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Determining the Osmolality of Seminal Fluid Aids in the Rapid Diagnosis of the Fertilizing Potential of Spermatozoa

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INTRODUCTION

The osmolality of semen in fertile men ranges between 360 and 380 mosmol (1). Alterations in the osmolality of the seminal fluid causes sperm agglutination (2). Exposure of spermatozoa to hypoosmotic solutions causes swelling of their cytoplasmic membrane (3); this phenomenon has been used by Jeyendran *et al.* (4) to assess the fertilizing potential of spermatozoa.

In the course of screening semen samples for our in vitro fertilization–embryo transfer (IVF–ET) program, we came across cases where the osmolality of the semen was significantly below the normal range observed in fertile men. Since osmolality plays an important role in sperm function, the characteristics of spermatozoa present in the hypoosmotic semen were compared with those present in the semen with normal osmolality.

MATERIALS AND METHODS

Semen samples were collected by masturbation in sterile beakers from 60 different men, and the physicochemical characteristics of the semen such as liquefication time, pH, and viscosity were determined by standard procedures. Osmolality was determined using a freezing-point osmometer (u-Osmette, Model 5004, Precision System Inc., USA). The threshold for normality of seminal plasma osmolality is 360–380 mosmol.

Sperm characteristics such as total number, motility, viability, and incidence of morphological defects, with particular reference to tail defect were determined as described in the WHO manual (5). Spermatozoa were also subjected to the hypoosmotic swelling test (HOS test) as described by Jeyendran *et al.* (4). Preovulatory oocytes aspirated by laparoscopy were graded according to the method described by Veeck *et al.* (6). Immature eggs were incubated for 24 hr, and mature and intermediate oocytes for 6–8 hr. Approximately 50,000 sperm processed according to the method described by Tartalzis and De Cherney (7) were used to inseminate each egg.

For statistical analysis, Students paired *t* test was used to compare groups.

RESULTS

Osmolality in 35 samples (Group A, Table I) showed a mean value of 371.3 (±8.9) mosmol compared with the remaining 25 samples (Group B, Table I), which showed a mean value of 303.8 (±31.4)

Table I. Characteristics of Seminal Plasma and Spermatozoa in Normal (Group A) and Hypoosmotic (Group B) Semen Samples

	Group A	Group B	Group C
Seminal plasma			
Osmolality (mosmol)	371.3 ± 8.9	303.8 ± 31.4	<0.001
Liquefication time (min)	25.9 ± 15.4	25.9 ± 15.5	NS
Viscosity	6.67 ± 3.60	7.4 ± 3.8	NS
pH	7.4 ± 1.3	7.4 ± 1.5	NS
Spermatozoa			
Count (10^6/ml)	85.6 ± 66.4	51.6 ± 46.1	<0.05
Normal morphology (%)	32.0 ± 12.18	15.57 ± 10.85	<0.001
Tail defects (%)	17.3 ± 8.2	25.7 ± 8.4	<0.001
Motility (%)[a]	56.3 ± 13.9	35.1 ± 16.2	<0.001
Viability (%)[b]	73.1 ± 13.3	57.7 ± 22.6	<0.002
Agglutination (%)	3.5 ± 3.31	3.07 ± 3.62	NS
HOS test (%)[c]	72.9 ± 12.8	51.2 ± 20.9	<0.001

[a] Incidence of motility <50% is considered infertile.
[b] Incidence of viability <60% is considered infertile.
[c] Incidence of HOS test <60% is considered infertile.

mosmol. There was no significant difference in any of the other physicochemical parameters studied between semen samples.

The incidence of various sperm characteristics, such as motility, viability, tail defects, and a normal response in the HOS test in semen having a normal osmolality, was similar to that observed in samples obtained from fertile men. The incidence of sperm characteristics observed in the hypoosmotic semen was significantly different from those seen in normosmotic semen and they were comparable to those observed in infertile semen samples (Table I). The morphologically normal forms were significantly reduced in the hypoosmotic group. There was no significant difference in the percentage of agglutinated spermatozoa.

Spermatozoa from 14 semen samples of normoosmotic semen were used for in vitro fertilization and they showed a fertilization rate of 63%, with two full-term pregnancies having occurred after transferring the embryos. None of the spermatozoa from any of the hypoosmotic semen was able to fertilize oocytes in vitro. Microscopic examination of the hypoosmotic semen failed to reveal the presence of bacterial pathogens.

DISCUSSION

The present study has revealed that hypoosmotic semen can occur spontaneously in some infertile semen. The reason for this is not known but it is possible that this may be due to physiological disturbances having occurred in the secretion of the various accessory reproductive organs in such a way as to affect the osmolality of the semen.

There was a significant reduction of morphologically normal forms in the hypoosmotic group. It may be pertinent to mention that the most striking feature of normal spermatozoa having a high fertilization potential is a coiling of the tail when they are exposed to hypoosmotic solutions (4). This coiling is due to the swelling of the sperm membrane and retraction of the axoneme fibers in the tail. The process of hypoosmolality-induced coiling of the sperm tail is reversed when the spermatozoa are reexposed to hyperosmotic solutions. It remains to be determined whether the incidence of coiled sperm found in hypoosomotic semen is reduced by transferring the sperm to semen having a normal osmolality or washing them with hyperosmotic solutions.

The incidence of motility, viability, agglutination, and those showing a positive reaction in the HOS test for spermatozoa present in hypoosmotic semen was similar to that observed in infertile semen samples. It would therefore seem that estimating the osmolality of the semen, which can be carried out more rapidly than determining other morphological characteristics, is a good and rapid diagnostic aid for determining the fertilizing potential of spermatozoa.

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