

Impediments to implantation

Dr Nathalie Lédée is investigating the immune partners implicated in embryo implantation – the major factors limiting successful birth. She is working to provide personalised treatments to enhance fertility and understand the importance of immune-endometrial interplay



Could you summarise your academic background?

My current research focuses on the immune mechanisms occurring at the time of embryo implantation. I received my MD in 1993, a Master's in Experimental Surgery in 1995 and a PhD in Reproductive Immunology in 2003. After a medical position in obstetrics and gynaecology, I became an assistant professor in reproductive medicine in 2004. Until 2008, I was in charge of the work package 'bench to bedside' in the European network of excellence – EMBryo Implantation Control (EMBIC). Since 2010, I have been Principal Investigator of a research team dedicated to 'cytokines and the materno-foetal dialogue' and, in 2012, I created an innovative start-up, MatriceLAB Innove (MLI).

You launched an innovative investigation into reproductive immunology. What are the main objectives of this project?

Our hypothesis was that the local environment and its immune equilibrium, which was previously poorly investigated, may be crucially deregulated in patients with an unexplained history of repeated embryo implantation failures after *in vitro* fertilisation (IVF)/intra-cytoplasmic sperm

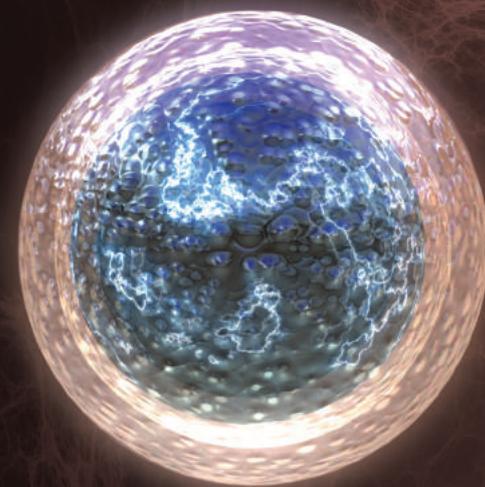
injection (ICSI). This is called recurrent implantation failure.

Based on analysis of the immune endometrial profile and its equilibrium before conception on a first cycle, our objective was to personalise the subsequent assisted reproductive technology to increase live birth rates. This immune method has been recently patented as a technique for increasing implantation success in assisted fertilisation.

Why is it important to establish an adequate dialogue between the embryo and the endometrium at the time of implantation?

Embryo implantation is still the main limiting factor of live birth success after fertility treatments, despite research progress. In Europe and the US, the majority of pregnancy losses occur before or during embryo implantation. Uterine remodelling events of the endometrium, required for a successful pregnancy, begin before implantation at each menstrual cycle, during the mild luteal phase. This is defined as the period of uterine receptivity and is a vital process for pregnancy as it prepares future maternal immune tolerance, protects the foetus and regulates the placentation process.

Within the endometrial environment, at a specific stage termed 'the implantation window', a peculiar influx of immune cells occurs, involving a quasi-complete switch of local immunity from an adaptive type to an innate one. This process provides nourishment to the embryo until a placenta is established and protects the semi-allogeneic embryo from attack by the maternal immune system. It is thought that the 'ideal' immune environment at the time of implantation should allow the development of local mechanisms promoting immunotrophism and angiogenesis, while downregulating inflammation and cytotoxic pathways, thus allowing the embryo to successfully implant.



Could you outline the potential difficulties that can arise during embryo transfer?

The most significant problems arise after the embryo transfer. Human implantation is a three-step process, starting with the apposition and adhesion of a competent embryo to the endometrial epithelium (attachment), followed by an extensive invasion in a receptive endometrium. Without conception, the endometrial surface is anti-adhesive to protect the uterus against infections. For embryo attachment to occur, local reactivity on the maternal side is required, as well as the development of a local immune tolerance, since the embryo is genetically different from his or her mother.

MLI is located in the heart of the Saint-Louis Hospital, Paris. What makes the lab conducive to world-leading research?

The Saint-Louis Hospital dedicates part of its activity to immunology, particularly exploring the role of innate immunology in health and disease. We therefore have all the resources necessary to understand the complex interactions between locally involved immune cells. Moreover, the hospital has developed a platform of automated biological diagnosis. Such a platform will be essential for the effective industrialisation of our innovative method.

Will you be attending any major congresses or seminars this year to present your findings?

Our current data have already been presented at the European and American Congresses of Reproductive Immunology. They will also be presented at Excellence in Medical Education (EXCEMED) conferences, dedicated to reproduction and implantation, in Budapest and Monaco next spring. Moreover, during 2012-13 we organised a number of meetings for French physicians to introduce immune concepts and the potential related applications for their practice.

Reproductive innovation

To combat infertility, researchers from the **Saint-Louis Hospital** in Paris, France, are studying the immunological components that are known to be involved, with a view to creating personalised treatments

RECENT YEARS HAVE seen incredible progress in fertility treatments, such that more women are now able to conceive than ever before. In particular, there have been great advances in assisted conception methods, including *in vitro* fertilisation (IVF) and intra-cytoplasmic sperm injection (ICSI). However, infertility remains a widespread problem and it can be a very difficult and emotionally trying time for those affected.

Despite ongoing progress in the field of embryo implantation, it remains the primary factor limiting live birth success following assisted conception. Indeed, every seventh couple in Europe and the US is affected by implantation disorders and the vast majority of pregnancy losses take place at this early stage.

Dr Nathalie Lédée is working to understand exactly what causes these failures in embryo implantation. She leads the MatriceLAB Innove (MLI), located in the Saint-Louis Hospital in Paris – a laboratory dedicated to research and innovation in reproductive medicine, with a particular focus on reproductive immunology. Through her work, Lédée hopes to increase fertility and live birth rates by improving understanding of the dialogue between the embryo and endometrium at the time of implantation – an area that has so far been insufficiently studied.

IMMUNE DIALOGUE

Based on a long history of research in reproductive immunology, Lédée established an innovative programme at MLI to understand how the actions of the immune system prior to conception can lead to recurrent implantation failures. “MLI objectives are to understand the immune processes at the endometrial site that may explain embryo implantation failures, in order to deliver personalised corrective actions,” she explains. “Once diagnosed, we give specific recommendations to personalise the assisted reproductive strategy, in order to counteract the deleterious mechanisms and promote effective implantation.”

Embryo implantation is the crux of assisted reproduction, and it requires an understanding of uterine receptivity and the interplay between the endometrium and the embryo. The endometrium can only accommodate

the embryo for a few days in each cycle – the implantation window – that occurs five to nine days after ovulation. During this time, important immune cells leave and enter the endometrium, and the newly created immune environment plays a key role in embryo implantation. Underactive immune cells fail to create the necessary implantation reaction; conversely, overactive immune cells can lead to the destruction of the endometrium and rejection of the embryo. This unique immune reaction is essential for promoting embryo adhesion, and its disruption is likely to obstruct implantation.

SURPRISING RESULTS

The MLI team conducted a prospective cohort study of over 300 women with a history of recurrent implantation failures. They performed an endometrial biopsy during the non-conceptual cycle in order to analyse a number of important immune factors. Firstly, they quantified the mobilisation of uterine natural killer (uNK) cells. Blood peripheral natural killer cells are a component of the immune system that help the body fight off infection, but there is some evidence to suggest that uNK cells are a unique subset – found in the womb lining during early pregnancy – that are essential for placentation but, when overactivated, are prone to attack the foetus as a foreign body. The researchers also analysed the mRNA expression of the cytokines interleukin (IL)-15, a marker of uNK cell activation and maturation, and IL-18, which is associated with the essential destabilisation of the uterine spiral arteries and of some local immunoregulators. Based on their findings, specific treatments were delivered to patients in order to counteract the deleterious mechanisms, such as reduced or excessive endometrial immune activation.

After the immune evaluation and treatment in patients, the team assessed the results according to pregnancy rate after the first embryo transfer, revealing some unexpected findings: “Surprisingly, we diagnosed local immune deregulation in 85 per cent of patients. Furthermore, 42.5 per cent of the deregulated treated patients became pregnant at the next embryo transfer, while patients with no diagnosed deregulation had a pregnancy rate of 19 per cent. This was higher than that observed in a cohort of infertile patients with good prognoses,” Lédée explains.

INTELLIGENCE

INNOVATION IN REPRODUCTION: METHOD FOR INCREASING LIVE BIRTH AND FERTILITY

OBJECTIVES

- To explore the unexplained failure of embryo implantation by investigating the immunological dialogue at the maternal-foetal interface
- To understand the mechanisms leading to the absence of embryo implantation
- To offer personalised fertility treatment to increase pregnancy rates

KEY COLLABORATORS

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NATHALIE LÉDÉE is CEO of MatriceLAB Innove at the Saint-Louis Hospital and Physician in Reproductive Medicine at Bluets Hospital – Pierre Rouques in Paris. She received her MD in Obstetrics and Gynaecology in 1993, and her PhD in Reproductive Immunology in 2003, both from the University of Paris VI. From 2004-08, she actively participated to the European network of excellence – EMBryo Implantation Control (EMBI) and, for four years, she was Principal Investigator of the Inserm research team dedicated to 'cytokine and materno-foetal dialogue'.



PROGRESSING TO CLINICAL TRIALS

Because the patients in this cohort were older than most women when they conceive – almost half were over 38 years old – and had a long history of infertility, the expected pregnancy rate at the subsequent embryo transfer, without any specific intervention was estimated to be 20 per cent. However, using a treatment specific to the patient's endometrial immune profile, the team reported a doubling of ongoing pregnancy success. This led Lédée to hypothesise that using this new innovative method in typical cases of infertility might increase expected pregnancy rates by as much as 100 per cent.

To verify this hypothesis, Lédée and her researchers are undertaking a randomised controlled trial, in September 2014, evaluating the interest of the immune endometrial profile with personalised strategies in 400 infertile women. This is only a small part of Lédée's plans for the future: "Our objective for the next five years is to demonstrate that a preventive approach, taking into account the immune endometrial profile to design personalised treatment, is able to raise live births by around 50 per cent for all infertile patients undergoing assisted conception".

Through her method of evaluating the endometrial ability of women to accommodate an embryo prior to conception, Lédée has developed an effective and affordable solution to infertility. The approach is truly innovative in two key ways: it is preventive (ie. applied before conception) and personalised (ie. based on the individual endometrial immune profile of the woman). The technology is already patented, and has the potential to help millions of couples worldwide.

FORLIFE

In October, Lédée's team will apply for EC funding for their FORLIFE project

The 'Method FOR increasing Live Birth and Fertility' (FORLIFE) project aims to make use of endometrial biomarkers – indicating the endometrial immune profile of infertile patients – a clinical reality.

The specific objectives of the project are to:

- Bring their pioneering *in vitro* diagnosis method to market
- Measure the societal and economic impact of the innovation based on data from longitudinal cohort studies
- Trial the *in vitro* diagnosis process in France and three other European countries
- Consolidate the strategy through robust research, including comparisons of circulating and uterine natural killer cells

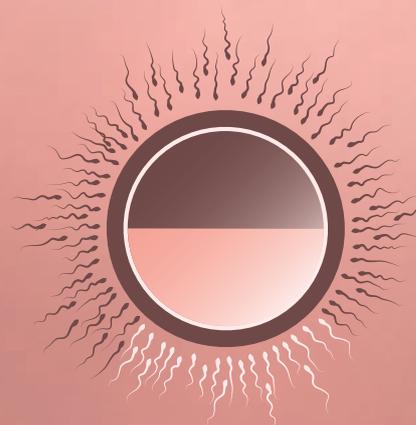
INFERTILITY AND ASSISTED REPRODUCTIVE THERAPY IN NUMBERS

EUROPE



PEOPLE ARE ESTIMATED
TO BE INFERTILE, OF THEM:

50%
SEEK MEDICAL ASSISTANCE



22%
RECEIVE FERTILITY TREATMENT

WORLDWIDE



5 MILLION
CHILDREN HAVE BEEN BORN THROUGH
IVF IN THE LAST 35 YEARS



US \$21.6 BILLION
ESTIMATED SIZE OF IVF MARKET
REVENUE BY 2020