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SHORT COMMUNICATION

Media viscosity affects post-thaw ram sperm rheotactic behaviour

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Abstract

The aim of this experiment was to assess the effect of media viscosity on ram sperm motility, kinematics and rheotaxis in vitro by using methylcellulose as a media thickener. Frozen–thawed semen of three rams was thawed and diluted in Tyrode's albumin lactate pyruvate (TALP) media supplemented with 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% and 0.7% w/v of methylcellulose. Sperm motility and kinematic characteristics were analysed using computer-assisted sperm analysis (CASA). The rheotactic behaviour was assessed in a microfluidic channel, and the number of spermatozoa that passed the 10 mm point of a microfluidic channel over a 2min period against a flow rate of 30 μm/sec was assessed. The use of media with higher viscosity (higher levels of methylcellulose) resulted in significantly lower ($p < .05$) sperm motility and kinematic parameters. Moreover, higher levels of methylcellulose reduced ($p < .05$) the number of spermatozoa that exhibited positive rheotaxis. In conclusion, viscosity affected the kinematic properties and rheotactic behaviour of ram sperm.

KEYWORDS
methylcellulose, ram sperm, rheotaxis, viscosity

1 | INTRODUCTION

Successful fertilization in vivo relies on the ability of sperm migration through viscous fluids in the female reproductive tract of mammals. Sperm must swim upstream against the outwards flow of viscous secretions, guided by mechanisms such as rheotaxis (Miki & Clapham, 2013). It has been proposed that sperm use rheotaxis to travel towards the oocyte by swimming against the secreted female fluids due to the spiral rotation of their tail (Miki & Clapham, 2013). Therefore, sperm motility plays a crucial role in sperm migration and gamete interaction (Fair et al., 2019; Mahé et al., 2021).

In recent years, microfluidics has been used in various species to simulate the swimming of spermatozoa against a fluid flow in order to evaluate sperm behaviour during rheotaxis (Eamer et al., 2015; El-sherry et al., 2014; Romero-Aguirregomezcorta et al., 2018). This relatively new tool facilitates the study of the movement of spermatozoa along the surface of microchannels in which small amounts of liquid are pumped through (Kantsler et al., 2014). Under these conditions, it has been demonstrated that fluid flow and viscosity influence sperm rheotactic movement and that positive sperm rheotaxis is a powerful guiding mechanism for spermatozoa in humans (Eamer et al., 2015; Kantsler et al., 2014), cattle (Eamer et al., 2015; El-sherry et al., 2014) and horses (Romero-Aguirregomezcorta et al., 2018).

Various macromolecules such as acrylamide, powdered plant extracts, polyvinylpyrrolidone, hyaluronic acid or methylcellulose have been used to increase the viscosity of media (González-Abreu

et al., 2017). Among these, methylcellulose has been most widely used because of its microbial growth resistance, reliable quality and consistent performance, and has been used in human in vitro studies (González-Abreu et al., 2017).

Although many studies have assessed ram sperm motility and kinematic parameters in non-viscous media, there are no studies that investigate the effect of microfluid media viscosity on motility and rheotactic response of ram spermatozoa. Therefore, the aim of the present study was to assess the effects of different viscosity media on ram sperm motility and kinematics parameters, including rheotactic behaviour.

2 | MATERIALS AND METHODS

2.1 | Animal ethics and reagents

All animal experimentation was conducted in line with the University of Limerick Animal Research Committee guidelines. All of the reagents were purchased from Sigma-Aldrich Ireland Ltd (Arklow, Ireland) unless otherwise indicated.

2.2 | Semen collection and cryopreservation

Semen was collected via artificial vagina from three Suffolk rams at a commercial semen collection centre. Subsequently, sperm was diluted to 800×10^6 sperm/mL in Biladyl® with 5% egg yolk and frozen in 0.25 mL straws following the protocol described by Papadopoulos et al. (2005).

2.3 | Thawing and evaluation of post-thawed sperm quality

Semen thawing was accomplished by placing the straws in a 37°C water bath for 30 s. Samples from individual rams were pooled and centrifuged in 4 mL TALP at 400 ***g*** for 8 min. The pellet was resuspended in TALP supplemented with 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% and 0.7% w/v of methylcellulose (4000 cP; Sigma) at 37°C to increase the viscosity of the media to 1, 1.8, 3.2, 5.2, 8.8, 14.1 and 21.0 cP., respectively.

2.4 | Sperm motility assays using computer-assisted sperm analysis

Sperm kinematics parameters were evaluated in samples with a final concentration of 10×10^6 sperm per mL. A pre-warmed (37°C) Leja chamber (20 μm depth; IMV Technologies, L'Aigle, France) was loaded with 4 μL of sample. A minimum of five fields of view were captured and analysed using the Sperm Class Analyser® (SCA 2002) software (Microptic S.L., Barcelona, Spain). A mean of approximately 70 spermatozoa were anlaysed per field of view. All samples were examined using a phase contrast microscope at 100× (BX60; Olympus, Centre Valley, USA) fitted with a heated stage. Recorded parameters were total motility (TM, %), progressive motility (PR, %), curvilinear velocity (VCL, μm/s), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s), lateral head displacement (ALH, μm) and sperm mucus penetration (SMP, %). Sperm mucus penetration (assessed by SCA) included spermatozoa exhibiting VSL >25 μm/s, STR >90% and ALH >2.5 μm (Mortimer, 1994).

2.5 | Sperm rheotactic response

To assess sperm rheotactic response, spermatozoa were loaded into the starting well (50 μL at a concentration of 10×10^6 spermatozoa per mL) of a specialized microfluidic device manufactured from polymethyl methacrylate (PMMA) at the University of Limerick (microchannel size of 300 μm wide, 100 μm deep and 30 mm in length). The microchannel was connected to a syringe pump and a flow rate of 30 μm/s used. The number of sperm that swam passed the 10 mm mark in the microchannel between 13 and 15 min was assessed using an inverted microscope at 200× magnification (CK40; Olympus, Center Valley, USA) fitted with a heated stage at 37°C. A total of five replicates were performed.

2.6 | Statistical analysis

Statistical analysis was performed using SPSS v. 23.0 software (IMB corp., Chicago, USA). Data were checked for normality of distribution. The effect of media viscosity on motility parameters and rheotactic behaviour of ram spermatozoa was evaluated using a univariate general linear model (GLM). Values are expressed as mean ± SEM, and comparisons of means were performed using the Bonferroni test. A p-value of <.05 was considered to be statistically significant.

3 | RESULTS

Regarding motility parameters, there was a decrease ($p<.05$) in total motility (TM) as media viscosity increased, coinciding with the highest concentrations of methylcellulose. Thus, lower concentrations of methylcellulose (0.1%, 0.2% and 0.3%) or the absence of this substance in the TALP media resulted in a higher percentage of TM compared with 0.4%, 0.5%, 0.6% and 0.7% of methylcellulose. Moreover, highly viscous media resulted in lower ($p<.05$) PR with the lowest values of PR for treatments above 0.4% of methylcellulose compared to 0.1, 0.2% and TALP (Table 1).

Regarding sperm velocity, VCL, VSL and VAP were affected ($p<.05$) by methylcellulose-induced viscosity. Sperm velocity decreased inversely with methylcellulose concentration with the lowest values ($p<.05$) at concentrations 0.5%, 0.6% and 0.7% compared

TABLE 1 Effect of media viscosity on post-thaw ram sperm motility and kinematic parameters.

Motility parameters							
% methycellulose	TM	PR	VCL	VSL	VAP	ALH	SMP
TALP	45.93 ± 5.03^{a}	28.45 ± 3.66^{a}	69.38 ± 2.64^{a}	54.58 ± 2.54^{a}	60.42 ± 2.52^{a}	2.26 ± 0.09^{a}	49.18 ± 4.02^{a}
0.1%	34.21 ± 5.03^{ab}	17.82 ± 3.66^{ab}	54.15 ± 2.64^{b}	42.91 ± 2.54^{ab}	46.97 ± 2.52^{b}	1.88 ± 0.09^{abc}	36.86 ± 4.02^{ab}
0.2%	45.43 ± 5.03^{a}	22.87 ± 3.66^{ac}	52.93 ± 2.64^{b}	39.29 ± 2.54^{b}	43.67 ± 2.52^{b}	2.09 ± 0.09^{bc}	41.81 ± 4.02^{ad}
0.3%	34.63 ± 5.03^{ab}	14.06 ± 3.66^{ab}	42.13 ± 2.64^{bc}	31.89 ± 2.54^{bc}	35.96 ± 2.52^{bc}	1.70 ± 0.09^{bd}	33.31 ± 4.02^{abc}
0.4%	21.57 ± 5.03^{abc}	6.29 ± 3.66^{bc}	37.25 ± 2.64^{c}	31.07 ± 2.54^{bc}	31.90 ± 2.52^{cd}	1.57 ± 0.09^{cd}	26.97 ± 4.02^{bcd}
0.5%	15.80 ± 5.03^{bc}	2.83 ± 3.66^{b}	32.66 ± 2.64^{cd}	23.03 ± 2.54^{cd}	26.72 ± 2.52^{cd}	1.62 ± 0.09^{cd}	20.08 ± 4.02^{bc}
0.6%	8.57 ± 5.03^{c}	0.79 ± 3.66^{b}	25.09 ± 2.64^{d}	18.02 ± 2.54^{d}	20.98 ± 2.52^{d}	1.37 ± 0.09^{d}	15.47 ± 4.02^{c}
0.7%	8.12 ± 5.03^{c}	0.67 ± 3.66^{b}	23.73 ± 2.64^{d}	15.42 ± 2.54^{d}	18.88 ± 2.52^{d}	1.44 ± 0.09^{cd}	15.31 ± 4.02^{c}

Note: Data are expressed as mean ± SEM. Different letters show differences between media viscosity for each parameter within each column ($p < .05$).

Abbreviations: ALH (μm), lateral head displacement; PR (%), progressive motility; SMP (%), sperm mucus penetration; TM (%), total motility; VAP (μm/s), average path velocity; VCL (μm/s), curvilinear velocity; VSL (μm/s), straight line velocity.

to 0.1, 0.2% and TALP (Table 1). Similarly, higher concentrations of methylcellulose provided lower values ($p < .05$) of ALH and SMP. Moreover, there were significant differences ($p < .05$) in the number of sperm that progressed passed a distance of 10 mm, with lower numbers at 0.6% and 0.7% compared to 0.1% and 0.2% methylcellulose (Figure 1).

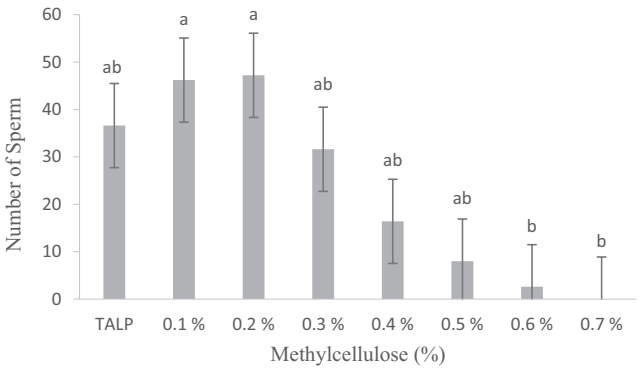

FIGURE 1 Number of spermatozoa that progressed a distance of 10 mm in the microfluidic channel over a 2-min period. Letters (a-b) illustrate significant differences ($p < .05$). Values are expressed as mean ± SEM.

4 | DISCUSSION

When semen is deposited in the female reproductive tract, spermatozoa start to migrate into mucus by positive rheotaxis. Although this phenomenon has been reported in several species such as mouse (Miki & Clapham, 2013), ram and stallion (Romero-Aguirregomezcorta et al., 2018), bull (El-sherry et al., 2014) and human (Eamer et al., 2015), there are a lack of studies that mimic the female reproductive tract environment by through the use of viscous media solutions in a microfluidic device. In the present study, the viscous secretions of the female tract were mimicked by adding increasing percentages of methylcellulose as a thickener. Sperm motility and kinematic parameters were evaluated to assess the effects of increasing media viscosity on ram spermatozoa Table 1.

As expected, our results showed that an increase in media viscosity gradually decreased sperm motility. This is in agreement with the study published by González-Abreu et al. (2017) which also found lower motility values when boar sperm were incubated with higher viscosity media. Furthermore, our results revealed that increasing media viscosity reduced sperm motility kinematic parameters such as VCL, VSL and VAP. This is in agreement with previous in vitro studies in which negative correlations between media viscosity and sperm kinematic parameters were described for bovine, mouse and human sperm (Eamer et al., 2015; Hyun et al., 2012; Pérez-Cerezales et al., 2016). In addition, we found that ALH was reduced when viscosity was increased. This decrease in ALH has been attributed to higher drag forces acting on the flagellum caused by increased viscosity (Eamer et al., 2015; Pérez-Cerezales et al., 2016).

This study reported for the first time the effect of different media viscosity on ram sperm rheotaxis. The results showed that ram sperm exhibited higher positive rheotaxis in media with lower viscosity. This is in agreement with other studies on human and stallion sperm, where an increase in viscosity resulted in a reduction of sperm progression (Kantsler et al., 2014; Romero-Aguirregomezcorta et al., 2018).

Interestingly, while sperm motility and kinematic parameters were gradually decreased with increasing media viscosity, the sperm migration parameter was only affected by media with the highest viscosity (0.6% and 0.7% of methylcellulose). These results suggest that other techniques to assess sperm migration could offer more insight into the effect of media viscosity on sperm behaviour. For example, sperm penetration tests using capillary tubes filled with cervical mucus or synthetic mucus. This technique facilitates the assessment of sperm progression through mucus including (i) the vanguard distance (distance of the foremost sperm in the capillary)

and (ii) the sperm count at various intervals along the tube, whereby, either the total number of sperm up to a point or the number of sperm per field of view alone (Aitken et al., 1992) or across the tube is quantified (Al Naib et al., 2011).

In summary, this study supports the concept that there is an association between fluid viscosity and positive rheotaxis as well as motility parameters of ram spermatozoa. Further in vitro sperm migration assays are needed to improve our understanding of how sperm behave in response to different physiological conditions in the female reproductive tract.

AUTHOR CONTRIBUTIONS

Alicia Martin Maestro: Experimental design, completion of experimental work, drafting the manuscript, data collection and critical revisions. Laura Abril Parreño: critical revisions. Ana Josefa Soler: Analysis data and critical revisions. Sean Fair: Experimental design and critical revisions.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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