PGM SUBTYPING OF SEMEN AND VAGINAL SECRETIONS

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INTRODUCTION

The polymorphism of human phosphoglucomutase (PGM 1), well known in blood, has been shown to be present in semen and vaginal secretions (Culliford 1971; Brinkmann and Koops 1971). The extended polymorphism of PGM 1 later revealed in blood by isoelectric focusing, was reported in semen as well (Sutton 1979).

PGM activity in vaginal secretions is usually very low (Price et al. 1976; Eastwood 1977) but increases in the presence of semen (Garlo 1985). Other alterations of the PGM pattern in body fluid mixtures have been reported. The PGM group of semen is modified by saliva (Sensabaugh et al. 1980); this modification being reversed by addition of reducing agents.

In this work, the stability of PGM subtypes was studied in semen stains and postcoital vaginal samples, in view of their application to sexual assault evidence.

MATERIALS AND METHODS

Samples: Semen samples were obtained from the Urology Clinic, vaginal secretions samples from the Gynecology Clinic; coupled blood/semen/vaginal samples were collected from volunteers. Most liquid semen were frozen at -18°C within no more than 10 hours. Vaginal samples were collected with sterile cotton wool swabs. Semen stains and vaginal swabs were stored dried at room temperature. The presence of semen in the vaginal samples was assessed by a quantitative ELISA for P30 (Kamenev et al. 1987).

Extraction: For PGM, 50 mm2 of semen stain or of vaginal swab were extracted in 50 μl of a 0.1 % (v/v) 2-mercaptoethanol aqueous solution, for 1 hour at 20°C or alternatively overnight at 4°C. Liquid semen was diluted 1:3 with water containing 2-mercaptoethanol at the same final concentration. This optimal concentration was previously assessed in semen samples of various PGM subtypes, mixed with saliva.

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Electrophoresis was run on cellogel, according to Sonneborn (1972) and stained according to Spencer et al. (1964).

Isoelectric focusing : polyacrylamide gels (5% T, 3% C, 220 x 110 x 0.5mm) were prepared with carrier ampholytes pH 4-6(LKB) 1 % (w/v) and pH 6-8 (LKB) 1 % (w/v). The anolyte and catholyte were 1M H_3PO_4 and 1M NaOH respectively. Samples were applied on Whatmann n° 1 filter papers, at 2 cm from the cathodal wick. Focusing was performed on the LKB Multiphor II, with Vmax: 1000 V, Imax: 10 mA, Pmax: 8.5 W, for 115 min. The gels were then PGM stained in the usual way. For some gels, the upper third was separately stained with Coomassie blue.

RESULTS

PGM Subtypes of Semen

72 samples of liquid semen were PGM subtyped and the observed phenotype frequencies were not significantly different from those found in the belgian population (Coosemans et al.,to be published). In 10 cases, the PGM subtypes of semen and blood were compared and no discrepancy observed.

Stability of Semen PGM in Experimental Stains

Semen stains of all PGM subgroups (except 2A2B) were made on cotton cloth with fresh semen and stored dried at room temperature. In all 72 recent stains the correct PGM subtype was determined. Out of 37 stains, analyzed after three months, 33 were PGM subtypable and 4 with initial lower PGM level in liquid semen were no more typable. After six months of storage, 11 stains out of 15 were still typable. After eight months, 5 stains out of 9 were still interpretable.

PGM Subtypes of Vaginal Secretions

23 semen-free vaginal swabs were analyzed. They were collected at least three days after sexual intercourse and were P30 negative. No blood was visible, although the benzidine test for blood was positive on some of them. Only 5 out of 23 samples had sufficient PGM activity to be subtyped. PGM activity was correlated neither with the delay before drying (immediate for 13 swabs, several hours for the others) nor with the delay before analysis (about four days for 10 swabs and about twenty five days for the others).

Postcoital vaginal swabs exhibited higher PGM activity since 5 out of 7 swabs collected within less than four hours after intercourse were PGM typable. Similarly 4 out of 5 swabs collected between 9 and 12 hours after intercourse were PGM typable, although semen PGM are known to be no more detectable after this delay. These swabs were dried after a few hours and typed after about 15 days.

Stability of Semen PGM in the Vagina after Sexual Intercourse

Eight couples provided a timed intervals set of post-coital vaginal swabs and a pre-coital semen-free swab as well. The swabs, immediately dried were analyzed within the next few days. Donors n° 4, 8 and 9 remained lying for eight hours after intercourse whereas the others were active. The results, summarized in the table, show that vaginal PGM activity was increased whereas seminal PGM subtypes could only be determined during a short period (seminal PGM was still typable after 6.5 h in donor n° 9). These findings are in agreement with those previously reported (Price et al. 1976; Eastwood 1977; Davies 1982; Garlo 1985). The PGM are optimally typable few minutes after intercourse. After 30 min., the enhancement of PGM activity often causes smears on the gels, affecting the bands'resolution. PGM activity presents individual variations. This could be partially due to the manner of collection of the samples, that were taken by the donors themselves. PGM activity was generally found to be associated to high amounts of semen as reflected by high P30 concentration..

Table 1: PGM subtypes in semen-positive vaginal swabs

donor	test	blood female	blood semen male	time after intercourse (h) prior	0.1	0.5	1.5-2.5	3.5-6.5	5.5-6.5	7.5-8.5	9-10
2)	PGM	2B1B	1A1B	neg	1A1B	(1A) (1B)	1A1B	neg	neg	neg	
	P30			neg	$>2^{-10}$	2^{-4}	$>2^{-10}$	2^{-6}	2^{-4}	2^{-3}	
3)	PGM	2A1B	1A	2A(1B)	2A(1B)1A	2A1B1A	2A1B1A	2A(1B)(1A)	neg	neg	
	P30			neg	$>2^{-10}$	$>2^{-10}$	$>2^{-10}$	$>2^{-10}$	2^{-7}	2^{-6}	
6)	PGM	1A1B	2B1B	neg	(1A) (1B) (2B)	1A1B2B	1A1B2B	neg	neg	neg	
	P30			neg	$>2^{-10}$	$>2^{-10}$	$>2^{-10}$	2^{-5}	2^{-5}	2^{-1}	
9)	PGM	1B	2A1A	neg	1B2A1A	1B2A(1A)	1B2A(1A)	1B2A(1A)	1B2A(1A)		neg
	P30			neg	$>2^{-10}$	$>2^{-10}$	$>2^{-10}$	2^{-9}	$>2^{-10}$		2^{-9}
4)	PGM	1A	1A	1A	1A	1A		1A	neg	neg	
	P30			neg	$>2^{-10}$	$>2^{-10}$		$>2^{-10}$	$>2^{-10}$	2^{-5}	
10)	PGM	1A	1A	neg	1A	(1A)	1A	1A	(1A)	(1A)	neg
	P30			neg	$>2^{-10}$	$>2^{-10}$	$>2^{-10}$	$>2^{-10}$	2^{-8}	2^{-7}	2^{-6}
8)	PGM	1A2B	1B	neg	(1)	neg	neg	neg	neg	neg	
	P30			neg	$>2^{-10}$	2^{-8}	2^{-6}	2^{-3}	2^{-2}	2^{-1}	
5)	PGM	1A1B	2A1B	neg	neg	neg	neg	neg	neg	neg	
	P30			neg	2^{-10}	2^{-9}	2^{-9}	2^{-2}	1	2^{-1}	

P30 : last positive two-fold dilution of the extract

( ) : weak but still PGM subtypable band.

DISCUSSION

Isoelectric focusing is a sensitive method but limitated by frequent distortions in the PGM pattern of semen and vaginal samples, due to their high and variable salt content. Dialysis of microsamples is not convenient. In order to be informed of a possible distortion, non polymorphic bands were stained with Coomassie blue, in the upper part of the gel where the locus 2 PGM bands are usually absent.

Seminal PGM is a highly polymorphic and stable protein. Nevertheless, results obtained in casework are disappointing, due to the complex situation in body fluid mixtures.

The success rate of seminal PGM typing in vaginal swabs is low, for different reasons: 1) the rapid elimination of seminal PGM, 2) the high vaginal PGM activity possibly masking seminal PGM and 3) the frequent occurrence of smears in isoelectric focusing patterns. Better results should be obtained from vaginal drainage stains and, when possible, even more from other semen stains.

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