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Immunoreactive Ubiquitin in Human Seminal Plasma

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ABSTRACT: The polypeptide ubiquitin, up to now almost exclusively discovered in intracellular spaces, was measured immunologically in a total of 187 samples of human seminal plasma. The values were between 1.83 and 19.11 μg/ml. In spermatozoa ubiquitin was detected too; the values, however, were significantly lower than in the seminal plasma. The origin and function of ubiquitin in human seminal plasma is still unclear. The possible role of ubiquitin in reproduction is discussed.

Key words: Human seminal plasma, ubiquitin.

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Ubiquitin was discovered as a protein capable of inducing B-lymphocyte differentiation in 1975 (Goldstein et al, 1975). In the following years, ubiquitin was found in all eukaryotic cells playing a central role in intracellular proteolysis. Various other functions are suggested by the findings of several stable ubiquitin–protein conjugates (Mayer et al, 1991).

Up to now ubiquitin research has almost exclusively been carried out with regard to its functions in the intracellular space. There is little information about the presence of ubiquitin in extracellular fluids. Therefore, it seemed to be of interest to investigate human seminal fluid for its ubiquitin content.

Materials and Methods

Semen specimens with normal and pathologic qualities were obtained by masturbation from 187 unselected men who attended the andrological outpatient clinic because of unexplained infertility. After liquefaction, 0.5–1.0 ml of each sample was frozen (−20°C) and stored. The ejaculated samples were defrosted and centrifuged at ca. 2,600 × *g* for 15 minutes, and the supernatant was frozen until measurement.

From 10 samples, spermatozoa were washed twice with 0.9% NaCl solution and then homogenized with a teflon homogenizer, centrifuged (ca. 2,600 × *g*), and frozen until measurement.

Ubiquitin concentrations were determined by a radioimmunoassay. Bovine ubiquitin (Fa. Sigma, Munich, Germany) was labeled according to the chloramine T method and porcine ubiquitin antiserum from sheep in a dilution of 1:400 served as antibody.

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Bovine ubiquitin standards were between 0.16 and 2.5 μg/ml. Standards and samples were incubated with tracer and antibody—each in a volume of 100 μl—at 4°C for 1 hour. The antigen–antibody complex was precipitated with a polyethylene glycol solution (17%) and centrifuged (2,600 × *g*, 30 minutes). The sensitivity limit of the assay was 10 ng/ml (B_o − 2 SD). Inter- and intraassay variation coefficients were 9.60 ± 2.30% and 7.14 ± 1.84%, respectively. Recovery rates with the addition of 0.25 and 1.25 μg/ml ubiquitin to samples (n = 10) were 107 ± 8.7% and 112.7 ± 14.6%, respectively.

Multiple dilutions of the samples were parallel to the standard curve.

Results

Ubiquitin could be detected in all human seminal plasma samples investigated. The values were between 1.83 and 19.11 μg/ml. The mean was 7.90 ± 3.23 μg/ml. Because there is no difference in the amino acid sequence of bovine and human ubiquitin, it can be assumed that ubiquitin concentrations can be measured accurately in human specimens with the assay used.

Figure 1 shows the distribution curve of the ubiquitin values. Statistical testing of the values for normal distribution according to the method of Kolmogoroff-Smirnow showed a P value of 0.48, i.e., the distribution of ubiquitin values is not significantly different from a normal distribution.

In 10 samples the values of seminal plasma ubiquitin and spermatozoa ubiquitin were compared with each other. For this purpose the spermatozoa were washed with physiological NaCl solution and the washing liquid was examined for its ubiquitin content. Table 1 shows the measured values of ubiquitin concentration in seminal plasma and the spermatozoa after homogenization. The sperm density is shown simultaneously. After the first

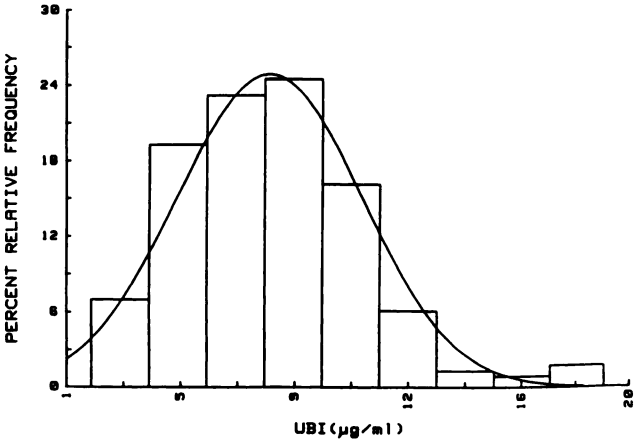

FIG. 1. Distribution (percent relative frequency) of ubiquitin (UBI) concentration in 187 samples of human seminal plasma.

Table 1. *Ubiquitin concentration (µg/ml) in seminal plasma and spermatozoa in comparison to sperm density*

Patient number	Ubiquitin (µg/ml)		Sperm density (million/ml)
	Plasma	Sperma-tozoa	
1	9.37	ND*	10
2	10.73	0.84	11
3	7.68	0.23	12
4	14.26	0.20	14
5	8.78	1.28	18
6	11.21	0.23	21
7	7.27	1.46	22
8	22.40	0.90	25
9	18.40	1.39	40
10	9.10	1.36	128

* Not detectable.

washing procedure only small amounts of ubiquitin were detected; after the second wash the ubiquitin concentration was below the detection limit. In the spermatozoa ubiquitin was found in 9 out of 10 samples. The percentage relation of spermatozoa to seminal plasma ubiquitin concentration was between 1.4 and 20.1%. No correlation of seminal plasma ubiquitin to spermatozoa ubiquitin and to sperm density could be found in the small group investigated.

Discussion

This study shows the presence of immunoreactive ubiquitin in human seminal plasma, in an extracellular space that is a cell-free body fluid. It seems to be the first observation of ubiquitin in high concentrations outside the cell. Surprisingly, there are only small amounts of ubiquitin in the spermatozoa. The question arises regarding the origin of ubiquitin in the plasma. One possible origin could be an outflow of cells, i.e., the spermatozoa. The samples, however, were treated very carefully, and the washing stage is a routine procedure without any greater risk for the cells. Therefore this origin seems to be unlikely. Secretion from the cell surface into the plasma before sperm extraction could be another possibility. In addition, ubiquitin could arise from the prostate or the seminal vesicles.

Contrary to Agell and Mezquita (1988), who observed no ubiquitin in mature chicken spermatozoa, we detected ubiquitin in human spermatozoa. One reason for this different result could be the higher sensitivity of the radioimmunoassay compared with the electrophoresis used by Agell and Mezquita. At least, we cannot exclude cross-reactivity of our radioimmunoassay.

The function of ubiquitin in seminal plasma is still open to speculation. Conceivably, ubiquitin could have an effect during reproduction. Earlier investigations showed a uterus-relaxing effect of ubiquitin (Lippert et al, 1987). Ubiquitin concentrations in the range that could be found in seminal plasma led to a 50% inhibition of spontaneous uterine contractions. Relaxation of the uterus seems to have an importance for the penetration of the sperm.

Further investigations are needed to clarify the origin and function of ubiquitin in seminal plasma.

Acknowledgment

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