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Commentary

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Comment on 'Importance of real-time measurement of sperm head morphology in intracytoplasmic sperm injection' by Fumiaki Itoi *et al.*

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Summary

We present a commentary on the article published in the *Zygote* FirstView: 'Importance of real-time measurement of sperm head morphology in intracytoplasmic sperm injection' by Fumiaki Itoi and colleagues. We comment on the importance of providing the microscope setup details whenever sperm morphology visualization is discussed. The claim of ×6000–10,000 magnification is misleading as such levels of magnification are impossible to achieve.

Dear Sirs,

We have read with great interest the article 'Importance of real-time measurement of sperm head morphology in intracytoplasmic sperm injection' by Fumiaki Itoi and colleagues (Itoi *et al.*, 2022).

We agree that this work is very much needed as it gives an overview of the current achievements in the automation of intracytoplasmic sperm injection (ICSI). The authors outline the directions for further development of methods to optimize the selection of sperm for fertilization, while keeping the cost of technology at a lower level through reducing the need for high-end microscopes and limiting the associated difficulties in performing ICSI such as lengthening the procedure and exposing both sperm and oocytes to unfavourable conditions associated with changes in their storage conditions (glass culture dishes for ICSI, the use of immersion oil, etc.).

However, we have to bring attention to the problem we have pointed out previously (Lukaszuk *et al.*, 2016), but which is unfortunately still repeated in many articles and shows the weakness of our field in the application of basic physics. Few publications (Wilding *et al.*, 2011; De Vos *et al.*, 2013) do not repeat the claim of using the ultra-high magnification in the range of ×6000–10,000. Itoi and colleagues do, however, state that the spermatozoa [in the intracytoplasmic morphologically selected sperm injection (IMSI) motile sperm organelle morphology examination (MSOME) and in automatic classification systems] were observed under ×6000–10,000 magnification. It is a very misleading claim that makes it seem that it is indeed possible to obtain such a magnification using a standard optical microscope (even a most advanced one). It is impossible to calculate the actual magnification as the basic microscope parameters such as the objective numeric aperture (NA), condenser NA or wavelength that was used to observe the spermatozoa is not provided.

If these details were available, the resolution could be calculated based on the following formula:

$$d = \frac{1.22\lambda}{(NAobjective + NAcondenser)}$$

where d = optical resolution; λ = wavelength of the illuminating light used; and NA = numeric aperture.

Simplifying it greatly, we can estimate that the real magnification of a microscope can be most easily given by multiplying the objective's NA by 1000.

This should all be taken into account when information about microscopes is included in publications and when used to rationalize data and results.

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