Institution: University of Birmingham

Unit of Assessment: UOA 10 – Mathematical Sciences

Title of case study: Bringing awareness of viscous fluid mechanics to clinical reproductive medicine

1. Summary of the impact (indicative maximum 100 words)

Deficiencies in sperm motility, the ability of cells to migrate actively through the female reproductive tract, are implicated in around half of all cases of subfertility. The biochemical regulation of motility is a subject of considerable interest in clinical science due to its potential for improvements in diagnosis and treatment of subfertility, however the accompanying physical aspects of motility have hitherto received less attention. In 2005, mathematicians Dr David Smith and Prof. John Blake began working closely with Dr Jackson Kirkman-Brown, Science Lead for Birmingham Women's Fertility Centre, as core investigators in the Centre for Human Reproductive Science (ChRS), a clinical research and development network centred at Birmingham Women's Hospital (BWH). The resulting mathematical models of sperm motility have been impacting clinical science in three ways. (1) Changing the awareness of practising and trainee clinical scientists, both nationally and internationally. (2) Assisting the capture of infrastructure funding for translational research by opening new scientific avenues. (3) Increasing public awareness through outreach, TV and the press, in turn encouraging changes to lifestyle. (4) Commercial impact through the marketing of a novel image-splitting device developed as part of this programme.

2. Underpinning research (indicative maximum 500 words)

We only partially understand the fundamental process in human reproduction by which a single sperm, from an initial population of 10^6-10^8, migrates through the high viscosity fluids lining the closely-opposed and convoluted internal architecture of the female reproductive tract and eventually fertilises an egg. Sperm are propelled through biological fluids by beating a single flagellum, a physical process; however the vast majority of clinical research effort focuses on molecular biology and biochemistry, physical aspects being relatively neglected by comparison.

Around one in six couples in the UK fail to conceive after 12 months and Assisted Reproduction Therapy has become routine. The number of IVF cycles performed annually is increasing steadily, reaching nearly 60000/year in 2010, every cycle incurring both financial cost and a physical and emotional toll; success rates however remain below 50%. Because physical aspects are relatively neglected, clinical diagnostics, research and development are usually restricted to visual assessments of sperm count, morphology and motility in laboratory media. These fluids used in laboratory assays are very different from secretions such as cervical mucus, a limitation also present in most clinical research on the biochemical regulation of motility.

The research is a multidisciplinary effort to elucidate the physical principles underlying sperm motility, through integrating mathematical models with the physiological features identified to be potentially important by clinical scientists. These include the shape of the DNA-carrying 'head' (morphology) and physiological fluid properties such as viscosity. A distinctive feature is the two-way exchange of knowledge, both the integration of clinical data and questions into mathematical models, and the communication of the results of models, which are often counterintuitive, to clinical scientists. The collaboration between Mathematics and ChRS was initiated by Professor John Blake (School of Mathematics) in 2004; the work was subsequently conducted by Dr David Smith (as MRC Fellow from 2006-2009, then Lecturer).

2.1 Mathematical modelling of the fluid dynamics of sperm motility

Sperm motility is a very low Reynolds number fluid dynamic process, involving static boundary conditions associated with bounding surfaces, and dynamic boundary conditions associated with the moving flagellum. The underpinning research involved the investigation of the disturbance flow field and energetic requirements, taking into account aspects identified by clinical scientists including the effect of the non-spherical head 'morphology' (shape), and the confined internal geometry of the female tract. The model [R5] allows efficient and accurate computation of the viscous-dominated flow and long timescale cell trajectories with different head shapes and

waveforms near surfaces, opening a further area for bioengineering [R1].

2.2 Nonlinear instability of flagellar movement at high viscosity

The next stage involved examining how the elastic structure of the internally-actuated flagellum couples with the surrounding viscous fluid, to alter the waveform and hence trajectory [R4]. The geometrically nonlinear model showed how viscosity alters the flagellar wavelength and cell trajectory through viscous-elastic interaction. The inclusion of nonlinearity revealed a symmetry-breaking bifurcation at high viscosity, demonstrating that experimentally-observed circling motion of cells is not necessarily due to biochemical signalling. Moreover the model showed how defective cell head shapes can radically alter cell trajectory through nonlinear interaction, an effect that would be impossible to predict from heuristic reasoning or intuition. This study was conducted jointly between the Universities of Birmingham and Oxford; a principal part of the work was conducted while the lead author (H. Gadêlha) conducted a research visit in Birmingham, jointly supervised by Smith and Kirkman-Brown.

2.3 Analysis of local mechanical activity in the flagellum in physiological viscosity fluid

The energetics of motility are of great interest, for example as a cause of subfertility, and as a potential contraceptive target. Existing dogma based on low viscosity observations held that energy need not be transported significantly along the flagellum. Refs. [R6, R3] describe the capture from imaging data of the movement of the flagellum in physiological viscosity fluid, and spatially-localised estimates of required activity (Smith's contributions to this work included these calculations). The above findings have been reported and commented on in clinical science publications, conferences and training courses, results being conveyed through images, videos and metaphors appropriate to the audience.

3. References to the research (indicative maximum of six references)

* Denotes sponsor.

[R1] Denissenko, Kantsler, Smith & Kirkman-Brown* (2012) Human sperm cells swimming in microchannels. Proc. Natl. Acad. Sci. USA 109, 8007-8010. doi:10.1073/pnas.1202934109

[R2] J. Kirkman-Brown* and D. J. Smith (2011) Sperm motility: is viscosity fundamental to progress? Journal publication: Mol. Hum. Reprod. 17, 539-544. doi: 10.1093/molehr/gar043

[R3] E. A. Gaffney, H. Gadêlha, D. J. Smith, J. R. Blake and J. Kirkman-Brown* (2011) Mammalian sperm motility: observation and theory. Annu. Rev. Fluid Mech. 43, 501–528. doi: 10.1146/annurev-fluid-121108-145442

[R4] H. Gadêlha, E. A. Gaffney, D. J. Smith and J. C. Kirkman-Brown* (2010) Non-linear instability in flagellar dynamics: A novel modulation mechanism in sperm migration? 2010. J. R. Soc. Interface, 7, 1689–1697. doi: 10.1098/rsif.2010.0136

[R5] D. J. Smith, E. A. Gaffney, J. R. Blake and J. C. Kirkman-Brown* (2009) Human sperm accumulation near surfaces: a simulation study. J. Fluid Mech. 621, 289-320. doi:10.1017/S0022112008004953

[R6] D. J. Smith, E. A. Gaffney, H. Gadêlha, N. Kapur, J. Kirkman-Brown* (2009) Bend propagation in the flagella of migrating human sperm, and its modulation by viscosity. Cell Motil. Cytoskel. 66, 220-236. doi:10.1002/cm.20345

References 3, 4 and 5 best indicate the quality of the underpinning research

4. Details of the impact (indicative maximum 750 words)

The underpinning research elucidated physical mechanisms by which viscosity radically alters the flagellar waveform, internal energy transport requirements, and resulting cell trajectory. This means that experimental work in the highly active field of sperm chemotaxis (chemical guidance to the egg) and also metabolic studies can only be interpreted accurately if physiological viscosity fluids are used. The research also provided mathematically-based computer codes with which flow fields and cell trajectories resulting from observed cell shapes, waveforms and surface interactions can be computed and visualised, the results of which have challenged intuitions regarding the fluid mechanics of motility based on inaccurate analogies in macroscale flow. Taken together, these findings have had a distinct and material impact on practices in clinical science. This impact has occurred in a short time-frame, following the first publications of the research in 2009 (relative to typical lab-to-bedside timescales of the order of 10-20 years). In more detail:

4.1 Clinical perspectives and training

Despite the relatively short time-frame since 2009, the work is impacting the medical science perspectives of world-leading laboratories by changing the focus of their work. Prof. David Clapham, Howard Hughes Medical Institute and Harvard University, a pre-eminent figure in biomedicine, states: '*Spermatozoa are short-lived mechanically driven cells and their biology is dominated by low Reynolds number fluid mechanics. Biologists without the training of Dr. Smith naturally have poor insight and understanding of mechanical forces on the microscopic scale... Without such collaborations across fields of expertise, the field will stall, and worse, go in the wrong direction due to misinterpretation of data... [Kirkman-Brown and Smith] have made remarkable inroads in areas of sperm research. I refer to your work often to understand the problems we are approaching in sperm biology. In particular, [Smith] helped us a great deal in understanding microscopic mechanics for our 2013 paper in Current Biology (Miki K and Clapham DE. 2013. Rheotaxis Guides Mammalian Sperm)...*' [S8] The latter study exemplifies fluid dynamics of cell motility, a new addition to a group specialising in cell electrophysiology.

The influence on clinicians' perspectives is also evidenced from a paper by the clinical group led by Prof. Chris Barratt (Ninewells Hospital and Medical School, Dundee) which give the following corroboration *'...experiments modelling and examining sperm behaviour in these physiologically relevant environments are essential for obtaining an accurate analysis, as recently demonstrated by Smith and colleagues'* – Barratt, Kay & Oxenham, J. Biol., 8:63, 2009.

The clinical science journal *Molecular Human Reproduction*, a publication which generally focuses on biochemical and molecular processes, summarised the concepts described above, with a mathematical modelling image from Smith and Kirkman-Brown being chosen to illustrate the journal front cover in a special issue on sperm motility published in August 2011.

Members of the ChRS team have disseminated these findings at meetings for clinical scientists internationally, including the following invited oral presentations, growing from more specialist meetings in 2009 to major international conferences in 2011 and 2013.

- 2009: International Symposium of Advanced Research Progress in Reproductive Medicine, Changzhou Women and Children's Health Hospital, China [S1], Maternal Communication with Gametes and Embryo COST Meeting, Alghero, Sardinia, Italy (convened by Dr Alireza Fazeli, a leading figure in British Andrology) [S10].
- 2011: Gordon Research Conference on Fertilization and Activation of Development, NH, USA (an internationally-leading meeting for specialists in the field) [S2], 1st International CAESAR Conference on Sperm Signaling and Motility, Bonn, Germany (including leaders such as Profs. U.B. Kaupp, D. Clapham and M. Eisenbach).
- 2013: Society for Reproduction and Fertility Annual Conference, Cambridge, UK, Society for the Study of Reproduction Annual Meeting, Montreal, Canada.

The work has further impacted the training of clinical and research scientists through Dr Kirkman-Brown's teaching, for example at *Frontiers in Reproduction*, the internationally-renowned six week residential course at the Marine Biology Laboratory in Woods Hole, Massachusetts. Each year this course trains 20 future leaders in reproduction; without the underpinning research, this strand of thinking would not have been part of the curriculum.

Kirkman-Brown continues to bring the awareness of these ideas to his work as British Andrology Society Secretary, and UK host for the Basic Semen Analysis course affiliated to the European Society of Human Reproduction and Embryology.

Finally, studies at BWH are underway applying the model to test motility drugs. A manuscript on the energetic effects of the drug 4-aminopyridine is in preparation. The mathematical approach developed allows calculation of energy expenditure and transport in the flagellum, quantifying how the metabolism of the cell is modified under drug stimulation.

4.2 Grant funding for the ChRS clinical research laboratory at Birmingham Women's Hospital

The research has brought two major grants to the ChRS labs at Birmingham Women's Hospital (MRC £379K and STFC £176K), allowing the laboratory to be equipped with imaging equipment for sperm motility research, including a high speed camera and an optical splitting device

(designed by Dr Kirkman-Brown in collaboration with SME CAIRN Research Ltd; for the work see 4.4).

The underpinning research motivated a sub-project as part of a much larger successful bid 'Experimental Medicine Network of Excellence' to Advantage West Midlands (the former regional development agency). The Reproductive Medicine project brings approximately £278K of infrastructure to ChRS. The new equipment ('BAMBI') allows high-throughput screening of samples, tracking and imaging of live cells, and precise spatial and temporal control of photo-releasable hormones and pharmacological agents [S9].

Combined with the fluid dynamic knowledge of the effect of viscosity on motility and chemical dispersion, these capabilities mean that Birmingham Women's Hospital now has a system unrivalled in the UK through which motility dysfunction and new therapies can be investigated. BAMBI is currently being used to trial a new potential motility drug 'Omega'.

4.3 Public awareness and behaviour

Findings have fed into ChRS TV work, including an episode of the 2008 BBC TV Series *Don't Die Young* on the Male Reproductive Organs [S4], designed to raise awareness of the challenge faced by sperm, and consequent need for men to be aware of the effect of poor lifestyle and prevalence of subfertility. These ideas also contributed to an internationally-screened 2009 TV programme *The Great Sperm Race* (Channel 4 and Discovery Channel) [S3]. Finally, work from refs. R4, R5 have received press attention internationally [for example, S5]. Dr Smith has drawn on this research in public engagement activities he has led including a 'Meet the Scientist' presentation at Birmingham *thinktank* (February 2010) and talks for local school children interested in STEM subjects, including the 2010 British Science Festival.

4.4 Commercial impact

The imaging device, referred to in 4.2, and designed by Dr Kirkman-Brown in conjunction with CAIRN Research Ltd, is now marketed by the company as the Optosplit III Image Splitter, a 3-way image splitter as a device for dividing an image into either one, two or three separate, spatially equivalent components which can be displayed side by side on a single camera chip. This device has generated around £50K of sales for CAIRN, in addition to prompting further independent developments by the company, including a 4-way image splitter. [S7]

5. Sources to corroborate the impact (indicative maximum of 10 references)

[S1] International Symposium of Advanced Research Progress in Reproductive Medicine, Changzhou Women and Children's Health Hospital, 18th-21st Sept. 2009, Pp. 61-66.
[S2] Gordon Research Conference on Fertilization and Activation of Development 2011. http://www.grc.org/programs.aspx?year=2011&program=fert
[S3] http://www.channel4.com/programmes/the-great-sperm-race
[S4] http://www.bbc.co.uk/programmes/b00cv2vd
[S5] http://www.abc.net.au/science/articles/2010/07/30/2969046.htm
[S6] Birmingham Programme - British Science Festival 2010 (p. 3)
[S7] Corroborating letter, Managing Director, Cairn Research Ltd, 21/11/2013
[S8] Corroborating letter, Howard Hughes Medical Institute dated 16/5/2013.
[S9] Birmingham Advanced Microscopy for Biomedical Imaging, Specification and Requirements document.
[S10] Maternal Communication with Gametes and Embryo (Proceedings of the 2nd Meeting of GEMINI) 1st-3rd Oct. 2009, p.30. ISBN 978-0-9563694-0-6, GEMINI COST ACTION FA0702.