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Seminal Plasma Glycodelin and Fertilization In Vitro

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ABSTRACT: Endometrium-derived glycodelin-A inhibits sperm-egg binding, whereas differentially glycosylated seminal plasma glycodelin-S does not. The difference has been ascribed to the specific type of glycosylation of glycodelin-A. We studied whether the total glycodelin concentration or the relative glycodelin-A concentration in seminal plasma are related to the in vitro fertilization rate of oocytes. We found that total glycodelin levels were significantly higher in a quartile of men with the lowest in vitro fertilization rate compared with the remaining 3 quartiles combined (P = .01). However, for predicting low fertilization capacity of sperm, combining the glycodelin and sperm concentrations by logistic regression analysis did not significantly increase the information obtained from sperm concentration alone. We used specific lectin-immunoassays to determine whether increased glycodelin-A-type glycosylation in seminal plasma would be related to failure to fertilize. No difference was found between the groups with high fertilization and no fertilization in vitro. It is concluded that, although high seminal plasma total glycodelin level has a tendency of being associated with a lower fertilization rate, the difference has limited value to predict fertilization in vitro.

Key words: Glycosylation, spermatozoa.

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Amniotic fluid-derived glycodelin-A (GdA) is a human glycoprotein expressed mainly in secretory/decidualized endometrium (Julkunen, 1986; Julkunen et al, 1986; Seppälä et al, 1997). This glycoform has been found to inhibit sperm-egg binding (Oehninger et al, 1995) and immune cell reactivity (Bolton et al, 1987; Okamoto et al, 1991). These observations and the fact that glycodelin synthesis is induced in the endometrium only after ovulation, particularly in the peri-implantation period, suggest that GdA may contribute to a natural contraceptive microenvironment in the uterus during the postovulatory phase and to embryonic defense mechanisms at the fetomaternal interphase (Seppälä et al, 1997). Peritoneal fluid of patients with endometriosis contains high levels of glycodelin (Koninckx et al, 1992), and this may have bearance on endometriosis-associated infertility because such fluid potently inhibits sperm-egg binding in the hemizona assay (Coddington et al, 1992).

Seminal plasma contains a high concentration of glycodelin that is immunologically indistinguishable from GdA (Koistinen et al, 1996). This glycodelin, named glycodelin-S (GdS), is differentially glycosylated and does not inhibit sperm-egg binding (Koistinen et al, 1996; Morris et al, 1996). GdS is synthesized in seminal vesicles (Koistinen et al, 1997) and its function is unknown. A negative correlation has been reported between glycodelin concentration and sperm motility (Julkunen et al, 1984), but this has not been observed in all studies (Glander et al, 1996). Because the primary and tertiary protein structures of GdA and GdS are identical (Koistinen et al, 1997, 1999), it has been suggested that it is the unique glycosylation pattern that accounts for the inhibitory sperm-egg binding activity of GdA (Morris et al, 1996). We studied whether abnormal glycosylation of seminal plasma glycodelin would exist and result in GdA-type oligosaccharides that would be related to reduced fertilization capacity of sperm. In view of the controversial literature we also studied whether total glycodelin concentration in seminal plasma is related to any changes in sperm quality or to fertilization rate in vitro.

Materials and Methods

The study was approved by the Institutional Review Board of Helsinki University Central Hospital and by the Scientific Board of National Hospital, University of Oslo.

Seminal Plasma Proteins and Sperm Parameters

With informed consent, 112 sperm samples were obtained from men in infertile couples who were participating in an in vitro fertilization (IVF) program at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital. The indications for IVF were tubal damage in 29 women (25.9%), endo-

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metriosis in 21 women (18.8%), polycystic ovarian syndrome in 5 women (4.5%), anovulation in 4 women (3.6%), abnormal sperm in 5 men (4.5%), at least 2 of the above indications in 11 couples (9.8%), and unexplained infertility in 37 couples (33%). After assessment of routine sperm parameters according to World Health Organization guidelines (1993), aliquots of seminal plasma were stored at −20°C for glycodelin and other analyses. Twenty-eight of the samples were oligozoospermic (sperm concentration $<20 \times 10^6$/mL) and 25 were asthenozoospermic (<50% spermatozoa with forward progression); of these, 10 were both oligozoospermic and asthenozoospermic.

The concentrations of glycodelin and total protein, volume of ejaculate, sperm concentration, and percentage of spermatozoa with forward progression were analyzed from the same samples as used for IVF (n = 112). In cases in which the same evaluation criteria were available, comparisons were made for sperm morphology (n = 19), round cells (n = 31), bacteria (n = 31), and antibodies (n = 31).

Total glycodelin concentration was measured by an immunofluorometric assay that detected both GdA and GdS (Koistinen et al, 1996). Total protein concentration was measured using the bicinchoninic acid protein assay (Pierce, Rockford, Ill).

In Vitro Fertilization

Spermatozoa were prepared using PureSperm gradient according to the manufacturer's instructions (NidaCon International Ab, Gothenburg, Sweden). Spermatozoa with forward progression were recovered for IVF from all men ($>10^6$). Oocytes were cultured in droplets of IVF-500 medium (Scandinavian IVF Science, Gothenburg, Sweden) under Ovoil-150 (Scandinavian IVF Science). Insemination was performed with 25 000 to 75 000 progressively motile spermatozoa 4 to 5 hours after oocyte retrieval. Degenerated oocytes and those with fragmented zona were not inseminated. Oocytes were examined for fertilization 17 hours after insemination. Maturity of oocytes was assessed by the appearance of the first polar body. An oocyte was considered to be normally fertilized when 2 pronuclei and 2 or 3 polar bodies were identified. Only normally fertilized and mature (metaphase II), unfertilized oocytes (Veeck, 1986) were considered in the calculation of the fertilization rate of individual oocytes.

Determination of GdA-Type Glycosylation by Lectin-Immunoassays

We used a separate set of normospermic medium-diluted (up to 5% dilution) sperm samples in which spermatozoa were separated by the swim-up procedure (n = 51). Samples were selected according to fertilization rate in vitro and were divided into 2 groups; those with no fertilization in vitro (n = 9), and those in which the fertilization rate was above the average of 57.6% (n = 42). For this set of samples collected in Oslo, sperm preparation and IVF procedures have been described in detail elsewhere (Åbyholm et al, 1990). Indications for IVF in this group included tubal infertility (50%), unexplained infertility (35%), and endometriosis (15%).

GdA-type glycosylation of affinity-purified seminal plasma glycodelin was studied using lectin-immunoassays employing monoclonal antibodies and agglutinins (lectins) from *Wisteria floribunda* (WFA) and *Sambucus nigra* (SNA; Koistinen et al, 1996). WFA reacts specifically with GalNAc and SNA with NeuNAcα2-6Gal(NAc) carbohydrate structures. We have previously shown that these lectins react with GdA but not with GdS (Koistinen et al, 1996).

Statistical Analyses

The Mann-Whitney *U* test was used for comparison of different groups, and Spearman rank correlation was used for determining linear relationships between the 2 variables (StatView 4.1 for Macintosh; Abacus Concepts, Berkeley, Calif). To evaluate parameters that would predict low fertilization capacity we compared the couples in the lowest fertilization rate quartile with the remaining combined 3 quartiles that had higher fertilization rates. Logistic regression analysis either with or without forward and backward stepwise selection was performed using SPSS 6.1 for Macintosh (SPSS Inc, Chicago, Ill). For logistic regression analyses we divided the samples according to fertilization rate (lowest quartile versus remaining cases) and logarithmically transformed the concentrations of sperm and glycodelin to correct for skewness. Receiver-operating characteristic (ROC) curves were drawn and areas under these curves were calculated to evaluate the validity of the variables in prediction of fertilization capacity of sperm.

Results

Seminal Plasma Proteins and Sperm Parameters

Percentiles and mean values of IVF of oocytes, concentrations of glycodelin and total protein, volume of ejaculate, sperm concentration, and percentage of spermatozoa with forward progression (categories a and b) are presented in Table 1.

No significant differences were found between the asthenozoospermic group and the group with normal sperm progression on any parameters, including glycodelin. The IVF rate in the oligozoospermic group and percentage of spermatozoa with forward progression were smaller (P = .0003, P = .0001, respectively), and volume of ejaculate was higher (P = .005) compared with the group with normal sperm concentration. As expected, concentration and motility of spermatozoa were lower in the couples belonging to the lowest fertilization rate quartile compared with the remaining couples (Table 2). There was no linear relationship between total immunoreactive glycodelin concentration in seminal plasma and fertilization rate in vitro (P = .14, $\rho = -0.14$; see Figure), whereas glycodelin levels were significantly higher in those men who belonged to the lowest quartile of fertilization rate compared with the remaining cases (P = .010; Table 2).

As expected, significant linear relationships were found between fertilization rate and sperm motility (P = .020), fertilization rate and sperm concentration ($P < .001$), sperm concentration and volume of ejaculate (P = .002), and sperm concentration and percent of sperm with for-

Table 1. *The percentile and mean values (±SD) of fertilization of the oocytes retrieved for in vitro fertilization, concentrations of glycodelin and total protein, volume of ejaculate, sperm concentration, and percentage of spermatozoa with forward progression (World Health Organization categories* a *and* b*)*

	Mean ± SD	10th	25th	Median	75th	90th
Fertilization (%)	73.8 ± 25.2	42.0	64.0	80.0	94.3	100
Glycodelin (mg/L)	47.8 ± 36.2	12.2	22.0	39.9	68.2	89.1
Total protein (g/L)	33.2 ± 7.9	23.4	27.6	33.4	38.8	42.1
Volume (mL)	3.3 ± 1.4	1.8	2.2	3.0	4.0	5.5
Sperm concentration (10^6/mL)	40.4 ± 28.2	10.0	20.0	32.0	56.5	80.0
Motility (%)	59.4 ± 14.5	43.6	52.0	63.0	69.0	75.4

ward progression ($P < .001$). In addition, we found significant positive correlations between fertilization rate and total protein concentration ($P = .017$). In samples in which full sperm analysis was available, glycodelin concentration did not correlate with morphological properties of sperm, presence of round cells, bacteria, or antibodies (results not shown). The percent of sperm with normal morphology correlated with sperm concentration only ($P = .014$, $\rho = 0.58$).

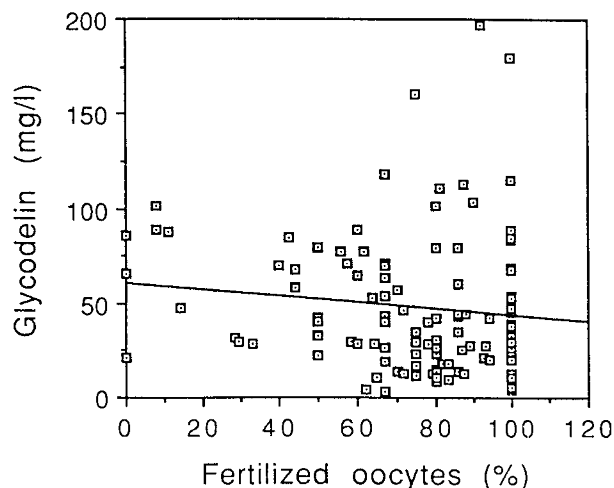
In logistic regression analyses with forward and backward stepwise selection, concentrations of glycodelin ($P = .011$) and sperm ($P = .007$) were the only independent variables predicting low fertilization potential (the quartile with lowest fertilization rate versus remaining cases). ROC plots showed that both the test using sperm concentration alone (area under curve = 0.71, $P = .0004$) and a test in which glycodelin and sperm concentrations are combined by logistic regression analysis (area under curve = 0.74, $P < .0001$) can be used to predict low fertilization capacity; however, these 2 approaches did not significantly differ from each other ($P = .60$). Therefore, it is concluded that measurement of total seminal plasma glycodelin concentration gives little additional information to sperm concentration alone in the clinical evaluation of fertilizing capacity of sperm.

GdA-Type Glycosylation in Total Seminal Plasma Glycodelin

The relative concentration of WFA-reactive glycodelin tended to be higher in the group with no fertilization (1.5 ± 1.8%, mean ± SD, n = 9) compared with the group with an above-average fertilization rate (1.0 ± 0.8%, n = 42), but the difference did not reach statistical significance ($P = .51$, Mann-Whitney *U* test). In the group with no fertilization, 1 man had an abnormally high concentration of WFA-reactive glycodelin in seminal plasma (6.2%). Even in this individual, the concentration of contraceptive GdA-type glycodelin was very low, only 0.53 µg/mL. Among those samples in which total glycodelin concentration was high, the highest GdA concentration (0.85 µg/mL) was still sixfold lower than the lowest concentration that inhibits sperm-egg binding (about 5 µg/mL; Oehninger et al, 1995; Morris et al, 1996). Likewise, the relative concentration of SNA-reactive glycodelin was not significantly different between the 2 groups (0.7 ± 0.6%, n = 9 and 0.6 ± 0.3%, n = 14, $P = .75$). However, there was a positive correlation between proportions of WFA- and SNA-reactive glycodelin concentrations ($\rho = 0.56$, $P = .009$). The WFA- or SNA-reactive, or total glycodelin concentrations were not different between the groups with fertilization rates above the average and the group with no fertilization at all (WFA-reactive glycodelin: 0.2 ± 0.15 µg/mL versus 0.29 ± 0.16 µg/mL; SNA-reactive glycodelin: 0.14 ± 0.04 µg/mL versus 0.12

Table 2. *Comparison between seminal plasma parameters in couples with the lowest quartile of fertilization rate (in this group, less than 64% of the oocytes were fertilized; n = 28) and the remaining couples (in this group, greater than or equal to 64% of the oocytes were fertilized; n = 84). The P values indicate differences between the lowest quartile and the remaining 3 quartiles combined (Mann-Whitney U test)*

	Glycodelin (mg/L)	Total Protein (g/L)	Volume (mL)	Sperm Concentration (10^6/mL)	Motility (%)
P	0.010	0.15	0.45	0.002	0.023
Median, lowest 25%	61.1	31.6	3.0	21.5	55.5
Median, highest 75%	33.9	34.5	3.0	37.0	64.0



Seminal plasma glycodelin levels in relation to fertilization rate of the oocytes retrieved from individual women (n = 112).

± 0.01 μg/mL; total glycodelin: 29 ± 22.3 μg/mL versus 32.5 ± 21.7 μg/mL, respectively; $P > .05$ for all).

Discussion

Clinical experience has shown that there are couples whose sperm parameters are overtly normal, yet fertilization fails in vitro. In many cases, conventional semen analysis and sperm function testing are of little diagnostic value, and new sperm function tests are needed (Barratt and St John, 1998). Poor fertilization in vitro may depend on either or both gametes, or on technical reasons. The role of seminal plasma constituents is not obvious, although previous studies have suggested that low concentrations of seminal plasma CA-125 (Matorras et al, 1995) and high α-glycosidase activity (Spiessens et al, 1998) are favorable signs of fertilization in vitro. Hemizona assay would be helpful for research purposes because it measures the ability of spermatozoa to bind to the zona pellucida (Burkman et al, 1988), but a paucity of human oocytes hampers routine clinical application of this method. The sperm penetration assay using zona-free hamster oocytes (Yanagimachi et al, 1976) is still used in some centers.

As in the present study, Glander and coworkers (1996) found no correlation between glycodelin concentrations and sperm motility. Given the different sites of origin of spermatozoa and seminal plasma glycodelin, the absence of correlations between glycodelin and morphological parameters of sperm is not surprising. Because endometrial GdA inhibits sperm-egg binding but the differentially glycosylated seminal plasma glycodelin does not do so (Oehninger et al, 1995; Morris et al, 1996), it was of interest to learn that total glycodelin concentration in seminal plasma was greater in the quartile of men who had the lowest in vitro fertilization rate. Indeed, this was of further interest because no similar difference was observed in total protein concentration between the same groups. Therefore, we examined the data by logistic regression analysis to develop a multiple parameter test for fertilization prediction. The only independent variables that predicted low fertilization rate were glycodelin concentration and sperm concentration; however, ROC plots showed that glycodelin concentration adds little to the predictive value of measuring sperm concentration alone.

Seminal plasma glycodelin contains a small proportion, usually less than 2%, of GdA-type glycosylation (Koistinen et al, 1996). Therefore, one may postulate that a high total glycodelin concentration would also carry a higher total amount of GdA-type glycodelin that would potentially interfere with fertilization. In order to address this we used the WFA and SNA lectin-immunoassays, which specifically identify GdA but not GdS (Koistinen et al, 1996). Although WFA- and SNA-reactive glycodelin concentrations were not significantly different between the groups with no fertilization and normal fertilization in vitro, the results did not rule out the rare possibility that an abnormally high relative concentration of GdA-type glycodelin would inhibit fertilization. The highest relative concentration of GdA-type glycodelin found in this study was 6.2% of the total glycodelin concentration, and in the total material, the highest absolute GdA concentration was 0.85 μg/mL. Even in these cases the concentration of GdA-type glycodelin remained sixfold to 10-fold smaller than what is needed to inhibit binding of sperm to the zona pellucida (Oehninger et al, 1995). Therefore, we conclude that glycodelin measurement from seminal plasma has little value in the prediction of fertilizing potential of sperm.

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