Decrease of both stem cell factor and clusterin mRNA levels in testicular biopsies of azoospermic patients with constitutive or idiopathic but not acquired spermatogenic failure

Ingrid Plotton, Pascale Sanchez, Philippe Durand and Herve Lejeune

BACKGROUND: Sertoli cells nurse germ cells during spermatogenesis, and alterations of Sertoli cell functions have been suggested in cases of spermatogenic failures. METHODS: In this work, we measured stem cell factor (SCF) and clusterin mRNA levels, by quantitative RT–PCR, in RNA extracted from testicular biopsies of 49 azoospermic patients classified according to testicular histology as having normal spermatogenesis or spermatogenic failure. RESULTS: When related to the percentage of Sertoli cells counted on a histological section of a neighbouring tissue sample, SCF and clusterin mRNA levels were significantly lower in the ‘spermatogenic failure’ group compared with the control group (P = 0.0297 and P = 0.0043, respectively). These levels were also significantly lower in the cases of ‘constitutive’ (cryptorchidism and Yq microdeletion) and ‘idiopathic’ spermatogenic failures when compared with the control group; conversely, they were not significantly decreased in the group with ‘acquired spermatogenic failure’ (orchitis, testicular traumatism, chemoradiotherapy and varicocele). CONCLUSIONS: These data further demonstrate an alteration of Sertoli cell functions in some human spermatogenic failures and suggest that a lack of Sertoli cell maturation may be involved in cases of constitutive or idiopathic spermatogenic failures.

Key words: azoospermia/clusterin/Sertoli/spermatogenesis/stem cell factor

Introduction

Sertoli cells nurse germ cells during spermatogenesis. The crosstalk between the Sertoli and the germ cells involves many paracrine/autocrine factors and has been studied mainly in animal models and rarely in the human species (Mruk and Cheng, 2004). Alterations of Sertoli cell morphology (Nistal et al., 1998) and protein production as revealed by immunocytochemistry (Sharpe et al., 2003) have been suggested in cases of spermatogenic failures. These alterations are important to consider when pursuing the aim of developing therapeutic procedures involving testicular paracrine factors. Indeed, if the production of a Sertoli cell-derived factor is altered in some case of spermatogenetic failure, its normalization, by a therapeutic intervention in vivo or in vitro, should improve spermatogenesis. Such factors have already been studied in human testicular biopsies by immunocytochemistry and in situ hybridization (Bar-Shira Maymon et al., 2000, 2002; Defamie et al., 2003; Sharpe et al., 2003). However, whereas these morphological methods allow cell localization of the factors or of their mRNA, they do not allow an accurate quantification of their production. The small amount of tissue available in clinical practice has precluded the use of peptide measurement. Hence, quantitative RT–PCR would be the method of choice to estimate the production of paracrine factors by the Sertoli cells. Furthermore, measuring Sertoli cell production of paracrine factors in pathological samples is difficult because of the cellular heterogeneity of the testicular tissue samples and the large variations in the cellular composition of the testis according to the degree and type of spermatogenic failure. Stem cell factor (SCF) is one of the paracrine factors produced by the Sertoli cells (Rossi et al., 1991). Mutations in the W (dominant White spotting) and Sl (Steel) loci in the mouse, encoding the c-kit receptor and the c-kit ligand (SCF), respectively, cause sterility as they are required for the migration and proliferation of primordial germ cells (Sette et al., 2000). Moreover, SCF stimulates spermatogonial proliferation (Rossi et al., 1993) and appears involved in regulating latter stages of spermatogenesis (Vincent et al., 1998; Yan et al., 2000) together with reducing germ cell apoptosis (Yan et al., 2000).

Clusterin, also referred to as sulphated glycoprotein 2 (Tenniswood et al., 1998), has been shown to be produced within the testis by the Sertoli cells (Morales et al., 1987). Although its role is far from being understood, an anti-apoptotic
effect of clusterin on germ cells has been reported (O’Bryan et al., 1994; Bailey et al., 2002). However, clusterin knockout (KO) mice have only subtle alterations of spermatogenesis and spermatid resistance to heat stress (Bailey et al., 2002). Clusterin mRNA levels have been shown to increase with Sertoli cell maturation between day 10 and day 20 of age in the rat and to remain very stable thereafter (Morales et al., 1987; Plotton et al., 2005). Clusterin might therefore be considered as a marker of Sertoli cell maturation.

In this work, we measured both SCF and clusterin mRNA levels by quantitative RT–PCR in RNA extracted from testicular biopsies of 49 azoospermic patients classified according to their testicular histology and the aetiology of their spermatogenic failure.

Materials and methods

Patients

A total of 49 patients (36.8 ± 7 years; mean ± SD) had a testicular biopsy for sperm extraction at the Département de Médecine de la Reproduction because of azoospermia. Azoospermic patients with an abnormal karyotype were not included in this study.

Medical history and clinical findings were recorded for each patient. Testicular volumes were measured using Prader’s orchidometer.

Sperm analysis was performed according to the World Health Organization’s recommendations (1999) including a centrifugation step of the semen. Azoospermia was confirmed by a second analysis. Plasma FSH levels were assayed by radioimmunooasay (normal range 1.2–7.6 IU/l); plasma Inhibin B levels were assayed by enzyme-linked immunosorbent assay (ELISA) (DSL-10684100, normal range 55–309 ng/l).

Testicular biopsies

Open testicular biopsies were carried out under general anaesthesia. One sample of testicular tissue was immediately immersed in RNA later AMBION® (Austin, TX, USA), (catalogue no. 7020) for RNA extraction. A neighbouring sample was fixed in Bouin’s solution for histological study. Spermatozoa were extracted by mechanical dilaceration for ICSI.

Ethics

The procedure has been authorized, according to the French legislation, by the ‘Comité Consultatif de Protection des Personnes soumises à la Recherche Biomédicale’. Informed consent was obtained from all participants.

Testicle histology

Fixed testicular tissue samples were paraffin-embedded, cut at 3 μm and stained by haematoxylin eosin standard procedure.

Histological classification

The seminiferous tubules were classified according to the Johnsen’s score (Johnsen, 1970). This allowed us to assign the patients to the following groups:

- Normal spermatogenesis: a majority (>50%) of the spermatogenic tubes had a Johnsen’s score of 10 (normal) or 9 (disorganized architecture, but with ≥10 sperm cells). This group was considered as the control group of this study.
- Spermatic failure: when less than 50% of the spermatogenic tubes exhibited a Johnsen’s score of 10 or 9. To investigate the role of the severity of the spermatogenic failure, this latter group was further divided into the following histological subgroups:
  - (i) Moderate hypospermatogenesis: less than 50% of the seminiferous tubes had a Johnsen’s score of 9 or 10 but ≥10% of the seminiferous tubes had a Johnsen’s score of 8 (<10 sperm cells).
  - (ii) Severe hypospermatogenesis with few sperm cells: no seminiferous tubes had a Johnsen’s score of 9 or 10 and ≤10% of the seminiferous tubes had a Johnsen’s score of 8 (<10 sperm cells).
  - (iii) Severe hypospermatogenesis without sperm cells: no mature sperm was seen but at least one seminiferous tube had a Johnsen’s score between 7 and 3 (presence of immature germ cells).
  - (iv) Sertoli cell-only syndrome: the seminiferous tubes had a Johnsen’s score ≤2 (no germ cells).

Aetiological classification

The aetiology of the spermatogenic failure was defined from the clinical records. Accordingly, the patients were assigned to the following aetiological subgroups:

- Acquired spermatogenic failure: in case of history of orchitis, testicular trauma, chemoradiotherapy or presence of a voluminous varicocele (detected at scrotal visual inspection);
- Constitutive spermatogenic failure: in case of history of cryptorchidism or in case of AZFc microdeletion;
- Idiopathic spermatogenic failure: when no aetiological factor could be identified.

Proportion of Sertoli cells in biopsies

Because the number and/or volume of Sertoli cells, germ cells and interstitial cells may vary according to the spermatogenic alteration, the variations in the proportion of Sertoli cells in the testis samples were roughly estimated by making the ratio of the number of Sertoli cells to the total number of cells counted on randomly chosen histological sections of the biopsies.

Vimentin immunoreactivity was used to identify Sertoli cells in histological sections. An anti-vimentin-specific monoclonal antibody (Dako, France) was used at a dilution of 1:1000. Vimentin immunoreactivity was revealed by a biotin-coupled goat anti-mouse antibody incubated with streptavidine-coupled horseradish peroxidase (Dako, Trappes, France) giving a brown colouration to the Sertoli cells after reaction with diaminobenzidine. The cell nuclei were then stained with haematoxylin.

The cells were classified according to the following criteria: Sertoli cells (vimentin-positive cells within the seminiferous tubule), germ cells (vimentin-negative cells within the seminiferous tubule) and interstitial cells (vimentin-positive cells outside the tubules) (Plotton et al., 2005).

For each patient, at least 1000 cells were counted in randomly chosen microscopic fields at 1000× magnification. The results (Table IV) were similar in our control group and in the subjects younger than 40 years in the study of Johnson et al. 1984.

RNA extraction

RNA extraction was performed with the AMBION® kit according to the manufacturer’s instructions. The quality of the total RNA preparation was checked by 1% agarose gel electrophoresis in the presence of ethidium bromide showing the typical 18S and 28S bands.

Competitive RT–PCR

As previously described in detail (Plotton et al., 2005), this method is based on the use of a synthetic RNA competitor which allows the
monitoring of both the reverse transcription and the polymerase chain reactions (PCRs). The stability of this RNA is increased by incorporating modified nucleotides, making the competitor RNA resistant to ribonucleases.

**Competitor synthesis and quantification**

The construction and quantification of the competitor RNAs with modified nucleotides was performed with the AMBION® RT–PCR competitor construction kit (catalogue no. 1356), according to the manufacturer’s instructions.

The sequences used for primer synthesis (Table I), spanning different exons, were chosen according to the published human clusterin, accession no. M64722 (Wong et al., 1993) and human SCF, accession no. M59964 (Martin et al., 1990).

**Competitive RT–PCR**

Single-strand cDNAs were synthesized by reverse transcription using the same primer (P4) from the mRNA to be quantified and from the competitor RNA added in a known amount in the same tube. The reverse transcription was performed for 1 h at 37°C, in a volume of 20 μl/tube: 200 units MLVT reverse transcriptase (Invitrogen, Cergy Pontoise, France), 10 mM DTT, 1.25 mM of each dNTP, 0.3 μM of P3 primer, 0.01 μg total RNA and the following amount of RNA competitor: 5 × 10^5, 1 × 10^6, 2 × 10^6, 5 × 10^6, 1 × 10^7 and 2 × 10^7 copies for clusterin or 0.1 μg total RNA and the following amount of RNA competitor: 2 × 10^5, 5 × 10^5, 1 × 10^5, 2 × 10^5, 5 × 10^5 and 1 × 10^6 copies for SCF.

Two microlitres of reverse transcription products were subjected to PCR in a volume of 100 μl/tube containing 50 pmole of P1 primer, 50 pmole of P3 primer and 0.25 mM of each dNTP. After a denaturation step (5 min at 94°C), 2 units/tube of Taq polymerase (Roche Diagnostics, Meylan, France) were added while the temperature was maintained at 80°C; then the PCR was performed for 40 cycles: 94°C for 1 min, 55°C for 2 min and 72°C for 1 min. The RT–PCR products obtained from the mRNA and from the synthetic RNA competitors were, respectively, 348 and 273 bp for clusterin and 429 and 322 bp for hSCF. They were resolved with 1.5% agarose gel electrophoresis in the presence of ethidium bromide. Photographs of ethidium bromide UV fluorescence were digitally scanned with NIKON-IMAGE software. The ratio of the RT–PCR products from the mRNA to the RT–PCR products from the competitor RNA was calculated and plotted against the initial competitor RNA concentration on a bi-logarithmic scale. The regression line was calculated and the initial concentration of mRNA was determined as the competitor concentration corresponding to ratio equal to 1.

The intra- and inter-assay coefficients of variation were, respectively, 14.4 and 29.3% for clusterin mRNA and 17.7 and 34.7% for SCF mRNA, as determined by repeated measurements both (n = 12) of the same RNA sample.

**Statistical analysis**

Because the number of observations in each group was insufficient to assess the normality of the distributions, the results of each group were compared with the ‘normal spermatogenic group’ by the non-parametric Mann–Whitney U-test. The relationship between the quantitative parameters and the severity of the spermatogenic failure was studied by the non-parametric Spearman’s Rank correlation test between the parameter values and the values 1, 2, 3, 4 and 5 affecting to the histologoic subgroups, ‘normal spermatogenesis’, ‘moderate hypospermatogenesis’, ‘severe hypospermatogenesis with few sperm cells’, ‘severe hypospermatogenesis without sperm cells’ and ‘Sertoli cell-only syndrome’ as defined above. P < 0.05 was considered significant.

**Results**

The distribution of the 49 azoospermic patients among the control and spermatogenic failure groups and among the histological and aetiological subgroups is given in Table I. The cross-tabulation of the histological and aetiological subgroups is given in Table II; it did not show a link between the severity and the aetiology of the spermatogenic failure.

In the 13 cases of ‘normal spermatogenesis’ and in the eight cases of ‘moderate hypospermatogenesis’, a reproductive tract obstruction was found. A seminal tract obstruction was also found in three cases of ‘severe spermatogenesis with few sperm cells’ and in one case of ‘Sertoli cell-only syndrome’.

As expected, FSH plasma levels were significantly higher and Inhibin B plasma levels and testis volumes were significantly lower in the ‘spermatogenic failure’ group than in controls.

---

**Table I. Nucleotide sequences used for the construction of RNA competitors and competitive RT–PCR**

<table>
<thead>
<tr>
<th>Clusterin (Wong et al., 1993)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>+821; +840</td>
</tr>
<tr>
<td>S2</td>
<td>+896; +915</td>
</tr>
<tr>
<td>S3</td>
<td>+1179; +1198</td>
</tr>
<tr>
<td>S4</td>
<td>+1225; +1254</td>
</tr>
<tr>
<td>S5</td>
<td>+1373; +1392</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>hSCF (Martin et al., 1990)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>−4; +21</td>
</tr>
<tr>
<td>S2</td>
<td>+129; +148</td>
</tr>
<tr>
<td>S3</td>
<td>+402; +425</td>
</tr>
<tr>
<td>S4</td>
<td>+440; +459</td>
</tr>
<tr>
<td>S5</td>
<td>+500; +520</td>
</tr>
</tbody>
</table>

hSCF, human stem cell factor.

The position of the first and the last nucleotide is given relatively to the start codon (+1) following the cDNA sequences. S1, sequence for the forward primer for the PCR (P1); S2, sequence used to construct the primer (P2) used for preparing the RNA competitor synthesis including successively the T7 polymerase-promoting sequences, a sequence of 10 nucleotides including only G and A (5′-GGGAGAGGG-3′), allowing the beginning of the in vitro translation without the incorporation of modified nucleotides (C and U), followed by S1, followed by S2. The succession of S1–S2 induced a deletion between these two sequences. The deletion was of 57 bp for clusterin and 107 bp for hSCF; S3, sequence located in a downstream exon, used for the reverse primer of the PCR (P3); S4, sequence for the reverse primer of the reverse transcription (P4); S5, sequence for the reverse primer used for the competitor template synthesis (P5). See (Plotton et al., 2005) for details.
Clinical and hormonal data

**Table II. Clinical and hormonal data**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>FSH M ± SEM (IU/l)</th>
<th>P value*</th>
<th>Inhibin B M ± SEM (ng/l)</th>
<th>P value*</th>
<th>Testis volume M ± SEM (ml)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal spermatogenesis (obstructive azoospermia)</td>
<td>13</td>
<td>4.5 ± 0.7</td>
<td>0.0001</td>
<td>161.8 ± 24.8</td>
<td>&lt;0.0001</td>
<td>19 ± 1.2</td>
<td>0.0009</td>
</tr>
<tr>
<td>Spermatogenic failure</td>
<td>36</td>
<td>19 ± 1.8</td>
<td>&lt;0.0001</td>
<td>156 ± 10.1</td>
<td>&lt;0.0001</td>
<td>12 ± 0.9</td>
<td>0.3080</td>
</tr>
<tr>
<td>Histological classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate hypospermatogenesis (with seminal duct obstruction)</td>
<td>8</td>
<td>10.6 ± 2.6</td>
<td>0.0204</td>
<td>111.3 ± 29.9</td>
<td>0.1655</td>
<td>15.8 ± 2.7</td>
<td>0.0035</td>
</tr>
<tr>
<td>Severe hypospermatogenesis with few sperm cells</td>
<td>11</td>
<td>19.1 ± 4.2</td>
<td>0.0011</td>
<td>73.6 ± 16.5</td>
<td>0.0099</td>
<td>13.1 ± 1.4</td>
<td>0.0116</td>
</tr>
<tr>
<td>Severe hypospermatogenesis without sperm cell</td>
<td>6</td>
<td>25.2 ± 3.2</td>
<td>0.0006</td>
<td>18.3 ± 2.3</td>
<td>0.0013</td>
<td>11.5 ± 1.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Sertoli cell only</td>
<td>11</td>
<td>21.6 ± 2.6</td>
<td>&lt;0.0001</td>
<td>22.3 ± 4</td>
<td>&lt;0.0001</td>
<td>10.7 ± 1.6</td>
<td>0.0019</td>
</tr>
<tr>
<td>Aetiological classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquired (varicocele, orchitis, traumatic, chemoradiotherapy)</td>
<td>12</td>
<td>21.8 ± 4</td>
<td>0.0001</td>
<td>56.7 ± 16.2</td>
<td>0.0019</td>
<td>12.3 ± 1.5</td>
<td>0.0018</td>
</tr>
<tr>
<td>Constitutive (cryptorchidism, AZFc microdeletion)</td>
<td>12</td>
<td>19.6 ± 2.7</td>
<td>0.0001</td>
<td>48.9 ± 12.9</td>
<td>0.001</td>
<td>11.8 ± 1.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>12</td>
<td>15.7 ± 2.7</td>
<td>0.0016</td>
<td>64.3 ± 23.3</td>
<td>0.0033</td>
<td>14.1 ± 1.8</td>
<td>0.0561</td>
</tr>
</tbody>
</table>

*Versus the normal spermatogenesis group (Mann–Whitney U-test).

**Table III. Cross-tabulation of histological and aetiological classifications of the azoospermic patients**

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>Aetiological classification</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate hypospermatogenesis (with seminal duct obstruction)</td>
<td>Acquired</td>
<td>2</td>
</tr>
<tr>
<td>Severe hypospermatogenesis with few sperm cells</td>
<td>Constitutive</td>
<td>6</td>
</tr>
<tr>
<td>Severe hypospermatogenesis without sperm cell</td>
<td>Idiopathic</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

(Table II). The percentage of Sertoli cells relative to the total number of testicular cells counted on histological sections was higher ($P = 0.0016$) in the ‘spermatogenic failure’ group (Table IV); it increased according to the severity of the spermatogenic failure assessed by histological evaluation in subgroups (rho = 0.705; $P < 0.0001$).

When related to the amount of total RNA, the SCF and clusterin mRNA levels were not different between the ‘spermatogenic failure’ and the ‘control’ groups. However, when related to the percentage of Sertoli cells, SCF and clusterin mRNA levels were significantly lower in the ‘spermatogenic failure’ group compared with the ‘control’ group ($P = 0.0297$ and 0.0043, respectively) (Table IV).

In each histological subgroup, SCF mRNA levels expressed relative to the percentage of Sertoli cells were lower than in the ‘control’ group, but the decreases did not reach statistical significance. By contrast, these levels were significantly lower in the ‘constitutive’ and in the ‘idiopathic’ groups than in the control group but were not decreased in the ‘acquired’ group.

The decreases of clusterin mRNA levels expressed relative to the percentage of Sertoli cells were significant for all the histological subgroups but not for the ‘moderate hypospermatogenesis’ subgroup. As for the aetiological subgroups, the pattern was similar to SCF mRNA levels, with a significant decrease in the ‘constitutive’ and the ‘idiopathic’ groups but not in the ‘acquired’ subgroup (Table IV).

**Discussion**

In this work, we measured the mRNA levels of SCF and clusterin (which are both produced by the Sertoli cells), by quantitative RT–PCR, in testicular biopsies obtained during a programme of testicular sperm extraction (TESE) in azoospermic patients. When the SCF and clusterin mRNA levels were expressed relative to the amount of total RNA, no statistical differences could be demonstrated between the ‘control’ group with normal spermatogenesis and the group with spermatogenic failure. However, the measured levels depend on both the amount of mRNA produced by the Sertoli cells and the proportion of RNA of Sertoli cell origin in the samples. Because hypospermatogenesis and especially Sertoli cell-only syndrome are characterized by a reduction in the number of germ cells, these pathologies should lead to an increase in the proportion of Sertoli cells in the testis tissue samples.

Indeed, when the number of Sertoli cells counted on histological sections of the biopsies was related to the total number of cells counted on the same sections, the ratio increased with the severity of the spermatogenic failure. Conversely, we showed in a previous work that the ratio of Sertoli cells in relation to the total number of cells counted on histological sections decreases during post-natal development of the rat, as the number of germ cells increases in the testis (Plotton et al., 2005).

It must be underlined that the ratio of the number of Sertoli cells to the total number of cells counted on histological sections was in no way aiming to be an estimation of the number of Sertoli cells in the testis; this requires much more sophisticated methodologies to be determined (Wreford, 1995). However, the ratio provides evidence that the proportion of Sertoli cells in testis samples varied according to the pathological conditions, as previously shown for experimentally-induced spermatogenesis alterations (Plotton et al., 2005).
When we took into account the proportion of RNA of Sertoli cell origin in the tissue samples by relating the SCF and clusterin mRNA levels to the ratio of Sertoli cell number to the total number of cells counted on histological sections, we found that both SCF and clusterin mRNA levels were lower in cases of spermatogenic failure than in the control group of patients with obstructive azoospermia and normal spermatogenesis at histology. These quantitative data demonstrate that the gene expression of clusterin and SCF by Sertoli cells was reduced in cases of spermatogenic failure as previously suggested by immunohistochemical studies (O’Bryan et al., 1994; Fujita et al., 2005).

As SCF acts on the germ cells to stimulate spermatogenesis, the decrease of its mRNA levels in cases of spermatogenic failure suggests strongly that the paracrine control of Sertoli cells on germ cells was decreased in many cases of human spermatogenic failure. However, it should be noted that whereas the levels of SCF mRNA measured in our study did not appear to be related to the intensity of the spermatogenesis alteration, a discrimination between ‘acquired’ and ‘constitutive/idiopathic’ pathologies could be made on this parameter because ‘acquired’ pathologies did not result in a decrease in the production of SCF by the Sertoli cells. Hence, it seems reasonable to suggest that in such cases Sertoli cell dysfunction is not the primordial cause of spermatogenesis failure. This hypothesis is strengthened by the fact that, in the ‘acquired’ group, the levels of clusterin mRNA also were not different from those of the ‘control’ group (discussed below).

Experimental alteration of spermatogenesis by methoxy-acetic acid or a long-acting GnRH agonist in the adult rat did not modify clusterin mRNA levels (Plotton et al., 2005). By contrast, we observed a decrease of clusterin mRNA levels in cases of ‘idiopathic/constitutive’ human spermatogenic failure. The only situation in which clusterin mRNA levels are low in the rat is the immature animal (Morales et al., 1987; Plotton et al., 2005). Thus, the decrease of clusterin mRNA levels in cases of ‘idiopathic/constitutive’ human spermatogenic failure may suggest an alteration of the maturation of Sertoli cells. This hypothesis has been already proposed on the morphological aspect of Sertoli cells in testicular biopsies of infertile men (Schulze et al., 1976; Nistal et al., 1990, 1998) as well as on the persistence of a strong immunostaining of those proteins which decrease during normal maturation of the testes: AMH (Tran et al., 1987), M2A antigen (Baumal et al., 1989) and cytokeratin 18 (Bar-Shira Maymon et al., 2000) or the maintenance of a weak immunostaining of proteins which increase during normal testis maturation: androgen receptor (Regadera et al., 2001) and Connexin 43 (Defamie et al., 2003). Hence, our results sustain the hypothesis of a deficit of maturation of Sertoli cells in some cases of human spermatogenic failure leading to a pattern of gene expression similar to that of immature Sertoli cells.

The alteration of the maturation process of Sertoli cells in cases of human spermatogenic failure could be related to the fact that experimental alterations of Sertoli cell maturation in animal models have been shown to induce a reduction of germ cells in adults (Sharpe et al., 2003). Thus, some human spermatogenic failure could be primarily because of a Sertoli cell dysfunction. Making the diagnosis of the cases in which
the Sertoli cell immaturity is the cause of the spermatogenic failure would be of clinical interest, opening the possibility of therapeutic action on Sertoli cells by treating the patients, or their germ cells in seminiferous tubule cultures, with factors inducing Sertoli cell maturation or by mimicking the microenvironment provided by mature Sertoli cells. Studies of the markers of Sertoli cell differentiation in experimental animal models and in cases of spermatogenic failure of known origin should therefore be developed.

Conclusion
When related to the proportion of Sertoli cells in the tissue sample, both testicular clusterin and SCF mRNA levels were decreased in cases of human ‘constitutive’ and ‘idiopathic’ spermatogenic failure. This might indicate a defect in the maturation process of the Sertoli cells. Further investigations are needed to determine whether, and in which cases, the alteration of Sertoli cell maturation is the cause of the spermatogenic failure, and whether therapeutic action enhancing Sertoli cell function might improve spermatogenesis.

Acknowledgements
The authors thank the staff of the Département de Médecine de la Reproduction and more especially for their specific help: M.Benchaib, R.Bouvier, B.Cuzin, J.Lornage, M.Mohammad and B.Salle. This work was supported by grants from INSERM, INRA, Université Claude Bernard Lyon 1 and Hospices Civils de Lyon.

References

Submitted on March 22, 2006; resubmitted on April 7, 2006; accepted on April 11, 2006.