LE STUDIUM
Loire Valley
Institute for Advanced Studies

LE STUDIUM Multidisciplinary Journal
2669-7807

www.lestudium-ias.com

FELLOWSHIP FINAL REPORT

Visualising Fertility: Imaging Reproductive Function to Improve Animal Breeding and deliver Immersive Education

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REPORT INFO

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Period of residence in region Centre-Val de Loire: Jun 2025 – Jan 2026

Keywords:
Reproduction; Sperm transport; Mass motility; Sheep; Horse; Fertility; Reproductive imaging; Ultrasound; MRI; Artificial insemination

ABSTRACT

A series of experiments were undertaken using advanced imaging technologies and quantitative analysis to investigate sperm transport, semen function and fetal development in sheep and horses, based on the principle that reproductive function can be better understood, taught and improved when the underlying biological processes are made visible.
Probe-based confocal laser endomicroscopy was used to compare sperm transport in the ewe and mare by tracking fluorescently labelled spermatozoa and inert fluorescent microbeads within the female reproductive tract. This demonstrated clear species-specific differences: in the ewe, sperm motility was essential for progression through a highly contractile and selective uterus, whereas in the mare, uterine contractions were the predominant driver of sperm transport. In ram semen, mass motility was investigated as an emergent property of dense sperm populations. Experimental manipulation of sperm concentration and percentage motility showed that high mass motility requires both sufficient sperm density and a high proportion of motile spermatozoa. A complementary image-analysis method was developed to objectively quantify mass motility from routine phase-contrast videos, providing a practical alternative to subjective operator scoring. Longitudinal ultrasound and MRI of pregnant ewes were also used to initiate a multimodal atlas of fetal development across gestation, providing the foundation for improved pregnancy diagnostics and future immersive training tools in reproductive education.
Together, these studies improved biological understanding of fertility and provided practical applications for livestock breeding, semen evaluation and veterinary training. The work demonstrates how imaging can move reproductive biology beyond indirect or subjective assessment toward more precise, mechanistic and educationally valuable interpretation.

Simon. P. de Graaf, E. Barakat, M. Van de Hoek, M. Golledge, E. Caldas, F. Reigner, P. Barrière, T. Blard, H. Adriaensen, S. Barbey, O. Lasserre, J. Cognié, G. Tsikis, T. Prudhomme, X.Druart. Visualising Fertility: Imaging Reproductive Function to Improve Animal Breeding and deliver Immersive Education, LE STUDIUM *Multidisciplinary Journal*, **2026**, 10, 5-11
https://doi.org/10.34846/le-studium.331.02.fr.01-2026

1- Introduction

Efficient reproductive management in livestock depends on accurate assessment of both male and female reproductive function. Fertility outcomes are determined by sperm quality, sperm transport through the female reproductive tract, successful fertilisation, and accurate diagnosis and management of pregnancy [1]. Many of these processes are dynamic, three-dimensional and difficult to observe directly, meaning they are often interpreted through indirect measurements, subjective assessments or final reproductive outcomes such as pregnancy rate.

A major limitation in reproductive biology is that important biological events, including sperm transport, collective sperm motion and fetal development, are largely hidden from direct observation. As a result, management decisions in artificial breeding programs often rely on practical but imperfect tools such as subjective semen scoring or operator-dependent ultrasound interpretation. Improving the visualisation of these processes provides an opportunity to better understand the biology underlying fertility and to improve both breeding outcomes and reproductive education.

A series of experiments were therefore undertaken using imaging technologies and quantitative analysis to investigate sperm transport, semen function and fetal development in sheep and/or horses. The central principle was that reproductive function can be better understood, taught and improved when the underlying biological processes are made visible.

Three linked research areas were examined. First, sperm transport within the female reproductive tract was investigated using probe-based confocal laser endomicroscopy to determine the relative roles of sperm motility and uterine contractility in the ewe and mare. Second, mass motility in ram semen was studied as a collective property of dense sperm populations to determine how sperm concentration and percentage motility contribute to wave motion and whether this process could be measured objectively using image analysis. Third, longitudinal ultrasound and magnetic resonance imaging were used to document fetal development in pregnant ewes and initiate a multimodal atlas for pregnancy diagnosis and immersive teaching applications.

These studies address both fundamental and applied questions in animal reproduction. Improved understanding of sperm transport informs species-specific approaches to insemination and sperm evaluation [2]. Better interpretation of ram semen mass motility improves semen selection in artificial breeding programs [3]. Multimodal imaging of fetal development supports more accurate pregnancy diagnosis and provides new resources for veterinary and animal science education [4].

1- Experimental details

In vivo imaging of sperm transport in ewes and mares

Sperm transport within the female reproductive tract was examined using probe-based confocal laser endomicroscopy (pCLE; [5]), which allows real-time microscopic imaging within living tissues. Fluorescently labelled spermatozoa were inseminated together with inert fluorescent microbeads, allowing active sperm movement to be distinguished from passive particle displacement.

The study compared two species with contrasting reproductive physiology. In the ewe, sperm must progress through a highly selective reproductive tract, with major barriers at the cervix and uterotubal junction [6]. In the mare, semen is deposited directly into the uterus and sperm transport is strongly influenced by uterine contractions and post-breeding uterine clearance [7]. The experiments aimed to determine the relative contribution of sperm motility and uterine contractility in each species.

Semen from Lacaune rams was collected using an artificial vagina, pooled, and labelled with R18 and MitoTracker Green FM before laparoscopic intrauterine insemination into synchronised Ile de France ewes. In mares, semen from Welsh Pony stallions was similarly labelled following optimisation of the staining protocol and deposited into the uterine body following ovulation induction. In both species, fluorescent microbeads were added immediately prior to insemination as inert reference particles.

To assess the role of uterine contractility, imaging was performed during both oestrus and the luteal phase. Oestrus represents a period of high uterine contractility under oestrogen dominance, while the luteal phase is characterised by reduced contractility under progesterone influence. Sperm and bead distribution were quantified across different regions of the reproductive tract using pCLE.

Quantitative analysis of ram semen mass motility

Mass motility, or wave motion, is a collective swirling movement observed when undiluted ram semen is examined under low magnification [3]. It remains one of the most widely used indicators of ejaculate quality in sheep artificial breeding programs, despite being assessed subjectively and without clear understanding of the factors governing its expression.

Two complementary studies were undertaken. The first examined the biological basis of mass motility by manipulating sperm concentration and percentage motility under controlled conditions. Ejaculates from Lacaune rams were diluted to defined sperm concentrations ranging from 500 to 2500 × 10^6 sperm/mL. Separate experiments altered the proportion of motile sperm by mixing live semen with snap-frozen immotile spermatozoa to create defined live:dead ratios while maintaining total sperm concentration.

Mass motility was assessed using standard subjective scoring on a 0–5 scale under phase-contrast microscopy. Parallel samples were analysed using computer-assisted sperm analysis (CASA) to measure percentage motility and velocity parameters. This allowed direct comparison between collective wave motion and individual sperm movement.

The second study developed an objective method for quantifying mass motility using routine phase-contrast video recordings. Videos of undiluted semen were recorded and independently scored by experienced technicians. Objective analysis was performed using frame-to-frame correlation analysis in Fiji, where similarity between sequential video frames was measured using Spearman's rank correlation coefficient. Lower frame-to-frame similarity reflected stronger collective motion. Temporal lag and the number of frame comparisons were systematically optimised to produce a stable and biologically meaningful objective metric.

Multimodal imaging of fetal development in sheep

Longitudinal fetal development was documented using ultrasound and magnetic resonance imaging to create a multimodal visual atlas of pregnancy in sheep. The aim was to improve interpretation of fetal development across gestation and provide a platform for future immersive educational tools in pregnancy diagnosis.

Synchronised Ile de France ewes (n=10) were artificially inseminated and monitored throughout pregnancy. Ultrasound examinations were performed at regular intervals from early gestation onward using both commercial sheep scanning equipment and higher-resolution veterinary imaging systems. Imaging included standard B-mode ultrasound and, where appropriate, functional modalities such as pulse wave and power Doppler.

Magnetic resonance imaging was performed using a 3 Tesla Siemens Magnetom Skyra

system at key gestational stages, including approximately Days 30, 50, 90 and 120. MRI provided high-resolution anatomical detail that could be directly linked to ultrasound appearances observed under field conditions. This created a more complete understanding of fetal growth, placental development and diagnostic interpretation across pregnancy. The resulting dataset forms the basis of a fetal development atlas and provides image resources for future virtual reality and mixed-reality teaching tools in reproductive biology and veterinary training.

2- Results and discussion

Species-specific mechanisms of sperm transport

Probe-based confocal laser endomicroscopy demonstrated clear species-specific differences in sperm transport between the ewe and mare.

In mares, spermatozoa and inert fluorescent beads were detected throughout the reproductive tract following uterine deposition, indicating that uterine contractions were sufficient to transport passive particles as well as spermatozoa. Sperm density showed a descending gradient from the uterine body toward the upper uterine horn, with marked selection occurring between the middle and upper horn. The recovery of beads throughout the tract, including the upper horn, showed that moderate uterine contractions play a dominant role in sperm transport in the mare.

In ewes, the opposite pattern was observed. Spermatozoa showed an ascending distribution gradient toward the upper uterine horn, whereas beads were largely displaced back toward the vagina and failed to progress effectively into the horn. This demonstrated that passive particles were unable to move successfully against the strong contractile forces of the ewe uterus, while motile spermatozoa were able to progress toward the uterotubal junction.

When uterine contractility was reduced during the luteal phase, bead distribution became more homogeneous in both species, confirming that oestrous uterine contractions strongly influence sperm transport patterns. In the ewe, the highly contractile uterus acts as an active selective barrier that requires sperm motility for progression. In the mare, uterine contractions themselves are the predominant transport mechanism, with sperm motility likely becoming more important only in final selection near the oviduct.

These findings demonstrate that sperm transport cannot be interpreted as a universal mechanism across species. Species-specific anatomy and uterine physiology determine whether sperm motility or female tract contractility is the dominant factor influencing fertilisation success. This has direct implications for artificial insemination strategies and interpretation of sperm function testing.

Biological interpretation of ram semen mass motility

Mass motility declined rapidly following semen collection, even while CASA-derived percentage motility and velocity remained relatively stable. This showed that the disappearance of visible wave motion preceded detectable declines in individual sperm motility, indicating that mass motility reflects an ejaculate-level property rather than simply individual sperm speed.

Increasing sperm concentration significantly increased mass motility scores. Mean scores increased from approximately 2.0 at 500×10^6 sperm/mL to more than 4.0 at 2500×10^6 sperm/mL. Scores of 4 or greater were consistently achieved only at concentrations of at least 2000×10^6 sperm/mL. Importantly, CASA measures of individual sperm motility did not differ across these treatments, confirming that the observed changes were driven by sperm density rather than altered sperm function. Reducing the proportion of motile sperm also significantly reduced mass motility, even when total sperm concentration was maintained. High mass motility scores were associated with percentage motility greater than

approximately 75%, whereas scores below 3 were typically observed when percentage motility was 60% or lower.

These results show that mass motility emerges from collective interactions between large numbers of actively swimming spermatozoa. Sufficient sperm density is required to generate coordinated movement, but percentage motility is the major determinant of variation in wave motion intensity.

Mass motility therefore provides information that is distinct from CASA, reflecting the functional behaviour of the ejaculate as a whole rather than the movement of isolated sperm cells. This explains why mass motility remains a valuable practical tool in ram breeding programs despite its simplicity and subjectivity. It captures biologically relevant collective behaviour that is closely associated with fertility outcomes.

Objective quantification of mass motility

Subjective scoring of mass motility showed substantial variation between experienced technicians. Complete agreement between all six scorers occurred for only 36% of videos, highlighting the limitations of operator-based assessment, particularly when decisions depend on practical threshold values for ejaculate acceptance.

Frame-to-frame correlation analysis provided an objective method for quantifying wave motion directly from routine phase-contrast videos. Increasing the temporal lag between compared frames decreased correlation values, reflecting stronger movement. A lag of 0.2 seconds and 10 frame comparisons per video provided the best balance between stability and discrimination between score categories.

Videos with strong wave motion produced lower frame-to-frame similarity, while those with weak or absent motion remained highly correlated. The objective metric clearly separated ejaculates below and above the operational threshold of mass motility score 4, which is commonly used in artificial insemination centres for semen acceptance.

This method is practical because it requires no change to normal semen handling procedures and uses standard microscopy recordings. It therefore offers a realistic pathway toward improved consistency of semen evaluation in commercial breeding programs. Rather than replacing the biological value of mass motility, objective quantification improves reliability and reduces operator bias.

Multimodal fetal imaging and reproductive education

Longitudinal ultrasound and MRI provided the foundation for a multimodal fetal atlas of sheep pregnancy. Repeated imaging across gestation captured major developmental transitions including early embryonic development, placental growth, fetal positioning and later structural development.

Ultrasound remains the primary practical tool for pregnancy diagnosis in sheep production systems, but interpretation depends heavily on operator experience and image familiarity. MRI provided high-resolution anatomical detail that is rarely available in livestock pregnancy studies and allowed ultrasound appearances to be interpreted against a more complete anatomical reference. Combining these modalities improved understanding of fetal development across pregnancy and provided a stronger basis for gestational age estimation, fetal number assessment and diagnostic confidence. The work also created a valuable image library for training purposes.

Pregnancy diagnosis is difficult to teach because students and trainees must interpret moving, low-contrast images that vary between animals and operators. Linking ultrasound and MRI datasets to immersive visualisation platforms such as virtual reality and mixed reality creates opportunities for structured training before live-animal practice. This improves both teaching efficiency and learner confidence. The fetal atlas therefore has both

scientific and educational value, extending the role of imaging beyond diagnosis to training and knowledge transfer.

3- Conclusion

A series of imaging-based experiments were undertaken to improve understanding of sperm transport, semen function and fetal development in domestic animals.

Probe-based confocal laser endomicroscopy showed that sperm transport is governed by fundamentally different mechanisms in sheep and horses. In the ewe, sperm motility is essential for progression through a highly contractile and selective uterus, whereas in the mare, uterine contractions are the predominant transport mechanism. This highlights the importance of species-specific reproductive physiology when interpreting fertility and designing insemination strategies.

Studies of ram semen mass motility demonstrated that wave motion is a collective property of the ejaculate requiring both sufficient sperm concentration and a high proportion of motile spermatozoa. Mass motility reflects biologically meaningful sperm function that is not captured by conventional CASA measurements alone. Objective image analysis further showed that mass motility can be quantified reliably using routine video recordings, improving consistency of semen assessment and supporting practical decision-making in artificial breeding programs.

Longitudinal ultrasound and MRI of pregnant ewes established the foundation for a multimodal fetal development atlas. This improves interpretation of pregnancy diagnosis and provides a platform for immersive educational tools in veterinary and animal science training.

Together, these studies show that reproductive biology can be strengthened when dynamic biological processes are made visible. Imaging moves reproductive assessment beyond indirect interpretation and subjective scoring toward more precise, mechanistic and educationally valuable understanding. The outcomes provide both fundamental biological insight and practical applications for livestock breeding, fertility management and reproductive education.

4- Perspectives of future collaborations with the host laboratory

The work undertaken during this project has strengthened an already productive collaboration between the University of Sydney and the INRAE PRC group in Nouzilly, particularly in the areas of sperm physiology, reproductive imaging and artificial breeding technologies. The complementary expertise of both groups creates strong opportunities for continued joint research.

Immediate collaboration will continue through completion and publication of the current manuscripts on sperm transport, ram semen mass motility, and objective assessment of wave motion. These projects provide a foundation for further work examining species-specific sperm transport mechanisms and the practical application of objective semen assessment tools within commercial artificial breeding systems.

The multimodal fetal imaging work also provides a clear pathway for future collaboration. Expansion of the fetal development atlas, integration of machine learning approaches for pregnancy diagnosis, and development of virtual and mixed-reality teaching tools remain important shared objectives. These areas combine INRAE's strengths in reproductive imaging with ongoing work in Australia on sheep pregnancy scanning, veterinary teaching and industry extension.

There is also strong potential for future collaborative projects focused on ram breeding soundness evaluation, sperm morphology standards and fertility prediction, particularly through integration of laboratory semen assessments with large commercial fertility

datasets from industry partners in Australia and Europe.

Beyond specific projects, continued exchange of students, visiting researchers and joint supervision of postgraduate research would be highly valuable. Maintaining this international collaboration will strengthen both scientific output and translational impact, particularly where research outcomes can be directly applied to livestock breeding programs and reproductive education.

5- Articles published in the framework of the fellowship

Barakat E, Caldas E, Reigner F, Barrière P, Blard T, Lasserre O, Cognié J, Tsikis G, de Graaf SP, Druart X (2026) Species-specific mechanisms of sperm transport: predominant roles of motility in ewes and uterine contractility in mares. Reproduction, submitted

Van de Hoek M, Barakat E, Prudhomme T, Rickard JP, Druart X, de Graaf SP (2026) High mass motility in ram semen requires sufficient sperm concentration but is primarily driven by the percentage of motile spermatozoa. Reprod. Fertil Dev, in preparation

Van de Hoek M, Prudhomme T, Barakat E, Rickard JP, de Graaf SP, Druart X (2026) Objective mass motility assessment in ram semen using frame-to-frame correlation analysis. Reprod. Fertil. Dev., in preparation.

Golledge M, Barakat E, Prudhomme T, Adriaensen H, Barbey S, Druart X, de Graaf SP (2026) Multimodal imaging of fetal development in sheep: integrating ultrasound, MRI and immersive training tools for pregnancy diagnosis. Reproduction, in preparation.

6- Acknowledgements

The authors gratefully acknowledge LE STUDIUM Loire Valley Institute for Advanced Studies for supporting the fellowship, the members of the Équipe Interactions Cellulaires et Fertilité, UMR Physiologie de la Reproduction et des Comportements, and the staff of the INRAE animal facilities and imaging platforms for their technical expertise, animal care and scientific support. We also acknowledge collaborators and students from the University of Sydney and INRAE who contributed to the semen analysis, sperm transport and fetal imaging studies described in this report.

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