UNIVERSITY OF CALIFORNIA, BERKELEY

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

POLINA V. LISHKO, Ph.D.
Associate Professor of Cell and Developmental Biology
Department of Molecular and Cell Biology
University of California, Berkeley
221A Life Sciences Addition
Berkeley, CA 94720-3200
Adjunct Associate Professor at the Center for Reproductive Longevity and Equality at Buck Institute for Research on Aging
lishko@berkeley.edu
Office: (510) 642-4687
Lab: (510) 664-4885

November 25, 2020

Dear *JoVEs* Editors,

We would like to thank all reviewers for the constructive and helpful criticism. We have extensively revised the manuscript by making it more structured, removed redundant parts and added missing references. As mentioned in our response below, we were not able to perform some of the additional experiments that were suggested due to the current COVID related situation that keeps our lab at low occupancy and prevented animal experimentation. Below is our point-to-point response (in italic) to the reviewers' suggestions.

Reviewers' comments:

Reviewer #1:.

Minor Concerns:
1) Line 235: you name HS solution: High Saline solution. Is this correct? The solution seems to be composed of a standard salt concentration.
A: corrected, thank you!

2) It is unclear how the U-tube itself affects positive and negative pressure (line 545).
A: correction and explanations added in Figure 11-12.

3) The legend of Figure 1 refers to the human sperm cells attached to the pipette being the left panel, when it is on the right (line 617).
A: corrected, thank you!

Reviewer #2:
Specific comments:
L97ff: The authors state in the abstract that the technique has also been instrumental to record chloride channels in sperm. Citation for the recording of Cl channels are missing in this list of citations. Please, either add a citation or edit the abstract.
A: we have removed all citations from the abstract, and included it in the line 108 in the introduction.

L 126: The authors state, that all attempts to form a gigaseal have been unsuccessful outside the CD. Nevertheless, there are papers describing recordings in the cell-attached configuration from the head of sperm cells (e.g. Gu Y, Kirkman-Brown JC, Korchev Y, Barratt CL, Publicover SJ. Multistate, 4-aminopyridine-sensitive ion channels in human spermatozoa. Dev Biol. 2004; 274:308-317.) and others. Please specify this.
A: we have now properly cited these papers

L149-140: Same comment also holds true for this.
A: see above

L173-175: It remains unclear for the reviewer, why only these two patch-clamp amplifier should work. Every

modern patch-clamp amplifier should be able to record these currents from sperm cells. Especially, since all these amplifiers are suited to record currents from single channels.
A: we have removed all vendor mentioning, but based on our experience we have used these two types successfully, and listed them in the methods.

L331ff: This procedure to prevent sperm cells from sticking to the glass seems to be quite complicated. In the field of motility analysis of sperm cells, which faces the same problem, usually HSA is used for this purpose.

A: we have not used HSA and therefore do not know whether this compound can alter sperm ion channel behavior by chelating lipids and/or cholesterol. We describe in details the method that has been working successfully in our hands. Besides, we apply coating solution made from the same donor sperm to the same sperm populations.

L396: Here the authors report a pipette resistance of 11-17 MΩ. In line 194 it is 11-13 MΩ. Please specify the value and keep it consistent throughout the protocol.
A: corrected, thank you!

L508: Here the authors say that the difference between pipette and bath solution should not exceed 10mOsm. In line 399 it is less than 15 mOsm. Please specify the value and keep it consistent throughout the protocol.
A: corrected, thank you!

L594: The study cited here as an example for high throughput Ca2+ imaging did not use Ca2+ fluorometry but motility and acrosome reaction as readout. There are much more and better suited studies that used medium- or high-throughput Ca2+ fluorometry in sperm cells. Moreover, the comparison between the two methods is not very precise. There is no doubt, that the direct study of ion channels via patch-clamp is best suited to characterize ion channels. Nevertheless, ionic imaging also has a lot of advantages, since it is much less invasive. Also the statement, that only change nd a proper calibration also allows to study Ca2+ concentrations in a quantitative manner.s in the Ca2+ concentration can be measured is incorrect. Using ratiometric dyes a proper calibration also allows to study Ca2+ concentrations in a quantitative manner.
A: Thank you for this statement. We have adjusted references and corrected this part.

Minor comments:
L153: 2µM has to be 2 µm
A: corrected

Table4: The amount of Tris-HCl has to be 25 µl.
A: corrected.

Reviewer #3:
Major Concerns:
My only criticism would be that it is not clear who the ms is aimed at. In parts it describes patch clamping from first principles and appears to be addressing readers with no background in electrophysiology, but the more technical parts may only really be understood by readers with some patching experience. It might be worth indicating some reading on basic patch clamp techniques and concepts, and suggesting that readers look into (and try) standard patching techniques first before tackling the particular problems of patching sperm. Similarly, it might be worth emphasising what is particular to sperm patching. For instance - sections 2.2 and 3 are not really specific to patching sperm.
A: corrected. We have added additional reading sources

Minor Concerns:
Lines 126-7 and lines 148-150 both state that seals cannot/have not been achieved outside of the CD. Though it is true that whole-cell patch has only been achieved via the CD, cell-attached seals on the sperm head have been reported a number of times and in several species. Alberto Darszon's group has done this on sea urchin (Guerrero et al, 1987), mouse (Espinosa et al, 1998), and human (Orta et al, 2012). Gorelick et al (2002) reported achieving seals on the head of sea urchin sperm using SICM (smart patch) and Jimenez-Gonzalez et al 2007 used cell-attached patches to map channel activity on the human sperm head.
A: corrected. We have added those citations.

Line 190 - might be worth explaining what 'fire polished' means
Line 444 - should this be step 5.2.4?
Line 506 - should read "The slightly hypotonic extracellular solution in comparison to the pipette solution"?

A: all above were corrected.

Sincerely,
Polina Lishko, Ph.D.