RNA dynamics of fertile and infertile spermatozoa

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Abstract
The presence of a complex population of mRNAs in human mature spermatozoa is well documented; among them, transcripts of aromatase and ERs (oestrogen receptors) have been described but their significance is not clear. Therefore, to clarify the role of this complex population of mRNAs in human ejaculated sperm, we have isolated on discontinuous density gradients two main fractions from the same sample: high- and low-motile spermatozoa. The levels of different transcripts coding for molecules involved in nuclear condensation [Prm-1 (protamine 1) and Prm-2], capacitation [eNOS (endothelial nitric oxide synthase), nNOS (neuronal nitric oxide synthase), c-myc], motility and sperm survival (aromatase) have been assessed using semi-quantitative RT (reverse transcriptase)–PCR. The viability of sperm as well as the percentage of apoptosis were identical in high- and low-motile fractions. No significant change in the c-myc/Prm-2 ratio between the two populations of spermatozoa was observed. Conversely the amount of Prm-1 mRNA was significantly higher in low-motile than in high-motile fraction; in most of the high-motile sperm samples analysed, eNOS and nNOS transcripts were undetectable, whereas they were observed in low-motile sperm. Moreover, a partial or complete disappearance of c-myc transcripts was observed after capacitation. As to the aromatase expression, a significant decrease in the amount of transcripts in immotile sperm fraction was recorded in all samples studied. To conclude, analysing mRNA profiles in humans could be helpful either as a diagnostic tool to evaluate male fertility, since they reflect spermatogenesis gene expression, and/or a prognosis value for fertilization, since these RNAs are delivered to oocytes.

Presence of mRNAs in human ejaculated spermatozoa: relationship to sperm motility and capacitation
Until recently, the male genome was thought to be in a transcriptionally dormant state, especially in the spermatozoa. The transcripts present were believed to be remnants of stored mRNAs from post-meiotically active genes especially from round spermatids which contained numerous RNAs either produced in early stages of spermatogenesis [1] or during spermiogenesis such as protamines and transition proteins [2]. During these last 10 years, not only mRNAs have been discovered in human ejaculated sperm but also some translational activities have been demonstrated, giving rise to proteins likely to be concerned in sperm quality but also in the first steps of embryonic development [3]. The presence of extremely varied transcripts in mature sperm of rodents and humans [for example c-myc, Prm-1 (protamine 1) and Prm-2, HSPs (heat-shock proteins) 70 and 90, β-integrins, phosphodiesterase isoforms and progesterone receptor] has been reported (for reviews see [3,4]). In addition we and others have provided data on the presence of aromatase and ERs (oestrogen receptors) both in human immature germ cells and ejaculated spermatozoa (for a review see [5]). However, the function of these various transcripts is not fully understood [4,6]. A lot of investigations have been performed with various techniques: RT (reverse transcriptase)–PCR, microarray technology, in situ hybridization, immunofluorescence, confocal microscopy and serial analysis of gene expression have been used recently [7] to identify and provide new information about these complex and specific population of RNAs in mature spermatozoa. Indeed, numerous controversies arose on their putative role in such mature germ cells believed to only deliver the paternal genome to oocytes. Therefore clarifying the role of these complex populations of RNAs in human spermatozoa will be helpful to understand the events concerned in sperm maturation and in fertilization. Sperm were obtained from healthy donors and the liquefied semen samples were fractionated on a discontinuous Pureperm gradient to isolate high-motile (>90%) and low-motile (<30%) sperm. One important issue is the quality of the sperm preparation, which should be devoid of any immature germ cells and leucocytes, indicated using specific markers [8]. Moreover, we have checked the viability of the sperm [EN (eosin/nigrosin) vital stain and HOST (hypo-osmotic swelling test)] as well as the percentage of apoptosis [TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) assay] in high- and low-motile fractions and found no differences.

The levels of different transcripts coding for molecules involved in nuclear condensation (Prm-1 and Prm-2),

Key words: aromatase, endothelial nitric oxide synthase (eNOS), protamine, RNA dynamics, spermatozoa

Abbreviations used: ER, oestrogen receptor; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; P450arom, P450 aromatase; Prm-1, protamine 1; RT, reverse transcriptase.

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capacitation [eNOS (endothelial nitric oxide synthase), nNOS (neuronal nitric oxide synthase) and c-myc], motility
and sperm survival [P450arom (P450 aromatase)] were ass-
essed using semi-quantitative RT-PCR [8,9]. No significant
change in the c-myc/Prm-2 ratio between the two populations
of spermatozoa was observed. Conversely, the amount of
Prm-1 mRNA was significantly higher in low-motile than
in high-motile fraction. We have also noted an almost
complete absence of eNOS and nNOS transcripts in motile
spermatozoa, whereas for the first time an intensive signal for
these two mRNAs was observed in the low-motile fraction.
As for c-myc, the amount of transcript was identical in both
fractions; however, a partial or complete disappearance of
these transcripts was observed after capacitation [8].

Concerning the aromatase expression, a significant
decrease (30%) in the amount of transcripts in the immotile
sperm fraction was recorded in all samples studied;
moreover, the aromatase activity determined in vitro was also
diminished by 34% [9,10]. In addition, we have amplified
aromatase mRNA by real-time PCR in asthenospermic
spermatozoa from infertile men and recorded a 57% decrease
in the amount of transcripts compared with controls (L. Said,
I. Galeraud-Denis and S. Carreau, unpublished work).

Significance of transcripts in spermatozoa
and future developments
It is generally accepted that spermatozoa are in an inac-
tive transcriptional state as supported by the histone-to-
protamine exchange and, consequently, the resulting chro-
matin condensation, which finally leads to the arrest of tran-
scription [11]. Herein, in sperm samples from healthy men we
have observed a differential distribution of some mRNAs be-
tween high- and low-motile spermatozoa, such as Prm-1,
eNOS and nNOS, whereas no variation was observed for
Prm-2 or c-myc transcript. In fact, an increase in Prm-1
mRNA in the low-motile population compared with the
high-motile fraction is recorded, whereas Prm-2 remains
identical. An important decrease in Prm-1 gene expression
has been observed in testicular biopsies from non-obstructive
azoospermia compared with obstructive azoospermia asso-
ciated with normal spermatogenesis [12]. In addition, the
data reported by Steger et al. [12] and us confirmed the ab-
sence of modification of Prm-2 transcripts, suggesting that
Prm-1 is one of the main factors that could be studied in male
infertility.

As evoked by the persistence of mRNAs coding for
transition proteins and protamines, the reported transcripts
could be considered as untranslated stored remnants and
as a ‘fingerprint’ of spermatogenesis quality [13]. However,
our results suggest a weak but a potential function of some
transcripts during capacitation and/or acrosome reaction.

c-myc, which was one of the first transcripts described
in spermatozoa [14] and the protein corresponding reported
in human sperm cells [15], has been carefully studied, and
we have observed an incomplete or total disappearance of
c-myc transcripts after 4 h of capacitation without modification
of the mRNA encoding Prm-2. Moreover, the levels of
c-myc transcripts are roughly identical with those before
capacitation when spermatozoa are incubated with cyclohexi-
ude. Recently, incorporation of labelled amino acids into
polypeptides during sperm capacitation has been shown [16].

The presence of other transcripts such as eNOS and nNOS
in low-motile spermatozoa, but not in motile sperm cells,
must be also taken into account. Nitric oxide synthesized
by NOS is a potential modulator of spermatozoa function,
mainly in the acquisition of motility and capacitation.
The high levels of eNOS and nNOS transcripts in low-motile
spermatozoa (our results) could be related to the excessive
production of nitric oxide responsible for inhibition of
sperm motility [17].

A number of investigations using conventional screening
strategies or microarray technology have shown that sper-
matozoa contain RNAs encoding proteins concerned in
signal transduction and cell proliferation. An interesting
question is to understand whether these transcripts are only
remnants from earlier steps of spermatogenesis and stored
in the nucleus for a long period or whether they could be
synthesized in the mature spermatozoa. What is rather puzz-
ling is the presence of residual DNA and RNA polymerase
activity within spermatozoa [18,19], as well as some features
of active chromatin (acylated) in the head of spermatozoa
[20]. We have indirectly shown using cycloheximide that c-
myc mRNA could be translated during capacitation, whereas
Prm-2 remains unchanged. In addition, the existence of
specific P450arom transcripts in spermatozoa, together with
the presence of a biologically aromatase protein (formation
of oestrone from androstenedione in vitro), is an additional
argument in favour of a role of some of these mRNAs
within ejaculated spermatozoa. This last observation is
in keeping with the presence of ERs in ejaculated sperm [21–
23] and therefore may suggest a positive role of oestrogens
in sperm survival/motility [10]. From our results collected
from infertile patients, a 2-fold decrease in the amount of
P450arom transcripts was observed, which is in agreement
with a recent study [24] and with the clinical observations
of aromatase deficiency in men consecutive to a P450arom gene
mutation, which leads to sterility with low sperm counts and
reduced sperm motility [25].

Indeed, in mouse [26,27], as well as in humans [28], it has
been shown that oestrogens are positively involved in sperm
capacitation and acrosome reaction. The existence of ERs on
sperm membrane is well known [29]; moreover, they have
been described in the mitochondria [30,31], which may be of
significance for the role of oestrogen in male gamete motility
as shown for another parameter from mitochondrial origin,
the transaldolase, which is concerned in fertility of mouse
spermatozoa [32].

However, one should keep in mind that contrary
to rodent spermatozoa, human spermatozoa express a
functional aromatase that is still active after ejaculation,
and together with the presence of ERs and especially ERβ
in the mitochondria of spermatozoa, these data open new
considerations about the role of oestrogens all along the
male genital tract and a likely role also in the mobility and fertilizing ability of sperm. Indeed, receptors are numerous in sperm cells but their role needs to be fully elucidated [33].

To conclude, analysing mRNA profiles in humans could be helpful either as a diagnostic tool to assess male fertility, since they reflect spermatogenesis gene expression, and/or as a prognosis value for fertilization and embryo development, since these RNAs are delivered to oocytes [34,35].

Male infertility is a modern world problem and consequently comparison of transcript ‘fingerprints’ in ejaculates between fertile and infertile males is one of our goals. Even though our results suggest a weak but potential function of some transcripts in spermatozoa during capacitation and/or acrosome reaction it is necessary to collect more data from larger groups of infertile patients in order to understand the role of these RNAs.

We thank Professor J.P. Dadoune (Centre Universitaire des St Pères, Paris, France) for helpful and ongoing discussions.

References

Received 3 November 2006