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Low Expression of Glycoprotein Subunit 130 in Ejaculated Spermatozoa from Asthenozoospermic Men

ZHI-MING CAI, YAO-TING GUI, XIN GUO, JING YU, LIAN-DIAN GUO, LI-BING ZHANG, HE WANG AND JIE YU

From the Laboratory of Male Reproductive Medicine, The Center for Reproductive Medicine, Peking University Shenzhen Hospital, Shenzhen 518036, China.

Correspondence to: Dr Yao-Ting Gui, Laboratory of Male Reproductive Medicine, The Center for Reproductive Medicine, Peking University Shenzhen Hospital, Shenzhen 518036, China (e-mail: guiyaoting{at}hotmail.com).

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Abstract

Previous studies showed that interleukin-6 (IL-6) was expressed in human Leydig and Sertoli cells and that it inhibited sperm motility. The aim of this study was to compare the expression of IL-6, IL-6R, and GP130 in ejaculated spermatozoa between normozoospermic and asthenozoospermic men. Human spermatozoa in the semen were purified by Percoll gradient technique to separate the seminal plasma and other round cells. RT-PCR, immunocytochemistry, and Western blot were used to detect the expression of IL-6, IL-6R, and GP130 in spermatozoa. With RT-PCR, only GP130 mRNA but not IL-6 and IL-6R mRNA was expressed in human ejaculated spermatozoa. The expression of GP130 mRNA was significantly lower in asthenozoospermic men than in normozoospermic men. The protein expression of GP130 was further confirmed by both immunocytochemistry and Western blot. Again, GP130 protein levels were significantly lower in asthenozoospermic men than in normozoospermic men. The results suggested that the decreased expression of GP130 in ejaculated spermatozoa could be associated with low sperm motility in asthenozoospermic men.

Key words: Interleukin-6, human spermatozoa, motility

Asthenozoospermia, or low sperm motility, is a common cause of human male infertility. About 20% of male infertility is associated with asthenozoospermia (Curi et al, 2003). However, the molecular mechanism of low sperm motility is not fully understood in those men. Some recent studies showed that soluble adenylyl cyclase (SAC) (Esposito et al, 2004; Cai et al, 2006), catsper (Nikpoor et al, 2004), aromatase (Lambard et al, 2003), testis-specific protein 1, and lactate dehydrogenase C (Wang et al, 2004) played very important roles in sperm motility.

Interleukin-6 (IL-6) is a proinflammatory cytokine with multiple functions and it shows biological effects by binding to its receptor, IL-6 receptor (IL-6R), and glycoprotein subunit 130 (GP130). It has been reported that IL-6 is produced in vitro by human Leydig cell– and Sertoli cell–enriched preparations (Cudicini et al, 1997), and both IL-6R and GP130 are expressed in human ejaculated spermatozoa (Laflammme et al, 2005). Others have reported that IL-6 affects motility and the fertilization capacity of human spermatozoa in vitro (Kocak et al, 2002; Camejo et al, 2003; Furuya et al, 2003; Huleihel et al, 2004). Furthermore, a combination of IL-6 and soluble IL-6 receptor (sIL-6R) significantly reduced human sperm motility in vitro (Yashida et al, 2004).

GP130 as a common receptor with multiple functions not only binds to IL-6, but also to other members of the IL-6 family, such as IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), and cardiotrophin 1(CT-1), which are all pleiotrophic and exhibit overlapping biological functions (Ernst et al, 2004). It has also been reported that GP130 was expressed in mouse spermatocytes and spermatids (Molyneaux et al, 2003) as well as human ejaculated spermatozoa (Laflammme et al, 2005). The aim of this study is to compare the expression of IL-6, IL-6R, and GP130 in ejaculated spermatozoa between normozoospermic men (with normal motility) and asthenozoospermic men.

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Sperm Samples

Sperm samples were obtained from normozoospermic men and infertile patients from the Center for Reproductive Medicine, Peking University Shenzhen Hospital. Semen was collected by masturbation after 3 days of sexual abstinence and allowed to liquefy for 30–60 minutes at room temperature. A semen analysis was performed according to WHO (World Health Organization 1999) guidelines. Eosin-nigrosin staining was used for assessing viability of selected sperm, and the samples with more than 5% of dead spermatozoa were excluded from this study. Diff-Quik staining was used to evaluate the sperm morphology. Normozoospermic (sperm concentration $\geq$ 20 x 10^6/ml, total of motility grades A and B $\geq$ 50%, normal sperm morphology $\geq$ 30%, n = 30) and asthenozoospermic semen (sperm concentration $\geq$ 20 x 10^6/ml, total of motility grades A and B $\leq$ 30%, normal sperm morphology $\geq$ 30%, n = 37) were selected for the study. Spermatozoa were purified by the Percoll gradient technique (4 layers: 47.5%, 57%, 76%, and 95%) to separate seminal plasma and also remove other round cells (Lambard et al, 2004). After centrifugation (20 minutes at 400 x *g*, 25 °C), the spermatozoa were collected from the interface 57%–76% and the under layer of 95%, then washed twice with PBS buffer. The washed spermatozoa were used for making the spermatozoa smear or stored at –80 °C for RNA analysis. In order to detect the purification of selected sperm, the samples were examined under microscope. No remaining round cells were observed. Immunocytochemistry was also used to check the contamination of round cells in the purified spermatozoa with the antibodies of CD45, c-kit, and E-cadherin (Zymed Laboratories Inc., South San Francisco, Calif), which are the markers for leukocytes, testicular germ cells, and epithelial cells, respectively. No signals of the markers were detected in the

samples (data not shown).

This study was approved by the ethical committee of the hospital, and all participants signed the consent form permitting to use their sperm samples in the study.

RT-PCR Assay

Total RNA from purified spermatozoa was extracted with TRIzol Reagent (Invitrogen, Carlsbad, Calif). Two µg of total RNA was reverse transcribed with Reverse Transcription System (Promega, Madison, Wis). PCR was performed on RT products 5 µl, 2.5 IU of Taq DNA polymerase, 10 x PCR buffer 5 µl, 25 mmol $MgCl_2$ 4 µl, 10 mM dNTP 1 µl, and 50 pmol of the forward and reverse primers in a final volume of 50 µl. Primer for IL-6, IL-6R, GP130, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was synthesized by Proligo Primers & Probes Inc (Boulder, Colo), and their sequences were listed in Table 1. The PCR conditions were as follows: initial denaturation at 94 °C for 5 minutes, then 30 cycles of 95 °C for 30 seconds, 55 °C for 45 seconds, 72 °C for 90 seconds, and 72 °C for 10 minutes. The PCR products were analyzed using a Rapid Agarose Gel Electrophoresis System (Wealtec Corp, Sparks, Nev) in 2.0% agarose gels in 0.5 x TBE buffer (1 hour at 70 V). The PCR products for IL-6, IL-6R, GP130, and GAPDH were 630, 485, 380, and 239 bp, respectively. The intensities of the bands were quantitated by Dolphin software (Wealtec). The relative values were calculated by dividing the densitometric signals for IL-6, IL-6R, and GP130 by the signal obtained with the internal standard GAPDH.

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Table 1. *The sequences of specific primers for RT-PCR*

Immunocytochemistry Assay

Spermatozoa from normozoospermic men and infertile patients, recovered from Percoll gradient, were rinsed 2 times with PBS buffer (pH 7.4) and allowed to settle onto slides for immunocytochemistry. The slides were fixed in acetone for 10 minutes at room temperature and washed for 5 minutes with PBS + 0.1% Triton X-100. With Histostain-Plus Kit (Zymed), the slides were incubated at 37 °C for 1 hour with 1:200 dilution of rabbit anti-GP130 primary antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) in PBS with 1% BSA. Omission of primary antibody was used for negative controls. Immunoreactivity was visualized by using biotinylated goat anti-rabbit secondary antibody and streptavidin-HRP with diaminobenzidine as the chromogen. Tissues were washed and mounted in 75% glycerol on Lab-Tek chambered coverglass (Nalge Nunc International, Rochester, NY). Images were captured using BX41 U-CMAD3 system (Olympus, Tokyo, Japan). The intensity of staining for each sample was scored by 0, 1, 2, and 3, which represented negative, weak, moderate, and strong staining, respectively (Gui et al, 1999).

Western Blot Analysis

For Western blotting, Percoll purified spermatozoa were homogenized in RIPA buffer [10 mmol/l Tris–HCl, pH 8.0, 10 mmol/l EDTA, 0.15 mol/l NaCl, 1% NP-40, 0.5% sodium dodecyl sulphate (SDS), 1 µg/ml Aprotinin, 1 mmol/l phenyl methyl sulphonyl fluoride (PMSF)]. The homogenate was clarified by centrifugation at 15 000 x *g* for 15 minutes, and the concentration of protein in homogenates was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, Calif). Aliquots (30 µg) of homogenate protein were separated by discontinuous 8% SDS–polyacrylamide gel electrophoresis (SDS–

PAGE). The separated proteins were electroblotted onto polyvinylidene fluoride membrane using a tank-blotting system at 200 mA for 2 hours. After blocking the nonspecific binding sites with nonfat dry milk in TBST buffer (5 mmol/l Tris–HCl, pH 7.4, 136 mmol/l NaCl, 0.1% Tween 20) for 1 hour at room temperature, the blots were incubated overnight at 4 °C with a 1:200 dilution of rabbit anti-GP130 antibody (Santa Cruz Biotechnology). The blots were then washed 3 times with TBST buffer, incubated for 2 hours at room temperature with horseradish peroxidase-linked goat anti-rabbit IgG (Santa Cruz Biotechnology) at a dilution of 1:5000, and after further washing, the immunoreactive proteins were visualized by chemiluminescence. (Pierce Biotechnology, Rockford, Ill) and quantified by densitometry using Dolphin software (Wealtec).

Statistical Analysis

The data were expressed as mean ± SEM. The relationship between the expression GP130 mRNA and sperm motility was determined by the Spearman (nonparametric) test. Differences of GP130 expression between normozoospermic and asthenozoospermic men were examined using Student's *t* test, and $P < .05$ was considered as statistically significant.

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A total of 67 (30 normozoospermic and 37 asthenozoospermic) semen samples were studied. Table 2 shows the data of semen analysis; there were no significant differences in sperm count, morphology, and viability between the 2 groups, but sperm motility in asthenozoospermic men was significantly lower than that in normozoospermic men.

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Table 2. *Mean results (±SEM) of semen analysis for both normozoospermic and asthenozoospermic men*

With RT-PCR, the expression of GP130 mRNA, but not IL-6 and IL-6R, was detected in human ejaculated spermatozoa (Figure 1A). GAPDH mRNA was used as an internal control (Figure 1B). Expression of GP130 mRNA was significantly lower in asthenozoospermic men than in normozoospermic men (Figure 1C, $P < .01$). There was a significant correlation between sperm motility and the expression of GP130 mRNA (Figure 1D, Spearman $r = .83$, $P < .01$)

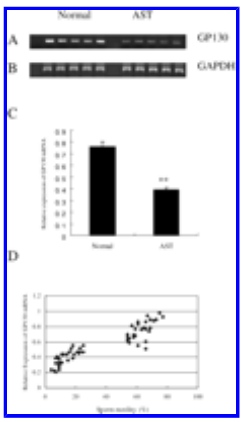

Figure 1. Expression of GP130 **(A)** and GAPDH **(B)** in ejaculated spermatozoa from normozoospermic (Normal, lanes 1–5) and asthenozoospermic men (AST, lanes 6–10). The PCR products for GP130 and GAPDH were 380 and 239 bp, respectively. **(C)** PCR products were visualized under UV light, photographed and quantitated by Dolphin software. Results were expressed as the ratio of GP130 to GAPDH signal intensities (mean ± SEM, $n = 30$). The expression of GP130 was significantly lower in asthenozoospermic men than in normozoospermic men (**$P < .01$). **(D)** Significant correlation between sperm motility (grade A+B) and the expression of GP130 mRNA (Spearman $r = .83$, $P < .01$).

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To further confirm the results of RT-PCR, immunocytochemistry was used to detect GP130 protein expression in the same samples. GP130 was specifically located in the membrane and cytoplasm of the spermatozoa (Figure 2). The staining intensity was stronger in the tail than in the head of the spermatozoa. In 30 normozoospermic samples, 4 were weak positive (1+), 6 intermediate positive (2+), and 20 strong positive (3+, Figure 2B). In contrast, the 37 asthenozoospermic samples contained 10 negative (0), 17 positive (1+), and 10 intermediate positive (2+, Figure 2C). There was no specific staining in the negative controls (Figure 2A). The expression of GP130 in the 2 groups was analyzed with a semiquantatitive method, as described above. The results showed that the expression of GP130 in ejaculated spermatozoa was significantly lower in asthenozoospermic men than in normozoospermic men ($P < 0.05$, Figure 2D).

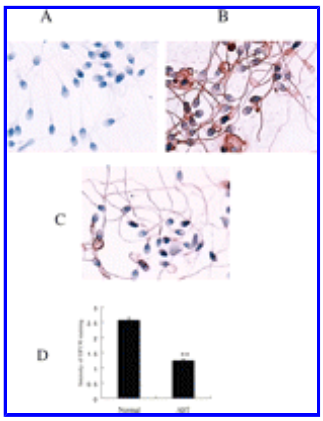

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Figure 2. Immunostaining of GP130 in human ejaculated sperm. **(A)** negative control; **(B)** normozoospermia (normal, $n = 30$); **(C)** asthenozoospermia (AST, $n = 37$); **(D)** The intensity of staining was scored by 0, 1, 2, and 3, which represented negative, weak, moderate, and strong staining, respectively. The expression of GP130 was significantly lower in asthenozoospermic than normozoospermic men (mean ± SEM, $^{**}P < .01$).

The protein expression of GP130 in ejaculated spermatozoa was also confirmed by Western blot, and a band at approximately 130 kd (Arrow) was detected (Figure 3A). Again, the expression of GP130 was significantly lower in asthenozoospermic men than in normozoospermic men ($P < .01$, Figure 3B), which was consistent with the results from both RT-PCR and immunocytochemistry.

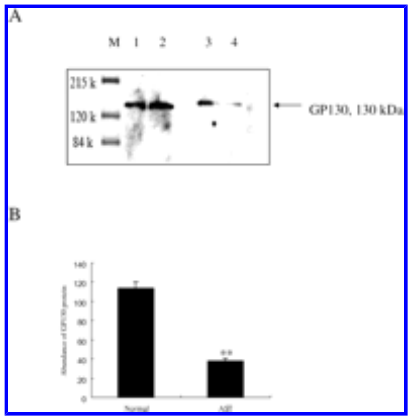

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Figure 3. Western blot analysis of GP130 in ejaculated spermatozoa between normozoospermic (Normal, lanes 1–2) and asthenozoospermic men (AST, lanes 3–4). The expression of GP130 was significantly lower in asthenozoospermic men than in normozoospermic men (mean ± SEM, $n = 6$, $^{**}P < .01$).

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Round cells, such as leukocytes, testicular germ cells, and epithelial cells, contain much more RNA than spermatozoa. In order to remove the contamination of those cells, we used a 4-layer discontinuous Percoll system to purify the mature spermatozoa. With this method, Lambard et al (2004) demonstrated that motile and immotile spermatozoa were respectively isolated from the 95% layer and from the interface 76%–57%. With RT-PCR, they did not detect the expression of CD45, c-kit, and E-cadherin mRNA in the purified spermatozoa, which are the markers for leukocytes, testicular germ cells, and epithelial cells, respectively. With immunocytochemistry, the present study got similar results. It suggested that the purified spermatozoa were free of round cell contamination. Since asthenozoospermic semen often contains a high proportion of nonviable spermatozoa, only samples with >95% of viable spermatozoa after Percoll selection were used in this study

The mature spermatozoa are usually considered to be tools only to transfer genetic messages. However, findings from several studies have demonstrated that the mature spermatozoa contain a complex repertoire of mRNAs, which play a key role in sperm motility, capacitation, and acrosomal reaction (Ostermeier et al, 2002; Wang et al, 2004; Dadoune et al, 2005; Rui Pires Martins et al, 2005). With microarray techniques, Ostermeier et al (2002) identified at least 2686 transcripts in ejaculated spermatozoa of normal fertile men, and Wang et al (2004) identified 149 genes which were expressed at higher levels in both testis and ejaculated spermatozoa. Among the 149 transcripts, the expression of testis-specific protein 1 and lactate dehydrogenase C in the spermatozoa of normal semen samples were significantly higher than in those of motility-impaired sperm. Esposito et al (2004) reported that the mice deficient for SAC were infertile because of a severe sperm-motility defect. Our previous data showed that the expression of SAC mRNA and the concentration of cAMP in ejaculated spermatozoa were significantly decreased in the patients with asthenozoospermia, and the expression of phosphodiesterase-4C mRNA significantly increased in the patients compared with normozoospermic men (Cai et al, 2006).

IL-6 is a member of the family of "interleukin-6 type cytokines" and plays an important role in immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging (Keller et al, 1996). The effects of IL-6 most likely occur through binding to its receptor, IL-6R, and GP130. IL-6 binds to IL-6R with lower affinity. The IL-6/IL-6R binary complex must bind to GP130 to form a ternary complex to achieve its function. In the present study, we demonstrated that GP130, but not IL-6 and IL-6R, was expressed in human ejaculated spermatozoa, which was similar to the report from Yoshida et al (2004). However, a recent study showed that IL-6R exists in human spermatozoa, and tyrosine kinase JAK1 became phosphorylated on tyrosine residues upon sperm treatment with recombinant IL-6, which suggests its activation by the IL-6 and IL-6 intracellular signaling machinery is present in human spermatozoa and might be involved in the acquisition of sperm fertilizing ability (Laflamme et al 2005).

With RT-PCR, immunocytochemistry, and Western blot assay, we also demonstrated that the expression of GP130 in ejaculated spermatozoa significantly decreased in patients with asthenozoospermia, and sperm motility was positively correlated to the expression of GP130. The data suggested that GP130

was beneficial to sperm motility, which was contradictory to a previous report by Yoshida et al (2004). They reported that addition of IL-6 or sIL-6R individually to the culture media had no affect on sperm motility, and a combination of IL-6 and sIL-6R dose-dependently reduced the percentage of motile and rapidly moving sperm. It suggested that the combination of IL-6 and sIL-6R may be associated with GP130 expression in the sperm and reduce sperm motility, and IL-6 and sIL-6R may contribute to the pathogenesis of endometriosis-associated infertility (Yoshida et al, 2004). GP130 as a common receptor not only binds to IL-6 but also to other members of the IL-6 family. Consequently, other members of the IL-6 family may mediate the response of sperm motility through GP130. It has been reported that LIF significantly increased human sperm motility (Attar et al 2003), and LIF, OSM, and CNTF could promote the differentiation of germ cells and the survival of Sertoli cells (De Miguel et al 1996; Jeong et al 2003). However, Molyneaux et al (2003) demonstrated that GP130-mediated signaling was not required for the early stages of PGC development by using the Cre-loxP system to generate germ-cell–specific ablations of GP130. Future work will investigate the expression of other members of the IL-6 family in ejaculated spermatozoa of asthenozoospermic and normozoospermic men

Overall, our study demonstrated that GP130 was expressed in human ejaculated spermatozoa and its expression was significantly lower in asthenozoospermic men than in normozoospermic men. The decreased expression of GP130 may be one possible reason for low sperm motility, which is worthwhile for further investigation.

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Footnotes

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Attar E, Ozsait B, Bulgurcuoglu S, Serdaroglu H, Arici A. Effect of leukemia inhibitory factor on long-term sperm motility and survival. *Reprod Biomed Online*. 2003; 7: 71 –74.[Medline]

Cai ZM, Gui YT, Guo LD, Zhang LB, Wang H, Yu J. Functional expression of adenylyl cyclases and phosphodiesterases in ejaculated human spermatozoa. *Chin J Androl*. 2006; 12: 195 –198.

Camejo MI. Relation between immunosuppressive PGE (2) and IL-10 to pro-inflammatory IL-6 in seminal plasma of infertile and fertile men. *Arch Androl*. 2003; 49: 111 –116.[Medline]

Cudicini C, Lejeune H, Gomez E, Bosmans F, Ballet E, Saez J, Jegou B. Human Leydig cells and Sertoli cells are producers of interleukins-1 and -6. *J Clin Endocrinol Metab*. 1997; 82: 1426 –1433. [Abstract/Free Full Text]

Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi LM, Repetto HE, Blanco AM. Asthenozoospermia: analysis of a large population. *Arch. Androl*. 2003; 49: 343 –349.[Medline]

Dadoune JP, Pawlak A, Alfonsi MF, Siffroi JP. Identification of transcripts by macroarrays, RT-PCR and in situ hybridization in human ejaculated spermatozoa. *Mol Hum Reprod*. 2005; 11: 133 –140. [Abstract/Free Full Text]

De Miguel MP, De Boer-Brouwer M, Paniagua R, van den Hurk R, De Rooij DG, Van Dissel-Emiliani FM. Leukemia inhibitory factor and ciliary neurotropic factor promote the survival of Sertoli cells and gonocytes in coculture system. *Endocrinology*. 1996; 137: 1885 –1893.[Abstract]

Ernst M, Jenkins BJ. Acquiring signaling specificity from the cytokine receptor GP130. *Trends Genet*. 2004; 20: 23 –32.[CrossRef][Medline]

Esposito G, Jaiswal BS, Xie F, Krajnc-Franken MA, Robben TJ, Strik AM, Kuil C, Philipsen RL, van Duin M, Conti M, Gossen JA. Mice deficient for soluble adenylyl cyclase are infertile because of a severe sperm-motility defect. *Proc Natl Acad Sci U S A*. 2004; 101: 2993 –2998. [Abstract/Free Full Text]

Furuya Y, Akashi T, Fuse H. Soluble Fas and interleukin-6 and interleukin-8 levels in seminal plasma of infertile men. *Arch Androl*. 2003;49: 449 –452.[Medline]

Gui Y, Zhang J, Yuan L, Lessey BA. Regulation of Hoxa-10 and its expression in normal and abnormal endometrium. *Mol Hum Reprod*. 1999;5: 866 –873.[Abstract/Free Full Text]

Huleihel M, Lunenfeld E. Regulation of spermatogenesis by paracrine/autocrine testicular factors. *Asian J Androl*. 2004;6: 259 –268.[Medline]

Jeong D, McLean DJ, Griswold MD. Long-term culture and transplantation of murine testicular germ cells. *J Androl*. 2003;24: 661 –669.[Abstract/Free Full Text]

Keller ET, Wanagat J, Ershler WB. Molecular and cellular biology of interleukin-6 and its receptor. *Front Biosci*. 1996; 1: d340 –357.[Medline]

Kocak I, Yenisey C, Dundar M, Okyay P, Serter M. Relationship between seminal plasma interleukin-6 and tumor necrosis factor alpha levels with semen parameters in fertile and infertile men. *Urol Res*. 2002;30: 263 –267.[CrossRef][Medline]

Laflamme J, Akoum A, Leclerc P. Induction of human sperm capacitation and protein tyrosine phosphorylation by endometrial cells and interleukin-6. *Mol Hum Reprod*. 2005; 11: 141 –150. [Abstract/Free Full Text]

Lambard S, Galeraud-Denis I, Bouraima H, Bourguiba S, Chocat A, Carreau S. Expression of aromatase in human ejaculated spermatozoa: a putative marker of motility. *Mol Hum Reprod*. 2003; 9: 117 –124. [Abstract/Free Full Text]

Lambard S, Galeraud-Denis I, Martin G, Levy R, Chocat A, Carreau S. Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation. *Mol Hum Reprod*. 2004; 10: 535 –541.[Abstract/Free Full Text]

Molyneaux KA, Schaible K, Wylie C. GP130, the shared receptor for the LIF/IL6 cytokine family in the mouse, is not required for early germ cell differentiation, but is required cell-autonomously in oocytes for ovulation. *Development*. 2003; 130: 4287 –4294.[Abstract/Free Full Text]

Nikpoor P, Mowla SJ, Movahedin M, Ziaee SA, Tiraihi T. CatSper gene expression in postnatal development of mouse testis and in subfertile men with deficient sperm motility. *Hum Reprod*. 2004;

19: 124 –128.[Abstract/Free Full Text]

Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz A. Spermatozoal RNA profiles of normal fertile men. *Lancet*. 2002; 360: 772 –777.[CrossRef][Medline]

Rui Pires Martins RP, Krawetz SA. RNA in human sperm, *Asian J Androl*. 2005; 7: 115 –120.[CrossRef][Medline]

Yoshida S, Harada T, Iwabe T, Taniguchi F, Mitsunari M, Yamauchi N, Deura I, Horie S, Terakawa N. A combination of interleukin-6 and its soluble receptor impairs sperm motility: implications in infertility associated with endometriosis. *Hum Reprod*. 2004; 19: 1821 –1825.[Abstract/Free Full Text]

Wang H, Zhou Z, Xu M, Li J, Xiao J, Xu ZY, Sha J. A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. *J Mol Med*. 2004; 82: 317 –324.[CrossRef][Medline]

World Health Organization, *WHO Laboratory Manual for the examination of human semen and sperm-cervical mucus interaction*. 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999 .