Viscosity and Refractive Index of Follicular Fluid in Relation to in Vitro Fertilization

BENJAMIN FISCH,[1,3] LINDA HAREL,[1] SHOSHANA AMIT,[1] RUTH KAPLAN-KRAICER,[1] NAOMI MOR,[2] YONA TADIR,[1] JARDENA OVADIA,[1] and PAUL MERLOB[2]

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Purpose: *To set the standard values of follicular fluid viscosity and refractive index, and to investigate a possible relationship between these physiological parameters and the outcome of in vitro fertilization treatment.*

Design and Results: *128 samples of follicular fluid were collected from 40 in vitro fertilization patients. Viscosity determinations (centipoise; mean ± SD) for shear rates of 23, 46, 115, and 230 were 2.04 ± 0.86, 1.84 ± 0.49, 1.48 ± 0.27, and 1.38 ± 0.22, respectively. The average (± SD) refractive index was 1.030 ± 0.002. There was no significant difference between the values of thawed frozen fluids and fresh samples of the same specimens. The data showed no correlation between follicular fluid viscosity or refractive index and the presence of oocytes, their maturation grade or their fertilizing capacity.*

Conclusions: *For the first time, values of the viscosity and refractive index of follicular fluid obtained during in vitro fertilization have been determined. However, these preliminary results did not reveal any relationship between the physiological parameters examined and the outcome of in vitro fertilization treatment.*

KEY WORDS: follicular fluid; viscosity; refractive index; in vitro fertilization.

INTRODUCTION

Various properties of follicular fluid (FF) obtained during oocyte retrieval for in vitro fertilization (IVF) have been studied and examined for correlation with treatment outcome (see Ref. 1 for a review). These include, among others, certain hormone and electrolyte concentrations, pH, osmolarity, and total protein and immunoglobin contents (2–8). The viscosity and refractive index (RI), which reflects the FF specific gravity, have yet to be determined. The present study was undertaken to evaluate the standard values of these two physiological parameters. Furthermore, we aimed at examining their possible correlation with oocyte morphology and fertilizing capacity as well as with treatment outcome. Since both these FF parameters can be easily assessed immediately following follicular aspiration, such a correlation, if it exists, would enable the identification of those oocytes with a higher fertilization potential.

MATERIALS AND METHODS

Patients

One hundred twenty-eight FF samples from 40 patients undergoing ovum pickup as part of IVF treatment at the Beilinson Medical Center were examined. All patients were under 41 years of age, had normal endocrine profiles, and ovulated regularly.

IVF Protocol

Superovulation was achieved using a combined regimen of clomiphene citrate (Ikaclomin; Ikapharm, Israel) and human menopausal gonadotropin (hMG; Pergonal; Teva Pharmaceutical Industries Ltd., Israel). Clomiphene citrate (100 mg) was given daily for 5 days, starting on the third day of the cycle. hMG (2 ampoules; 150 IU) was administered daily from cycle day 4, until at least two follicles reached a diameter of 15 mm. Ovulation was induced with 10,000 IU

[1] IVF and Infertility Unit, Department of Obstetrics & Gynecology, Beilinson Medical Center, Petah-Tikva, and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
[2] Department of Neonatology, Beilinson Medical Center, Petah-Tikva, and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
[3] To whom correspondence should be addressed at Infertility and IVF Unit, Department of Obstetrics & Gynecology, Beilinson Medical Center, Petah Tikva 49100, Israel.

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human chorionic gonadotropin (Chorigon, Ikapharm, Israel). Transvaginal ultrasound-guided follicular aspiration was performed 32–36 hr later.

FF Preparation

Following oocyte isolation, FF was centrifuged at 600*g* and the supernatant divided into two parts: one part was frozen at −20°C, while the other was tested immediately. Oocytes were classified according to the morphologic appearance of the oocyte–corona–cumulus complex (OCCC) (9). FF samples that contained flushing medium and those grossly contaminated with blood were excluded.

Viscosity Measurement

Viscosity was measured at 37°C on 1.2-ml samples in a Wells–Brookfield microviscometer equipped with a CP-7 spindle (cone, 1565°). Triplicate viscosity measurements were taken at shear rates of 23, 46, 115, and 230 sec^{-1}.

Refractive Index (RI) Measurement

The RI correlates closely with the specific gravity of a liquid and therefore reflects the relative properties of dissolved solid components to the total volume of the specimen. It is defined as the ratio of the velocity of light in air to the velocity of light in solution. This ratio varies directly with the number of dissolved particles in a liquid and, as such, varies similarly with the specific gravity. The hand refractometer provides a means for simple, yet quick and accurate determination of the RI of various body fluids (10). In the present work, the RI was determined by the Goldberg refractometer (American Optical Company) for both fresh and frozen specimens. Statistical analysis was performed using chi-square and *t* tests.

RESULTS

FF viscosity determinations (cP; mean ± SD of 128 FF samples) were 2.04 ± 0.86, 1.84 ± 0.49, 1.48 ± 0.27, and 1.38 ± 0.2 for shear rates of 23, 46, 115, and 230, respectively. The average (±SD) of RI was 1.030 ± 0.002, for both fresh and frozen specimens. No correlation was found between the FF viscosity at the different shear rates and the presence or absence of an oocyte in the sample (Table I). Moreover, FF viscosity and RI were similar for follicles containing immature, intermediate, and mature oocytes (Table II). Following insemination, no relationship was found between fertilization and FF viscosity or RI. Likewise, pregnancy following embryo replacement could not be predicted using these parameters (data not shown).

DISCUSSION

Systematic studies of FF viscosity have not yet been described. It appears that this physical parameter changes during follicular growth, although differently in various species (11). These changes have been attributed to alterations in glycosaminoglycan concentration prior to ovulation. Similarly, limited data are available concerning FF specific gravity. Since this property reflects the degree of concentration or dilution of a specimen, it would be affected in this case by follicular wall permeability to water and to substances that derive from the blood as well as by granulosa cell secretory function. FF contains granulosa cell secretions, mainly steroids and glycosaminoglycans, as well as plasma exudate, especially proteins (12–14). It accumulates between granulosa cells in growing follicles and is separated from the blood by the basement membrane. Plasma and FF concentrations of small molecules equilibrate rapidly, whereas the blood–follicle barrier is practically impermeable to proteins with a molecular weight higher than 850,000 (8). The permeability of the basement membrane changes as ovulation approaches (14) when larger molecules can be detected in the FF.

In contrast to FF, blood viscosity has been investigated extensively and may serve as a model for investigating FF viscosity. Factors known to influence whole-blood viscosity include the hematocrit, plasma viscos-

Table I. Follicular Fluid Viscosity Correlated with the Presence or Absence of Oocytes

	Viscosity (cP)	
Shear rate (sec^{-1})	Follicles with oocytes (n = 90)[a]	Follicles without oocytes (n = 38)[a]
23	1.99 ± 0.81	2.16 ± 0.97
46	1.84 ± 0.46	1.86 ± 0.56
115	1.48 ± 0.26	1.47 ± 0.28
230	1.37 ± 0.20	1.40 ± 0.25
Refractive index	1.0305 ± 0.0023	1.0305 ± 0.0021

[a] n represents number of follicles.

Table II. Follicular Fluid Viscosity Correlated with the Maturity of Oocytes Obtained from the Same Follicles

	Viscosity (cP)		
Shear rate (sec^{-1})	Immature oocytes (n = 17)[a]	Intermediate oocytes (n = 29)[a]	Mature oocytes (n = 44)[a]
23	1.99 ± 0.77	2.02 ± 0.83	2.01 ± 0.92
46	1.81 ± 0.42	1.74 ± 0.45	1.84 ± 0.52
115	1.44 ± 0.25	1.46 ± 0.27	1.47 ± 0.31
230	1.36 ± 0.28	1.38 ± 0.17	1.38 ± 0.1
Refractive index	1.0303 ± 0.0024	1.0305 ± 0.0027	1.0308 ± 0.0022

[a] n represents number of follicles.

ity (15), shear rate (15,16), red cell deformability (16), plasma protein concentration (16,17), pH (5), P_aO_2, and temperature (16). Plasma viscosity has been investigated in pregnant women and shown to increase with advanced gestation (3). In the same study, a positive correlation was found between plasma viscosity and increasing fibrinogen content but not with circulating hormone levels. Increased concentrations of certain plasma proteins, especially fibrinogen or macroglobulins, enhance cell aggregation and may greatly increase whole-blood viscosity at low shear rates. Therefore, it appears from this model that, of all the factors which influence the viscosity of body fluids, the protein content is probably the main determinant of FF viscosity. Whole blood behaves as a non-Newtonian fluid at low shear rates, reflecting the effect of reversible adhesion of red cells via fibrinogen (18,19). Our results suggest that FF also behaves as a non-Newtonian fluid at the shear rates studied (its viscosity changes at different shear rates), possibly reflecting the interaction between fibrinogen and cells (erythrocytes and granulosa) present in the fluid.

Various constituents of FF have been examined as markers of follicular and oocyte maturation. In non-stimulated ovarian cycles, FF estradiol and progesterone concentrations rise progressively with advancing follicular maturation, while androstenedione correlates inversely with normal development of preovulatory follicles (4–7). In stimulated cycles, higher FF progesterone levels and higher progesterone/estradiol ratios have been found to be related to successful fertilization *in vitro* of the oocytes contained in these follicles (19–22). In stimulated cycles, high FF luteinizing hormone levels were found to be associated with higher rates of pregnancy following IVF (23). FF prolactin levels have been shown to reciprocate with oocyte maturity and fertilization by some authors (24) but not by others (25). Of follicular proteins studied, only α-1-antitrypsin and fibrinogen levels were found to correlate with fertilization and pregnancy (26). As FF fibrinogen is a major determinant of FF viscosity, we expected to obtain a positive correlation between FF viscosity and fertilization. Had that been the case, the simple measurement of FF viscosity could have been used to predict IVF outcome. However, no such correlation was demonstrated. In conclusion, this study has determined for the first time the viscosity and refractive index of FF obtained from hormonally stimulated ovaries. However, these preliminary results do not reveal a relationship between the physiological parameters of FF and the outcome of IVF treatment.

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