Author’s response to reviews

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

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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 version of our manuscript.

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

I will be ready for any suggestion or further modification that you need me to make.

**I- Responses to reviewer’s comments and recommendations**

1- Responses to reviewer I: Maciej Kurpisz

- Responses to Major Compulsory Revisions

  * In the methods, page 6 lines 120–122, we have listed the values of standard semen variables of semen: “Oligospermia was defined by a sperm concentration < 20 x 10^6/ml, asthenospermia by sperm motility <50 %, necrospermia by a sperm viability < 50 % (category [a + b]) and teratospermia by a normal morphology <30 %”.
  * In the abstract, page 2 lines 324-327, we have mentioned a negative correlation between sperm concentration and the detection of *M. genitalium* “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”.
  * In the discussion, page 15 lines 321-322 and lines 326-328, we have mentioned a negative correlation between sperm concentration and the detection of *M. genitalium or M. hominis* “Yet, a correlation was reversely observed between sperm concentration and the detection of this organism in semen samples”, “A negative correlation was also found between sperm concentration and the detection of *M. genitalium* in semen samples of infertile men.”
• Responses to Minor Essential Revisions

The language of the manuscript was revised by proficient in English. We have listed below all corrections.

* In the abstract, the sentences page 2 lines 23-28 “Reports are, however, controversial regarding the effects of these microorganisms infections in the sperm seminological variables. This study aimed at determining the frequency of genital ureplasmas and mycoplasmas in semen specimens collected from infertile men, and at comparing the seminological variables of semen from infected and non-infected men with these microorganisms.” replace the sentences “Reports are however controversial about the effects of these microorganisms infections on sperm seminological variables. The aims of this study were to determine the frequency of genital ureplasmas and mycoplasmas in semen specimens collected from infertile men, and to compare the seminological variables of semen from infected and non-infected men with these microorganisms.”

* In the abstract, the sentences pages 3 and 4 lines 38-45 “Comparison of the parameters of the standard semen analysis between the male partners of the infertile couples with and without genital ureplasmas 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 \((p = 0.03)\) and a negative correlation between sperm concentration and the detection of \(M. \ genitalium\) in semen samples of infertile men \((p = 0.05)\). The mean values of seminal volume, pH, vitality, motility and leukocyte count were not significantly related either to the detection of genital mycoplasmas DNA or to the detection of ureplasmas DNA in semen specimens.” replace the sentences “Comparison of the parameters of the standard semen analysis between the male partners of the infertile couples with and without genital ureplasmas 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). The mean values of seminal volume, pH, vitality, motility and leukocyte count were not significantly related either to the detection of genital mycoplasmas DNA or to the detection of ureaplasmases DNA in semen specimens.”

*In the background,* the sentences page 4 lines 57-63 “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 which makes it difficult to detect them. We also note their high frequency in healthy persons and the poor design of research studies attempting to deepen the association with the disease as regards the mere presence of the organisms in the lower urogenital tract. Besides, we fail to consider the multifactor aspects of diseases; and thus considering these genital mycoplasmas only as a last resort (3).” replace the sentences “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).”

*In the background,* page 5 lines 80 we have delete the word “either.”

*In the background,* the sentences page 5 lines 86-89 “However, given the time-consuming culture which requires to 2 to 5 days for *Ureaplasma* spp. and *M. hominis*
and up to 8 weeks for *M. genitalium* culture, infectious agents can be detected in less than 8 hours by nucleic acid amplification techniques.” replace the sentences “However, giving the time-consuming culture, as a case in point 2 to 5 days of *Ureaplasma* spp. and *M. hominis* culture and up to 8 weeks of *M. genitalium* culture, infectious agents can be detected in less than 8 hours by nucleic acid amplification techniques.”

* In the Methods, the sentences page 9 and 10 lines 189-197 “Means were calculated for the separate groups for sample volume, pH, sperm concentration, vitality, rapid progressive motility, total progressive motility, normal morphology and leukocyte count for semen samples.

All the variables were initially inspected for normally distribution, and the sample volume and pH were all found to be approximately normally distributed. Therefore, standard parametric techniques (*t*-tests) were used to test significance of factors. Because sperm concentration, vitality, rapid progressive motility, total progressive motility, normal morphology and leukocyte count were not normally distributed and nonparametric techniques (Wilcoxon tests) were used to test the significance of the differences between groups regarding these factors.” replace the sentences “Means were calculated for the separate groups for sample volume; pH; sperm concentration; percent vitality; percent rapid progressive motility; percent total progressive motility; percent normal morphology and leukocyte count for semen samples.

All the variables were initially inspected for normally distribution, and the sample volume and pH were all found to be approximately normally distributed; therefore, standard parametric techniques (*t*-tests) were used to test significance of factors. Because of sperm concentration; vitality; rapid progressive motility; percent total progressive motility; percent normal morphology and leukocyte count were not normally distributed and nonparametric techniques
(Wilcoxon tests) were used to test the significance of the differences between groups regarding these factors.”

* **In the results**, page 10 and 11 lines 219-221 “either”, “or to the detection of *M. genitalium* DNA” and “table 3” were deleted.

* **In the discussion**, page 13 lines 271-272 “in isolating it” replace “of isolation its”

* **In the discussion**, the sentence page 13 lines 284-287 “In our study, although *M. genitalium* was not separately isolated among patients, it was detected together with *U. urealyticum + M. Hominis* in one patient, with *M. hominis* in one patient, with *U. parvum* in two patients and with *U. urealyticum* in two patients.” replace the sentence “In our study, although *M. genitalium* was not isolated alone in any patient, it was detected together with *U. urealyticum + M. Hominis* in one patient, with *M. hominis* in one patient, with *U. parvum* in two patients and with *U. urealyticum* in two patients.”

* **In the discussion**, the sentences page 15 lines 320-322 “In our study, a positive correlation was found between sperm morphology and the detection of *M. hominis* in semen samples of infertile men. Yet, a correlation was reversely observed between sperm concentration and the detection of this organism in semen samples.” replace the sentences “In our study, positive correlations were found between sperm concentration and sperm morphology and the detection of *M. hominis* in semen samples of infertile men.”

* **In the discussion**, the sentences page 15 lines 326-328 “A negative correlation was also found between sperm concentration and the detection of *M. genitalium* in semen samples of infertile men.” replace the sentences “We have a positive correlation found between sperm concentration and the detection of *M. genitalium* in semen samples of infertile men.”

* **In the discussion**, page 15 lines 328 the word “Although” replace the word “Despite”

* **In the discussion**, the sentences page 15 lines 331-335 “The comparison of the sperm seminological variables between semen with mixed infections and semen without mixed
infections or without infections showed 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 additional effect on seminology.” replace the sentences “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 the discussion, page 16 lines 341 “among our studied patients.” replace “the study groups”

* In the conclusion the last sentence “However, this does not imply that mycoplasmas and ureaplasmas do not affect semen parameters in some cases, mainly *U. urealyticum.*” was deleted.

2- Responses to reviewer II: Marcia Hobbs

- Responses to Minor Essential Revisions

We have eliminated the term “colonisation” throughout the manuscript and we have used the term “infection”.

The language of the manuscript was revised by proficient in English. We have listed below all corrections.

* In the abstract, the sentences page 2 lines 23-28 “Reports are, however, controversial regarding the effects of these microorganisms infections in the sperm seminological variables. This study aimed at determining the frequency of genital ureplasmas and
mycoplasmas in semen specimens collected from infertile men, and at comparing the
seminological variables of semen from infected and non-infected men with these
microorganisms.” replace the sentences “Reports are however controversial about the effects
of these microorganisms infections on sperm seminological variables. The aims of this study
were to determine the frequency of genital ureplasmas and mycoplasmas in semen specimens
collected from infertile men, and to compare the seminological variables of semen from
infected and non-infected men with these microorganisms.”

* In the abstract, the sentences pages 3 and 4 lines 38-45 “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 \((p = 0.03)\) and a negative correlation
between sperm concentration and the detection of \(M.\) genitalium in semen samples of
infertile men \((p = 0.05)\). The mean values of seminal volume, pH, vitality, motility and
leukocyte count were not significantly related either to the detection of genital
mycoplasmas DNA or to the detection of ureaplasmas DNA in semen specimens.” replace
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 \((p = 0.05)\). The mean values of seminal
volume, pH, vitality, motility and leukocyte count were not significantly related either to the
detection of genital mycoplasmas DNA or to the detection of ureaplasmas DNA in semen
specimens.”
In the background, the sentences page 4 lines 57-63 “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 which makes it difficult to detect them. We also note their high frequency in healthy persons and the poor design of research studies attempting to deepen the association with the disease as regards the mere presence of the organisms in the lower urogenital tract. Besides, we fail to consider the multifactor aspects of diseases; and thus considering these genital mycoplasmas only as a last resort (3).” replace the sentences “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).”

In the Methods, the sentences page 9 and 10 lines 189-197 “Means were calculated for the separate groups for sample volume, pH, sperm concentration, vitality, rapid
progressive motility, total progressive motility, normal morphology and leukocyte count for semen samples.

All the variables were initially inspected for normally distribution, and the sample volume and pH were all found to be approximately normally distributed. Therefore, standard parametric techniques (t-tests) were used to test significance of factors. Because sperm concentration, vitality, rapid progressive motility, total progressive motility, normal morphology and leukocyte count were not normally distributed and nonparametric techniques (Wilcoxon tests) were used to test the significance of the differences between groups regarding these factors.” replace the sentences “Means were calculated for the separate groups for sample volume; pH; sperm concentration; percent vitality; percent rapid progressive motility; percent total progressive motility; percent normal morphology and leukocyte count for semen samples.

All the variables were initially inspected for normally distribution, and the sample volume and pH were all found to be approximately normally distributed; therefore, standard parametric techniques (t-tests) were used to test significance of factors. Because of sperm concentration; vitality; rapid progressive motility; percent total progressive motility; percent normal morphology and leukocyte count were not normally distributed and nonparametric techniques (Wilcoxon tests) were used to test the significance of the differences between groups regarding these factors.”

* In the results, page 10 and 11 lines 219-221 “either”, “or to the detection of M. genitalium DNA” and “table 3” were deleted.

* In the discussion, page 13 lines 271-272 “in isolating it” replace “of isolation its”

* In the discussion, the sentence page 13 lines 284-287 “In our study, although M. genitalium was not separately isolated among patients, it was detected together with U. urealyticum + M. Hominis in one patient, with M. hominis in one patient, with U. parvum
in two patients and with *U. urealyticum* in two patients.” replace the sentence “In our study, although *M. genitalium* was not isolated alone in any patient, it was detected together with *U. urealyticum* + *M. Hominis* in one patient, with *M. hominis* in one patient, with *U. parvum* in two patients and with *U. urealyticum* in two patients.”

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male partners without infections. Our findings show that the mixed infections have no additive affect on seminology.”

* In the discussion, page 16 lines 341 “among our studied patients.” replace “the study groups”

* In the conclusion the last sentence “However, this does not imply that mycoplasmas and ureaplasmas do not affect semen parameters in some cases, mainly U. urealyticum.” was deleted.

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