

1 **Hyperactivated stallion spermatozoa fail to exhibit a rheotaxis-like behaviour,**
2 **unlike other species**

3 Jon Romero-Aguirregomezcorta, Emer Sugrue, Lucía Martínez-Fresneda, David

4 Newport and Sean Fair*

5

6 **Supplementary Information**

7

8 **Supplementary Video S1. Non-hyperactivated frozen-thawed stallion sperm in**
9 **a microfluidic channel exhibiting rheotaxis.** Sperm detect the flow and swim
10 against it during rheotaxis. The arrow indicates the direction of the flow.

11

12 **Supplementary Video S2. Non-hyperactivated frozen-thawed stallion sperm in**
13 **a static environment.** This video was recorded as a control in order to compare the
14 movement pattern of hyperactivated and non-hyperactivated sperm.

15

16 **Supplementary Video S3. Hyperactivated frozen-thawed stallion sperm in a**
17 **static environment.** After incubation with procaine 5 mM sperm showed high-
18 amplitude flagellar waves and asymmetrical flagellar beating when assessed in a
19 static droplet.

20

21 **Supplementary Video S4. Non-hyperactivated fresh stallion sperm in a static**
22 **environment.** This video was recorded as a control in order to compare the
23 movement pattern of hyperactivated and non-hyperactivated sperm.

24

25 **Supplementary Video S5. Hyperactivated fresh stallion sperm in a static**
26 **environment.** After incubation with procaine 5 mM sperm showed high-amplitude
27 flagellar waves and asymmetrical flagellar beating when assessed in a static droplet.

28

29 **Supplementary Video S6. Hyperactivated frozen-thawed stallion sperm in a**
30 **microfluidic channel.** Stallion sperm incubated with procaine (5 mM) were non-
31 progressive and unable to exhibit rheotaxis. The arrow indicates the direction of the
32 flow.

33

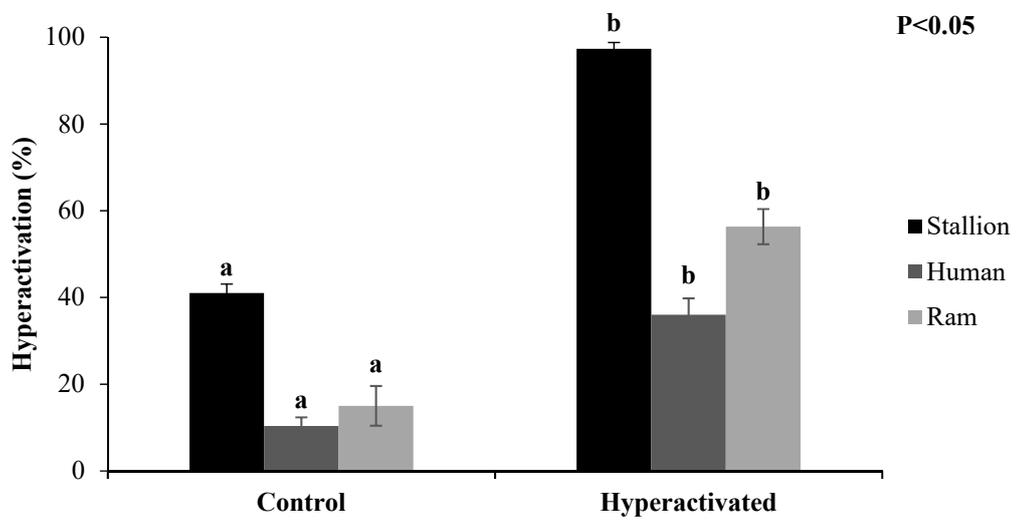
34 **Supplementary Video S7. Non-hyperactivated fresh stallion sperm in a**
35 **microfluidic channel exhibiting rheotaxis.** Sperm detect the flow and swim
36 against it during rheotaxis. The arrow indicates the direction of the flow.

37

38 **Supplementary Video S8. Hyperactivated fresh stallion sperm in a microfluidic**
39 **channel.** Stallion sperm incubated with procaine (5 mM) were non-progressive and
40 unable to exhibit rheotaxis. The arrow indicates the direction of the flow.

41

42 **Supplementary Figure S9. The percentage of hyperactivated sperm compared to**
43 **the control treatment following incubation with the hyperactivation agonists,**
44 **procaine (stallion), progesterone (human) and caffeine (ram) as characterised by**
45 **high-amplitude flagellar waves and asymmetrical flagellar beating and assessed**
46 **subjectively in a static saline droplet (Experiment 4). Vertical bars represent the**
47 **s.e.m. (n=3 replicates). ^{ab}Differing superscripts differ significantly within species**
48 **(P<0.05).**



49

50