Author's response to reviews

Title: Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis and Mycoplasma genitalium infections and semen quality of infertile men.

Authors:

Radhouane Gdoura (gdourar@yahoo.com)
Wiem Kchaou (wiemkchaou@yahoo.com)
Chiraz Chaari (chirazchaari@yahoo.fr)
Abir Znazen (abirznazen@yahoo.fr)
Leila Keskes (leilakeskes@yahoo.fr)
Tarek Rebai (tarek.rebai@fmsf.rnu.tn)
Adnane Hammami (adnane.hammami@rns.tn)

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Author's response to reviews: see over
Dear Editor,

I am very grateful to you for your response to our manuscript entitled "The correlation of *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections with male infertility" MS: 8064124521424744

Would you please find enclosed the revised form of our manuscript.

I- Responses to editor's notes

- **Ethics**
  Statement ethical and consent approval for the study were mentioned in the methods section in page 6 line 104 "**Formal consent was obtained from all men of infertile couples tested for infertility in this study**" and in page 6 lines 109-110 "**Prior approval by the ethics committee (Association d'Enregistrement et de Lutte Contre le Cancer du Sud Tunisien) was obtained for this study**"

  A statement from the ethical committee was send by fax.

- **Abstract**
  Abstract was structured according to the guidelines provided at the [http://www.biomedcentral.com/info/ifora/abstracts](http://www.biomedcentral.com/info/ifora/abstracts)

- **Manuscript sections**
  Manuscript sections include (in the following order): Abstract; Background; Methods; Results; Discussion; Conclusions; Competing interests; Authors' contributions; Acknowledgements; References; Tables.
II- Responses to reviewer’s comments and recommendations

We have listed below all revisions to the manuscript according to the reviewer’s comments and recommendations.

1- Responses to reviewer I: Takashi Deguchi

- Responses to major Compulsory Revisions

  * In the methods, page 6 line 104, we have mentioned the formal consent obtained from all men of infertile couples tested for infertility “after they gave informed consent” and in page 6 lines 109-110 we have mentioned the ethic committee approval for this study “Prior approval by ethic committee (Association d’Enregistrement et de Lutte Contre le Cancer du Sud Tunisien) was obtained”.

  A statement from the ethical committee was send by fax.

  * In the results, page 10 lines 212-216, the subjects enrolled in this study was divided to normal and abnormal categories based on seminological variables according to the WHO criteria and the frequency of each mycoplasma or ureaplasma was compared between the normal and abnormal categories “The analysis of the semen specimens based on seminological variables according to the WHO criteria (WHO 1999) had shown that 5.8 % (7/120) were normal categories and 94.2 % (113/120) were abnormal categories. The Frequency of genital ureaplasmas and mycoplasmas DNA in semen samples were not significantly different between the normal and abnormal categories (p > 0.05)”

  * In the discussion, page 14 lines 297-309, we have include the data of urine examination and we have discuss the sites where the mycoplasmas and ureaplasmas infected or colonized “We have compared semen and first void urine specimens from the 120 infertile men for the detection of genital ureaplasmas and mycoplasmas infections using in-house PCR (unpublished data). We have found a very high concordance (> 95 %) and a very good agreement (K > 0.8) between the detection of genital mycoplasmas and ureaplasmas DNA in semen and corresponding first void urine specimens. Several studies have shown that nucleic acid amplification tests performed on first void urine samples are able to detect as many or more infected patients than traditional swabs from the urethra or cervix or semen (Chernesky et al,
1994; Pannekoek et al, 2003; Gaydos et al, 2004; Jensen et al, 2004; Hamdad et al, 2004). In some cases, we have found discrepancies between the detection of genital mycoplasmas and ureplasmas DNA in semen and corresponding first void urine specimens. The presence of genital mycoplasmas and ureplasmas DNA in first void urine samples and its absence in semen specimens may indicate an asymptomatic urethral infection. The detections of genital mycoplasmas and ureplasmas DNA only in semen may indicate that these organisms are harbourd in the epididymis or seminal vesicles.”

- Response to Discretionary Revisions
  I ignore

2- Responses to reviewer II: Maciej Kurpisz

- Responses to Major Compulsory Revisions

  * The findings of the influence of *M. genitalium* on sperm concentration were mentioned:
  - in the results, page 11 lines 224-227, “The sperm concentration of spermatozoa in the male partners of infertile couples with *M. genitalium* DNA in semen specimens were significantly lower than that of the male partners without *M. genitalium* DNA (21.74 x 10⁶/mL vs 49.87 x 10⁶/mL; *p* = 0.05) (Table 3).”
  - in the discussion, page 15 lines 324-327, “We have found a positive correlation between sperm concentration and the detection of *M. genitalium* in semen samples of infertile men. Despite the sperm count with the presence of *M. genitalium* was within a normal range, a decrease in sperm concentration was significant”.

  * The findings of the additive effect of mixed infections on seminology were mentioned:
  - In the results, page 11 line 230-236, “The comparison of the sperm seminological variables between semen with mixed infections and semen without infections and between semen with mixed infections and semen without mixed infections demonstrated no significant differences in the mean values of seminal volume, pH, sperm vitality, sperm motility, sperm morphology and leukocyte count (Table 4). Only the sperm concentration in the semen specimens of infertile men with mixed infections were significantly lower than that of the semen specimens without infections (14.94 x 10⁶/mL vs 55.30 x 10⁶/mL; *p* = 0.02)”
- In the discussion, page15 lines329-324, “The comparison of the sperm seminological variables between semen with mixed infections and semen without infections and between semen with mixed infections and semen without mixed infections had demonstrated that only the sperm concentration in the male partners of infertile couples with mixed infection in semen specimens were significantly lower than that of the male partners without infections. Our findings show that the mixed infections have no additive affect on seminology”

- In table 4 page 25

Table 4: Seminological variables of semen with mixed mycoplasmas and ureaplasmas species infections

<table>
<thead>
<tr>
<th>Variable</th>
<th>Semen with mixed infections (n = 8)</th>
<th>Semen without infections (n = 87)</th>
<th>P value</th>
<th>Semen without mixed infections (n = 25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>3.74 ± 0.60</td>
<td>3.35 ± 0.17</td>
<td>0.52</td>
<td>3.23 ± 0.32</td>
<td>0.45</td>
</tr>
<tr>
<td>pH</td>
<td>7.56 ± 0.06</td>
<td>7.63 ± 0.03</td>
<td>0.55</td>
<td>7.55 ± 0.08</td>
<td>0.95</td>
</tr>
<tr>
<td>Sperm concentration (x10^6/mL)</td>
<td>14.94 ± 7.27</td>
<td>55.30 ± 7.26</td>
<td>0.02</td>
<td>35.37 ± 6.96</td>
<td>0.064</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>64.50 ± 11.53</td>
<td>62.86 ± 2.19</td>
<td>0.63</td>
<td>59.50 ± 3.76</td>
<td>0.50</td>
</tr>
<tr>
<td>Total progressive motility (%)</td>
<td>28.75 ± 9.66</td>
<td>30.82 ± 1.44</td>
<td>0.97</td>
<td>28.8 ± 2.28</td>
<td>0.51</td>
</tr>
<tr>
<td>Rapid progressive motility (%)</td>
<td>12.50 ± 4.33</td>
<td>10.13 ± 0.89</td>
<td>0.50</td>
<td>8.86 ± 1.83</td>
<td>0.32</td>
</tr>
<tr>
<td>Morphology (Normal forms) (%)</td>
<td>12.25 ± 6.97</td>
<td>14.76 ± 1.33</td>
<td>0.34</td>
<td>9.14 ± 1.28</td>
<td>0.91</td>
</tr>
<tr>
<td>Leukocyte count (x10^6/mL)</td>
<td>0.717 ± 0.240</td>
<td>1.239 ± 0.319</td>
<td>0.10</td>
<td>0.886 ± 0.284</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*Note: Data are means (± Standard Error).

*a Difference of seminological variables between semen with mixed infections and semen without infections.

*b Difference of seminological variables between semen with mixed infections and semen without mixed infections.

# Unless indicated, variables were tested by the Wilcoxon rank-sum test.

* Tested by t-test.
In the discussion, page 15 lines 314-318, we have explained that the discrepancies in the result of the influence or the lack of influence of mycoplasmas and ureaplasmas on infertility may come from ethnic differences, geographical location (population sensitivity to microbial agents) or histocompatibility haplotypes. “The influence or the lack of influence of mycoplasmas and ureaplasmas on seminology may come from the capability of bacterial species to attach to spermatozoa and to affect directly via cellular interactions their vitality, motility, morphology, cellular integrity and their molecular structure or the development of protective immunity to genital infection by the host (population sensitivity to microbial agents) or other host factors.”

In the conclusions – the sentence page 16 lines 357-359 “Therefore, it can be concluded that the screening of mycoplasmas and ureaplasmas species in routine semen analysis is not clinically relevant in our specific population” replace the sentence “Therefore, it can be concluded that the screening of mycoplasmas and ureaplasmas species in routine semen analysis is not clinically relevant”

• Responses to Minor Essential Revisions

* In Tables 2 and 3 – pages 23 and 24– we have stated under total progressive motility (category \([a + b]\)) and rapid progressive motility (category \([a]\)).

* In all the manuscript the expression "prevalence" was replaced by "frequency" and "andrological variables" by "seminological variables".

* The sentence Page 11 lines 221-224 was cross-referenced with Table 3 “The sperm concentration and the percentage of normal forms of spermatozoa in the male partners of infertile couples with \(M.\ hominis\) DNA in semen specimens were significantly lower than that of the male partners without \(M.\ hominis\) DNA (14,14 x 10⁶/mL vs 52,63 x 10⁶/mL; \(p = 0.007\) and 8.56 % vs 13.98 %. \(p = 0.03\) respectively) (Table 3).”

* "RCR" in Page 9 line 143 was replaced by "PCR".

* In the discussion, the sentences page 15 lines 320-329 “The sperm concentration (14.14x10⁶/ mL) was lower than the normal reference of WHO manual (≥20x10⁶/ ml) in semen of \(M.\ hominis\) -positive infertile men and higher (52.63x10⁶/ mL) in semen of \(M.\)
hominis-negative infertile men. The present data show that \textit{M. hominis} may affect sperm concentration and sperm morphology of infertile men. We have found a positive correlation between sperm concentration and the detection of \textit{M. genitalium} in semen samples of infertile men. Despite the sperm count with the presence of \textit{M. genitalium} was within a normal range, a decrease in sperm concentration was significant. However, we have failed to demonstrate a correlation between sperm concentration and sperm morphology and the detection of genital ureaplasmas in semen samples.” replace the sentences “The sperm concentration (14.14 \times 10^6/ \text{mL}) was lower than the normal reference of WHO manual (\geq 20 \times 10^6/ \text{mL}) in semen of \textit{M. hominis} -positive infertile men and higher (52.63 \times 10^6/ \text{mL}) in semen of \textit{M. hominis}-negative infertile men. The present data show that \textit{M. hominis} may affect sperm concentration and sperm morphology of infertile men. However, we have failed to demonstrate a correlation between sperm concentration and sperm morphology and the detection of \textit{U. urealyticum}, \textit{U. parvum} and \textit{M. genitalium} in semen samples.”

* \textbf{In the discussion}, page 14 line 291, ”\textit{Previous studies have reported.”} replace ”Previous studies have described.”

* \textbf{In the methods}, page 9 line 190, expression “\textbf{normally distributed}” replace expression 'normality'

3- \textbf{Responses to reviewer III: Marcia Hobbs}

\begin{itemize}
  \item Responses to major Compulsory Revisions

  * The title “\textit{Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis and Mycoplasma genitalium infections and semen quality of infertile men}” replace the title “The correlation of \textit{Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis} and \textit{Mycoplasma genitalium} infections with male infertility.”

  * The limitation imposed by the study design was mentioned in page 11 lines 237-240 “\textbf{We couldn’t get semen samples from fertile men and so we have limited our comparison for seminological variables between semen from infected and non-infected infertile men with genital mycoplasmas and ureaplasmas.”
In the abstract, page 1 lines 39-44, the sentences “Comparison of the parameters of the standard semen analysis between the male partners of the infertile couples with and without genital ureaplasmas and mycoplasmas infection showed that the presence of *Mycoplasma hominis* DNA in semen samples is associated with low sperm concentration ($p = 0.007$) and abnormal sperm morphology ($p = 0.03$). A positive correlation was also found between sperm concentration and the detection of *M. genitalium* in semen samples of infertile men ($p = 0.05$).” replace the sentence “Comparison of the parameters of the standard semen analysis between the male partners of the infertile couples with and without genital ureaplasmas and mycoplasmas infection showed only the presence of *Mycoplasma hominis* DNA in semen samples can affect sperm concentration ($p = 0.007$) and sperm morphology ($p = 0.03$).”

In the Background, Page 4 lines 56-64, we have mentioned the different interpretations of the relationships between genital mycoplasmas orureaplasmas and the host. “The genital mycoplasmas represent a complex and unique group of microorganisms that have been associated with a wide array of infectious diseases in adults and infants. The lack of conclusive knowledge regarding the pathogenic potential of *Mycoplasma* and *Ureaplasma* spp. in many conditions is due to a general unfamiliarity of physicians and microbiology laboratories with their fastidious growth requirements, leading to difficulty in their detection; their high frequency in healthy persons; the poor design of research studies attempting to base association with disease on the mere presence of the organisms in the lower urogenital tract; the failure to consider multifactorial aspects of diseases; and considering these genital mycoplasmas only as a last resort (3). The situation is now changing because of a greater appreciation of the genital mycoplasmas as perinatal pathogens and improvements in laboratory detection, particularly with regard to the development of powerful molecular nucleic acid amplification tests (3).”

References 4, 5, 6, 7 replace references in line 69 in the Background

*The conclusion was restructured “The results of our study demonstrate that the genital mycoplasmas and ureaplasmas seem to be widespread among male partners of infertile
couples in Tunisia. The study of the comparison of the semen parameters of infertile men with and without genital ureaplasma and mycoplasmas has not shown any significant differences, apart from the sperm concentration in the colonisation of *M. hominis* and *M. genitalium* and sperm morphology in the colonisation of *M. hominis*. Our results also indicate that PCR-microtiter plate hybridization assay method provides a rapid and effective measure to detect human genital mycoplasmas and ureaplasmas which is useful for etiological and epidemiological studies of these pathogens.

Little information was however available regarding the effect of mycoplasmas and ureaplasmas on the sperm quality, as well as their relationship with the leukocyte count. Therefore, it can be concluded that the screening of mycoplasmas and ureaplasmas species in routine semen analysis is not clinically relevant in our specific population. It should be restricted for men undergoing complete evaluation of infertility, genital infection and male partners from couples undergoing IVF. However, this does not imply that mycoplasmas and ureaplasmas do not affect semen parameters in some cases, mainly *U. urealyticum*.

* The sentences of the conclusion “During the past decade, evidence for damage caused by *U. urealyticum* to the development and vitality of human embryos has accumulated. In human *in vitro* fertilization systems, the presence of *U. urealyticum* in either semen or female genital tract resulted in a decline in pregnancy rate per embryo transfer (12, 13).” were transferred in pages 4-5 lines 74-77 and the sentence ”The dual effect of *U. urealyticum* on the sperm activity (inhibition of sperm motility at low pHs and increase of sperm velocity at higher pHs, depending on sperm metabolism) has been recently demonstrated (17)”. In page 5 lines 81-83.

I will be ready for any suggestion or further modification that you need me to make.
Looking forward to having my manuscript published in your Journal, I would like to send you my highest respect.

Sincerely yours

GDOURA Radhouane