	43.70 ± 4.95 (20–65)	30.8 ± 3.58 (10–40)	25.5 ± 3.40 (10–45)	28.6 ± 1.34 (24–36)	17.87 ± 1.0 (11.44–21.44)
2	64.5 ± 3.20 (58–80)	31.5 ± 3.81 (20–55)	33.0 ± 4.03 (15–60)	35.5 ± 3.20 (20–50)	26.67 ± 1.71 (18–36)	15.0 ± 2.14 (4.28–21.44)
4	53.0 ± 2.60 (40–65)	24.50 ± 5.66 (5–50)	28.5 ± 3.66 (15–45)	47.0 ± 2.61 (35–60)	27.6 ± 1.36 (22–34)	14.44 ± 1.12 (8.56–21.44)
8	37.7 ± 2.33 (20–45)	11.88 ± 2.66 (5–20)	25.84 ± 2.73 (15–40)	62.3 ± 2.33 (55–80)	26.8 ± 1.84 (22–38)	13.82 ± 1.73 (6.72–24.3)
12	19.5 ± 2.85 (5–35)	6.6 ± 1.22 (5–15)	12.9 ± 2.93 (5–35)	80.5 ± 2.85 (68–97)	28.0 ± 1.69 (22–40)	12.64 ± 1.73 (6.72–20.02)
24	9.8 ± 2.77 (0–25)	3.0 ± 2.99 (0–15)	6.8 ± 2.47 (0–20)	90.2 ± 2.77 (75–100)	28.89 ± 1.38 (24–36)	15.09 ± 1.74 (7.14–22.88)

The details are given in Table 1 and Fig. 1. Significant ('*p*') values were seen when each parameter was compared of two different timings and is given in Table 2. No significant difference was seen in case of levels of calcium and magnesium.

4. Discussion

Detailed study of human semen was conducted by many research workers.[18–20] Many led to new line of thoughts. We chose two areas, to study, (1) changes taking place in sperm motility in 24 h time and (2) level of calcium and magnesium in seminal plasma during this time to understand whether changes in sperm motility was due to changes in these elements crossing sperm cell membrane.

Utmost care was taken in selection of subjects collection of samples, followed by detailed evaluation of it. Donors were healthy as normal health was essential for production of semen.

Subjects were within young age group. Advancement in age decreased sperm count and motility.[21,22] Subjects belonged to a limited geographical area. Fernandez et al.[24] reported quality of semen differed among people from place to place.

More number of days of abstinence deteriorated semen quality.[24] We advised 2–5 days abstinence which was necessary to get normal semen[25,26] and others advised 0[27] to 2–7 days.[18,28] Studies had shown the difference in quality of semen in daily[20,21] and in more frequent ejaculation. Frequency of collection was having a significant effect on the composition of semen, with respect to volume, sperm density, seminal plasma constituents and other parameters.[25,26,29–32]

Mode of collection of sample was masturbation. Coitus interruptus was not advised due to chances of losing one or two drops during collection as well as mixing the sample with vaginal secretions and vaginal cells.[33] Total semen was important for the study. Also portion of semen differed in total sperm count and pattern of its motility.[34–36]

Providing a place next to laboratory for sample collection suited not to damage sperms during the transition period caused by movement as well as change in temperature and to learn sample from almost collection time. Others also opted for similar way.[19,28] In this study, liquefaction time was below 15 min. Sample collected at different places required minimum 30 min to 1 h even more time to submit it to laboratory. Sperm motility decreased with slight change in biophysical condition[37] and also deteriorated as time lapsed.[3,21] We studied sperm motility from 30 m after collection (Table 1). Others advised to perform within 1 h[18] or 2–4 h[24,38,39]m.

We provided wide mouthed container for collection which permitted not to lose any drop.[40] Each portion of semen differed.[34,35] Semen collected in narrow mouthed bottle or test tube may lead to missing a small portion of sample.[24] Well cleaned containers supplied were to exclude unknown chemicals possibly present in containers otherwise used. For the same reason condom was not suitable for collection of sample as which was known to contain chemicals.[24]

Devices for semen collection were designed and discussed by different authors.[39,40] All type of containers were not suitable for storage of sample used for chemical study.[41,42]

Subjects collected sample at 8 am (±30 m). Statistically significant difference in semen quality was observed when samples were collected at different timings of the day.[38] Similar chronobiological changes in body was known.[43,44] Present study was restricted to one month period. Singh et al.[46] reported seasonal changes in semen electrolytes.

Veena et al.[38] had shown sperm motility was better in dark than in light. Considering this, we maintained constant wave length of light in laboratory and a fixed level of light on stage of microscope throughout our study. The importance of sample and the slide to remain near 37 °C while for studying sperm motility was taken care following Freund.[46] The effect of temperature in samples was known since 1962.[46] Semen study was carried out as per WHO.[18] A minimum amount of antibiotics was added to sample as it was known that seminal plasma was not a good medium for sperm survival.[3]

Total semen volume, liquefaction time, pH and test for presence of fructose were within normal range. Total sperm count and percentage of total, PR and NP motility were also within normal range (Table 1).

One of the two aspects of present study was on sperm motility. Percentage of sperm motility was significantly correlated with total sperm count.[47] Results on total sperm motility (%) showed a fall in it from 75.6 ± 3.22 (1/2 h) to 9.8 ± 2.77

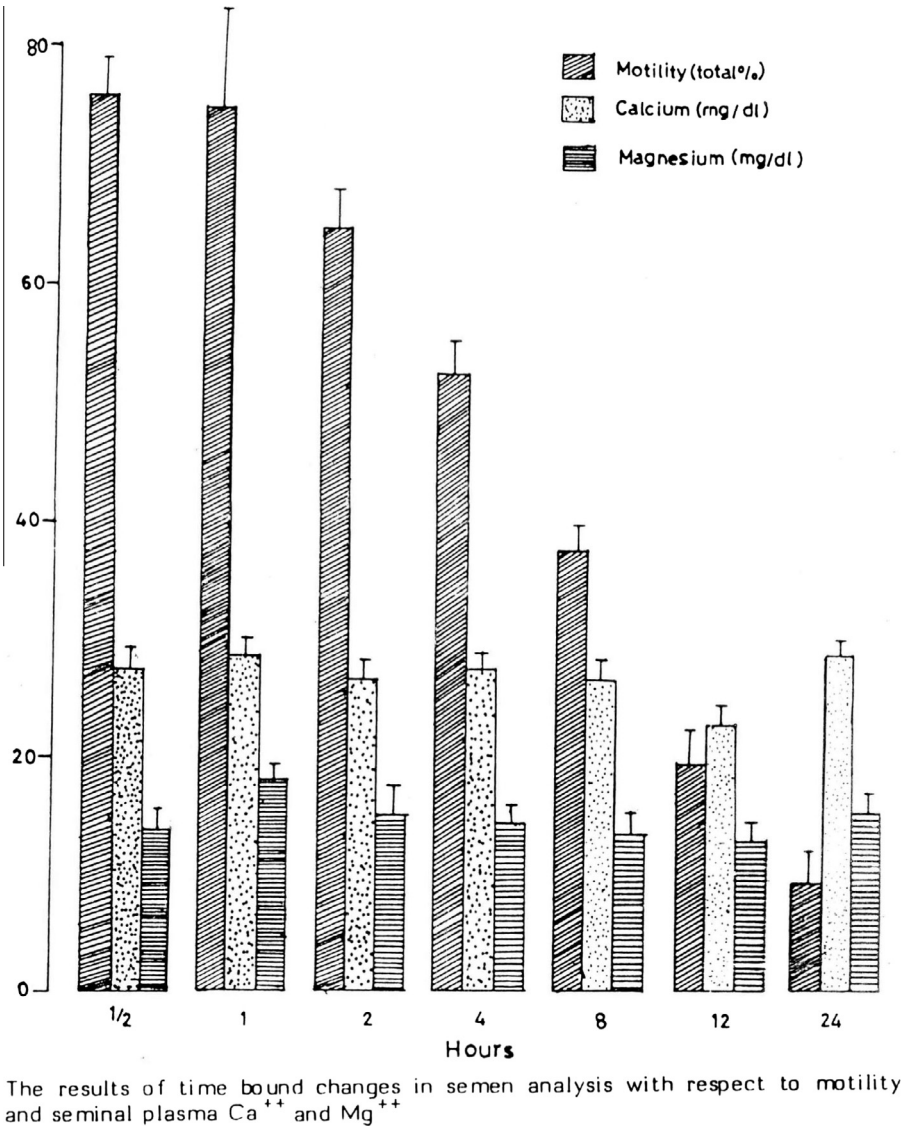

Figure 1 The changes observed in time bound study in semen analysis with respect to motility and seminal plasma calcium and magnesium.

(24 h). Percentage of total motility was decreasing from 1 h of study though statistically significant difference (<0.01) was seen from 2 h. Significant fall (<0.01) was seen in PR motile sperms from 1 h (Table 2). Eliasson[34] and Freund[47] observed a significant correlation between sperm motility and rate of forward progressing motile sperms. Number of NP sperms increased from 1 h (<0.05) to 4 h. time, compensating the fall in PR (Table 1). Our study was likely to be the first one of its kind to show that total sperm motility was $>50\%$ at 8 h. after ejaculation (Table 1 and Fig. 1). Normally, the overall motility reached a minimum of 60 percentage after 2–3 h of ejaculation.[48] A significant decrease in sperm motility over 2–4 h period indicated a serious problem even if the sperm count and original motility were good.[48] One reason for decrease in sperm motility was due to the change in organic and inorganic elements.[5,49] Sperm motility inhibitory factors present in semen were also responsible which were removed from semen after its deposition in vagina.

We estimated calcium and magnesium by using colorimetry.[17] It was shown statistically this method was equivalent to atomic absorption spectrophotometry for estimating calcium and magnesium.[15]

Major contributor of calcium and magnesium in semen was prostate gland.[2] X-ray diffraction analysis[9] and Energy dispersive X-ray analysis employed on electron microscope[10] confirmed that both elements were present throughout the length of human male genital tract. Determination of calcium and magnesium, in seminal plasma was important to understand the functional capacity of accessory glands of genital system.[50,51]

The result showed the level of calcium and magnesium was similar to earlier studies.[14] Calcium in seminal plasma exceeded 3 times that in blood plasma.[1] Calcium was the most abundant cation seen in semen after sodium and potassium.[51] Major function of male accessory organs, prostate and seminal vesicle, was to maintain an optimum free calcium concentra-

Table 2 Showing the significant '*p*' value in 24 h time bound study of sperm motility and seminal plasma calcium and magnesium.

Timing (hours)	Parameter	'*t*'	*p*
½/2	Motility total	4.01	< 0.01
½/4	"	7.26	< 0.001
½/8	"	23.80	< 0.001
½/12	"	10.49	< 0.001
½/24	"	19.17	< 0.001
½/1	Motility active	4.22	< 0.001
½/2	"	4.55	< 0.001
½/4	"	4.42	< 0.001
½/8	"	10.48	< 0.001
½/12	"	8.38	< 0.001
½/24	"	9.22	< 0.001
½/1	Motility sluggish	2.41	< 0.05
½/12	"	2.35	< 0.05
½/24	"	3.81	< 0.01

No significant difference was seen in level of Ca^{2+} and Mg^{2+}.

tion in the fluid surrounding sperms.[52] The participation of calcium in activities of a cell was important, like that in case of cyclic AMP dependent protein kinase. Animal experiments showed calcium as essential for sperm motility.[53,54] Excess calcium exposure may damage male reproductive system and its functions.[55] Electrolytes may enter sperm cell or may get released. The present study showed the level of calcium inside and outside sperm remained same throughout 24 h study (Fig. 1 and Table 1). Table 2 shows there was no statistical difference in values at different timings to conclude there was no movement of these electrolytes across the membrane. Rufo et al.[56,57] showed an inhibitory protein present in seminal plasma and on sperm membrane was secreted by seminal vesicle which inhibited calcium uptake by ejaculated sperm. Similar to our observation Pleban and others[59] found there was no change in level in a 5 h study on trace metals such as cadmium, copper, iron, lead, selenium and zinc, though movement of zinc ion across the sperm cell was reported.[59] Supporting our study, others observed that sperm cell membrane was impermeable to calcium unlike some organic substances.[27]

The steady state of calcium inside sperms was maintained by calcium pump[60] as well as certain proteins present on sperm membrane which repel calcium.[61]

High concentration of magnesium was reported in human testis,[62,63] prostate gland, seminal vesicles and seminal plasma.[65] Dietary magnesium was shown to effect testis directly.[66] Lack of magnesium in direct was an evidence to this.[67] Magnesium level in seminal fluid was helpful in the diagnosis of chronic prostatitis.[68] The study by Sorensen et al.[69] did not see any inhibitory effect on sperm motility with high magnesium concentration. Both calcium and magnesium did not show any correlation with any conventional semen parameters. The magnesium concentration was more in sperm than in seminal plasma.[14,65] Magnesium was positively correlated with calcium concentration.[68] Functions of magnesium are several including its role in sperm motility.[70,71] In presence of magnesium ions, sodium and potassium stimulate ATPase, a hydrolyzing enzyme present in human sperm membrane. It was established ATPase was the main source of energy available for propulsion of spermatozoa and that any alteration of ATP pool either directly or indirectly affected sperm motility.[72,73] In 2nd fraction of split ejaculate sperm motility was less[35,74] to which addition of magnesium slightly improved motility.[74] In in vitro study it was shown magnesium moved in and out of the cell.[59] Similar movement was not seen in this study (Table 2, Fig. 1) and we did not observe change in the level of magnesium in seminal plasma during different timings of the study.

In conclusion of 24 h. study, we showed that after ejaculation in 8 h the total sperm motility deteriorated to 50%. PR sperms were seen reduced to 50% in 4 h. by increasing number of NP sperms. In 24 h. percentage of PR was 3.0 and of NP was 6.8. The level of calcium and magnesium in seminal plasma during the study period did not show any significant change. This proved these electrolytes did not move across cell membrane or they move equally to both sides and which was not responsible for deterioration in motility. Results of other studies showed there was no association between calcium concentration and progressive motility was reported.[69,75]

Conflict of Interest

Authors state that there is no conflict of interest.

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