Dear Mr. Kushnir,

I have read with great interest the manuscript by Liu and collaborators (Liu et al., 2016) on the genital bacterial load in African men and the claimed relationship of their results with the risk of tetanus following the use of PrePex for nonsurgical circumcission.

In this manuscript, the authors attempted to provide a straightforward comparison between the genital microbiome of two groups of uncircumcised and circumcised men from Rakai, Uganda. The differences between the biomes of these two groups have been then discussed in light of the twelve cases of tetanus reported in individuals that underwent voluntary medical male circumcision (VMMC), eight of them after receiving conventional VMMC surgery, and four after being treated for nonsurgical male circumcision with PrePex (Grund et al., 2016).

The currently FDA approved PrePex device allows circumcision without the need for surgery and local anesthesia. One of its main advantages of Prepex is that it is a mono-use device, which does not need complex sterilisation procedures. It is very simple and comprised of only three plastic elements (placement, inner ring and elastic ring) and a verification thread, which can be deployed by paramedical personnel and easily discarded upon usage. The radial compression operated by the elastic ring gently stops the blood flow in the foreskin, thus causing a localised necrosis yielding to the formation of granulation tissue on the compression area and detachment of the necrotic foreskin.
In spite of the claims, the study by Liu et al. does not shed any light on the possible contamination of the genital area with *Clostridium tetani* or its spores and/or the association of VMMC with clinical tetanus. In this regard, I believe that the abstract and discussion of this manuscript are misleading since no steps have been taken to assess the real risk of tetanus in Prepex-treated individuals. Although the microbiological assessment is sound, it lacks specific test for *C. tetani* or its spores and it is based on patient groups that are not directly comparable. Moreover the suggested mechanism of intoxication by tetanus neurotoxin allegedly produced in the necrotic foreskin upon application of the Prepex device (to date, a completely undemonstrated claim) is scientifically unsound as it contradicts our present knowledge of the process leading to dissemination of tetanus neurotoxin in the patient’s nervous system.

Specific points:

1. The Prepex-treated and control groups are unsuitable for a direct comparison. Indeed, the **group size** (22 PrePex-treated individuals versus 145 uncircumcised controls), their **average age** (median age 20.0 years for PrePex-treated individuals and 30.0 years for uncircumcised controls), their **social status** (50% of men circumcised with the PrePex device were never married and 13.6% reported no sexual partner in the past year, whereas all uncircumcised controls were married and reported being sexually active in the past year) are very different between the two groups, which may justify the observed differences. In this light, the uncircumcised group cannot be considered a bona-fide reliable “control for the Prepex treated pool of individuals.

2. The analyses of the samples collected from the two groups were performed at the same time, yet the collections of the specimens were done in 2004 (uncircumcised controls) and much more recently (2014 or later for uncircumcised controls). Hence these groups cannot be directly compared.

3. No longitudinal sampling of PrePex-treated individuals (before and after application of the Prepex device) was performed. This type of analysis is much more powerful in detecting the specific effect of a given treatment (e.g. PrePex device than any population studies, especially if underpowered.

4. The bacterial load of the sub-preputial area of PrePex-treated individuals appears to be enriched in non-pathological anaerobes. However, as the authors of this study did not find any evidence of vegetative forms of *Clostridium tetani* or tested for the presence of its spores (page 8; Liu *et al.*, 2016). Hence the claim that “this anaerobiosis with the PrePex device might explain the possible increased risk of tetanus in PrePex users with no or incomplete tetanus toxoid immunization” (page 8; Liu *et al.*, 2016) is **completely unfounded**.

5. As mentioned above, the operating principle of the Prepex device is that the elastic o-ring completely abolishes the blood flow in the application area, thus inducing localised necrosis followed by the detachment of the foreskin. Hence the application of the Prepex device generates two barriers, the first mechanical (the o-ring pressing on the plastic inner ring), and the second physiological (the formation of granular tissue proximal to site of application), which block the entry of any agent (chemical or bacteriological) eventually present in the necrotic area. This includes any toxin, including tetanus neurotoxin, possibly generated by the low oxygen condition present in the necrotic foreskin. In addition, the compression caused by
the o-ring together with the resulting anaerobic conditions would determine a rapid
degeneration of any nerve terminals and axons located distally to the Prepex
application site, making impossible the entry of any neurotropic agents in the
nervous system. This is particular important for tetanus neurotoxin, which requires
an intact axon and axonal transport machinery to be transported into the spinal cord
and upper brain structures to elicit the clinical symptoms of tetanus (Bercsenyi et
al., 2013).

6. Even in the eventuality of contamination of the necrotic foreskin with Clostridium
tetani spores, their germination would be halted by the implementation of the new
WHO recommendations for cleansing of the foreskin prior and after non-surgical
circumcision with an appropriate disinfectant, such povidone-iodine. This
disinfectant would inhibit spore germination and inactivate any tetanus neurotoxin
which may form in the necrotic foreskin.

After completing the critical analysis of the manuscript by Liu et al., I had the opportunity to
read the commentary published by Professor I. Brook (Brook, 2016) on this study. I fully
endorse the point raised by Professor Brook in this commentary, which are perfectly
aligned to the issues highlighted above. The additional point raised are fully compatible
with the pathophysiology of tetanus and the biology of tetanus neurotoxin, which is
produced uniquely by the vegetative form of Clostridium tetani and it is the sole
responsible of the clinical symptoms of tetanus.

Please do not hesitate to contact me for additional information about this matter.

Your sincerely,

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