Protamine and Fertilin mRNA: Potential Biomarkers of Assisted Reproductive Technology Outcomes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 4, pp. 504-506, April, 2012
Original article submitted December 23, 2011

We studied the relationship between the levels of protamines 1 and 2 (*PRM1* and *PRM2*) and fertilin-β (*ADAM-2*) mRNA expression and outcomes of infertility treatment using assisted reproductive technologies was studied. Analysis of the relationships between the outcomes of *in vitro* fertilization and embryo transfer and profiles of the expression of seminal genes *PRM1*, *PRM2*, *ADAM-2* mRNA, evaluated by reverse transcription quantitative PCR was carried out in 79 couples. Significant differences in the expression of seminal *PRM1*, *PRM2*, *ADAM-2* mRNA were detected in couples with different outcomes of *in vitro* fertilization and embryo transfer. The levels of seminal gene expression are potential predictors of the efficiency of *in vitro* fertilization and embryo transfer.

Key Words: *assisted reproductive technologies; protamines (PRM1, PRM2); fertilin-β (ADAM-2); reverse transcription quantitative PCR*

The priority significance of infertility problem and creation and development of new modern approaches to its diagnosis and treatment stimulate the development of assisted reproductive technologies.

The impact of the male factor for early ontogenesis processes is now in the focus of the attention [9]. Presumably, "paternal contribution" to embryo development consists in the genetic material granted by the father and in delivery of seminal mRNA stored in the cytoplasm until translation process has to be realized and the essential molecules obtained [4,7,8]. It was previously assumed that transcripts for the early embryo are stored exclusively in oocytes. Since S. M. Wykes *et al.* described seminal mRNA in 1997, the paternal contribution to fertilization and embryo development became an important object of research [4,7].

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The spermatozoon transcriptome contains more than 400 mRNA types [3,4]. Presumably, spermatozoon transmits these mRNA to the oocyte during fertilization [3,4,8]. In addition, as a part of the nuclear matrix, the seminal mRNA perform structural function [8,9].

Chromatin remodeling (replacement of histones and histone-like proteins packing DNA with protamines through a series of transistor proteins) is realized in the course of spermatogenesis [1,2]. As a result of this process, chromatin becomes transcriptionally inert because of high DNA condensation. Recent studies demonstrated the relationship between the quantity of protamine transcripts, protamine concentration, spermatozoon mobility, and fertilization capacity [4,7,11]. Protamine deficiency can result from transcription and translation disturbances during spermatogenesis [3,4].

Spermatozoon penetration capacity is one more essential factor for the outcome of assisted reproductive technology. A Disintegrin And Metalloprotease Domain (ADAM) proteins, also known as fertilin-α

0007-4888/12/1534-0513

(*ADAM-1*) and fertilin-β (*ADAM-2*), play the key role in the spermatozoon-oocyte interactions. These proteins are located in the equatorial region of the spermatozoon head, while the fertilin soluble domains bind to the oocyte microvillous region. The findings of the British scientists indicate that fertilin-α gene in humans is inert and is a pseudogene; hence, we studied the expression of fertilin-β mRNA [6]. Fertilin-β potentiates binding of spermatozoon head and promotes gamete fusion. However, the eventual role and contribution of fertilin-β to the zygote formation and embryo development remain unknown.

Hence, mRNA profile may characterize spermatozoon status and is essential for the early stages of embryogenesis. We studied the relationships between the levels of types 1 and 2 protamines (*PRM1* and *PRM2*) and *ADAM-2* mRNA expression and outcomes of sterility treatment by assisted reproductive technologies.

MATERIALS AND METHODS

The *PRM1*, *PRM2*, *ADAM-2* mRNA profiles were studied by reverse transcription quantitative PCR in 79 couples with different results of infertility treatment by *in vitro* fertilization (IVF) and embryo transfer (ET). The IVF and ET outcomes were effective in 13 couples (group I; control) and failed in 66 couples (group II). Of these, 10 couples had spontaneous abortions before week 11 (subgroup IIa), only biochemical pregnancy was recorded in 19 couples (subgroup IIb), and there was no pregnancy in 37 couples (subgroup IIc).

Specimens of native seminal fluid for PCR were collected on the day of transvaginal puncture of the ovaries. The expression of protamine and fertilin mRNA was evaluated by the ΔΔCq method by comparing standard values of the studied genes threshold cycles. Standardization by the reference genes *HPRT1*, *TBP*, and *B2M* was carried out. The median (Me) in the control group was taken for a unit.

The median was selected as a measure of the central trend in quantitative signs and the upper and lower quartiles were used for interval evaluation for statistical processing of the results. The significance of differences between the groups was evaluated using Mann–Whitney's *U* test.

RESULTS

A significant decrease in *ADAM-2* (fertilin-β) mRNA expression in native seminal fluid was found in couples with failure of IVF and ET programs: 6.3 times drop in subgroup IIb (Me=0.16, p=0.002) and 2.6 times drop in subgroup IIc (Me=0.38, p=0.012) in comparison with the control group (Table 1). A similar trend was traced in subgroup IIa: by 2.2 times lower than in the control (p=0.077).

Analysis of differences in the levels of *ADAM-2* mRNA expression in the control and group II subgroups showed a statistically significant reduction (2.6 times) of *ADAM-2* in group II (p=0.003).

Significant differences in protamines mRNA expression in groups I and IIb were detected: *PRM1* mRNA expression was by 3.7 times (p=0.023) and *PRM2* by 7.1 times (p=0.008) below the control. A similar trend was traced in subgroup IIc: by 1.2 (p=0.13) and 1.9 (p=0.15) times below the control, respectively. No significant differences or trends to reduction of *PRM1* and *PRM2* mRNA expression in subgroup IIa (spontaneous abortions) in comparison with the control group were detected.

ADAM-2 (fertilin-β) plays the key role in gamete fusion. The main function of protamines is realized in chromatin remodeling. The biological sense of this process consists in compact delivery of the paternal

TABLE 1. Expression of mRNA in Groups (*Me* (*L-H*))

Group		ADAM-2	PRM1	PRM2
I (control, n=13)	clinical pregnancy or delivery	1.0 (0.6-4.0)	1.0 (0.7-1.6)	1.0 (0.4-1.7)
IIa (n=10)	miscarriage before week 11	0.4 (0.4-0.5) p=0.077	1.8 (0.4-3.0) p=0.73	0.8 (0.4-3.5) p=0.92
IIb (n=19)	biochemical gestation	0.2 (0.1-0.7) p=0.002	0.3 (0.1-0.7) p=0.023	0.1 (0.1-0.6) p=0.008
IIc (n=37)	IVF failure	0.4 (0.1-1.4) p=0.012	0.8 (0.2-1.3) p=0.13	0.5 (0.2-0.8) p=0.15

Note. Significant difference in comparison with the control.

genetic material to the oocyte without risk of injury to DNA molecules. A different quantity of protamines leads to inadequate compactization of chromatin and violation of structural integrity of DNA. Spermatozoa with high DNA fragmentation retain the capacity to fertilize oocytes under conditions of assisted reproductive technologies, but subsequent embryonic development can be blocked at different stages. A significant correlation between the degree of chromatin condensation and embryo survival at different stages of development has been detected [5]. This can be explained by involvement of protamines in interactions between the maternal and paternal genomes and in organization of chromosome packing during the first division of the zygote and subsequent first divisions of the embryo.

Our data indicate a negative effect of low protamine and fertilin-β mRNA levels on the outcomes of assisted reproductive technologies, which was in line with other reports [4,7].

Hence, evaluation of the expression of seminal mRNA, characterizing the paternal contribution to embryo development, can be used as a predictive marker of the efficiency of assisted reproductive technologies.

The study was supported by the Ministry of Education and Science (State Contract of the Russian Federation No. 16.22.12.2009 of 29.09.2011).

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