

# Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation

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**The existence of a complex population of mRNA in human sperm is well documented but their role is not yet elucidated. Using discontinuous density gradients, we have isolated high and low motile sperm from the same semen sample. The levels of different transcripts coding for molecules either involved in nuclear condensation (protamines 1 and 2) or in capacitation [endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS) and *c-myc*] were then assessed in the two populations using semi-quantitative RT-PCR. Sperm viability was estimated by eosin–nigrosin staining and by hypo-osmotic swelling test; apoptosis percentage was measured by the TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling technique. The contamination by somatic and germ cells was assessed by looking for specific molecular markers of these cells, respectively CD-45 and E-cadherin for somatic cells and *c-kit* for germ cells. The viability of sperm was unchanged in high and low motile fractions, as well as DNA fragmentation percentage. The amount of Prm-1 mRNA was significantly higher in low density motile than in the high motile fraction. In most of high motile sperm samples eNOS and nNOS transcripts were undetectable whereas they were present in the low motile sperm. In contrast, no significant variation was found in the *c-myc*/Prm-2 mRNA ratio between the two populations. Moreover, a partial or complete disappearance of *c-myc* transcripts was observed after capacitation. Thus analysing mRNA profiles could be helpful as a diagnostic tool and prognosis value for fertilization.**

*Key words:* capacitation/human/motility/RNA/sperm

## Introduction

During the last decade, mRNA have been discovered either by gene-specific RT-PCR or *in situ* hybridization in human ejaculated sperm. A non-exhaustive list of transcripts including *c-myc*, HLA class 1, protamines 1 and 2, heat shock proteins 70 and 90,  $\beta$ -integrins, variants of phosphodiesterase, progesterone receptor and aromatase reveals a wide range of transcripts in mammalian sperm (Lambard *et al.*, 2003; reviewed in Miller, 2000; Wykes *et al.*, 2000). However, their putative roles are unknown, but several hypotheses could be suggested to explain the presence of mRNA in sperm.

The most common idea is to consider that these transcripts represent remnants of stored mRNA from post-meiotically active genes. Spermatogenesis consists of a complex and dynamic process of proliferation and differentiation leading to the transition of diploid spermatogonia to haploid sperm. During this process, nucleosomal histones, present in spermatogonia, are gradually replaced by transition proteins (Tnp-1 and Tnp-2) in round spermatids, and subsequently by protamines (Prm-1 and Prm-2) in elongating spermatids and sperm. These proteins are responsible for a highly stable chromatin condensation leading to a sperm genome transcriptionally inactive (reviewed in Grootegoed *et al.*, 2000). Haploid post-meiotic germ cells are then characterized by a temporal uncoupling of translation and transcription (see for reviews: Dadoune, 2003; Steger, 2003). Translation of some mRNA into

proteins could then occur for up to several days after the cessation of transcription before the completion of spermiogenesis. Thus, the mRNA present in mature sperm seem to be roughly similar to those found in testis and could reflect the accurate development of spermatogenesis.

It has also been reported that there is the persistence of a low but detectable level of transcription in mature sperm cells (Miteva *et al.*, 1995). More recently, Naz (1998) has described the existence of transcriptional and translational activities in human sperm during capacitation and acrosome reaction, which could also explain the presence of mRNA in mature sperm.

Finally, one can argue that these stored mRNA may be used during the first steps of fertilization, which are not sensitive to transcriptional inhibitors, and then contribute to the paternal printing (Braude *et al.*, 1988; Siffroi and Dadoune, 2001).

Indeed, as also reported for the PAF receptor mRNA (Roudebush *et al.*, 2000), we have recently shown a significant decrease of aromatase mRNA level in low motile sperm compared to high motile sperm isolated from the same semen sample of normospermic patients (Lambard *et al.*, 2003). These results suggest that the establishment of sperm mRNA profiles could be used as a genetic fingerprint of normal fertile men.

We have therefore tested the hypothesis that sperm quality could be related to its mRNA distribution by comparing the levels of different transcripts coding for molecules either involved in nuclear

condensation (protamines 1 and 2) or in sperm function [endothelial nitric oxide synthase (eNOS), nNOS and *c-myc* respectively; Naz *et al.*, 1991; Herrero *et al.*, 2003] between low and high motile sperm isolated from the same sample.

## Materials and methods

### Human sample preparation

Sperm samples ( $n = 41$ ; mean age:  $33.4 \pm 4.1$  years) were obtained from healthy donors by masturbation after 3 days of sexual abstinence and allowed to liquefy for 30–60 min at room temperature before processing. Informed patient consent was obtained for the use of sperm samples in this study. The selected specimens had normal semen parameters according to the World Health Organization (1999) guidelines. Infectious sperm and samples with  $> 1 \times 10^6$  round cells/ml were excluded. A spermocytogram was performed to eliminate samples with cytoplasmic droplets. To rule out the possibility of any contamination by residual cells (germ cells or polynuclear cells), the liquefied semen samples were fractionated on a discontinuous Puresperm gradient (JCD, France) consisting of four successive layers with the following densities: 95, 76, 57 and 47.5%. After centrifugation (20 min at 300 g, 25°C), high motile ( $> 90\%$ ) and low motile sperm ( $< 30\%$ ) were isolated respectively from the 95% layer and from the interface 76–57%. Sperm obtained from the interface 76–57% showing motility  $> 30\%$  were excluded. These two fractions were then washed twice with Earle's medium (Eurobio, France) before being used. A microscopic examination of the enriched sperm fractions (95 and 76–57%) was performed to control the quality of cell preparations. No remaining round cells and cytoplasmic droplets were observed as described by Lambard *et al.* (2003). Human granulosa cells (positive control for eNOS and *c-myc*) were obtained by collection of human follicular fluid from pre-ovulatory follicles in the IVF centre (CHRU Clemenceau). Human sperm samples contaminated by either testicular germ cells or leukocytes were used as positive controls respectively for *c-kit* and CD45 (Lambard *et al.*, 2004). E-cadherin, a positive marker for epithelial cells, was also included in the quality control of our sperm cell preparations (Andersson *et al.*, 1994).

### Evaluation of sperm viability

Methods for evaluation of the sperm plasmalemma integrity in motile and immotile fractions included both EN vital stain and hypo-osmotic swelling test (HOST). For the EN test, smears were prepared by mixing 20  $\mu$ l of sperm fraction and an equal volume of EN solution (4 g eosin plus 5 g nigrosin per litre of distilled water; Sigma Chemical Co., France; BDH Chemical Ltd, UK) (Blom, 1950). The percentage of membrane-intact sperm identified by EN was determined by counting 200 sperm under magnification ( $\times 400$ ) with bright-field microscopy. Unstained sperm were viable whereas sperm showing partial or complete purple staining were considered as dead cells.

The HOST was performed by incubating 100  $\mu$ l of sperm fraction with 100  $\mu$ l of a hypo-osmotic solution (7.35 g sodium citrate plus 13.5 g fructose per litre of distilled water; Sigma) at 37°C for 60 min. Two hundred sperm were evaluated under magnification ( $\times 400$ ) with bright-field microscopy. Sperm without swollen or coiled tails were considered as non-viable cells.

### TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) assay

The DNA fragmentation was assessed using the Apo BrdU Kit (Phoenix Flow Systems, USA). Sperm diluted to  $5 \times 10^6$  cell/ml were centrifuged at 600 g for 5 min, fixed and permeabilized in 70% ethanol at  $-20^\circ\text{C}$  for  $> 12$  h. After two washes with 1 ml of phosphate-buffered saline (PBS), the elongation reaction was performed by incubating sperm in 50  $\mu$ l of labelling solution containing the TdT enzyme and dUTP for 1 h at 37°C. For each experimental set, a negative control was prepared by omitting TdT from the reaction mixture. Two subsequent washes were performed to stop the reaction. To perform the labelling reaction, the fluorescein-PRB1 antibody, providing high specificity for dUTP, was incubated with sperm for 30 min at room temperature in the dark. Before flow cytometry analysis, sperm was

washed twice with PBS, labelled with 150  $\mu$ mol/l of propidium iodide, and filtrated. Positive controls were prepared as described before but with an additional treatment with 10 IU DNase I for 1 h at 37°C before the labelling reaction.

Analysis was performed using the cell-sorter FACS Vantage SE (BD Biosciences, USA). Fluorochromes were excited with the 488 nm line of the Enterprise laser (Coherent, USA). Green and red fluorescences were detected using FL1 and FL3 detectors respectively through a BP 530/30 nm and a BP 695/40 nm filters.

For each sample, 15 000 events were recorded at a flow rate stabilized at 200–300 cells/s. Cell doublet and debris were excluded using a FL3-A versus a FL3-w gate. Analysis of DNA fragmentation was performed using a FL3-A versus FL1-H cytogram. FL1 and FL3 fluorescence signals were respectively recorded before logarithmic and linear amplifications. Data were analysed with Cell Quest Pro Software (BD Biosciences).

### Capacitation

Purified sperm were capacitated in Earle's medium supplemented with BSA (3 mg/ml; Roche, France) for 4 h at 37°C in 5% CO<sub>2</sub> and 95% air.

### RNA extraction

Total RNA from purified sperm fractions and granulosa cells were extracted using the guanidium thiocyanate-derived method (Chomczynski and Sacchi, 1987). Briefly, after centrifugation the cell pellets were homogenized on ice in a 1 mol/l Tris buffer containing guanidinium thiocyanate 4 mol/l. The RNA was then isolated with a phenol–chloroform–isoamyllic acid solution and was precipitated from the aqueous phase with isopropanol. The RNA was again re-extracted and precipitated with isopropanol, washed with 75% ethanol, dried on speed-vac and dissolved in Diethyl pyrocarbonate (DEPC)-treated water and then stored at  $-80^\circ\text{C}$ . The purity of RNA samples was checked spectrophotometrically at 260 and 280 nm.

### RT-PCR assay

Four hundred nanograms of total RNA were reverse-transcribed to first strand cDNA as follows: 1 h at 37°C with 200 IU M-MLV-RT (Promega, France), 500  $\mu$ mol/l dNTP, 0.2  $\mu$ g oligo-dT (12–18 mers) and 24 IU RNasin in a final volume of 10  $\mu$ l, then 5 min at 94°C. The DNA were further amplified by PCR using selected oligonucleotides. PCR were performed in the presence of 1.5 mmol/l MgCl<sub>2</sub>, 200  $\mu$ mol/l dNTP, 1.5 IU Taq polymerase and 50 pmol of the forward and reverse primers (Life Technology, France) in a final volume of 50  $\mu$ l. The applied PCR primers and the expected lengths of the resulting PCR products for eNOS, nNOS, *c-myc*, Prm-1 and Prm-2 are shown in Table I. All primers have been chosen in different exons to eliminate a possible contamination by genomic DNA. The different cycle profiles are summarized in Table II. For all PCR amplifications, negative control (only water) and positive control were included. All cDNA fragments were run on a 1.5% agarose gel stained with ethidium bromide and visualized under UV transillumination.

### Semi-quantitative RT-PCR

In order to quantify different transcripts (Prm-1, Prm-2 and *c-myc*) in human sperm samples, we have determined the optimal conditions for the RT-PCR. The PCR amplifications were carried out for 26, 22 and 37 cycles for Prm-1, Prm-2 and *c-myc* respectively (Figure 1). The resulting PCR products were analysed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Gels were photographed using Polaroid film under UV light and analysed using AGFA Snap-Scan 1200U Scanner, Photoexpress software and the NIH image computer program (<http://rsb.info.nih.gov/nih-image>). The intra- and inter-assay coefficients of variation were  $< 12\%$ .

### Statistical analysis

Results are means  $\pm$  SEM. Statistical analyses were determined by two-way analysis of variance (ANOVA).  $P < 0.05$  was considered significant.

**Table I.** Oligonucleotide sequences used for RT-PCR

Gene	Sequence (5'-3')	Size of PCR product (bp)
Protamine-1	5'-GCCAGGTACAGATGCTGTGCGCAG-3' 5'-TTAGTGTCTTCTACATCTCGGTCTG-3'	153
Protamine-2	5'-GGATCCACAGGCGGCAGCATCGCT-3' 5'-GCATGTTCTTCTCCTGGTTCTGCA-3'	104
CD45	5'-TGCAGATGCCTACCTTAATGC-3' 5'-CACATTGCAGCACTTCCATT-3'	844
c-kit	5'-AGTACATGGACATGAAACCTGG-3' 5'-GATTCTGCTCAGACATCGTCG-3'	780
c-myc	5'-TCCTCGGATTCTCTGCTCTC-3' 5'-TCGCCTTTGACATTCTCCT-3'	416
E-cadherin	5'-TCTACAGCATCACTGGCCAAGGAGCTG-3' 5'-AGCTTGAACCACCAGGTATACGTAGG-3'	745
eNOS	5'-AAGGTCTTCTCCTGGTAATGC-3' 5'-CAGTGTCCAACATGCTGCTGGAAATTG-3'	477
NOS	5'-TTTCCGAAGCTTCTGGCAACAGCGCAATT-3' 5'-GGACTCAGATCTAAGCGGTTGGTCACTTC-3'	471

## Results

### Characterization of the high and low motile sperm fractions

We have checked the purity of the two fractions by controlling for the absence of round cells and cytoplasmic droplets in the two selected populations as described by Lambard *et al.* (2003). Moreover, we have been unable to amplify either CD45, *c-kit* or E-cadherin mRNA which are positive markers for leukocytes, testicular germ cells and epithelial cells respectively, thus demonstrating the purity of our preparations (Figure 2).

The viability of the low motile fraction has been estimated both by EN test and HOST. The percentage of live cells was >80% in low motile sperm and was not significantly different with respect to the method used (EN:  $76.7 \pm 4.3\%$  versus HOST:  $79.8 \pm 4.4\%$ ,  $n = 10$ ) (Figure 3A).

The percentages of non-apoptotic and live cells, respectively estimated by TUNEL technique and EN staining, were similar whatever the technique used ( $85.7 \pm 3.1$  versus  $86.6 \pm 1.9\%$ ) (Figure 3B).

The percentage of non-apoptotic cells in motile and low motile sperm was  $92.7 \pm 1.8\%$  in the high motile fraction versus  $85.7 \pm 3.1\%$  in the low motile fraction ( $P < 0.005$ ;  $n = 6$ ) whereas the motility was >90 and <30% respectively (data not shown).

### Protamine mRNA status

The quantification of Prm-1 and -2 mRNA has been performed by a semi-quantitative RT-PCR. Prm-1 mRNA levels were significantly higher in the low motile fraction compared to the high motile fraction (Figure 4). No significant variation was observed between the two groups for Prm-2 mRNA levels. Thus, the low motile population showed a significant increase (43%) of the Prm-1/Prm-2 mRNA ratio compared to the high motile fraction.

### Expression of NOS mRNA

The presence of eNOS and nNOS proteins has been demonstrated in human sperm (Herrero *et al.*, 1996), but there is no report about the presence of eNOS and/or nNOS transcripts. Our RT-PCR results demonstrated a large difference in eNOS and nNOS mRNA distribution between the two fractions of sperm (Figure 5). All sperm samples analysed presented a similar and intensive signal at the expected size for eNOS (477 bp) and nNOS (471 bp) in the low motile population of sperm. In the high motile fraction, the

expression of eNOS and nNOS mRNA was more variable: no signal was observed for eNOS and nNOS in respectively seven and eight different samples, whereas a weak signal was detected in the others (Figure 5).

### Expression of c-myc transcripts

*C-myc* transcripts were the first mRNA described in human ejaculated sperm (Kumar *et al.*, 1993). No significant variation of *c-myc* mRNA levels was observed between the two populations of sperm. Therefore *c-myc* mRNA:Prm-2 mRNA ratio was not different between high motile sperm and low motile fraction (Figure 6).

### Expression of c-myc and Prm-2 transcripts in capacitated sperm

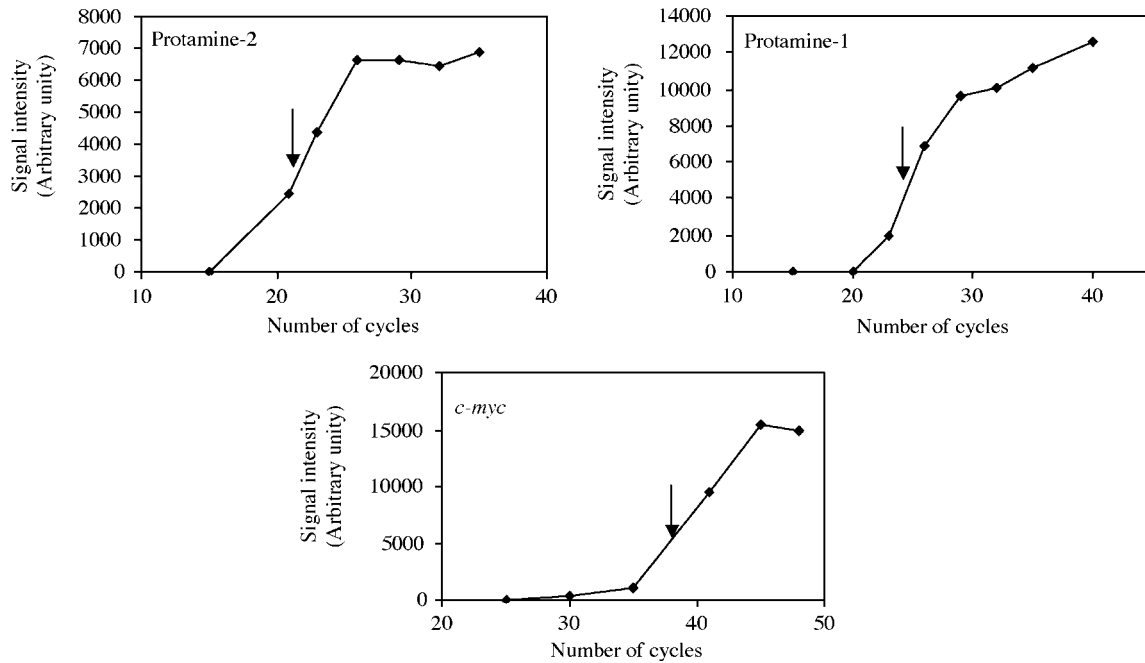
Because *c-myc* mRNA has a short half-life in a great number of cell types, we have analysed its expression during capacitation, together with that of Prm-2 mRNA. After capacitation for 4 h, the expression of Prm-2 transcripts remained constant whereas the signal intensity corresponding to the *c-myc* mRNA decreased in capacitated sperm compared to uncapacitated sperm cells (Figure 7).

## Discussion

In this study, semen samples from normospermic patients were fractionated on a four layer discontinuous Puresperm gradient in order to separate high motile from low motile sperm from the same sample. This technique has already been used by several authors (Weng *et al.*, 2002; May-Panloup *et al.*, 2003; Mengual *et al.*, 2003; O'Connell *et al.*, 2003) and is commonly utilized in assisted reproduction protocols. Herein we have observed a differential

**Table II.** Cycle profiles of the different primer pairs

Gene	Cycle profile
Protamine-1	95°C/45 s; 58°C/45 s; 72°C/45 s
Protamine-2	95°C/45 s; 56°C/45 s; 72°C/45 s
CD45	95°C/45 s; 60°C/45 s; 72°C/1 min
c-kit	95°C/1 min; 52°C/1 min; 72°C/1 min
c-myc	95°C/45 s; 56°C/45 s; 72°C/1 min
E-cadherin	95°C/45 s; 60°C/45 s; 72°C/1 min
eNOS	95°C/1 min; 62°C/1 min; 72°C/1 min
nNOS	95°C/1 min; 56°C/1 min; 72°C/1 min



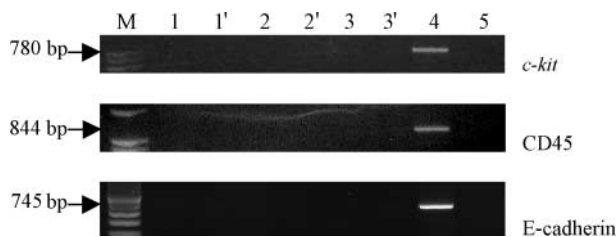
**Figure 1.** Kinetics of PCR amplification of protamine-1, protamine-2 and *c-myc* cDNA. Four hundred nanograms of total RNA were transcribed into cDNA and amplified with an increasing number of cycles. After migration on a 1.5% agarose gel, cDNA were visualized under UV light, photographed and analysed with NIH software. Arrows indicate the number of cycles for quantification of the PCR products.

distribution of some mRNA between high and low motile sperm, such as Prm-1, eNOS and nNOS, whereas no variation was observed for other transcripts such as Prm-2 or *c-myc*.

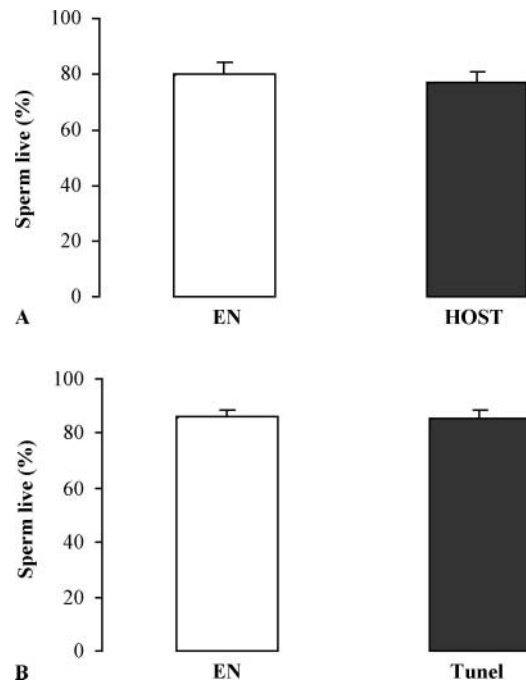
One explanation for the discrepancies observed between the two populations of sperm might lie in the presence of round cells (germ cells, leukocytes and epithelial cells), which contain much more RNA than sperm. In order to avoid this, we have used a four layer gradient, and in the various sperm preparations obtained, the absence of specific molecular marker of leukocytes, germ cells and epithelial cells (respectively CD45, *c-kit* and E-cadherin) has been demonstrated. It is of note that only N-cadherin mRNA is present in mature sperm whereas E-cadherin mRNA is absent (Goodwin *et al.*, 2000). Moreover, the viability of the high and low motile fractions was estimated and the percentage of apoptotic cells measured to control for the integrity of the sperm cells.

The discrepancies observed in this study between low and high motile sperm could be due to the presence of immature sperm with residual cytoplasm in the low fraction. In fact, it has been shown that the morphology of sperm recovered from the bottom of the

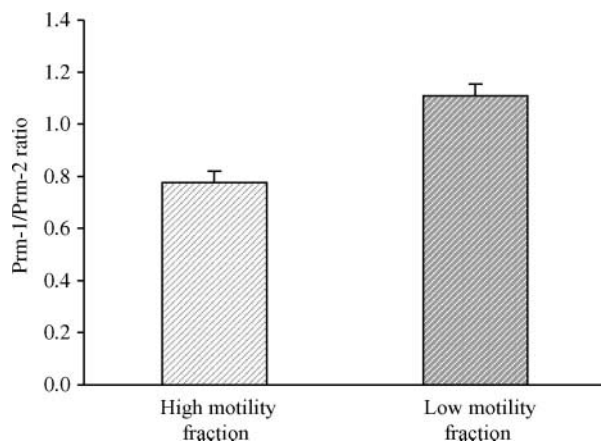
gradient is improved compared to the lower density layers (Chen and Bongso, 1999; Gil-Guzman *et al.*, 2001). In order to rule out this hypothesis, a spermocytogram was systematically performed to eliminate sperm with anormal morphology or with an excess of cytoplasmic droplets.



**Figure 2.** Agarose gel showing the PCR products of *c-kit*, CD-45 and E-cadherin. Five sperm samples were prepared individually by density gradient purification. A representative photograph is shown for three samples. High motile sperm were obtained from the 95% Puresperm fraction (lanes 1–3) and low motile sperm from the 76–57% interface (lanes 1'–3'). Granulosa cells and sperm contaminated with germ cells or leukocytes were respectively used as positive control for E-cadherin, *c-kit* and CD-45 (lane 4). RNA was omitted in lane 5. M corresponds to DNA ladder (100 bp).



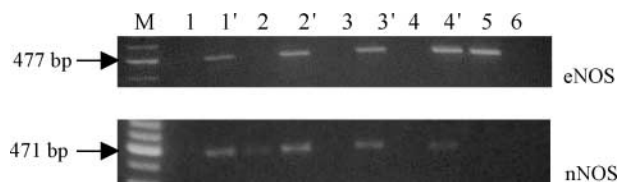
**Figure 3.** Evaluation of sperm viability in low sperm motile fraction by eosin–nigrosin staining (EN), hypo-osmotic swelling test (HOST) and apoptosis by TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL). (A) Percentage of live sperm evaluated by EN and HOST ( $n = 10$ ). (B) Percentage of viable and non-apoptotic sperm respectively measured by EN and TUNEL ( $n = 6$ ). The percentages were not different whatever the technique used.



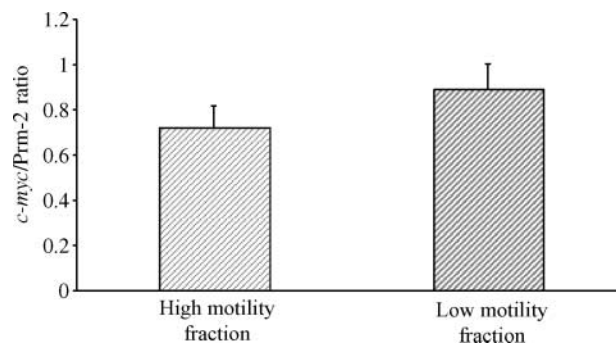
**Figure 4.** Comparison of Prm-1:Prm-2 mRNA ratio in the two different populations of sperm. Ten sperm samples were prepared individually by density gradient purification. High motile sperm were obtained from the 95% Puresperm fraction and low motile sperm from the 76–57% interface. Total RNA was extracted and amplified by RT-PCR. The resulting amplification products were analysed as described in Materials and methods. Results are expressed as the ratio of Prm-1 to Prm-2 signal intensities. \* $P < 0.001$  ( $n = 10$ ).

In our study, differences in the levels of Prm-1 and Prm-2 mRNA have been observed between low and high motile sperm (increase of Prm-1 mRNA in low motile population). An important decrease of Prm-1 gene expression is also observed in testicular biopsies from non-obstructive azoospermia compared to obstructive azoospermia, associated with a normal spermatogenesis (Steger *et al.*, 2003). Data from Steger *et al.* (2003) and our group reported the absence of modifications of Prm-2 transcripts, suggesting that Prm-1 is one of the main factors that could be studied in male infertility. Contrary to our work and that of Steger *et al.* (2003), it has been assumed that both Prm-2 protein and mRNA could be other markers of male fertility since a complete absence of Prm-2 mRNA in round spermatids (Ziyyat *et al.*, 1999) or P2 protein (deYebra *et al.*, 1993) has been noted. No significant differences in the P1:P2 ratio in the different populations of sperm isolated from Percoll fractions have been observed (Mengual *et al.*, 2003) but these data are in disagreement with other studies (Colleu *et al.*, 1996; de Yebra *et al.*, 1998).

We have also noted an almost complete absence of eNOS and nNOS transcripts in motile sperm whereas for the first time an intensive signal for these two mRNA is observed in the low motile fraction. Nitric oxide synthesized by NOS is a potential modulator of sperm function, especially in the acquisition of motility and capacitation. The motility of human sperm is increased by low NO concentrations (Hellström *et al.*, 1994) but inhibited in the presence of



**Figure 5.** eNOS and nNOS transcripts in the two different populations of sperm. Ten sperm samples were prepared individually by density gradient purification. A representative photograph is shown for four samples. High motile sperm were obtained from the 95% Puresperm fraction (lanes 1–4) and low motile sperm from the 76–57% interface (lanes 1'–4'). Granulosa cells were used as positive control for eNOS (lane 5). RNA was replaced by water in lane 6. M corresponds to DNA ladder (100 bp).

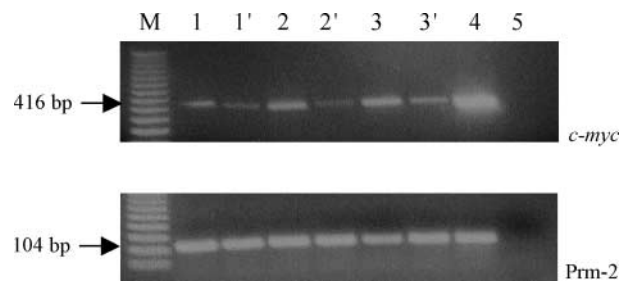


**Figure 6.** Comparison of *c-myc*/Prm-2 mRNA ratio in the two different populations of sperm. Ten sperm samples were prepared individually by density gradient purification. High motile sperm were obtained from the 95% Puresperm fraction and low motile sperm from the 76–57% interface. Total RNA was extracted and amplified by RT-PCR and the resulting amplification products were analysed as described in Materials and methods. Results are expressed as the ratio of *c-myc* and Prm-2 signal intensities.

high NO levels (Rosselli *et al.*, 1995). In the presence of NOS inhibitors, the motility is increased in low motile sperm initially characterized by a very elevated NO production (Perera *et al.*, 1996). The administration of high amounts of the NOS substrate, L-arginine, induces infertility in male rats (Ratnasooriya and Dharmasiri, 2002). The high levels of eNOS and nNOS transcripts in low motile sperm can be related to the excessive production of NO responsible for an inhibition of sperm motility (Rosselli *et al.*, 1995).

Contrary to the previous tested mRNA analysed herein (Prm-1, eNOS and nNOS), no significant difference in *c-myc* transcripts has been observed between high and low motile sperm fractions. mRNA coding for *c-myc* was first described in human sperm by Kumar *et al.* (1993). The *c-myc* protein could play a potential role in capacitation and/or acrosome reaction, and in oocyte fertilization (Kumar *et al.*, 1993). It could also modify characteristics of sperm movement such as head lateral amplitude and velocity (Naz *et al.*, 1991).

The presence of a complex population of mRNA in human mature sperm is well documented, but their roles are unknown. It is generally accepted that sperm are in an inactive transcriptional state. The histone-to-protamine exchange and, consequently, the resulting chromatin condensation finally leads to the cessation of transcription. Recently, protamine haplo-insufficient mice have been shown to be infertile by preventing chromatin assembly (Cho *et al.*, 2001). The mechanisms of translational control ensuring the absence of mRNA translation until several days after gene expression, involve



**Figure 7.** Levels of *c-myc* and Prm-2 transcripts before and after capacitation. RNA was extracted from three different samples before (lanes 1–3) and after (4h) (lanes 1'–3') capacitation. Granulosa cells were used as positive control (lane 4). RNA was replaced by water in lane 5. M corresponds to DNA ladder (50 bp).

either protein repressors binding specific sequences located in the 3'-UTR (untranslated region) or to 160–180 residues counting the poly-A tail of mRNA (Steger, 1999; Grootegoed *et al.*, 2000). Transgenic male mice lacking the normal Prm-1 3'-UTR are infertile, resulting in a premature translation of protamine mRNA and therefore in an arrest of transcription (Lee *et al.*, 1995). At first, most of the translationally silent mRNA stored as polyadenylated forms in messenger ribonucleoprotein particles (Schmidt *et al.*, 1999) become translationally active when the poly-A tails are shortened by a partial deacylation (Domenjoud *et al.*, 1991).

As evoked by the persistence of mRNA coding for transition proteins and protamines, the reported transcripts could be considered as untranslated stored remnants and as a fingerprint of spermatogenesis quality (Miller *et al.*, 1999).

Protein phosphorylation, transcription and translation steps have been observed in human sperm (Naz, 1998; Sakkas *et al.*, 2003). Indeed, the histone-to-protamine exchange process seems to be incomplete in human spermatids compared to rat or mouse spermatids (Tanphaichitr *et al.*, 1978; Gatewood *et al.*, 1987) which therefore suggest a putative transcription and/or translation in sperm. However, until now there has been no evidence for the presence of ribosomal RNA in human sperm.

Our results observed during capacitation could be explained by a modification of the *c-myc* half-life leading to the disappearance of *c-myc* transcripts. The existence of a 70 kDa polysome-associated protein binding specifically to a coding region of *c-myc* mRNA and serving to protect it from endonucleolytic action, thus regulating mRNA stability, has been reported (Prokipcak *et al.*, 1994; Ross, 1995). Recently, it has been shown that mouse sperm contain an endogenous reverse transcriptase (Giordano *et al.*, 2000), which can reverse-transcribe RNA into cDNA that are transmitted to offspring upon fertilization (Sciamanna *et al.*, 2003). This observation could also explain the diminution of *c-myc* mRNA level in the capacitated sperm. Sperm nuclei also have endogenous endonuclease activity (Maione *et al.*, 1997) that could reduce RNA carriage in capacitating sperm.

Using a microarray analysis, Ostermeier *et al.* (2002) have reported concordant mRNA profiles from testes and sperm of normospermic patients, suggesting that the study of sperm could reflect past events of spermatogenesis and/or spermiogenesis. However, some mRNA distinct from those found in oocytes but similar to those observed in zygotes seem to be testis- and sperm-specific and essential to the early embryo development. The identification of SMARS (sperm-specific nuclear matrix attachment regions) on the Prm-1-Prm-2-TNP-1 domain associated with the nuclear matrix and the DNase I sensitivity are in favour of an alternative chromatin structure, and can represent the initiation point for replacing protamines by histones during the male pronucleus formation (Wyke and Krawetz, 1999). As proposed by Ostermeier *et al.* (2002), the establishment of sperm mRNA profiles could be used as a genetic fingerprint of normal fertile men. But the presence of transcripts such as eNOS and nNOS in low motile sperm, but not in motile sperm cells, must be taken into account. Motility was thus found to be an important physiological marker, especially for ICSI procedures. The development of new investigation methods such as the analysis of mRNA profiles in high and low motile sperm and the understanding of the transcripts significance could be helpful as additional diagnostic tools and be of prognostic value to fertilization and pregnancy.

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