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A Review of the Physical and Chemical Properties of Human Semen and the Formulation of a Semen Simulant

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ABSTRACT: A fluid medium was developed to simulate the salient physical and chemical properties of human semen. The composition of the medium was based upon an extensive review of the literature on constituents of human semen. In choosing the ingredients for this medium, the goal was to emphasize properties that influence interactions of human semen with topical contraceptive, prophylactic, or therapeutic products. Among these properties, pH and buffering capacity, osmolarity, ionic strength, and rheological properties play dominant roles in the physico-chemical processes that govern drug release kinetics and delivery vehicle distribution.

Key words: Composition, human, semen, simulant, microbicide.
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When therapeutic, contraceptive, or prophylactic formulations are applied to the vagina, they encounter a variety of fluids with widely varying physical and chemical properties. These fluids include those that originate in the vagina and those that flow into it (eg, cervical mucus and semen). The fluid actually present at any location within the vagina is a mixture, to a varying extent, of these fluids. The flow, retention, drug delivery kinetics, and bioactivity of vaginal formulations depend upon their interactions with these resident fluids. Work done by our group has determined that the physical and chemical properties of both the delivery vehicle and the surrounding environment are important factors in determining product performance (Katz et al, 1998; Owen et al, 1999a, 2000, 2001, 2003). Understanding of these interactions can, therefore, aid in the design and development of new and improved formulations.

One component of such research is the in vitro testing of formulations with fluids representative of those that will be encountered within the vagina. Our laboratory has been developing and applying in vitro assays that focus on how the deployment and delivery of contraceptive and prophylactic compounds are affected by the properties of the delivery vehicle and its interactions with the surrounding fluids. To develop these assays using standardized materials of sufficient volume, we have found it useful to employ simulants of ambient biological fluids. Our formulation of a vaginal fluid simulant has been described in a previous publication (Owen and Katz, 1999b), and our semen simulant (and earlier versions) was presented in a number of studies of contraceptive and microbicidal gels (Owen et al, 2003, 2004; Geonnotti and Katz, 2004; Geonnotti et al, 2005a; Geonnotti et al, 2005b). Here we describe the formulation of a semen simulant embodying salient physical and chemical components and properties of human semen. This is based on a comprehensive review of the literature, and we present here, as well, an updated summary of the constituents of human semen.

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Materials and Methods

The quantity and composition of human semen have been studied for a variety of reasons (eg, for the diagnosis of conditions such as prostatitis, infertility, and cancer), and the results of these studies were used in the development of our simulant. It is designed to embody salient biochemical and rheological properties of human semen, with particular emphasis on those properties most likely to influence the performance of vaginally applied topical therapeutic, contraceptive, and prophylactic formulations.

Human semen is a mixture of components produced by several different glands. These components are incompletely mixed during ejaculation and, hence, the initial ejaculate is not an entirely homogeneous mixture. The first portion of the ejaculate, about 5% of it, is made up of secretions from the Cowper (bulbourethral) and Littre glands. The second portion derives from the prostate and contributes from 15% to 30% to the ejaculate. There follow small contributions of the ampulla and epididymis and, finally, of the seminal vesicles, which contribute the remainder, and majority, of the ejaculate (Polakoski et al, 1976; Mann and Lutwak-Mann, 1981; Coffey, 1995).

The secretions of the organs contributing to the ejaculate differ in composition, and there has been a longstanding interest in

evaluating the composition of semen from a diagnostic point of view (Eliasson, 1982). The prostate is the main source of the acid phosphatase, citric acid, inositol, calcium, zinc, and magnesium found in the ejaculate. The seminal vesicles' contribution is rich in fructose, ascorbic acid, and prostaglandins, while the concentrations of L-carnitine and neutral alpha-glucosidase are indications of epididymal function (WHO Manual, 1999). A small portion of the fructose present originates from ampulla of the ductus deferens.

The data used in formulating our simulant derive from a large number of articles describing the composition of semen and seminal plasma. The choice of composition, pH, viscosity, and buffering capacity of our simulant was complicated by a number of factors that made direct comparisons between studies difficult. The most important difference across the studies was in the choice of donors used. Many studies were performed on patients attending infertility clinics or on patients who were being screened for a variety of pathological conditions. In our work, studies in which the donors were demonstrably infertile or pathological were excluded. Included were studies in which donors were normozoospermic patients at infertility clinics or in which donors were fertile controls in studies involving semen. The number of donors used in each study varied; however, in our study, the data are not weighted by sample size, since in comparing studies, particularly those that used different measurement techniques, a larger sample size does not necessarily imply greater accuracy.

Some studies were performed on whole semen and others on seminal plasma alone. Sperm make up only a small portion of the whole semen, from 1% to 5% of the total volume (Mortimer, 1994). Most studies of the composition of both seminal plasma and whole semen indicate that the presence of sperm does not significantly influence the results; for instance, they make little contribution to the total ionic content of the semen (Bondani et al, 1973). In reviewing previous studies, it was sometimes difficult to determine if whole semen or plasma was used. Whole semen is assumed unless some separation procedure is described. Except where noted, composition data for whole semen and seminal plasma are considered together.

Another factor influencing the results of the various studies reviewed was the method of sample collection and preparation. The most important variable here was usually the length of time since ejaculation. Time after collection is particularly important for pH measurements, since the pH changes as a result of increased CO_2 concentration and lactic acid production. The rheological properties of the semen sample also change with time, as the material first coagulates and then liquefies. This process is accompanied by biochemical changes in composition. If sperm are present in the sample, over time they may influence the measured composition, as a result of binding of plasma components to the sperm and to sperm metabolic activity. Sperm metabolic activity can alter composition due to fructolysis, glycolysis, and the excretion of metabolic wastes. In addition, after ejaculation, some of the sperm cell contents may leak out into the surrounding plasma (Mann and Lutwak-Mann, 1981). Other postejaculatory concerns include proteolysis and the rise of free choline accompanied by the crystallization of insoluble spermine phosphate (Mann and Lutwak-Mann, 1981). Results can even be influenced by the type of sample container used. Studies have shown that high-quality polypropylene is the best material (Balerna et al, 1985) and that glass can contain enough zinc to influence zinc concentration measurements (Colleen et al, 1975).

A few of the articles reviewed measured composition on a per-ejaculate basis, and from a diagnostic point of view, per-ejaculate results may be more useful (Grizard et al, 1985). The overwhelming majority of the articles reviewed, however, measured composition on a per-volume basis, and this is the basis used throughout our study. When possible, per-ejaculate data were used here after conversion to a per-volume measurement.

The techniques used to measure particular semen components or properties sometimes differed from study to study. These different methods will be discussed below as each component and property is reviewed. A detailed description of the various assays used to measure semen composition and properties can be found in Mortimer (1994). Almost all of the property measurements used in this study were performed on semen obtained by masturbation. For interesting comparisons of semen composition measured on samples collected by masturbation vs coitus, see Hotchkiss et al (1938) and Purvis et al (1986).

The semen simulant proposed in this article is intended to model the properties of semen produced by healthy male donors after complete liquefaction of the semen. The proposed semen simulant is designed to incorporate information about chemical composition determined by previous researchers, with an emphasis on modeling the pH and buffering capacity, ions, osmolarity, sugars and protein composition, and viscosity of the material. We now consider each of these in turn.

pH and Buffering Capacity

Semen has a very high buffering capacity, much higher than that of most other fluids in the body. Semen maintains its pH near neutral in the acidic vaginal environment, providing the sperm with the opportunity to enter the neutral pH cervical mucus. The pH of human semen is a matter of some debate (Meacham, 2002); there is considerable variation in the pH measurements reported by different researchers. Most researchers have used one of two techniques for measuring semen acidity—pH indicator paper/colorimetry or a pH electrode (in almost all cases, whole semen was used). One study comparing the two methods found slightly higher values when pH paper was used (Haugen and Grotmol, 1998). The measured pH can depend on the length of time since ejaculation, and it tends to increase shortly after ejaculation as a result of loss of CO_2 (Makler et al, 1981; Wolters-Everhardt et al, 1986). Further aging of whole semen can result in a substantial decrease in pH resulting from fructolysis and the production of lactic acid (Shedlovsky et al, 1942; Searcy and Simms, 1967).

The high buffering capacity of semen has been reported in a number of studies, each of which presents its results in a different way. Tynen (1939) reported that the pH of 1 mL of semen can be reduced to 6.0 by the addition of 5.5 mL of 0.01 N HCl. A study conducted by Shedlovsky et al (1942) reported results as a curve of measured pH vs the volume of 0.50 N HCl solution added. A similar study (Mandal and Bhattacharyya, 1987b) measured buffering capacity as the change of pH resulting from adding 0.4 mL of 0.1 N HCl to 0.3 mL of seminal plasma. In a

Table 1. *Semen pH and references*

pH	References
7.8	Baker, 1939
7.83	Balerna et al, 1985
8.3	Bhushan et al, 1978
7.49	Chaudhari et al, 1990
7.60	Cooper et al, 1991
7.65	Ford and Harrison, 1984
7.43	Gonzales and Sanchez, 1994
7.4	Gopalkrishman et al, 1989
8.40	Haugen and Grotmol, 1998; paper, 30 min
8.23	Haugen and Grotmol, 1998; meter, 30 min
8.47	Haugen and Grotmol, 1998; paper, 60 min
8.28	Haugen and Grotmol, 1998; meter, 60 min
7.67	Hirsch et al, 1991
7.62	Homonnai et al, 1978
7.7	Homonnai et al, 1980
8.2	Hotchkiss et al, 1938
7.45	Hubner et al, 1985
7.26	Huggins and Johnson, 1933
7.19	Huggins et al, 1942
7.81	Jeyendran et al 1989
7.58	Kilic et al, 1996
8.1	Lindholmer, 1973
7.43	Magnus et al, 1990
7.65	Makler et al, 1981
7.53	Mandal and Bhattacharyya, 1987b
7.7	Nagy et al, 1986
7.38	Nikkanen, 1979
7.64	Paz et al, 1977
7.63	Prien et al, 1990
7.60	Raboch and Skachova, 1965; meter, 5 min
7.64	Raboch and Skachova, 1965; meter, 20 min
7.424	Searcy and Simms, 1967
8.04	Vaishwanar and Abhyankar, 1971
7.48	Wolters-Everhardt et al, 1986

study of infertile men, Wolters-Everhardt et al (1986) measured a buffering capacity of 41.1 slyke. A slyke is the number of micromoles of HCl added to 1 mL of test solution to get a 7 to 6 pH change. It is somewhat difficult to directly compare these results. However, expressed as micromoles of H+ per milliliter of semen required for a 1 pH unit change, they are approximately: Tynen, 25; Shedlovsky, 20; Mandal and Bhattacharyya, 15; and Wolter-Everhardt et al, 40.

There is some debate as to the source of the high buffering capacity of semen. Searcy and Simms (1967) proposed that citrate is an important source of seminal buffering capacity. A study by Wolters-Everhardt et al (1987) of the contribution of HCO_3/CO_2 to buffering capacity concluded that HCO_3/CO_2 contributes 24.9%, protein contributes 28.5%, and that the other half is due to low–molecular weight components such as citrate, inorganic phosphate, and pyruvate. After considering the results of the studies discussed above, we formulated our semen simulant from a phosphate buffer solution (containing citrate and protein) to have a target pH of 7.7 and a target buffering capacity of 25 slyke. A summary of the studies considered of semen pH and their results is given in Table 1.

Table 2. *Semen citrate concentration in mg/100 mL and references*

Citrate (mg/100 mL)	References
400	Coffey, 1995
534	Cooper et al, 1991
510.3	Dondero et al, 1972
523	Ford and Harrison, 1984
751	Gonzales, 1994
596	Grizard et al, 1985
479	Harvey, 1951
657	Jathar et al, 1977
646	Kavanagh, 1985
678	Mandal and Bhattacharyya, 1987a
304	Mandal and Bhattacharyya, 1990
376	Mann, 1964
546	Paz et al, 1977
446	Purvis et al, 1986
480.3	Videla et al, 1981

Citrate

Citrate is one of the most important anions present in human semen. Although citrate has high affinity for calcium, magnesium, and zinc, the citrate concentration is more than double the divalent metal concentration; consequently, much of the seminal citrate is strongly anionically charged (Arver, 1982a; Arver and Sjoberg, 1982; Kavanagh, 1985). Semen may owe its high calcium ion buffering capacity to citrate, and citrate is probably the major regulator of ionized calcium levels in seminal plasma (Fong et al, 1986; Magnus et al, 1990). Most studies measuring citric acid concentration used either enzymatic or spectrophotometric techniques. Our simulant is formulated to have a target citrate concentration of 528 mg/100 mL. The studies used are summarized in Table 2.

Ions

The measurement of calcium concentration in semen is of great interest as a result of its relation to sperm motility, metabolism, the acrosome reaction, and fertilization itself (Sorensen et al, 1999). Only a small portion, 2%–4%, of the calcium in semen is present in ionized form (2%, Arver, 1982b; 4%, Ponchietti et al, 1984). Measurement of calcium concentration can be complicated, since exposure to air results in a temperature-dependent decrease in ionized calcium levels (Arver and Sjoberg, 1983). In addition, binding with other compounds (citrate, phosphate, proteins, etc) may reduce calcium activity (ionization); semen has a very high calcium buffering capacity (see Citrate above) (Mann and Lutwak-Mann, 1981; Arver and Sjoberg, 1982; Ford and Harrison, 1984; Fong et al, 1986; Magnus et al, 1990). Calcium also binds to the sperm surface, which can lead to differences between measurements on whole semen vs seminal plasma (Mann and Lutwak-Mann, 1981).

The other important ions found in human semen are magnesium, potassium, sodium, and zinc. The concentrations of calcium, magnesium, and zinc are highly correlated (Homonnai et al, 1978; Adamopoulos and Deliyiannis, 1983). Studies measuring salt concentrations in semen indicate substantial variation among donors (Girgis et al, 1980). Measurements of magnesium,

potassium, sodium, and zinc concentrations are complicated by the tendency of those elements to form complexes with other components of the semen. Magnesium and zinc are also found complexed with other molecules, which can sometimes be bound to the surface of the sperm cells (Lindholmer and Eliasson, 1974; Mann and Lutwak-Mann, 1981; Hirsch et al, 1991). Zinc is excreted from the prostate as a low–molecular weight complex with citrate. After ejaculation, 50% is redistributed and bound to medium– and high–molecular weight compounds from the seminal vesicles (Lindholmer and Eliasson, 1974; Arver and Eliasson, 1982; Mandal and Bhattacharyya, 1990). Citrate is probably the main low–molecular weight zinc ligand (Arver, 1982a).

One study, Quinn et al (1965), examined the differences in salt concentrations measured in whole semen vs plasma. It found a higher concentration of calcium and magnesium in plasma vs whole semen; for sodium, the concentration in whole semen was greater than in plasma. Measurements of salt concentrations in the studies reviewed were usually conducted using atomic absorption spectrophotometry, flame photometry, or ion-selective electrode analyzers. Our simulant is formulated to have target calcium, chloride, magnesium, potassium, sodium, and zinc concentrations of 27.6, 142, 11.0, 109, 300, and 16.5 mg/100 mL, respectively, (see Table 3).

Osmolarity

Semen is notable for its high osmolarity, which is substantially higher than that of blood plasma. The osmolarity of semen depends greatly on the concentration of sugars and other organics concentrations as well as ionic salt concentrations (Mandal and Bhattacharyya, 1987b). Some researchers have noted that osmolarity increases measurably with semen aging (Velazquez et al, 1977). After a review of the relevant literature, we formulated our semen simulant to have a target osmolarity of 354 mosmolar. A summary of the studies considered and their results is given in Table 4.

Fructose and Glucose

Fructose concentration, because it is considered a measure of seminal vesicle function, has been studied in great detail. Studies indicate that there is a wide variation in fructose concentration (Nun et al, 1972), and this concentration can be a function of a number of factors, including time since collection and the age of the donor (Mauss et al, 1974; Kothari et al, 1977). Fructose is an important source of energy for the sperm, and, hence, measurements of fructose concentration in whole semen can change over time as a result of fructolysis, the primary source of lactic acid in semen (King and Mann, 1959; Mann and Lutwak-Mann, 1981). Fructose is also likely involved in protein complexes, particularly in coagulated semen (Montagnon et al, 1982). Glucose may also be an important source of energy to spermatozoa (Peterson and Freund, 1971; Martikainen et al, 1980) and is present in substantial concentrations.

Most of the studies reviewed measured fructose concentration using the resorcinol method. Sheth and Rao (1959) showed that the resorcinol method is not accurate and that the chromatographic method should be used. The chromatographic method measures only fructose, while other methods can measure other reducing substances as well. Sheth and Rao also showed that the concentration of reducing substances increases in semen with time and that this can confound fructose measurements made using the resorcinol method.

One group of researchers has proposed that absolute fructose concentration is not the best method for measuring seminal vesicle function and has further proposed a ‘‘corrected fructose’’ value, which is the fructose concentration (mg/mL) multiplied by the log of the sperm count (mil/mL) (Gonzales et al, 1988, 1993). Gonzales et al showed that this corrected fructose value correlates well with measures of seminal vesicle dysfunction. This derived measure has not been universally accepted.

The mean average fructose and glucose concentrations in the studies we reviewed were 272 mg/100 mL and 102 mg/100 mL, respectively. We have used these concentrations in our simulant, but it should be noted that the variation in the measured values among studies is very large (range of 136–628 mg/100 mL for fructose, range of 4–300 mg/100 mL for glucose). These studies are summarized in Table 5.

Protein

The bulk of the proteins found in semen derive from the seminal vesicles, although albumin is mainly of prostatic origin (Hirsch et al, 1991). A review of the literature indicates that albumin makes up about one third of the protein content of semen. The amino acid content of semen is much higher than that of plasma, and it increases rapidly (particularly glutamic acid) in the hours following ejaculation (Keil et al, 1979; Frohlich et al, 1980).

Protein concentration is difficult to accurately measure and depends greatly on the measurement technique employed. Hernvann et al (1987) showed that measurements made using the Biuret reaction and Lowry’s methods produce much higher values than do Meulemans’ method. Spectrophotometric methods produce even lower values and are only useful for measuring relative rather than absolute protein concentrations (Polak and Daunter, 1989).

In the studies reviewed, the average albumin concentration was 1550 mg/100 mL, and the average total protein concentration was 5040 mg/100 mL. We formulated our simulant with a protein content of 5040 mg/100 mL, with the entire protein contribution made up of bovine serum albumin (see Table 6).

Viscosity

The rheological properties of semen change dramatically after ejaculation; the initial ejaculate quickly coagulates into a gelatinous material, and this material then liquefies. Liquefaction occurs over a period of 5 minutes in vivo, but may take 20–30 minutes in vitro (Montagnon et al, 1982; Polak and Daunter, 1989). The biochemical mechanisms of this coagulation and liquefaction have been investigated by numerous researchers (eg, Mandal and Bhattacharyya, 1985; Polak and Daunter, 1989); the coagulation factors derive from the seminal vesicles, while liquefying factors come from the prostate (Gonzales et al, 1993).

Given the biological importance of this process, it is surprising how little quantitative data there are on the physical properties of semen. A measurement of ‘‘viscosity’’ is frequently performed as recommended in the WHO Manual (1999), which defines an abnormal viscosity sample as one that can be drawn

Table 3. *Semen ion concentrations in mg/100 mL and references*

	References
Ca (mg/100 mL)	
16.7	Abou-Shakra et al, 1989
24.9	Adamopoulos and Deliyiannis, 1983
23	Arver and Sjoberg, 1982
32	Bondani et al, 1973
38	Fong et al, 1986
44.5	Ford and Harrison, 1984
25	Gershbein and Thielen, 1988
24.5	Hirsch et al, 1991
31.9	Homonnai et al, 1978
26.1	Homonnai et al, 1980
20.8	Huggins and Johnson, 1933
24.9	Huggins et al, 1942
26	Jeyendran et al, 1989
30	Kavanagh, 1985
22.0	Kilic et al, 1996
40.9	Mandal and Bhattacharyya, 1987a
23.3	Mandal and Bhattacharyya, 1990
16.0	Ponchietti et al, 1984
13.7	Prien et al, 1990
20.6	Quinn et al, 1965; whole semen
28.2	Quinn et al, 1965; plasma
25.8	Rosecrans et al, 1987
53.3	Sorensen et al, 1999
33	Stegmayr et al, 1982
24.5	Umeyama et al, 1986
Ca^{2+} (mg/100 mL)	
0.68	Arver and Sjoberg, 1982
0.64	Fong et al, 1986
0.96	Ford and Harrison, 1984
1.5	Kilic et al, 1996
0.986	Magnus et al, 1990
0.92	Prien et al, 1990
Cl (mg/100 mL)	
130	Gershbein and Thielen, 1988
157	Hirsch et al, 1991
151.0	Huggins and Johnson, 1933
152	Huggins et al, 1942
112	Jeyendran et al, 1989
133	Kavanagh, 1985
158	Rosecrans et al, 1987
K (mg/100 mL)	
80	Bondani et al, 1973
125	Gershbein and Thielen, 1988
73.75	Girgis et al, 1980
129	Hirsch et al, 1991
89.5	Huggins et al, 1942
88.0	Jeyendran et al, 1989
106	Kavanagh, 1985
247.7	Mendiratta et al, 1980
81.6	Nag and Chaudhuri, 1978
91	Quinn et al, 1965; whole semen
88.0	Quinn et al, 1965; plasma
113	Rosecrans et al, 1987
50	Schirren, 1961
126	Sheth and Rao, 1962a
154	Sheth and Rao, 1968
96	Skandhan et al, 1978
113.9	Skandhan and Mazumdar, 1981
113	Wood et al, 1982

Table 3. *Continued*

	References
Mg (mg/100 mL)	
5.44	Abou-Shakra et al, 1989
8.7	Adamopoulos and Deliyiannis, 1983
11.4	Bondani et al, 1973
6.45	Colleen et al, 1975
13.1	Homonnai et al, 1978
11	Jeyendran et al, 1989
10	Kavanagh, 1985
9.97	Papadimas et al, 1983
31.8	Ponchietti et al, 1984
6.5	Quinn et al, 1965; whole semen
9.0	Quinn et al, 1965; plasma
11.3	Rosecrans et al, 1987
8.6	Sorensen et al, 1999
14	Stegmayr et al, 1982
7.89	Umeyama et al, 1986
Na (mg/100 mL)	
296	Bondani et al, 1973
290	Gershbein and Thielen, 1988
236.6	Girgis et al, 1980
512.0	Hirsch et al, 1991
269	Huggins et al, 1942
274.5	Jeyendran et al, 1989
271	Kavanagh, 1985
326.42	Mendiratta et al, 1980
267.6	Nag and Chaudhuri, 1978
308	Quinn et al, 1965; whole semen
258	Quinn et al, 1965; plasma
235.6	Rosecrans et al, 1987
273	Schirren, 1961
352	Skandhan et al, 1978
329.1	Skandhan and Mazumdar, 1981
Zn (mg/100 mL)	
10.5	Abou-Shakra et al, 1989
13	Arver and Sjoberg, 1982
20.2	Carpino and Siciliano, 1998
6.78	Colleen et al, 1975
19	Cooper et al, 1991
17.2	Hirsch et al, 1991
16.5	Homonnai et al, 1978
14	Jeyendran et al, 1989
16	Kavanagh, 1985
11.76	Lewis-Jones et al, 1996
16.1	Mandal and Bhattacharyya, 1986
16.7	Mandal and Bhattacharyya, 1987a
8.4	Mandal and Bhattacharyya, 1990
14	Mann and Lutwak-Mann, 1981
15.0	Marmar et al, 1975; atomic absorption
18.9	Marmar et al, 1975; neutron activation
13.4	Mawson and Fischer, 1956
9.26	Mendiratta et al, 1980
19.0	Papadimas et al, 1983
16.6	Paz et al, 1977
14.64	Ponchietti et al, 1984
14.7	Rosecrans et al, 1987
69.29	Schoenfeld et al, 1979
10.6	Sorensen et al, 1999
19.5	Stankovic and Mikac-Devic, 1976
13	Stegmayr et al, 1982
12.4	Umeyama et al, 1986
14.4	Wood et al, 1982

Table 4. *Semen osmolarity in mosm and references*

Osmolarity (mosm)	References
254	Gershbein and Thielen, 1988
371.3	Gopalkrishman et al, 1989
422.7	Hirsch et al, 1991
330	Lindholmer, 1973
337	Makler et al, 1981
382	Mandal and Bhattacharyya, 1987b
369.2	Polak and Daunter, 1984
366	Velazquez et al, 1977

out to more than 2 cm with a rod or pipette. This procedure is not a true measurement of viscosity, but rather a measure of the combined elastic and viscous properties of the material. However, it is still used frequently to separate semen samples into normal vs high-viscosity or ''high-consistency'' samples (eg, Dube et al, 1989; Carpino and Siciliano, 1998). More rigorous measurement methods usually involve comparison of the viscosity of semen to that of water using a capillary tube viscometer. This method is useful in comparing samples, but it provides little quantitative information about the rheological properties of the material, as it ignores elasticity, thixotropy, shear thinning, yield stresses, and other important non-Newtonian properties.

The studies comparing the viscosity of semen to that of water are summarized as follows. Tjioe and Oentoeng (1968), using a Hellige viscometer (a 2-capillary system), measured an average viscosity of whole semen of 3.92 centipoise (cP), with a wide range of values (1.3–23.3 cP). Note that the viscosity of water at 25°C/37°C is 0.8904/0.6915 cP. Ray et al (1977) measured the viscosity of previously frozen semen using a capillary device and determined that semen specimens from normal, azoospermic, and vasectomized patients tended to have the same viscosity, while the viscosity of oligospermic patients was higher. We can infer from their results a viscosity of about 3.2 cP for normal whole semen at 37°C. Nag et al (1979) used a U-tube capillary viscometer to study the viscosity of seminal plasma and whole semen from normal, azoospermic, and vasectomized patients. They concluded that only about a third of the differences in viscosity were due to sperm, with most of the remainder due to differences in plasma content. We can infer from their results a viscosity for normal semen of about 3.1 cP at 37°C. Another similar study comparing capillary flow of semen to water was conducted by Moulik et al (1989). They did not report any quantitative information but noted that high viscosity can be an indication of antibodies in the plasma and/or genital tract infection.

The most complete rheological characterization of semen (although only one donor was studied) was performed by Dunn and Picologlou (1977a,b). The material was shown to behave as a viscoelastic material shortly following ejaculation. After full liquefaction it behaved as a Newtonian fluid with a viscosity of 3.37 cP at 33.2°C. These researchers tracked the time course of liquefaction by measuring viscoelastic properties and found that at ejaculation, following coagulation, the material has a viscosity about 100 times its final value. Hubner et al (1985) used a concentric cylinder viscometer to measure the viscosity of previously frozen semen samples at 3 different shear rates. They measured a viscosity of 6.71, 6.11, and 5.77 cP (23°C) at shear rates of 45, 90, and 135 seconds^{-1}, respectively. They concluded that there is no correlation between viscosity and sperm motility and that viscosity was only influenced by sperm count when the count was very high. A study by Lin et al (1992), conducted

Table 5. *Semen fructose and glucose concentrations in mg/100 mL and references*

	References
Fructose	
330	Arver and Sjoberg, 1982
240	Biswas et al, 1978
274	Carpino and Siciliano, 1998
200	Coffey, 1995
352.7	Colleen et al, 1975
256	Cooper et al, 1991
203.8	Davis and McCune, 1950
234	Gonzales et al, 1988
288	Gonzales, 1994
155	Gregoire and Moran, 1973
277	Grizard et al, 1985
278	Harvey, 1951
226.3	Hirsch et al, 1991
290	Homonnai et al, 1980
374.2	Hubner et al, 1985
381	Jathar et al, 1977
232	Jeyendran et al, 1989
154	King and Mann, 1959
251	Kothari et al, 1977
464	Landau and Loughhead, 1951
256.4	Lewin et al, 1976
280	Lewis-Jones et al, 1996
296	MacLeod and Freund, 1958
286	Mandal and Bhattacharyya, 1985
285	Mandal and Bhattacharyya, 1990
224	Mann, 1964
231	Martikainen et al, 1980
179	Montagnon et al, 1982
222	Moon and Bunge, 1968
139.9	Nun et al, 1972
166	Oforofuo et al, 1997
363	Paz et al, 1977
237.3	Peterson and Freund, 1971
352	Pryde, 1946
326	Purvis et al, 1986
231.1	Rosecrans et al, 1987
248	Schirren et al, 1977
628	Schoenfeld et al, 1979
225	Sheth and Rao, 1962b
302	Tauber et al, 1975
136	Tomaszewski et al, 1992
337	Tyler, 1955
295.7	Videla et al, 1981
259	Wolters-Everhardt et al, 1986
Glucose	
18.3	Hirsch et al, 1991
295	Huggins and Johnson, 1933
291	MacLeod and Hotchkiss, 1942
4.3	Martikainen et al, 1980
71	Montagnon et al, 1982
5.41	Peterson and Freund, 1971
25.8	Tomaszewski et al, 1992

Table 6. *Semen protein concentration in mg/100 mL and references*

	References
Albumin	
1100	Gershbein and Thielen, 1988
2000	Hirsch et al, 1991
Total protein	
4000	Carpino and Siciliano, 1998
3700	Gershbein and Thielen, 1988
5727	Gregoire and Moran, 1973
3900	Hernvann et al, 1987
4400	Hirsch et al, 1991
7100	Hubner et al, 1985
4500	Huggins et al, 1942; by difference
5800	Huggins et al, 1942; gravimetric
4620	Mandal and Bhattacharyya, 1985
4140	Mandal and Bhattacharyya, 1990
7460	Montagnon et al, 1982
4200	Nun et al, 1972
5350	Purvis et al, 1986
5449	Srivastava et al, 1984
5195	Verma et al, 1993

using a rotational cone and plate viscometer, found a viscosity of 6.84 cP for normospermic semen, although no shear rate was given for this measurement. Gonzalez-Estrella et al (1994), using a Brookfield viscometer, demonstrated that 90 minutes following ejaculation, viscosities in 2 separate studies of normal consistency samples (as determined by the WHO standard) were 7.4 and 7.7 cP at 25°C. The shear rates experienced by the semen in the capillary viscometers described above are much higher than in the rotational viscometers, and so the difference in the results between these two types of instruments (viscosities of 3–4 at high shear rate vs 6–7 at low shear rate) can be explained by the shear thinning nature of the fluid.

Volume

The volume of a human ejaculate has been extensively studied. In almost all the studies we reviewed, the researchers measured volumes following masturbation. One study found that volumes were significantly higher, 4.99 mL vs 3.92 mL, when collection occurred during coitus (Purvis et al, 1986), while another study by Hotchkiss et al (1938) found a difference in the other direction (3.0 mL by withdrawal, 2.3 mL by condom). Our review of over 30 articles in the literature concluded that the average volume is 3.4 mL, and this is the volume of our simulant referenced in our studies of vaginal gel formulations. These results are summarized in Table 7.

Other Semen Components

Two other components have been included in our simulant, lactic acid at 62 mg/100 mL (Goldblatt, 1935; MacLeod and Hotchkiss, 1942; Lundquist, 1949) and urea at 45 mg/100 mL (Goldblatt, 1935; Srivastava et al, 1984; Hirsch et al, 1991). Materials that are found in small quantities, such as trace elements, have been excluded from our simulant as biophysically, if not biochemically, unimportant. Several components present in substantial quantities have also been excluded for reasons of simplicity and practicality. These include lipids, usually present as ''lipid bodies'' (Mann, 1964), choline, sialic acid, inositol, and spermine, pyruvate, creatine, and ascorbic acid.

Table 7. *Semen volume in mL and references*

Volume	References
4.7	Arver and Sjoberg, 1982
3.8	Balerna et al, 1985
2.7	Bhushan et al, 1978
3.7	Biswas et al, 1978
3.0	Bondani et al, 1973
3.9	Carpino and Siciliano, 1998
3.7	Cooper et al, 1991
2.85	Falk and Kaufman, 1950
3.08	Gonzales et al, 1993
3.09	Gonzales and Sanchez, 1994
3.27	Gregoire and Moran, 1973
3.9	Grizard et al, 1985
3.24	Harvey, 1951
3.0	Haugen and Grotmol, 1998
3.78	Hirsch et al, 1991
3.7	Homonnai et al, 1980
3.0	Hotchkiss et al, 1938; by withdrawal
2.3	Hotchkiss et al, 1938; by condom
2.79	Lewin et al, 1976
3.4	MacLeod and Heim, 1945
3.33	MacLeod, 1950
3.0	Mandal and Bhattacharyya, 1985
3.4	Mandal and Bhattacharyya, 1987b
2.8	Mandal and Bhattacharyya, 1990
3.4	Mortimer et al, 1982
2.9	Nag and Chaudhuri, 1978
4.16	Nikkanen, 1979
4.1	Oforofuo et al, 1997
3.19	Paz et al, 1977
3.92	Purvis et al, 1986; by withdrawal
4.99	Purvis et al, 1986; by condom
3.19	Raboch and Skachova, 1965
3.2	Rehan et al, 1975
3.0	Schoenfeld et al, 1979
3.3	Smith et al, 1996
2.89	Tauber et al, 1975
3.9	Tynen, 1939
3.10	Vaishwanar and Abhyankar, 1971
3.05	Velazquez et al, 1977
3.85	Wolters-Everhardt et al, 1986
3.8	Wood et al, 1982

Simulant Formulation

It was not possible to formulate our simulant and match the concentration of every component and every physical parameter to the literature values. The final recipe for 100 mL of simulant and the properties of the final formulation are described below.

First we mixed the following: 5.46 mL of 0.123 M sodium phosphate monobasic, monohydrate with 49.14

mL of 0.123 M sodium phosphate dibasic, anhydrate. Next, we added the following: sodium citrate dehydrate (813 mg); potassium chloride (90.8 mg); potassium hydroxide (88.1 mg); fructose (272 mg); glucose, anhydrous (102 mg); lactic acid (62 mg); urea (45 mg); and bovine serum albumin (5.04 g). Separately we mixed the following: 101 mg of calcium chloride dihydrate in 15.13 mL of water; 92 mg of magnesium chloride hexahydrate in 15.13 mL of water; and 34.4 mg of zinc chloride in 15.13 mL of water. We slowly added first the calcium solution, then the magnesium solution, and finally the zinc solution to the phosphate buffer solution. We raised the pH with sodium hydroxide to 7.7, sterile filtered the formulation, and froze it until ready for use.

This recipe results in a semen simulant with the following properties, as compared to those measured for human semen. Measured values are mean average values from literature: ph: semen, 7.7; simulant, 7.7; citrate (mg/100 mL): semen, 528; simulant, 523; chloride (mg/100 mL): semen, 142; simulant, 142; calcium (mg/100 mL): semen, 27.6; simulant, 27.6; magnesium (mg/100 mL): semen, 11.0; simulant; 11.0; potassium (mg/100 mL): semen, 109; simulant, 109; sodium (mg/100 mL): semen, 300; simulant, 484; zinc (mg/100 mL): semen, 16.5; simulant, 16.5; osmolarity (mosm): semen, 354; simulant, 340; fructose (mg/100 mL): semen, 272; simulant, 272; glucose (mg/100 mL): semen, 102; simulant, 102; protein (g/100 mL): semen, 5.04; simulant, 5.04; lactic acid (g/100 mL): semen, 62; simulant, 62; urea (g/100 mL): semen, 45; simulant, 45; buffering capacity (slyke): semen, 25; simulant, 38; and viscosity (cP): semen, 3–7; simulant, see following.

Our semen simulant, as formulated above, has a viscosity of about 1.3 cP. The viscosity of the simulant can be elevated to 4 cP by the addition of 0.17% methyl cellulose. Methyl cellulose was chosen because the properties of its aqueous solutions have been shown to be relatively insensitive to changes in pH and ionic strength (Rossi et al, 1995; Ghannam and Esmail, 1997) and have been demonstrated to be viable media in human sperm penetration studies (Ivic et al, 2002).

There is a long history of interest, both clinical and biological, in the composition of human semen. Many reviews have been published, including the classic books by Mann (1964) and Mann and Lutwak-Mann (1981). Taken as a review article, the present article contributes to our understanding of the physical and chemical properties of semen from normal men. However, the motivation for our work derives primarily from the need to create a standardized simulant fluid for human semen for use in research studies related to drug delivery to the human vagina. As noted in the introduction to this article, drug delivery formulations (for therapeutic, contraceptive, and prophylactic applications) may encounter semen during their residence within the vagina, and the resulting interactions can affect biological functionality of the formulations. The rational development of efficacious vaginal formulations requires standardized use of fluids that embody salient properties of ambient fluids within the human vagina. This semen simulant was developed to have the same physical and chemical properties known to influence intravaginal drug delivery gel efficacy and has proven useful in research into contraceptive and prophylactic drug delivery. We should note that this medium was not developed as a culture medium for spermatozoa or other microorganisms; it could, however, be adapted for such purposes.

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We dedicate this article to the memory of Drs Celia Lutwak-Mann and Thaddeus Mann.

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