swimming near the channel walls or in the centre at 5, 10, 15 and 20 mm distance from the sample inlet. A flow rate of 30 μm/s and a viscosity of 1 mPa s were used in experiments 2–4. Data were transformed where appropriate and analysed using repeated measures ANOVA. There was an effect of flow velocity and time ($P < 0.001$) on sperm progression. Sperm progressed furthest at a velocity range of 20–50 μm/s, with the optimum velocity for sperm progression determined to be 30 μm/s. Sperm progression was not influenced by change in the media viscosity within the range tested ($P > 0.05$). Sperm that were hyperactivated with caffeine were able to swim further than non-hyperactivated sperm ($P < 0.05$). The percentage of sperm swimming near the channel walls was higher than in the channel centre ($P < 0.001$) and the percentage of sperm swimming along the walls increased as they progressed upstream ($P < 0.001$). This study provides novel insights into bull sperm rheotaxis behaviour within a microchannel with varying flow velocities/viscosities and while undergoing hyperactivation.

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P22

The effect of dietary supplementation of algae rich in docosahexaenoic acid on boar fertility

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The objective of this study was to assess the effects of dietary supplementation of a commercial algal product (All-G-Rich, Alltech, Dunboyne, Co Meath, Ireland) rich in docosahexaenoic acid (DHA) on boar fertility. Purebred maternal and terminal line boars of proven fertility, were individually housed and fed one of three experimental diets for 19 weeks: (i) Control (Ctl) diet ($n = 31$), (ii) Ctl diet plus 75 g All-G-Rich per day ($n = 31$) or (iii) Ctl diet plus 150 g All-G-Rich per day ($n = 30$). Parameters assessed were (i) raw semen quality; volume, sperm concentration, motility and morphology; (ii) liquid semen quality; progressive motility, viability, hypotonic resistance and acrosomal integrity; (iii) frozen-thawed semen quality; motility, thermal stress, viability, membrane fluidity and mitochondrial activity; (iv) sperm and seminal plasma (SP) fatty acid composition (FAC); (v) total antioxidant capacity (TAC) of SP and (vi) farrowing rates and litter sizes of sows ($n = 1158$) inseminated with liquid semen. Data were analysed in the Statistical Package for the Social Sciences (SPSS software; version 20.0, IBM, Chicago, IL). Boars consuming 75 g All-G-Rich had a larger semen volume ($P < 0.05$) and a higher total sperm number ($P < 0.01$) than the Ctl treatment, however, there was no effect of treatment on any of the other raw semen quality parameters ($P > 0.05$). There was no effect of dietary treatment on the *in vitro* parameters assessed on liquid or frozen-thawed semen, the FAC and TAC of SP or on farrowing rate and litter size ($P > 0.05$). There was an effect of dietary treatment on the FAC of sperm, represented by an 1.71 and 1.75 fold increase in the DHA content for 75 g and 150 g treatments, respectively, compared to the Ctl treatment. In conclusion, dietary supplementation of boars with All-G-Rich successfully altered the FAC of sperm, increased semen volume and total sperm number but had no effect on sperm quality as assessed *in vitro* and *in vivo*.

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P23

Epididymal spermatozoa from *Hippopotamus amphibious*

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The common hippopotamus was first included in the list of endangered species in 2006 and listed as vulnerable to extinction. The aim of this study was to describe the morphology, morphometry and condensation of chromatin from spermatozoa of the epididymis. Testis–epididymis complexes were obtained from a male *Hippopotamus amphibius*, 37 years old, which belonged to the Araçatuba Zoo and died on 21/07/2015 due to Total Intestinal Obstruction. The epididymides were dissected and divided into caput, corpus and cauda. Semen samples from each region were diluted in phosphate buffered saline (PBS) solution for subsequent analysis. Sperm morphometry and chromatin changes were evaluated by the toluidine blue technique; sperm morphology by Cerovsky staining. Results underwent analysis of variance and means were compared according to Tukey's test ($P < 0.05$). Concerning sperm morphology, higher levels of proximal cytoplasmic droplets were observed in the caput (25.0%), compared to the corpus (9.5%) and cauda (2.0%) of the epididymis. The opposite was observed for the distal cytoplasmic droplets, revealing higher levels in the cauda (43.0%) than in the corpus (28.0%) and caput (17.0%). Few other morphological

defects were noted (excluding droplet defects) which also decreased during the epididymal transit: caput (12.0% total defects), corpus (16.5%) and cauda (9.5%). Concerning morphometry, the measurements (pixels) of area (A), perimeter (P), width (W) and length (L) of the sperm caput were significantly greater ($P < 0.0001$) in the cauda ($A = 4584.71 \pm 691.89$; $P = 15.58 \pm 1.15$; $L = 3.46 \pm 0.42$; $C = 4.36 \pm 0.35$) than in the caput ($A = 4148.59 \pm 604.96$; $P = 14.85 \pm 1.01$; $L = 3.25 \pm 0.42$; $C = 4.21 \pm 0.29$) and the corpus ($A = 4091.89 \pm 555.38$; $P = 14.80 \pm 0.91$; $L = 3.18 \pm 0.44$; $C = 4.26 \pm 0.30$), and there was no difference between the last two regions. Chromatin compaction did not differ among regions ($P = 0.1934$); however, chromatin was most heterogeneous in the corpus (8.22 ± 2.66), followed by the caput (6.78 ± 2.13) and the cauda (6.07 ± 2.42) ($p < 0.0001$). Although results are different from those already presented for other species, our data are novel and do not allow comparisons at this moment, waiting for future studies.

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P24

Effects of 17-β estradiol and progesterone on ram sperm functionality

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The central aim of this study is based on the need of further study about the non-genomic action of certain hormones usually found in the female reproductive tract, such as steroid hormones, on spermatozoa. The presence of receptors for these hormones in the mammal sperm surface leads to speculatation about a relevant role of the mentioned hormones on sperm functionality. However, the information about their specific effects on ram spermatozoa is scarce. Here, we report the effect of different concentrations of progesterone (P4) or 17-β estradiol (E2) on ram sperm motility (by CASA), membrane integrity (Carboxifluorescein (CFDA)/Propidium Iodide (PI) staining) and changes in intracellular calcium distribution (Clortetracycline (CTC) staining) and protein tyrosine phosphorylation (Western-blot). For this purpose, swim-up-selected spermatozoa (4×10^7 mL^{-1}) were diluted in TALP medium and incubated with two different doses of P4 or E2 for 3 h at 39 °C under 5% CO_2 and saturated humidity. Both hormones were dissolved separately in PBS with DMSO and added to the sperm samples to yield final concentrations of 1 μM or 100 pM. A control group containing the same DMSO concentration (0.1‰) was included.

The results obtained showed that the incubation with P4 or E2 did not affect the percentage of sperm with progressive motility or integral plasma membrane (PI−). However, incubation with these hormones resulted in a decreased non-capacitated sperm (CTC staining, $p < 0.01$) rate, concomitant with a higher ($p < 0.01$) percentage of acrosome-reacted sperm compared with control samples. A quantitative study of the effect of both hormones on protein tyrosine phosphorylation is currently in progress. As capacitation and the acrosome reaction are related to changes in cytoskeletal organization, we also carried out indirect immunofluorescence assays using an anti-actin antibody, which revealed changes in the actin distribution on sperm incubated with hormones.

The elucidation of the action mechanism of these hormones acting via membrane receptors would allow the use of agonist/antagonist of these receptors for controlling some specific effects in artificial insemination, without including hormones in the refrigeration diluent.

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P25

Changes in the localization of MT_1 and MT_2 melatonin receptors in ram spermatozoa during maturation in the testis and epididymis

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Although spermatozoa are produced in the testis, their final maturation takes place in the epididymis. In previous studies we have revealed the presence of melatonin receptors MT_1 and MT_2 in ejaculated ram spermatozoa, and described several immunotypes for each receptor (Casao et al., Reprod Fertil Dev. 2012; 24(7):953–61). In order to determine whether the melatonin receptor distribution varies during ram sperm maturation, spermatozoa from the testis and epididymis (head, body and tail) were collected by tissue mechanical homogenization. Indirect immunofluorescence with specific antibodies was used to evidence the localization of melatonin receptors. The percentage of each immunotype was analyzed by ANOVA.

In the testis, most spermatozoa showed a mid-piece location of MT_1 ($57.9 \pm 6.9\%$). This immunotype decreased during sperm maturation in the epididymis (47.1 ± 3.4, 31.3 ± 7.7 and $32.5 \pm 1.4\%$ in head, body and tail, respectively; $P < 0.05$ when compared with the testis). Concomitantly, the percentage of spermatozoa that showed a postacrosomal location of this receptor progressively