**Your comment:**   
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Our Response:

We checked the revised manuscript thoroughly for spelling or grammar errors

**Your comment:**   
2. Please provide an email address for each author.

Our Response:

The following email address for each author has been provided in the revised manuscript (lines 5-26).

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**Your comment:**   
3. Keywords: Please provide at least 6 keywords or phrases.

Our Response:

We added the following 2 new key words in the revised manuscript (line 29)

**KEYWORDS:** Percoll; sperm; mobility; density gradient; discontinuous; Accudenz

**Your comment:**

4. Please remove commercial language, like Eppendorf.

Our Response:

We changed the word “Eppendorf” to “polypropylenemicrocentrifuge” as follows in the revised manuscript:   
Page 4, Line 143: Pipette 0.5 mL semen into polypropylenemicrocentrifuge tube.

Page 5, Line 188: sterile polypropylenemicrocentrifuge tube.

**Your comment:**

5. 1.1: What is dH2O? What is the concentration of HCl?

Our Response:

dH2O means distilled water. The concentration of HCl was 0.1N

We revised our manuscript as follows:

Page 4, Line 136: 0.24 g KH2PO4 to 800 mL distilled water (dH2O). pH the solution to 7.4 using 0.1 N HCl

**Your comment:**

6. 1.2: What is the concentration of NaOH?

Our Response:

The concentration of NaOH was 1M.

We revised our manuscript as follows:

Page 4, line 141: ….using 1 M NaOH and bring solution to 1 L using dH2O.

**Your comment:**

7. 3.2, 3.4: Please provide more details so that these steps can be adequately filmed.

Our Response:

We included detailed protocols for the steps in 3.2 (lines 209-219 in the revised manuscript) and 3.4 (lines 231-249 in the revised manuscript).

**Your comment:**

8. Figure 4-6: Please define error bars in the figure legend. Also, what statistical tests were used?

Our Response:

Error bars indicate the standard error of the mean (SEM). All statistical analyses were performed with the Statistical Analysis System “R” software package. Data for the absorbance value, live and dead sperm, and hole number were subjected to one-way ANOVA, followed by the Tukey-Kramer test. Values are presented as the mean ± SEM (n = 5) of two independent experimental replicates, and five replicates were considered for the test.

**Your comment:**

9. Figure 5: Please include a space between the number and its unit (i.e., 550 nm).

Our Response:

We revised the figure 5: Absorbance [550 nm], and figure 6: No. holes/0.25 mm2

**Your comment:**

10. Please reference Figures 2 and 3 in the manuscript.

Our Response:

We revised the manuscript as follows:

Page 5, line 223: Pipet 1.0 mL 6 % Accudenz solution into polystyrene cuvettes, as illustrated in Figure 2.

Page 5, line 241: Incubate sperm in motility buffer with IPVL for 15 min at 37 °C, as illustrated in Figure 3.

**Your comment:**

11. Figure legends: Details of the methodology should not be in the Figure Legends, but rather the Protocol.

Our Response:

**Your comment:**

12. Please revise the Acknowledgements section to include any acknowledgments and all funding sources for this work.

Our Response:

There is none but the listed authors who contributed to this study. There was no funding source for this study.

**Your comment:**

13. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

Our Response:

As suggested by you, we have removed trademark (™) and registered (®) symbols from the Table of Equipment and Materials.