Biochemical Society membership

Discover the benefits including:

- Access to a range of grants and bursaries to support your research and travel
- Savings of up to £375 on Open Access article publishing fees in Portland Press journals*
- Up to £100 discount on registration fees at Biochemical Society events
- Opportunities to submit proposals for Biochemical Society events
- Up to £1500 to run a seminar series in your institution
- Personal online access to *Biochemical Journal* and *Biochemical Society Transactions*
- Automatic membership of the Federation of European Biochemical Societies (FEBS) and access to FEBS Fellowships

Join now at biochemistry.org/join

* Portland Press Limited (Company Number 2453983) is the wholly owned trading subsidiary of the Biochemical Society (registered charity No. 253894) Vat No. GB 523 2392 69
Fertility

Editorial 3

Features

The road to 1978: a brief history of fertility research 4
Michael Carroll

Meiosis and fertility: an old wives’ tale 8
Geraldine Hartshorne

Seminal reactive oxygen species, a novel biochemical assay for testing male fertility? 12
Anastasia Dimakopoulou and Channa N. Jayasena

Infertility as a matter of communication: getting the message from sperm to oocyte 14
A. Filipa Santos, Celine Jones and Kevin Coward

Manipulation of fertility to enhance productivity of cattle 20
Michael K. Holland and Michael McGowan

PGS: a four-letter word? 26
Darren Griffin and Çağrı Öğur

Interview 33
Putting fertility research into motion Allan Pacey

Regulars

Lifelong Learning
Feedback: Completing the cycle 36
Emily Coyte

The motivation to experiment — an art and science exchange 40
James Brown

Careers 42
A day in the life of a bioinformatician
James Campbell

Policy Matters
Driving developments in diversity 44
Emma Sykes

News

Biochemical Society 2019 Award Winners 46
Events and Membership News 50
Royal Society of Biology News 52
CEO Viewpoint 53
Book reviews 54
Cartoon 55
Prize Crossword 56

Coming up in 2018

Food Production 42
The Brain
Science and Space

June 2018 © Biochemical Society
A BIOCHEMICAL SOCIETY
TRAINING COURSE

R for Biochemists 101
Starts 10 September 2018

This online training course aims to equip participants with the information, tools and techniques to use R. It is suitable for beginners who want to use this programming software but have little or no experience.

Topics include:
- Drawing a protein standard curve
- Extracting data from objects
- Drawing an enzyme kinetics plot
- Customizing and reusing plots
- Getting your data into R for exploration

Lead Educator:
Dr Paul Brennan (Cardiff University, UK)

Find out more at
bit.ly/RforBiochemists101Sept18
Forty years of IVF

by Chris Willmott, Science Editor

One of the unsettling aspects of growing older is the realization that events which occurred within your own lifetime are considered by others to be history. This experience struck me for the first time when one of my children was studying the fall of the Berlin Wall for their GCSE course.

2018 marks the 40th birthday of Louise Brown, the first baby produced by IVF (in vitro fertilization). For many readers of The Biochemist this pre-dates their own birth, and definitely falls into the category of history. In 1978, I was a schoolboy who hadn't quite qualified for long trousers. I was sufficiently news-savvy to appreciate that a significant breakthrough had occurred but without being clear on the details. (In truth, I rather suspect this caveat could also have been applied to my understanding of the more traditional route to conception). In the intervening period, IVF has become the cornerstone of a broader array of assisted reproductive technologies (ART), some of which are discussed in more detail in articles in this issue.

Of course we should not forget that IVF was initially highly controversial. Objections were based, in part, around the status of the embryo, and, in part, that a Pandora’s box had been opened which would unleash a plethora of unexpected consequence. The former concern persists amongst those who take a high moral view of the human embryo. However, with more than 6 million children conceived via IVF, and with improvements in technology that have not only decreased the number of ‘spare’ embryos produced, but also facilitated the freezing of eggs as well as sperm, this worry has receded for many people. Additionally, the demonstration that many adults who were themselves conceived by IVF have gone on to have their own natural children, has offered further reassurance.

It is certainly true that there has been an escalation of realized or near-possible developments in reproductive science that would have been impossible without the original IVF breakthrough. Existing PGD (Preimplantation Genetic Diagnosis) can hand-pick embryos free from a certain inheritable condition, but it could equally be used to select for other characteristics (for example, a deaf couple undergoing PGD to ensure that their child is also deaf). Particular worries now surround the potential application of genome editing tools such as CRISPR. Basic research, altering human embryos to better understand development, have already been conducted, including at the Francis Crick Institute in London. However, concerns remain about the generic safety of the approach, even before consideration of the ethics of any specific modifications or enhancements are brought into play. There are probably no other areas of science in which innovation is so explicitly double-edged.

On a separate theme, I would like to send thanks and best wishes to Helen Albert, former editor of The Biochemist, who has recently left the Society to pursue a new science communication role based in Berlin.
The road to 1978: a brief history of fertility research

An explanation for the mechanism of reproduction was first proposed by the ancient Greeks. The philosopher and ‘Father of Medicine’, Hippocrates (460–370 BC) proposed the ‘two seed theory’ where both males and females produce seeds that combine to give rise to a new human. However, Aristotle (384–322 BC) held the view that the unborn child was preformed, consisting of fluids and menstrual blood (catamenia) in the womb. This catamenia awaited the male’s semen to trigger its development.

He considered that the preformed foetus grew like ‘the seeds of plants’ eventually developing into the baby. This theory of preformation or ‘epigenesis’ dominated the understanding of reproduction for over 1000 years until the latter part of the European Renaissance. Scholars such as the anatomist, Hieronymus Fabricius (1537–1619) turned his attention to the mystery of reproduction. Studying the domestic hen, Fabricius noticed that fertile eggs were laid some time after mating with a cockerel. This led him to believe that semen from the cockerel was stored in a little sac near the cloaca in the hen, where it rendered the whole uterus and eggs fertile. This sac was later named the Bursa of Fabricius (although it is not actually used for semen storage but B-cell development in birds). A student of Fabricius, William Harvey (1578–1657) – better known as the discoverer of blood circulation – was also interested in reproduction. He expanded the work of his mentor and investigated the role of semen through dissection of female deer, dogs and rabbits. His dissection of female deer soon after mating, found no ‘evidence’ of a physical role for semen in reproduction but ascribed its role as more ethereal, a conclusion closer to Aristotle’s preformationist idea. Harvey concluded ‘that all things come from the egg’ (ex ovo omnia) which he published in *Exercitationes de Generatione Animalium* (Exercises on the Generation of Animals), in 1651.

The first microscopes

Discoveries in science frequently follow innovations in technology, and the emergence of the microscope is a fine example. Improvements of Gutenberg’s printing press made printed books and manuscripts more accessible,
and this in turn generated a market for ground lenses to aid reading. The Dutch spectacle makers, father and son Hans and Zacharias Janssen, and Hans Lipperskey are credited with inventing the first microscope, during the 1600s. They placed two convex lenses at each end of an adjustable tube, with one end functioning as an eyepiece, and the other the objective. This was rather rudimentary, but offered some magnification of objects. Later the English polymath, Robert Hooke (1635–1703) improved on the design, exploring the micro-world in great detail. He published his findings in the magnificent *Micrographica* (published 1665). This masterpiece contained 38 plates, including a large pullout illustration of a flea, and included the first biological reference to cells.

Antonie van Leeuwenhoek (1632–1723) worked as a draper and acted as a minor city official in the Dutch town of Delft. He was a meticulous observer and keen craftsman, designing and making hundreds of simple microscopes, much different to the compound double-lens microscopes of Janssen and Hooke. Leeuwenhoek’s were mostly small handheld, single-lens microscopes with impressive magnifying and resolving powers (Figure 1). Through his observations, he made some remarkable discoveries, including the first observations and descriptions of small bacteria and protist in samples of water, which he called ‘animalcules’. He sent many letters describing his observations to the Royal Society in London, whose members (including Hooke) were impressed with his microscopic skills and meticulous descriptions. For this, he was elected as a foreign member in 1680.

Leeuwenhoek is most famous for the discovery of sperm. He believed that the generation of animals was from these ‘animalcules in the male sperm’ and noted their presence in abundance in his own semen and that of the dog, rabbit, and cockerel (Figure 2). Leeuwenhoek had the preformationist view of generation, stating his discovery ‘the parts and membranes of the fetus’, including the head and the shoulders. He postulated that these animalcules travel to the uterus where they grow and develop – ‘the female served only to afford nourishment to the animalcules of the male sperm’, akin to a seed planted in nutrient soil (for his letters to the Royal Society see vanleeuwenhoek.com). Nicolas Hartsoeker (1656–1725) a fellow Dutchman, described what he perceived these preformed little men would look like, as depicted in the drawing of the ‘homunculi’ in these animalcules (Figure 3).

During the seventeenth and eighteenth century, there were two preformationist concepts of reproduction – the ovists, who argued that generation is derived from the preformed foetus residing in the ovum or egg; and the spermists who proposed the idea of the homunculi and the ‘planting of the male seed’.

---

**Figure 2.** Sperm from rabbits and dogs, as described by Antonie van Leeuwenhoek in 1678. Taken from https://commons.wikimedia.org/wiki/File:Sperm_Anton_van_Leeuwenhoek_Rabbit_dog.jpg, available under Creative Commons Public Domain Mark 1.0.

---

**The role of the egg**

Through studies of insects, amphibians, fish and mammals – the ovists view of the importance of the egg in the generation of animals was further cemented by the work of Dutch biologists, Nicolaus Steno (1638–1683), Jan Swammerdam (1637–1680) and Reinier de Graaf (1641–1673). Steno dissected dogfish and noted that the ovaries were similar to the female ‘testis’ he observed in women and sheep. This led him to conclude “…I have no doubt that the testicles of women are analogous to the ovary…” , which he believed contained the eggs. Swammerdam and de Graaf were gifted anatomists, and were both keen to confirm the existence of eggs in the woman. They battled to be the first with to be accredited with this discovery. However, de Graaf first published his detailed work, *De Mulierum Organis Generationi Inservientibus Tractatus Novus* (New treatise concerning the generative organs of women), in 1672. In it he describes the follicle and its contents – exclaiming that this organ contains the egg (from where the Graaffian follicle is derived). He also postulated that a ‘seminal vapour’ reached the eggs through the uterus and fertilized them. However, the true role of both egg and sperm in the generation of animals was unraveled by the careful and conscientious work of the Italian Catholic priest, Lazzaro Spallanzani (1729–1799). Spallanzani was a great experimentalist and thinker, with a broad interest, including physics, chemistry, geology, and biology. He carefully designed controlled experiments,
describing the methodology in enough detail for others to repeat his work, and rebutted any critics with more controlled experimental data.

Spallanzani turned his attention to fertilization and reproduction. In one experiment, he examined the nature of the sperm’s aura spermatica. This was one prevailing preformationists theory, proposing that a ‘vapour’ emanating from semen triggered embryonic development. Spallanzani set out to investigate this by placing semen from a toad in a watch glass while eggs from the female were placed in the bottom of another watch glass turned upside down. The eggs were separated from the sperm by a few millimetres. After several hours, he noted the eggs were covered ‘as if by a dew’, from the condensation of the evaporated seminal fluid. However, none of the eggs developed. This disproved the property of the aura spermatica of sperm. In another, ingenious experiment, he prized male frogs from their mating amplexus and fitted them with tight taffeta britches, after which he replaced them back in their mating position. With the taffeta barrier, none of the eggs developed. However, when he scraped the semen from the britches and added it to the eggs, they all developed into tadpoles. Hence, Spallanzani was able to demonstrate unequivocally, the role of the egg and semen in the generation of animals. This was one of the earliest demonstrations of IVF and the proof that fertilization took place by the physical contact between semen and egg. However, he, erroneously, concluded that semen had the fertilizing property, not the sperm. It would be another 100 years before direct interaction of sperm and eggs could be characterized.

Fertilization described

By the mid-1800s, improvements in optics and microscopic techniques, together with the elucidation of the cell theory by Virchow, Schwann, and Schleiden paved the pathway for a more accurate explanation and description of the process of fertilization. In 1879, Oscar Hertwig (1849–1922) and Hermann Fol (1845–1892), who both trained under Ernst Haeckel, independently described sperm entry into the egg and the subsequent union of male and female nuclei in the starfish.

It took nearly 200 years after Leeuwenhoek’s discovery of sperm that it was finally confirmed that both egg and sperm had to fuse together, during the process of fertilization, to trigger embryo development.

The nineteenth century saw advances in the study of mammalian reproductive biology.

The Swiss physician Jean-Louis Prevost (1838–1927) and the French scientist, Jean-Baptiste Dumas (1800–1884) concluded that the testis produced sperm and in other experiments, they demonstrated that sperm was essential for fertilization in the frog. It was the embryologist, Carl Ernst von Baer (1792–1876), in 1826 identified the mammalian egg during his studies on the ovary of a dog and described early embryo development.

The practice of artificial insemination can be dated back to Spallanzani, who successfully artificially inseminated a bitch. However, Dr John Hunter is cited as having performed the first documented artificial insemination in humans (circa 1790). He carried out this with sperm derived from a man with hypospadia using a syringe.

The first reported attempted mammalian IVF was carried out in the 1930s. Gregory Pincus demonstrated this in the rabbit in 1934 stating "that first certain demonstration that mammalian eggs can be fertilized in vitro". However, his work was criticized and the ‘fertilized’ eggs were suggested to be parthenotes, as spontaneous egg activation was common in cultured rabbit eggs. The main impediment to mammalian in vitro fertilization was the physiology of sperm. Sperm must undergo biochemical and physiological changes in the female reproductive before they have the capacity to fertilize an egg. This process is called capacitation and was first identified by both Colin Austin (1914–2004) and M.C. Chang (1908–1991), who both published their findings separately in 1951. Austin further described another essential sperm process, the acrosome reaction, which enables the sperm to penetrate the outer layer of the egg (the zona pellucida).

In 1959, Chang collected mature unfertilized eggs from albino female rabbits. Sperm used for insemination were collected from uteri of albino females mated with albino males 12 hours previously. The fertilized eggs were cultured until the 4-cell stage, where they were then transferred to black surrogate females, that subsequently delivered live albino young. Chang’s work represented a significant advancement. However, the necessity to pre-incubate sperm in the uterus of a pregnant female prior to attempting to fertilize the eggs complicated this process. It was Chang and Austin’s work on capacitation that led to the belief that sperm must reside in the female reproductive tract in order to contribute to fertilization in vitro. However, Ryuzo Yanagimachi and Chang in 1963 showed that this was not essential, when they developed experimental in vitro conditions through which sperm (from hamster) without prior in vivo activation could fertilize eggs in vitro, with subsequent embryo development to the 2-cell stage. Following this, many other mammalian species (mouse, guinea pig, cat, sheep and pig) were fertilised in vitro. These important contributions laid the groundwork that would eventually lead to successful human IVF.
Combining advances in molecular genetics and embryology has given rise to techniques such as Preimplantation Genetic Diagnosis (PGD) and Preimplantation Genetic Screening (PGS), which are used to screen and test for a specific genetic disease that may be embryo lethal or result in severe abnormalities and miscarriages.

Developments in sperm, egg and embryo cryostorage (freezing) has enabled couples and single would-be parents to preserve their fertility, especially after cancer treatment.

The history of baby making is rich and diverse – with many important contributors playing essential parts. This article is a synopsis of some of the key events that took place over the course of history.

Human IVF

In 1963 Robert Edwards (1925–2013) set up a laboratory at Cambridge University to investigate human fertilization. His energy, enthusiasm and ambition to obtain successful human IVF were admirable. However, in trying to achieve this he encountered a number of scientific obstacles. He needed a regular supply of human eggs and a means to capacitate sperm in vitro.

His meeting, and eventual long collaboration with Patrick Steptoe (1913–1988), solved the problem with obtaining human eggs. Steptoe was a Consultant Obstetrician at Oldham General Hospital, Greater Manchester, where he had been pioneering the development and use of the laparoscope in gynaecological surgery. Edwards’ interest in collaborating with Steptoe was to initially to remove capacitated sperm from the oviduct using laparoscopy. However, that problem was solved by his appointment of Barry Bavister (Austin’s PhD student) on to the project. Bavister developed a culture media that produced higher rates of fertilization in hamster eggs (Bavister’s media). Steptoe would become the valuable source for human ovarian tissue and eggs.

This collaborative work between Edwards, Steptoe and Bavister led to their landmark 1969 Nature paper, which described convincingly for the first time the fertilization of human eggs in vitro. This paper ended with the humble concluding remarks, “Human oocytes have been matured and fertilized by spermatozoa in vitro. There may be certain clinical and scientific use for human eggs by this procedure”.

The continuing work of Edwards, Steptoe and an additional team member, Jean Prudy (1946–1985) eventually led to the birth of the first IVF baby, Louise Brown, on the 25 July 1978. Since then approximately 6 million babies have been born through ART.

Summary

Since the birth of Louise Brown, the field of ART has advanced both in our understanding of human reproduction, the causes of infertility and developments in ART.

Infertility is defined as the inability to conceive naturally after 12 months of actively trying and affects 1 in 7 people in the UK (15% of the global population according to the World Health Organization).

The advent of a variant of IVF, Intracytoplasmic Sperm Injection (ICSI), in which a single spermatozoon is selected and injected into the egg cytoplasm with a fine pipette, has revolutionized the treatment of male infertility, in particular in cases where sperm numbers are very low or their swimming ability (motility) is impaired.

Further reading

Meiosis and fertility: an old wives’ tale

Geraldine Hartshorne
(Warwick Medical School, UK)

For a variety of different reasons, women are delaying attempts to get pregnant until later in their lives. However, age-related decline in egg quality is a major factor in otherwise-unexplained infertility. What are the underlying biochemical processes contributing to this loss of fertility?

Current trends on childbearing in the UK

Women in Western nations are in control of their own lives, as never before. Career opportunities and financial independence are making real progress towards gender equality. Alongside this, a revolution has taken place in fertility choices. Many women plan pregnancies around their jobs, education, housing and lifestyle priorities. Life partners may be selected at a later age, and changes of partner are more frequent.

The Office for National Statistics regularly publishes data about the ages of women giving birth. Their most recent edition shows the expected trends; more women aged 40+, 35–39 and 30–34 are having babies than in earlier years, while fewer teenagers and 20-somethings are having children (Figure 1). A radio commentator, that I happened to hear discussing the latest dataset, thought this was good as children born would have more secure homes and more experienced parents. But he did not mention the hidden costs of this revolution, because biology is now at odds with society. Many women planning for a child in their later reproductive years will need assistance to become pregnant, while many others will spend a lot of time, money and energy trying unsuccessfully to have a baby.

The problems of trying to be an older mum

Around 1 in 7 UK couples will experience difficulty in conceiving when they start trying to do so. The later they start trying, the lower their fertility is likely to be, purely as a result of being older. Reproductive ageing is particularly abrupt in women, with fertility declining from around 30 years of age, the decline accelerates steeply at 37 years, reaching very low levels in the mid-40s. Figure 2 shows the live birth rates after IVF treatment in the UK, highlighting the association between a woman’s age and the chances of success, when using her own eggs.

How eggs form

Meiosis is the highly specialized cell division that produces haploid eggs or sperm from a diploid stem...
Older eggs come unstuck

While there are several hypotheses, one of the most popular is that molecules holding the meiotic chromosomes together deteriorate as time goes by, thus allowing chromosomes to drift apart, predisposing to difficulties when segregation of chromosomes is required just before ovulation. It is believed that these cohesive molecules are laid down before birth as the eggs enter meiosis, however, they are not replenished. So by the time the egg is preparing to ovulate, the chromosome structure has been...
waiting in an arrested state for many years. Inevitably, the older the woman, the longer the chromosomes have been in this arrested state. The first meiotic division, that occurs a few hours before ovulation, is complex and error-prone. This division started in the foetus, when homologous pairs of replicated chromosomes came together, synapsed and underwent genetic recombination, resulting in four intertwined chromatids with crossing over points. For these to be separated into haploid sets of chromosomes, such as are present in eggs or sperm, two divisions (meiosis 1 and meiosis 2) are required which each halve the number of chromosomes present. Therefore, in meiosis 1, two chromatids have to arrive at the same spindle pole, so some of the connecting proteins between them have to remain strong, whereas others have to dissolve to allow progression to anaphase 1. Protein clusters (known as kinetochores) which attach the chromosomes to the tubules comprising the spindle, control the orientation of the chromosome attachments. These kinetochores are critical to ensuring that chromosomes are pulled in the right direction at meiosis 1. In this way, meiosis is distinctly different to mitosis, where chromosomes must separate from their partners, as shown in Figure 3.

**Highlighting the problem**

Our work uses molecular markers to highlight key parts of the kinetochores and the chromosomes, and then applies high power microscopy to identify and reconstruct the detailed structure and relative orientations of the component parts. This reveals the organization of the kinetochores (Figure 4), and helps to identify where errors with chromosomal movements may have occurred.

For this research, we study eggs that are not appropriate for use in IVF treatment, either because they are immature, or because they didn’t fertilise when first inseminated with sperm. We fix and stain them using antibodies, or we highlight chromosomes in live cells and follow them using high power time-lapse microscopy. We then challenge the eggs to continue maturing, or to fertilize, and observe the movements of chromosomes in real time using live cell imaging. Many of these divisions show errors, allowing us to study the mechanisms by which abnormalities arise.

In eggs from older women having IVF treatment, we have found that the kinetochores are further apart in their pairs at metaphase of meiosis 1 than is the case in younger women (Figure 5). Moreover, kinetochores in humans appear different in structure from those in other animal species, notably mice where most study has been undertaken. Kinetochores in humans are distinctly separate, while those in mice and other species may be fused together, which would presumably help to hold them in place during the prolonged meiotic arrest since before birth, possibly explaining some of the differences between animals and humans in terms of the incidence of chromosomal anomalies in embryos.
The increasing distance between kinetochore pairs with maternal age suggests that the chromosomes to which they are attached are moving apart with time.

**So what can we do about it?**

There is nothing with current technology that can be done to avoid or correct errors in meiosis 1. Older women whose own eggs are unable to generate a pregnancy can receive donated eggs from a younger woman, but any resulting baby is therefore genetically unrelated to themselves. It is also an expensive, emotionally challenging and arduous option, so its avoidance by having children at a younger age, or perhaps by banking eggs when young for personal use in future, may be worth considering. Young and aspiring professional women need access to this information to help inform their decisions when planning their future families.

In the fertility centre, some women have a clinical problem which may affect egg quality, but many do not have a defined cause of infertility, and often age is the main contributing factor to their inability to become pregnant. One reason for writing this article is to highlight the difficulty that some older women may experience when attempting pregnancy. I would like to encourage any woman who wishes to have children, to balance the timing of that decision against other important aspects of her life, in full knowledge of the facts around fertility and reproductive ageing.

**Acknowledgements**

I wish to thank Dr Jess Patel for her excellent work on this project. I gratefully acknowledge all those patients who have provided material for us to use, and colleagues in Coventry, Leicester and Bath who have collected the eggs for our study. I also wish to acknowledge and thank all those who have stimulated my interest in female meiosis and collaborated over the years, in particular, Professor Bob Edwards, Professor Maj Hultén, Professor Eva Hoffmann and Professor Andrew McAinsh.

**Funding**

The author is funded in part by MRC grant MR/M000664/1, and parts of the work presented were funded by a grant from the Montreal Foundation for Regenerative and Reproductive Medicine. The research is carried out under Human Fertilisation and Embryology Authority Research Licence R0155.
Seminal reactive oxygen species, a novel biochemical assay for testing male fertility?

Anastasia Dimakopoulou and Channa N. Jayasena (Imperial College London, UK)

Infertility is defined as a failure to achieve a positive pregnancy test over 12 months of regular unprotected sex. It is a devastating condition affecting 15% of couples and is socially marginalizing. Despite significant focus on the female, nearly half of cases are in fact due to poor sperm function in the male partner. It is therefore surprising that despite the existence of numerous diagnostic tools and management options currently available for female infertility, very few exist for their male counterparts. So, what can we do to diagnose men with impaired fertility promptly and direct couples to effective management strategies?

Male infertility

Couples who are unable to conceive naturally are routinely referred by their GP for hospital investigations to determine whether either partner has impaired fertility. Approximately half of assisted reproduction treatments (ART) are related to male infertility and the Human Fertilisation and Embryology Authority reports that this number more than doubled between 2009 and 2013. Factors which can impair male fertility are numerous, ranging from infection, testicular pathologies and genetic disorders to endocrine or other systemic diseases. Lifestyle features such as cigarette smoking, illicit drug use, alcohol consumption, a high fat diet or increased scrotal temperature play an additional negative role on male reproduction. However, despite improvements in diagnostic examination, the cause remains unknown in 40% of cases.

Table 1. Lower reference limit and their 95% CI for semen parameters from fertile men whose partners had a time-to-pregnancy of 12 months or less [WHO Criteria, Cooper et al. (2010)].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower reference limit (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>1.5 (1.4–1.7)</td>
</tr>
<tr>
<td>Sperm concentration (106 per ml or M/ml)</td>
<td>15 (12–16)</td>
</tr>
<tr>
<td>Total motility (PR* + NP**, %)</td>
<td>40 (38–42)</td>
</tr>
<tr>
<td>Progressive motility (PR, %)</td>
<td>32 (31–34)</td>
</tr>
<tr>
<td>Sperm morphology (normal forms %)</td>
<td>4 (3.0–4.0) %</td>
</tr>
<tr>
<td>PR* progressively motile, NP** non-progressively motile</td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis and current limitations

Currently, the only NHS diagnostic tool for male infertility is semen analysis. Semen analysis involves the assessment of spermatozoal concentration, motility and morphology under specific World Health Organization criteria (Table 1). WHO criteria for the evaluation of human semen provide reference values for seminal characteristics and are associated with a couple’s likelihood to achieve pregnancy within 12 months. Results above or below the reference values are essentially a standardized guide regarding a man’s fertility status. However, changes in male fertility are not detected with standard semen examination in 15% of couples. In these cases, couples find the lack of diagnosis distressing as no explanation can be given for their presentation.

Seminal reactive oxygen species: an important diagnostic marker of male infertility

Conventional semen analysis can only evaluate male reproductive capacity to a certain extent, therefore it is clinically important to develop novel methods for the assessment of seminal quality. Over the last few years the role of seminal reactive oxygen species (ROS) have gained considerable interest because early diagnosis of high oxidative stress could potentially guide couples to effective therapeutic approaches. ROS detection could be a key factor when advising couples about optimal sperm quality before natural or assisted conception.
ROS are generated in seminal plasma from endogenous sources such as leucocytes or immature spermatozoa, and are physiologically required for sperm movement and fertilization of the oocyte. However, in men with testicular pathology such as varicocele (enlargement of blood vessels in the scrotum), sexually transmitted infections (STIs) or metabolic imbalance due to high energy diets or toxic environmental factors, ROS production is disproportionate (Figure 1).

Once excessive ROS production surpasses the antioxidant capacity of free radical scavengers within the semen oxidative stress is generated, sperm plasma membrane fatty acids undergo lipid peroxidation by ROS and multiple DNA defects can occur. DNA is damaged via fragmentation both in the sperm nucleus as well as the mitochondria and, as a result, sperm become dysfunctional and can impede fertilization of the oocyte, affect embryo development or lead to pregnancy loss and miscarriage.

**How can we practically measure seminal ROS?**

Indirect assays to measure ROS include analysis of sperm chromatin and evaluation of DNA damage via fragmentation. The Department of Andrology at Hammersmith Hospital recently developed a direct assay based on chemiluminescence. Light is emitted when luminol (5-amino-2,3-dihydro-1,4-phtalazinedione) is oxidized. Mean chemiluminescence is measured over a 10-minute period and compared to a negative control to eliminate background variation that may confound readings. Results are reported as relative light units per second (RLU/sec). A raised ROS is defined as >3.8 RLU/sec/106 sperm.

**Clinical implications and future avenues**

Testing for ROS can be particularly relevant in men with genitourinary infections, varicocele or exposure to harmful lifestyle factors. For example, increased seminal oxidation due to STIs can be managed with antibiotics and metabolic causes such as high fat diet or smoking can be eliminated with lifestyle changes. In addition, men with varicocele exhibiting high ROS through testing could be at substantial risk for DNA damage and may benefit from surgical varicocele repair. Finally, ROS levels may be higher in couples with recurrent miscarriage, therefore seminal ROS measurement may have diagnostic and therapeutic potential for couples with unexplained recurrent pregnancy.

Male infertility is increasingly viewed as a marker of a man’s general health. Biochemical assays such as seminal ROS may open new avenues for evaluating and treating couples with infertility. It is critical to build links with specialist clinicians to develop new services, and for further laboratory research to improve the diagnostic tools available to help a couple start a family. ■

**Further reading**

- WHO laboratory manual for the examination and processing of human semen (2010)

Anastasia Dimakopoulou is a Clinical Research Fellow in Endocrinology and Diabetes at Hammersmith Hospital, Imperial College London. Her research focuses on male infertility associated with obesity. Her team studies the development of diagnostic methods for couples with recurrent pregnancy loss. In addition, Anastasia has ongoing interest in the management of male cancer patients with affected fertility status. Email: a.dimakopoulou@imperial.ac.uk

Channa Jayasena is Clinical Senior Lecturer and Consultant in Reproductive Endocrinology and Andrology at Imperial College and Hammersmith Hospital, London. Dr Jayasena qualified in medicine at the University of Cambridge, after which he undertook specialist training in Diabetes & Endocrinology in London and performed research at Imperial College as a Wellcome Trust Clinical Research Training Fellow and subsequently NIHR Clinical Lecturer. Dr Jayasena has received several awards, including the prestigious 2014 Young Endocrinologist Prize Lecture, the highest honour to a trainee clinician by The UK’s Society for Endocrinology (SFE). Dr Jayasena has national expertise in the treatment of male reproductive disorders and is a member of the invited faculty for Clinical Update Meetings held by the SFE and European Society for Endocrinology. He is currently Clinical Lead for Male Infertility / Andrology at Hammersmith Hospital which offers specialist support for a range of male reproductive disorders. Email: c.jayasena@imperial.ac.uk
Infertility as a matter of communication: getting the message from sperm to oocyte

A. Filipa Santos, Celine Jones and Kevin Coward
(University of Oxford, UK)

Fertile and infertile states of reproductive health

Birth is often referred to as “the miracle of life” and very rightly so, since successful pregnancy represents a complex, highly coordinated succession of biological processes. In this regard, it is remarkable that pregnancy ever occurs at all.

In order for pregnancy to occur, the two gametes (sperm and oocyte) must meet (Figure 1) and the oocyte must be released from meiotic arrest. However, when sperm is deposited inside the female reproductive tract, an acidic vaginal pH, the presence of viscous cervical mucus and attack by the immune system ensure that only a few sperm make it successfully to the narrow utero-tubal junction. Not surprisingly, sperm suffer considerable damage during this dangerous journey, including DNA fragmentation. Therefore, before moving into the labyrinthine oviduct lumen, sperm undergo a form of biological selection, which is not yet completely understood but is thought to depend upon particular proteins carried on their surface. Sperm then attach to a very specific portion of the Fallopian tubes known as the reservoir, where they undergo a final maturation process called capacitation, in which, amongst other biological features, they acquire a hyper-motile state. After this, sperm detach and respond to chemical and temperature gradients to swim up along the Fallopian tube up into the ampulla, where the arrested oocyte awaits. Herein, sperm must encounter the zona pellucida, a protective glycoprotein coat that surrounds the oocyte. Contact with the zona pellucida elicits a mechanism referred to as the acrosomal reaction, in which the sperm membrane fuses with a cap-like structure in its own head (the acrosome), releasing its enzymic contents to break down the zona pellucida and thus allow the sperm to bind to the oocyte membrane and induce the process of fertilization.

Quite understandably, there are many steps along the way in which this complex process can go wrong, all of which represent a risk to fertility. Generally, infertility can arise because of a problem with the male, the female or a combination of both. Unfortunately, there is also a large proportion of infertility that remains unexplained (Figure 2).

So how can we treat infertility?

Luckily, the answer to this question is continually evolving, as the diagnostic and therapeutic tools at our disposal are constantly expanding and improving. Collectively, these techniques are referred to as Assisted Reproductive Technologies (ARTs) and refer to the in vitro handling and manipulation of sperm, eggs or embryos in attempt to achieve successful fertilization, implantation and clinical pregnancy. A brief description of some core ARTs is given in Figure 3.

The application of such techniques has completely revolutionized the way doctors and scientists view conception. ARTs have been responsible for over 5 million live births and may represent our only hope to balance global demographics in the future, particularly in Europe. However, their use has understandably drawn attention from ethical, legal and regulatory bodies across the world. Moreover, while ARTs have answered the dreams of millions of couples, the truth of the matter is that their success rates rarely exceed 35%. Additionally, these techniques often require multiple rounds of treatment in order to be successful, at great personal and economical cost for the couples involved. The reasons underlying this disappointing figure predominantly lie in the artificial methods and environments used, thus creating a significant challenge for the scientific/clinical research community to address. In this context, male infertility stands out as a particularly prominent candidate for improvement,
as our ability to diagnose and treat this condition is currently compromised by rather rudimentary morphological or biological parameters, which do not allow us to consider key genes or proteins. A classic example of this is a protein called PLCζ.

**Communication between oocyte and sperm: the critical role of phospholipase C zeta (PLCζ)**

The application of ARTs depends upon diagnosis on a case-by-case basis and also upon a specific couple’s response to previous treatment. In terms of assessing male infertility, the first step involves obtaining an ejaculated sperm sample from the patient and carrying out a series of specific tests designed to garner information relating to sperm count, motility, morphology, pH and volume. These parameters are then compared to reference figures published by the World Health Organization (WHO). The patient’s sperm can hence be classified into different categories of abnormality. Abnormalities include low sperm count (oligozoospermia), an absence of sperm (azoospermia), reduced motility (asthenozoospermia), a significant proportion of dead sperm (necrozoospermia), and morphology abnormalities (teratozoospermia) (Figure 4). These tests are used in clinics across the world, and generally lead to a clear diagnosis and the advocating of a specific treatment, that is, the one that most simply and effectively bypasses the defect presented by the patient. However, there is increasing concern that such assessments are rather superficial and do not provide any specific information on DNA quality or the competence

**Figure 1.** Overview of reproductive events leading to fertilization.

**Figure 2.** Infertility aetiologies. Pie chart representing the proportion of infertility by causative factor (according to the 2018 ART fact sheet from the European Society for Human Reproduction and Embryology).
of particular proteins that play critical biological roles. This is especially pertinent in infertile patients who exhibit normozoospermia, which, despite meeting the standard WHO tests’ requirements (Figure 4), are still unable to conceive.

Such normozoospermic patients can be infertile because reaching the oocyte is not the end of the race. After penetrating the egg, the sperm then plays a crucial role in alleviating the oocyte from meiotic arrest. Known as oocyte activation, this process also causes the oocyte to release cortical granules (the so-called ‘cortical reaction’), which cause the oocyte membrane to harden and thus prevents penetration by any further sperm. The nucleus of each gamete must then fuse to generate a zygote, and divisions must begin in a coordinated manner to produce an early embryo which must travel to the uterus and successfully attach to its wall (implantation).

For over a century now, scientists have debated how a sperm could elicit this absolutely fundamental mechanism of oocyte activation. It is currently accepted that there is a specific trigger within the sperm that initiates conversation between the two gametes when they meet; this is referred to as the “sperm factor” and was identified as a unique form of phospholipase C, phospholipase C zeta (PLCζ), which resides within the sperm’s cytoplasm. While inside the sperm, PLCζ remains inactive, but upon fertilization, and via an as yet unknown mechanism, it becomes active and initiates a series of events that ultimately culminate with the release of calcium ions (Ca2+) from the oocyte’s endoplasmic reticulum. This ionic release adopts a characteristic, oscillatory pattern, which controls the events that release the oocyte from meiotic arrest, activate it and initiate embryonic development (Figure 5).

Given the fundamental role that PLCζ plays in activating an oocyte, it is not surprising that male fertility has an implicit relationship with the level, localization and functional activity of PLCζ. Indeed, our own research, and that of other groups, has shown that the sperm of some infertile patients have low levels of PLCζ or are completely devoid of PLCζ. In other patients, PLCζ shows abnormal localization in the sperm head and/or impaired functionality due to genetic mutation or other problems at the molecular level.

This knowledge has led to the development of a variety of tools which we can use for the diagnosis and treatment of oocyte activation deficiency (OAD). However, PLCζ is not the only factor that may impact fertility. Other proteins, such as phospholipase C ε (PLCε), have also been implicated in the activation process. Further research is needed to fully understand the role of these proteins in fertility and to develop effective treatments for infertile patients.
cannot be investigated in patients using the current suite of WHO-controlled semen analyzes. Patients afflicted by oocyte activation problems have had to wait for the scientific community to ‘catch up.’

**Using PLCζ to diagnose and treat male infertility**

Our group, and others, have already mapped the localization, expression and function of PLCζ in normal fertile males. But how exactly can our knowledge of PLCζ be of use in treating infertility? First of all, PLCζ has great potential as a diagnostic tool, especially for patients who experience recurrent, unexplained intracytoplasmic sperm injection (ICSI) failure (which is estimated to happen 1-5% of the time). Indeed, our team has been able to link abnormalities in the localization, expression and function of PLCζ to infertility. However, the molecular mechanisms underlying these abnormalities are complex and require further investigative research. As part of a wider research project, and exciting collaborative ventures with Oxford Fertility (Oxford, UK) and the Assisted Conception Unit, Ninewells Hospital (Dundee, UK), we have developed simple, yet efficient, research assays with which to investigate PLCζ expression and localization in infertile males who comply with a series of specific inclusion criteria. These assays provide us with important information relating to the oocyte activation ability of a particular patient’s sperm, which can then be used by clinicians to develop appropriate clinical management strategies.

But what are the therapeutic options open to such patients? Currently, the only possible option is a technique known as Artificial Oocyte Activation (AOA), in which an artificial agent (commonly a Ca²⁺ ionophore) is applied to the oocyte during conventional ICSI to induce the release of Ca²⁺. While this successfully bypasses the sperm’s inability to release Ca²⁺ within the oocyte and therefore initiate activation events, there is a potential problem. Unfortunately, these chemical agents cannot accurately reproduce the specific pattern of Ca²⁺ oscillations that occur naturally. Instead, they lead to a single Ca²⁺ transient, akin to unleashing an uncontrolled tsunami of Ca²⁺ within the oocyte. It is important to remember that the actions of PLCζ do not just activate the oocyte, they are also involved in regulating gene expression in the early embryo via Ca²⁺-sensitive transcription factors.


**Figure 5.** The molecular pathway of PLCζ. After the sperm penetrates the zona pellucida and the two cytoplasmic membranes fuse, PLCζ gets inside the oocyte where I) it suffers an as yet unknown mechanism of activation. II) PLCζ then Hydrolyze oocyte vesicle membrane component PIP2, yielding InsP3 (membrane bound) and DAG (soluble). III) InsP3 goes on to bind the InsP3 receptor on the endoplasmic reticulum membrane, allowing the release of Ca²⁺ ions. Ca²⁺ release elicits different responses (a, b and c) within the cell: a) In the presence of Ca²⁺, PKC binds the membrane-bound DAG and becomes active, eliciting a pathway that culminates with the cortical reaction. b) Ca²⁺ activates CaMKII, which in turn inhibits CSF from inhibiting APC. The liberated APC degrades CNB1, which, together with CDK1, forms a complex responsible for maintaining meiotic arrest. CNB1 degradation inactivates the complex, allowing for the meiosis to resume. c) MAPK actively inhibits pronucleus formation; upon binding with calcium ions, MAPK is inactivated, allowing the oocyte’s pronucleus to form, which then goes on to fuse with the sperm’s pronucleus.

(PIP2 = phosphatidylinositol 4,5-biphosphate; InsP3 = inositol-1,4,5-triphosphate; DAG = diacylglycerol; PKC = protein kinase C; MARCK = myristoylated alanine-rich C-kinase; CaMKII = calmodulin-dependent protein kinase II; CSF = cytostatic factor; APC = anaphase-promoting complex/cyclosome; CNB1 = cyclin B1; CDK1 = cyclin-dependent kinase I; MAPK = Mitogen-activated protein kinase).
characteristic oscillatory pattern of Ca$^{2+}$ release is therefore important in terms of the regulation of gene expression. With this in mind, and despite the fact that artificial oocyte activating agents have been used by some clinics for several years, there are some concerns over the potential effects of abnormal gene expression and research effort is understandably gaining pace in this key area.

A more effective, and safer, approach might therefore be the administration of a recombinant version of PLCζ which has been pre-tested for its ability to release Ca$^{2+}$ in an appropriate oscillatory manner. However, this is not proving easy. Although PLCζ was first identified as far back as 2002, there is still no clinically-approved recombinant PLCζ protein available on the commercial market and a wide array of questions remain for the researchers working in this area: how do we make a pure and functionally active PLCζ protein? How do we store it without losing activity? How do we test it? How and when do we deliver it to an oocyte? Could there be any negative effects associated with its use? Until these questions are answered, the only way to treat oocyte activation deficiency is by using a Ca$^{2+}$ ionophore. Within the UK, the fertility sector’s regulatory body, the Human Fertilisation and Embryology Authority, has consented for the use of AOAs within the UK but only if there is appropriate evidence to justify that there is a specific activation problem, such as PLCζ deficiency.

While we cannot yet administer an endogenous recombinant PLCζ protein to oocytes in the clinic, we can at least now screen appropriate patients with our clinical assays and provide clinicians with evidence of PLCζ deficiency. These current methods, however, are rather crude and cannot test for the functional ability to cause Ca$^{2+}$ release. Further improvement in our diagnostic assays, for example, by elucidating the three-dimensional structure of PLCζ and creating PLCζ antibodies that exhibit far better specificity, will undoubtedly increase our ability to diagnose problems that could potentially be fixed by AOAs, and in future, by recombinant PLCζ.

**Summary**

Zeta (ζ) might have been the last letter for the ancient Greeks; but in the infertility arena, the ζ in PLCζ is rapidly becoming known as the first way-marker to restoring communication between sperm and oocyte. While it is still early days, and our work is purely research-oriented at the moment, our assays may serve to lay down the foundation for new clinical protocols in the future. However, the story is still unfolding: a recombinant PLCζ is urgently required for clinical testing and it is entirely conceivable that a range of other proteins and molecular pathways are involved in the process of oocyte activation. It is, however, important to highlight that the work described herein relates to just one sperm protein but clearly shows how collaboration between scientists and clinicians can serve to develop new diagnostic and therapeutic options for patients who might otherwise give up their dreams of parenthood. Given that there are over 1700 functional proteins in a single mature human sperm, the PLCζ story is a reminder for us all that each of these proteins might serve as a way forward and that the established WHO guidelines for sperm analysis provide only a very basic indication of sperm quality.
Further reading

Manipulation of fertility to enhance productivity of cattle

Michael K. Holland and Michael McGowan
(University of Queensland, Australia)

Fertility is most simply defined as the natural capacity to produce offspring. More technically, it is the ability of male and female animals to produce viable germ cells, mate, conceive, carry and deliver normal living young. Fertility is affected by genetic factors, health status and environmental factors which includes management practices. These factors can affect one or more of the key steps in fertility which are: production of the gametes (the sperm or eggs), ovulation, fertilization, implantation, gestation or parturition. Over the last century, we have learnt to manipulate fertility in cattle by targeting many of these key steps with the goal of producing animals that more efficiently produce meat and milk to meet the demands of the increasing human population. This has required developing and integrating our knowledge in the key disciplines of reproductive biology and genetics, and while much has been achieved, much more is possible.

Background

Genetic and archaeological evidence both support the contention that our current cattle breeds are the result of multiple, independent domestication events of wild aurochs beginning approximately 10,000 years ago. Broadly, Bos taurus breeds originated from the Middle East and Europe whilst Bos indicus originated in the region of India and Pakistan. Cross breeding occurred almost from the earliest times. Modern genetic analysis shows Bos indicus characteristics first integrated into Bos taurus in Southern Europe where North African cattle could easily be traded. Much of the early breeding was driven by opportunistic natural mating but exactly when and where human intervention in cattle breeding began is less clear.

Initially, this involved simply mating cows to a particular bull which had some desirable characteristic(s) which were passed onto the offspring. Thus the understanding of the importance of fertility and its manipulation to produce offspring with desirable characteristics is almost as old as the domestication of cattle. Consequently, integration of reproduction and genetics was a driver of breeding almost from the earliest times. The picture rapidly changed as our knowledge of bovine reproductive biology became more sophisticated. An expanding World population which is expected to reach 9 billion by 2050, together with significant changes in diets in developing countries where there is an increasing consumption of animal protein, has resulted in increased demand for meat and milk products. Overall, the UN’s Food and Agriculture Organization (FAO) has suggested there will need to be a 100% increase in food production in the next 40 years. This provides an economic driver that pushes scientific innovation in cattle breeding to both improve the quantity and quality of animals produced.

Although, manipulation of reproductive biology can increase the numbers of offspring produced in seedstock herds and flocks it should be accompanied by improvements in genetic merit. The sequencing of the bovine genome in 2009 has provided a stimulus to bovine genetics and allowed development of new tools for genetic selection both for the beef and dairy industry. Simply put, reproductive technologies combined with advances in bovine genetics allows us to select which bulls to breed with which cows to produce calves with desirable characteristics such as more rapid growth rate, superior milk production, better carcass quality etc. in ways not possible previously.

Improvements in male reproduction

The discovery of sperm cells in 1678 was the beginning of the serious study of male reproduction. Initial progress was slow and it took until 1780 before the role of sperm in fertilization was demonstrated through the first successful artificial insemination (AI) of a dog. Detailed description of the many steps in fertilization took almost another century and the work of a number
of scholars. It wasn’t until early in the 20th century that Russian scientists first harnessed this knowledge and developed practical methods for AI in cattle. The economic value of AI was quickly realized and livestock producers in both Europe and the United States had formed breeding co-operatives by the mid-1930s. The 1930s and 1940s saw further expansion with improved ways to collect semen by electroejaculation and the development of extenders which allowed semen to be diluted so that a single, quality ejaculate could be extended and used to impregnate a large number of cows. Subsequently, the addition of antibiotics was shown to improve the storage life of semen and when this was combined with the discovery in 1949 that glycerol added to extended semen permitted sperm to be stored frozen and retain useful fertility, the modern artificial breeding industry was established.

Since then, there have been important advances in both the basic understanding of male reproduction (including the discovery of sperm capacitation) and in applied reproduction, as well as continued improvement of semen extenders – replacing glass ampoules with plastic straws for cryopreservation of semen and the use of insulated liquid nitrogen tanks for storing cryopreserved semen for the long term all being important. In the last 45 or so years the development of in vitro fertilization (IVF) using both fresh and cryopreserved sperm, yielding embryos which developed to blastocysts efficiently and which gave acceptable rates of pregnancy when transferred into hormonally synchronized recipient females have been important advances in reproduction. More recently, use of computer analysis to objectively quantify semen “quality” has also become routine. The successful separation of X-bearing sperm from Y-bearing sperm using flow cytometry – which relies on small differences in DNA content (usually 2–4% depending on species) and thus weight of X- and Y- bearing sperm - has meant that the sex of the offspring produced from AI or IVF with sexed semen can be predicted with >90% accuracy. The early problems with slow speed of sorting semen, its poor cryopreservation properties and lower fertility after separation are largely issues of the past. However, a problem which still needs a solution is the variation between individual bulls in the success with which their semen can be sexed. At the extreme, there remains a low percentage of bulls who produce semen which cannot readily be sexed at all.

Challenges also persist with regard to the use of sexed semen in IVF systems, including the need to use greater numbers of sperm to ensure a satisfactory proportion of inseminated oocytes develop into blastocysts and subsequently normal pregnancies.

**Improvements in female reproduction**

Many of the developments in reproductive technologies involve improvements in both male and female reproduction operating hand in hand. For example, for the maximum value of cryopreservation of sperm and
AI to be realized, better techniques for detecting oestrus, which allowed determining the optimum timing of insemination of the females, were needed. This involved development of devices to detect oestrus. These early devices have been significantly further developed, so that other important properties such as the time an animal spends feeding, ruminating, resting and even its temperature can be monitored. The occurrence of natural oestrus in a herd occurs at a different time for individual animals meaning insemination would need to be performed almost on a daily basis as different cows came into oestrus if maximum pregnancy rates were to be achieved. In large herds this is hardly practical and so research into using hormones to induce oestrus and ovulation at predictable times was undertaken which facilitated insemination of many animals on the same day. This not only meant more efficient use of time and resources but it also means the resultant calf drop occurred over a tight interval with the associated advantages this gave for management and ultimately marketing of the offspring. Maximizing the number of cows cycling during the breeding period and the attendant minimization of the calving period has, for the industry, led to the modern 365 day intercalving interval.

Superior methods of detection of pregnancy by either using transrectal ultrasound, which could also be used to determine foetal sex, or detection of specific conceptus or maternal-derived pregnancy-associated proteins in blood serum, were developed to further increase the overall efficiency of artificial breeding programmes in particular. Cattle which had not conceived at first insemination could be included in a latter round of insemination thus improving overall herd pregnancy rates. This is critically important in the dairy industry where milk production depends on lactation, which requires cows to become pregnant.

The merits of sexed semen

The use of sexed semen has also been an important stimulus for research into some aspects of female reproduction focusing on areas such as defining the minimal numbers of sperm which can be inseminated to achieve pregnancy, and the value of the different sites where semen could be deposited (for example cervix vs uterus). To the dairy industry, being able to bias the sex of calves towards females has meant replacement heifers are readily available either to replace non-productive cows or to expand the size of the herd milked. This has led to a significant increase in herd size which has in turn impacted on the number of dairy farmers. Perhaps the most significant stimulus to
research has been the development of MOET (multiple ovulation and embryo transfer) programs. In MOET programs, superior performing females are stimulated to superovulate by injection of hormones. The females can then be inseminated using semen from a superior male, and the resultant embryos, which are the product of superior males and females, are then transferred to recipient cows who have had their oestrus cycles synchronized by hormone treatments. These recipient cattle are standard herd animals but their progeny are the offspring of the superior males and females. This allows propagation of superior offspring at a much faster rate than is naturally possible. In a variation in this approach, after collection of the oocytes from the superior donor female they can be divided into groups and then each group can be fertilized by semen from different bulls. Thus several bulls are effectively bred to the same female at the one time. The resultant embryos are cultured to blastocysts in vitro and then transferred to recipient females as previously described. This permits a breeder to evaluate the different offspring for performance measures such as growth rate, carcass composition, which are attributable to the different sires.

A variation of MOET called JIVET (juvenile in vitro fertilization and embryo) involves superovulation and collection of oocytes from juvenile females which are known to be of high genetic merit, their fertilization in vitro with sperm from high value bulls and the transfer of the fertilized eggs to standard recipient females. This shortening of the generation interval further contributes to acceleration of genetic gain.

In all cases embryos undergo a selection process before transfer with only the best quality embryos being transferred to ensure the resultant offspring are of the highest quality. Generally, selection of embryos is based on morphology alone. Unfortunately, this is not always a totally reliable procedure and there is an increasing demand for more objective measurements of quality, such as might be provided by quantitative gene expression. Such methods involve taking a biopsy from the embryo for analysis. This itself can be a damaging process with a percentage of biopsied embryos failing to develop. In addition, we need to understand much more about which genes are important for development and what differences in timing of expression of these genes means for successful development. There is no doubt that genetic analysis of biopsied embryos will become a means for embryo selection in the future, because of the amount of information about the embryo which can be obtained.

What is emerging from both in vitro and in vivo studies is that the future of the embryo is highly influenced by the environment in which it develops. This begins as early as post-fertilization and continues through to implantation and perhaps even beyond. After natural mating, AI and also embryo transfer the greatest period of pregnancy failure is in the first 45 days. Mismatch between embryo development and the uterine environment is the primary mechanism by which non-viable embryos are naturally eliminated. Changes in gene expression as a result of in vivo or in vitro environment induced epigenetic changes needs more research as does the whole overall picture of the normal changes in gene expression seen during pre-implantation development.

We have already identified several aspects of the road forward which need to be clarified in order to enhance the overall benefits that can be achieved from manipulation of fertility. However, many others remain to be resolved (particularly since some of the potential issues have yet to be identified, let alone solved). Developments in microscopy and imaging will permit more accurate morphological assessments of developmental competence of embryos, stem cell technologies will impact especially through the prospect, already partially realized, of producing gametes from stem cells and the use of gene editing to effectively "shape" the developing embryo, are but three possibilities for the foreseeable future.
Integration of genetic selection and reproductive technologies to accelerate genetic gain

Perhaps the most significant advances for the reproductive technologies and their impact on animal breeding have occurred in the sister fields of bioinformatics and molecular genetic technologies. These have led to advanced genomic selection as a powerful new tool increasing genetic gain in livestock, particularly cattle. Their combination with reproductive technologies to decrease generation interval have opened new possibilities for identification and selection of animals with desirable genotypes.

Whole genome sequencing is now routinely performed in a number of species and as the technology improves and the cost decreases sequencing the genome of individuals will become routine. Smith, in 1967, first suggested using genomic data to improve selection. As genes either linked to quantitative trait loci (QTLs) or major genes were identified especially with traits with low heritability, such as fertility, or where large phenotype databases did not exist, marker assisted selection (MAS) initially offered the possibility to significantly improve the precision of selection of animals for an array of production or health traits. That this was not realized in most cases was because of adverse cost:benefit ratios (i.e. the cost of collecting the data outweighed the benefit gained). However, with advances in genomics, and decreasing costs of obtaining data, the movement away from using genetic loci with large effects on specific traits to using genomic selection (GS) based on genomic breeding values (GEBVs) has occurred.

The National Center for Biotechnology Information (NCBI) holds full or partial sequences for an ever increasing number of species including at least 50 mammalian species. For example, the cattle genome is 2,670,422,299 base pairs in size and contains about 22,000 genes of which almost 14,000 are common to all mammalian species. Dense marker maps can be compiled based on quality sequence data and knowledge of the genome, and these serve as the basis for whole genome screening. Across species single nucleotide polymorphisms (SNPs) are the most common, generally biallelic variation in DNA which are both easy to detect and interpret. The cattle genome contains some 13,146,622 SNPs. SNPs could thus be used to support development of genotyping arrays and permit the sort of scale of genotyping to support breeding decisions. The SNP genotypes permit collection of information on linkage disequilibrium which allow identification of specific QTLs or large effect genes. Companies such as Illumina and Affymetrix have played a vital role by producing bovine SNP arrays with different formats. These initially had a few thousand SNPs, but more recent arrays include 650 to 800,000 SNPs. This permits routine screening for genetic evaluation as well as more refined molecular genetic research. Development of SNP chips drove a reconsideration of what constituted a breeding value and how whole genome information could be utilized. Genomic selection seeks to: 1) employ marker data to image the genome, 2) link genotype and phenotypic data to estimate marker effects and 3) use GEBVs to aggregate marker effects for selection. Estimation of reliable GEBVs requires large populations of animals which have detailed genotype data and trait phenotypes. Calculation of prediction equations can be set up and the genetic merit of ANY individual can be predicted from its SNP genotype. This can be done easily using biopsies from embryos which can yield DNA and so the genetic merit of embryos can be calculated as early as prior to implantation or indeed at any foetal age from either sex as long as the reference population is sufficiently closely related. These genotyped embryos can then be cryopreserved before being thawed and implanted when needed.

Breeding selection often includes traits with low heritability such as fertility, or traits which cannot be measured directly because they are limited to one sex (such as milk traits in dairy cattle) despite being highly desirable characteristics upon which to base selection. This can be achieved with reliable weighting formulae. Reproductive techniques such as MOET, JIVET and preimplantation embryo biopsy have reduced generation intervals dramatically making selection far faster. When using these advanced reproductive techniques coupled to genetic selection the inbreeding co-efficient must be carefully monitored to prevent deleterious genetic outcomes. Optimal contribution selection, which places a penalty on inbreeding while maximizing genetic gain, has been used to model the outcome of different selection strategies. Assuming a 1% increase in inbreeding rate per generation and when combined with genomic selection MOET programs were estimated to increase the rate of genetic gain by 38–76% compared to programs based on AI.

A particularly important need is to identify both male and female genes which impact fertility, to enable them to be selected together with desirable production traits. Analysis shows that genetic regions associated with fertility are present on all 31 bovine chromosomes emphasizing the complexity of this polygenic trait. Regions on chromosomes 1, 5, 14 and 16 were associated with female fertility traits, whereas the X chromosome was associated with male fertility traits. There are almost certainly other regions which remain to be mapped for this complex trait.
What then of the future?

Cattle are the source of two vital components of the human diet – meat and milk. Management of product image and market risk is thus vital. It is therefore essential that in our desire to increase productivity that there is strong public acceptance and understanding of changes being made. The issue of the use of hormone releasing implants to promote animal growth in the beef industry is an example of what happens when industry doesn’t adequately address the concerns of the public. More recently the issue of the cloning of elite bulls and cows caused significant public debate with the result that cloning is presently not considered a commercially-viable strategy (although it remains a research tool). In part this was because key research goals, viz to produce greater numbers of elite, healthy animals efficiently and thus at reasonable cost, could not be achieved.

Genome editing

Currently the issue of genome or gene editing technology is under consideration. With this approach, a chosen bovine genomic locus can be precisely modified in somatic cells, to knock out (KO) or knock in (KI) a gene via homologous recombination, a gene-targeting strategy that to date has used experimentally in mouse embryonic stem cells. The development of designer nucleases—such as zinc finger nucleases (ZFNs) and transcription activator-like effector nuclease (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9)—has enabled highly efficient and more facile genome engineering in cattle. Genomes can be engineered with this technology at single-nucleotide precision. This is completely distinct from the previous methods of creating genetically modified cattle, where foreign DNA was randomly integrated into the animal genome, sometimes accompanied by integration of bacterial or viral DNAs. This sometimes resulted in deleterious effects on the transgenic animals through disruptions to their genomes. The goal of transgenic animals producing valuable medical compounds in their blood can be achieved. This nbroadened to an interest in fertilization. This is important to promote animal growth in the beef industry.

We hope this has given you a sense of how two different research areas, i.e. reproductive biology and genetics, have become intertwined to deliver cattle which can produce better quality meat and milk to meet the demands of a constantly increasing global population.

Further reading


Michael Holland is a Professorial Research Fellow is the School of Veterinary Science at the University of Queensland. Professor Holland has some 39 years working in various aspects of fertility and reproductive biology working in part this was because key research goals, viz to produce greater numbers of elite, healthy animals efficiently and thus at reasonable cost, could not be achieved.

Professor Michael McGowan completed his undergraduate veterinary training at The University Sydney in 1979 and after spending several years in rural practice completed an internship in food animal medicine and surgery and then a residency in theriogenology at the Western College Veterinary Medicine (Saskatoon, Canada). He undertook his Phd studies at The University Sydney studying the impact of pestivirus (BVDV) infection on the reproductive performance of cattle. He then joined the School of Veterinary Science at The University of Queensland where his research has included defining the pathogenesis and impacts of leptospirosis, heat stress and neosporosis on dairy and beef cattle fertility, development of bull selection and management strategies, defining factors affecting the reproductive performance of beef and dairy cattle and development of methods of synchronizing estrus and ovulation to enable AI at a fixed time. He was appointed Professor of Farm Animal Medicine and Surgery at The Royal Veterinary College (University London) in 2000 and was invited to become a founding diplomat of the European College of Animal reproduction in 2001. Currently he is Professor of Livestock Medicine in the School of Veterinary Science at The University Queensland and Honorary Professor of Farm Animal Medicine and Surgery at The Royal Veterinary College. Email: m.mcgowan@uq.edu.au

Further reading

Preimplantation genetic screening (PGS) involves the identification of chromosome abnormalities in IVF embryos (rather than targeting diagnosis to a specified gene). Chiefly employed for couples with advanced maternal age, recurrent miscarriage or recurrent IVF failure, it aims to improve IVF success, and reduce miscarriage and affected live birth rates. The process involves the sampling of cells by embryo biopsy, cytogenetic diagnosis, then selective transfer of an apparently chromosomally normal embryo in the hope of establishing a pregnancy. Although PGS is the most common variant of PGD (preimplantation genetic diagnosis), accounting for 80% of cases it has, from the outset, been one of the most controversial areas of reproductive medicine. The subject of intense debate, it attracts opinions ranging from recommendations that it should be applied in all IVF cases, through to the suggestion it should be discontinued completely. What do you think? Should it continue or not?

At its broadest, the term preimplantation genetic diagnosis (PGD) can be defined as the diagnosis of genetic disease or chromosomal abnormality in human IVF embryos. The starting point involves counseling for, and testing of, a couple at risk of transmitting a genetic disorder, or who have manifest a need for assisted reproduction. Standard IVF treatment then follows and the embryo (or sometimes the oocyte) is biopsied, i.e. material from it is removed for sampling. Genetic diagnosis on the biopsied piece can then be genic, (i.e. looking for a disease-causing mutation in a single gene) or chromosomal, (i.e. looking for extra or missing chromosomes [or parts of them]). This article will focus primarily on the latter, most commonly referred to as “PGS” (preimplantation genetic screening). The terms PGD for aneuploidy (PGD-A) and PGT-A (preimplantation genetic testing for aneuploidy) are accepted alternatives for the same suite of techniques, but for the purposes of this article we will stick to PGS.

PGS exists to try and improve IVF success rates, to reduce the incidence of spontaneous abortions and avoid the births that might be affected with chromosomal disorders. For reasons explained below, PGS remains a controversial area of reproductive medicine.

**The problem with chromosomal disorders**

For the most part, chromosome abnormalities do not end well. The vast majority of humans have 46 chromosomes in each cell; 23 pairs of autosomes numbered 1–22 then, in females, a final pair that we call “X” and, in males an “odd couple” – one of which is an X, and the other a tiny Y. Any deviation from that norm usually has a consequence, which can be as relatively mild as impaired fertility or as severe as the embryo not making it past the first few divisions. By far the best known of chromosome disorders involves three copies (trisomy) of the smallest autosome – number 21. Down syndrome (the most common cause of mental retardation in humans) frequently ensues, however, eight times out of ten, trisomy 21 leads to a first trimester pregnancy loss. Indeed, that is the fate of the vast majority of embryos with extra chromosomes, whereas those with missing chromosomes don’t even make it to the point of clinical recognition. Taken together, chromosome disorders are the leading cause of mental retardation, pregnancy loss and IVF failure. They are one of the leading causes of birth defects and stillbirths. They can lead to infertility, high birth weights, low birth weights, abnormal placentas and obstetric complications. On the face of it, you don't want to be knowingly transferring IVF embryos with chromosomal disorders.

**Who potentially benefits from PGS?**

Couples presenting for PGS generally do so for reasons of advanced maternal age, previous recurrent miscarriage, previous recurrent implantation failure or severe male infertility. All of these referral categories can typically carry a higher risk of producing chromosomally abnormal conceptuses. Global figures are becoming increasingly difficult to collate but a reasonable estimate is that around 80,000 PGS cycles have been performed worldwide since its inception, accounting for 4 out of 5 of all PGD cycles.

The theory is straightforward; if you detect a chromosome disorder, do not transfer that embryo. However, real life tends to be rather more complicated.

**An overview of embryo biopsy for PGS**

It is a fact of PGS that, if you want to do genetic diagnosis, you need genetic material and, for the most part, that...
The process of PGS from biopsy to diagnosis

1. Three types of biopsy to sample genetic material for PGS: Polar body, cleavage stage (blastomere), and blastocyst (trophectoderm).
2. Polar body biopsy: the polar bodies are the by-products of meiosis and are thus not used in the subsequent embryo. This is therefore the least invasive of the methods.
3. Cleavage-stage (blastomere) biopsy. One cell is removed from the embryo at around day 3 post-fertilization, when the embryo is around eight cells. This has been the most used of approaches but has recently fallen out of favour as evidence emerged that the embryo could be damaged.
4. Blastocyst (trophectoderm) biopsy. At blastocyst stage (about day 5) the embryo consists of the inner cell mass (which will form the foetus), the fluid filled blastocyst cavity (which could perhaps in future be used as a source material for PGS) and the surrounding trophectoderm, from which 5–10 cells can be biopsied for PGS. This is now the most commonly used approach for PGS.
5. Cells were initially fixed to glass slides for diagnosis.
6. FISH (fluorescence in-situ hybridization). In this preparation, chromosome 13 is labelled in green, chromosome 16 in aqua, chromosome 18 in blue, chromosome 21 in red and chromosome 22 in yellow. The three red dots indicate trisomy 21.
7. Whole genome amplification: Methods include degenerate oligonucleotide primed PCR (DOP-PCR), multiple displacement amplification (MDA) and MALBAC (multiple annealing and looping based amplification cycles).
8. Array CGH (comparative genomic hybridization) microarray.
9. Output of a-CGH analysis showing multiple chromosome gains and losses.
10. NGS (next generation sequencing).
11. Output of NGS analysis showing the same multiple chromosome gains and losses as a-CGH but with a greater dynamic range.
12. SNP chip microarray.
13. Karyomapping analysis showing the origin of chromosome abnormalities, chromosome exchange events and extra/missing chromosomes.

means accessing embryonic cells. Sampling cells from an embryo (or oocyte) is, of necessity, quite invasive. Herein lies one of the core problems of PGS; if you compromise, in any way, the future development of the embryo by performing the biopsy then you have defeated the object of doing PGS in the first place.

Typically, there are three sources of cellular material available for biopsies prior to PGS: polar bodies (the by-products of maternal meiosis) from oocytes; single cells from cleavage-stage (roughly eight-cell, day-three) embryos, and trophectoderm cells from blastocyst-stage (day-five or six) embryos.
The polar bodies (PBs) are the consequence of asymmetric meiotic division in mammalian eggs and thus do not make a contribution to further embryonic development. There are usually two of them (although the first one can divide), the products of the first and second meiotic division. Polar body biopsy is less invasive than biopsying cells that the embryo might otherwise have used. The main drawback for PGS is that it can only detect those chromosome abnormalities that arise in maternal meiosis and, although this is actually the majority, some errors arise in the sperm, and others arise in the embryo after fertilization. The other issue is that randomized controlled trial (RCT, see later) data involving PGS by this approach does not appear to increase the likelihood of a live birth within one year compared to regular IVF, but it does appear to decrease the miscarriage rate. As a general rule, however, polar body biopsy has not been as widespread as cleavage-stage nor blastocyst-stage biopsy.

Cleavage-stage biopsy involves the removal of one or two cells (referred to as blastomeres) on embryos 3 days after fertilization, which, by then is usually around the six-to-ten cell stage. Surrounding the embryo is a glycoprotein layer called the zona pellucida and, in order to get to the cells, one needs to dissolve a little window of it with acid, or else make a hole with a laser. A biopsy pipette removes the cell or cells in question. This approach is the oldest and was, until recently, the most widely used approach for PGS. There is an inherent problem with cleavage-stage biopsy; remove only one cell and that is all you've got on which to make your diagnosis. Remove two or more, and you’re significantly reducing your embryonic volume, which can impair its future development. When it was first used for PGS, the signs were good, however the RCTs were not. Some very famous studies even suggested that it made things worse, i.e. it resulted in IVF success rates going down. Opponents of PGS argued that this was sufficient evidence to discontinue PGS completely, the proponents argued that the opponents were just doing it wrong, i.e. they were being too rough with the embryos. Back in 2007–2008, it is probably fair to say that the opponents won the day and PGS was in the doldrums.

At that stage, the possibility for blastocyst biopsy was already known. The reason why it did not immediately become popular was essentially that IVF in the nineties and noughties was not producing enough blastocyst embryos in culture. Blastulation is the first stage of embryonic differentiation where the inner cell mass (which will go on to form the foetus) and the trophectoderm (which will go on to form the placenta) are first seen as distinct structures. Removal of five to ten cells from the trophectoderm is therefore an attractive option (as the future foetus is left untouched), provided (as is now the case due to improved culture conditions) a sufficient number of blastocysts can be generated in each IVF cycle. With blastocyst (trophectoderm) biopsy you have a number of advantages. First, with >100 cells to go at, the removal of less than a tenth of them is less likely to harm the embryo. Second, with more cells on which to make the diagnosis, you can be more confident of your result. Third, the blastocyst seems to "sort itself out" chromosomally and day five embryos tend to be more chromosomally normal than day three embryos on average. Finally, there seems to be some evidence that this approach could be less operator-dependent and thus more reproducible between clinics.

Whereas RCTs for cleavage-stage biopsy have produced mixed results, some suggesting a benefit whereas others reporting that they can cause a reduced implantation rate, there seems to be no such problem after trophectoderm biopsy. Indeed, most RCTs demonstrating the benefits of PGS are after blastocyst biopsy.

Alternatives to embryo biopsy

Less invasive approaches that do not involve cell removal include blastocentesis, the aspiration of blastocoelic fluid (the cavity surrounded by the trophectoderm in the blastocyst embryo). The fluid certainly contains DNA, it has been found in over 80% of the samples tested and experiments have shown >97% concordance with results from trophectoderm biopsy. However, the confounding effects of both false-positive and false-negative outcomes suggest that it is a while before this will become mainstream. Analysis of spent culture medium for DNA is also a possibility, reportedly making the right diagnosis over 90% of the time in recent studies. This remains a little too inaccurate to enter mainstream diagnostics. Finally, time-lapse imaging and morphokinetics for
PGS has been looked at for some time. Time-lapse devices (basically a microscope and still camera inside an incubator) are very popular in IVF clinics as they can monitor embryos closely and minimize the need for embryologists to take the embryos out of the incubator quite so much. There are some suggestions that chromosomally abnormal embryos can display different kinetic parameters to chromosomally normal ones, with the latter following a more defined pattern. Definitive diagnosis is not clear, however, with many groups not showing significant differences. Thus, morphokinetic time-lapse monitoring alone is probably not sufficient to assess the chromosomal status of an embryo, but it may have a role in assessing implantation potential.

Taken together therefore, non-invasive approaches may well be promising, but they currently lack sufficient reducibility to replace standard PGS technology.

**FISHy beginnings**

Unlike use of PGD to screen for single gene mutations, detection of chromosome abnormalities (PGS) is less targeted. The aim is to try and detect as many chromosomes as possible. The first approach to be employed for this purpose was fluorescence in-situ hybridization (FISH). From the very early stages, PGS was both the most popular form of PGD, but was also the most controversial. With the stated aim to improve IVF success, decrease the time to pregnancy, reduce the risk of pregnancy loss and the birth of chromosomally abnormal children, PGS started out by screening a limited number of chromosomes. FISH is a multicolor technique directly on the nuclei of embryo blastomeres first used to sex embryos for families at risk of passing on sex-linked diseases like muscular dystrophy. Soon thereafter 5 to 12 colour FISH approaches were used. Nonetheless, from the outset, the approach had a number of drawbacks. Most significantly, not all chromosomes were screened. There was potential to generate both false-negative and false-positive results and these seemed to increase with the more chromosomes that were screened. Other problems included inter-centre variation in techniques such as embryo biopsy, single cell fixation to slides and analysis protocols. Around 2008–2010, following unfavorable RCT data, FISH was discontinued and replaced with whole genomic approaches.

**Whole genome analysis**

As the starting material for PGS is just a few cells (6 pg of DNA/cell), if whole genome analysis is going to be effective then the DNA needs to be amplified. Without going into specifics, there are an assortment of approaches, variously called degenerate oligonucleotide primed PCR (DOP-PCR), multiple displacement amplification (MDA) and MALBAC (multiple annealing and looping based amplification cycles). These methods can, if used correctly, be uniform, specific and reproducible.

Once amplified, two very accurate approaches for analyzing chromosome copy number for every chromosome has been described. The first, array-comparative genomic hybridization (a-CGH) is similar in some ways to FISH in that it involved DNA hybridization on a glass slide. Here, however, it co-hybridizes differentially-labelled test (amplified embryonic) and reference (normal) DNA on microarrays. Analysis with specialized software enables an automated ratio comparison of the ratio of red and green intensity information of fluorescent colours at each genetic locus along the chromosome. Chromosomal gains and losses are accurately detected as subtle swings towards the red or green. This approach has been applied successfully in polar body, cleavage-stage and blastocyst PGS but is limited in its ability to detect some abnormalities. Next Generation Sequencing (NGS) is now rapidly replacing a-CGH. This involves whole genome amplification, breaking down to small fragments, parallel DNA sequencing (low coverage) then “binning” to represent the number of sequence reads per chromosome. Using this approach, multiple samples can be processed simultaneously, thereby, reducing cost and workload. An added advantage of this approach is that it can also be used to detect copy number of mitochondrial DNA which is proposed as a biomarker for the estimation of the viability of the embryo (this is, in fact, another controversial area we won’t expand upon here). Real time quantitative PCR (RT-qPCR) can be robust, rapid, accurate and cost-effective however there are a number of types of abnormalities that could be missed by this system such as mosaicism (see below) and abnormalities caused by parts of, not whole, chromosomes.
Simultaneous PGD and PGS (Karyomapping)

Certain types of microarrays (called SNP [single nucleotide polymorphism] chips) detect genetic variation across each chromosome and have been applied to embryo biopsies. By using the SNP chip output for each parent plus a genetic relative of known disease status (this is usually an affected child), four distinct sets of markers can be identified. These basically represent each of the parental chromosomes. An analysis protocol called “Karyomapping” essentially detects the inheritance of these blocks of chromosomes. By comparison with the relative of known disease status (affected child), the inheritance of a disease gene can be detected, as can the presence of extra or missing chromosomes. The test is therefore relatively universal, being able to detect any single gene disorder and every chromosome disorder simultaneously. This is now a relatively mainstream approach, at time of writing, used to treat 6,000 patients.

To freeze or not to freeze?

Traditionally, those clinics performing PGD and PGS were in a race against time to perform the diagnosis within a 24 hour (or ideally same day) time scale so that the embryo could get transferred before blastulation happened (the point being that blastocyst transfer success rates were historically low compared to cleavage stage). Given that success rates for frozen embryos were also lower compared to fresh, the motivation to freeze PGS embryo was low. However, concurrent development of enhanced culture conditions and much improved fast-freezing (vitrification) approaches has led to a general acceptance of freezing as part of the PGS procedure. Indeed, more recently, strategies that involve freezing all embryos are becoming popular, with embryo transfer in the later menstrual cycles, giving the opportunity for the endometrium to be more physiologically receptive to the embryo.

The issue of mosaics

It would be very wrong to imagine that all cells in a human embryo are uniformly normal or abnormal. Mosaicism is a frequently observed phenomenon, where more than one chromosomal constitution is observed in the same embryo. Obviously, this presents a challenge for PGS given that the diagnosis is made on just a small sample of cells. Mosaicism can arise with an embryo that was previously normal, undergoing a post-zygotic chromosome segregation error or an originally abnormal embryo that underwent “correction” in a proportion of the cells. There are a variety of mechanisms that have been proposed to explain mosaicism – all or most probably occur to a greater or lesser extent. The number of human IVF embryos that are mosaic to some degree is not completely established but even conservative estimates suggest that the number is around 75%. Indeed, as mentioned, cleavage-stage embryos are thought to be more chromosomally abnormal (and hence more prone to mosaicism) on average than trophectoderm-stage embryos. Moreover, while the incidence of chromosomal abnormalities in general tends to be associated with maternal age, this does not appear to apply to mosaicism per se. Other factors may be involved, such as ovarian stimulation protocols, embryo culture conditions, genetic background and environmental pollutants.

The medical outcomes of mosaicism are dependent upon many factors, such as the timing of the error, the proportion of the embryo that is involved and which chromosome is affected. As a general rule, an embryo would most likely be more severely affected when the error occurs at earlier stages of development, or in meiosis, as a greater number of cells are likely to be affected. Sadly, nobody has a definitive answer to the incidence of mosaicism in IVF embryos and, in fact, it tends to be reported only when it is identified. As mentioned, these days this is usually from a five to ten cell biopsy of an embryo that has about 50–150 cells in it. Of all the available techniques, NGS is really the only one that can accurately detect mosaicism from a trophectoderm biopsy and, of course, this depends on whether a mixture of normal and abnormal cells is actually present in the sample taken. In an ideal world, a rigorous “cell by cell” approach to assess the relative incidence of chromosome abnormalities in trophectoderm and inner cell mass in a large cohort of embryos should be performed. To the best of our knowledge however, this has yet to happen. Whether diagnosis of a sample of trophectoderm (which will go on to form the placenta) is, by and large, representative of the inner cell mass (which will go on to form the baby) is still an open question but a body of evidence is now pointing to reasonable concordance (about 95% of the time when all studies are taken into account). The issue of whether or not mosaic embryos (as defined by a least one variant cell being detected in the trophectoderm biopsy) should (or could) be transferred (especially when there are no “normal” diagnoses) is a subject of much discussion. Indeed, at least one recent study has reported unaffected live births following the transfer of mosaic embryos. The debate continues and guidelines evolve. However, whether or not PGS should be performed at all remains a bone of contention.

What is the argument about?

There seems to be little doubt that a combination of a widespread move to trophectoderm biopsy plus, for diagnosis, aCGH (and later NGS) coupled with the
greater willingness to adopt freezing strategies has improved the situation. PGS is certainly a lot more satisfactory than in the days of cleavage-stage biopsy and FISH. There is now a growing body of evidence that PGS can be beneficial, in the right hands, and under certain circumstances. Some studies including meta-analysis and systematic reviews have demonstrated some improvements in pregnancy rate, live births, and miscarriage rate. However, the most recent trial [Single Embryo TrAnsfR of Euploid Embryo - STAR Trial (NCT02268786)] gave encouragement to both supporters and opponents of PGS, reporting a small, but not statistically significant, overall IVF success rate, but nonetheless significant improvements in the advanced maternal age category.

In essence, the debate revolves around what we consider to be a sufficient body of evidence-based medicine. If you Google “evidence-based medicine” (EBM) you will find a definition along the lines of “an approach to medical practice intended to optimize decision making by emphasizing the use of evidence from well designed and conducted research.” Nobody would argue with that. But the questions remain, what do we mean by “well-designed” and “well-conducted”? The problem with reproductive medicine in general (not just PGS) is that, conceptually, it is unlike any other areas of medicine for the following reasons. Firstly, patients undergo pretty radical treatments with an intention that does not always involve benefiting their own health. Secondly, there are few, if any other, medical disciplines where so many different academic fields combine. Thirdly, reproductive medicine is a field in which barely perceptible “good gardening” skills are so crucial. Technical ability, for example, in performing embryo biopsy and general handling of embryos, can have a significant impact on outcomes. Fourthly, it is the only form of treatment where the physiologies of two individuals combine (sometimes “couples” don't even meet in the case of donor eggs or sperm) and where the sole intention is to produce another human being.

So, at what stage is the evidence-base sufficient enough for everyone to agree that PGS is a good thing? Opponents of PGS base their argument on the assertion that any intervention should only be introduced into the clinic after at least one favourable double-blind randomized clinical trial. But, in PGS, it’s hard to imagine how we could introduce a placebo. Also, a poor embryologist could single-handedly ruin an RCT by inadvertently performing the embryo biopsy badly. So, here is the paradox: for PGS, are retrospective, single-centre studies equally important as randomized trials to the evidence base? Indeed, a meta-analysis of multiple centres could mask especially good (or bad) practice of specific clinics. An RCT can be beautifully designed but badly performed. What single-centre retrospective studies lack in prospective design, they could compensate for due to the rigour with which they are performed. Opponents of PGS argue that even the most recent studies are not sufficiently robust since clinics are motivated by the need to be seen to be innovating (and possibly by the money that comes in by billing patients for “the latest” therapy). A perception that a clinic is “cutting edge” can be crucial to their survival. Arguments in favour of PGS (and to be fair, these are the views of most of the IVF community) say that there is enough EBM to support PGS in that there are few areas of reproductive medicine where we can wait for RCTs.

Quite a number of widely used procedures have never been subject to an RCT, yet their benefits are obvious without one. The best example would be intracytoplasmic sperm injection (ICSI), the most comment treatment for male factor infertility.

If you want an insight into how a relatively impartial observer sees the argument between the proponents and opponents of PGS written by one of the current authors (Griffin) and Sally Sheldon of the Kent Law School (see further reading below). In this article “Jacob and Giuseppe” are introduced as imaginary scientists created to represent the extreme sides of the PGS argument. The exposition is a little “tongue in cheek” but paints a picture of two individuals, increasingly more polarized and entrenched in their own opinion, regardless of how much evidence appears. In this context, the authors ask the reader to consider the couple’s perspective: if they are looking to have a child by IVF (and PGS) they would perhaps choose a clinic that is dedicated to making that treatment work, not one that has an open mind based on the result of an RCT. If this were you and your partner, would you not want to find out the success rates of that particular clinic, rather than an RCT? From an ethico-legal perspective: what are the implications of not putting PGS into practice? What about the harm (both physically and psychologically) that could be inflicted on a couple who had a pregnancy (or a baby), assuming that they could, and would, have chosen to avoid this if they had been given the option of PGS? These questions are not easily answered.
Conclusions

In our opinion, nobody should ever take the extreme view of either a “Jacob” or a “Giuseppe.” Whichever way one’s opinion leans, more research needs to be done. PGS will never be perfect, but we can make it better. Conceptually it “should” work but an RCT involving many centres of varying quality may not necessarily show that. Through more research and more quality control, however, the benefits could ultimately be realized. New technologies should always be considered and the only way to get better at something is to practice it.

Additionally, study of IVF embryos can tell us so much about our early development, information that is also relevant to naturally conceived pregnancies. For instance, chromosome mosaicism can have severe clinical consequences, and there is a great opportunity to study this phenomenon in far more depth than was previously possible. Interested readers are directed to our article in the journal Reproduction for a list of biological questions that we might have answered via study of this material (see further reading).

As noted earlier, PGS is controversial, but it’s unlikely to go away. Study of model organisms (e.g. pig and cattle embryos) and conduct of basic research will add to our understanding. Put bluntly, both “Jacob” and “Giuseppe” need to grow up and listen more to one another. We need to appreciate that the evidence base will never be perfect but to be honest with patients about where it is and where it might be, if only we carry on working on it.

So, here’s a final plea (and apologies for repetition from our previous article). Companies, governments, research councils and charities: please work together to generate more funding for basic research into the chromosomes of IVF embryos. Everyone will benefit. For the furtherance of medical research, a greater understanding of chromosome abnormality in general and mosaicism in particular will lead to improved patient care. As the mean age of families get older, this is one of the most important challenges in the medical world.

Further reading


Darren Griffin is Professor of Genetics at the University of Kent. A graduate of the University of Manchester and University College London he has performed research at Case Western Reserve University, the University of Cambridge and Brunel University. In a 30-year research career involving ~200 publications he performed the first chromosomal PGD and was instrumental in the development of Karyomapping. His research interest mostly centres around chromosome behaviour in early development and in evolution and he is a champion of public engagement in science. Email: d.k.griffin@kent.ac.uk

Çağrı Oğur is a senior clinical scientist working in the field of preimplantation genetic diagnosis (PGD) and genetic testing for more than 15 years. Çağrı is also doing her PhD in the bioengineering department at Istanbul Yildiz Technical University. Her interests are PGD for chromosomal structural rearrangements, aneuploidy, mosaicism, and preimplantation HLA-typing. She is currently a lecturer at the Istanbul Arel University in Turkey. Email: cagribeyaziyurek@gmail.com.
Putting fertility research into motion

Allan Pacey is Professor of Andrology and Head of the Department of Oncology and Metabolism at the University of Sheffield, UK. He was previously Chair of the British Fertility Society from 2012 to 2015 (the first non-clinician to hold that post) and is currently Editor-in-Chief of their official journal Human Fertility. Professor Pacey has recently worked with the World Health Organization, and is active in public engagement activities around both fertility and cancer. He was awarded an MBE in 2016, for services to Reproductive Medicine. Science Editor Chris Willmott spoke to him about his work.

Thank you for sparing the time to talk to us. Can I ask you to begin by telling us about your own journey into the field of fertility research?

I grew up in rural Yorkshire in the 1970s and it’s true to say going to university wasn’t the kind of thing that rural boys did at the time. So, in modern parlance, I’d now be seen as a “Widening Participation” success story. In fact, I only applied to university because my best friend did. I went to study life sciences at the University of Hull. It was whilst I was there that I got really fired up about reproductive biology.

What was it that triggered your interest?

I think there were three things. The first was reading all 5 volumes of Roger Short’s textbook on Reproduction in Mammals in the Hull University library where, incidentally, the poet Philip Larkin was still head librarian; we would see him walking around. The second was the influence of an inspiring young lecturer John Robinson, who went on to set up the Hull IVF centre. The third was reading the Warnock report [The 1984 Report of the Committee of Inquiry into Human Fertilisation and Embryology, chaired by Mary Warnock], which struck me as a well-written and thoughtful paper.

So what was the next step?

After Hull I went to St Andrews to do my PhD, under the supervision of Matthew Bentley. At that stage I still didn’t have an active interest in human infertility; the research was in cell biology, specifically on sperm maturation in the lugworm. This led into a Royal Society research fellowship which allowed me to spend a year at a research station near Nice, in France. I was still working on the lugworm but also sea urchins as well. The main focus was on the influence of calcium on sperm movement. I didn’t clone any channels or anything like that, but we did make some significant observations, including stumbling onto the importance of certain wavelengths of light in directing the movement of these sperm.

From France, you went to Sheffield, where you still work?

Yes, that was the point at which my direct interest in human fertility began. The team in Sheffield were interested in how sperm behave in the female reproductive tract, or rather in model systems of it. I worked with clinical staff for the first time, consenting women who were undergoing hysterectomy in order to obtain Fallopian tube cells to culture in the lab. We did make some discoveries, but the work was eventually abandoned because it fell out of fashion. However, not before a certain amount of press coverage.

An aeronautical engineer at Glasgow, Richard Green, saw something on TV about the work I was doing and recognized that there was potential overlap with the kind of studies he was doing in aeronautical engineering (using fluid dynamics). This led into a very fruitful partnership in which we were able to show much more clearly than previously how the movement of the flagellum achieved the swimming motion of sperm.

Jumping to the present, what is the focus of your current research?

I’m still working on improving our understanding of aspects of male infertility, including the basic biology of human sperm. We have some very promising work developing an NMR-based screen for sperm health. At the moment tests for sperm quality are destructive, it’s like saying “oh, that was a good one” by which time it is too late. We are studying live sperm using various methods of magnetic resonance spectroscopy. The fundamental principles are the same as those involved in an MRI scan, so we are confident the technique is safe to the sperm.
Sperm need to generate ATP to sustain movement. The tail and mid-piece of sperm are relatively rich in enzymes associated with lactate metabolism, but there has been debate about the relative roles played by glycolysis within the cytosol and oxidative phosphorylation in the mitochondria. The kind of NMR we are doing doesn’t provide structural information, but as we follow the processing of molecules like 13C-pyruvate we can get ‘snapshots’ of the relative proportions of metabolic intermediates. This gives us a handle for looking at differences between healthy sperm and poor swimmers, and the influence of local conditions on motility. The test isn’t instantaneous but, as I said, it is non-destructive and, of course, sperm are fairly robust since they may take up to 6 days from ejaculation before they reach the egg. If you want to know more, we have a SPERM research website dedicated to this aspect of our work (http://spermmnr.group.shef.ac.uk).

Alongside your scientific and clinical research, you’ve been heavily involved in some ground-breaking TV programmes about fertility. No-one who has seen the Great Sperm Race (2009) will forget the image of thousands of men and women in white boiler suits charging down a valley and up a staircase as they recreated the journey of sperm seeking an egg. Can you tell us a bit about how that came about?

The first thing to say is that there wasn’t really a cast of thousands, there were only about 25 actors, the rest was CGI trickery. The evolution of the Great Sperm Race is actually quite interesting. In 2006 I’d published a review article with Susan Suarez in the journal Human Reproduction Update. A young TV producer read the review and contacted me about the possibility of making a documentary. She made a pitch to Channel 4 and managed to secure some funding from the Wellcome Trust. The programme was very well received around the world and I still get emails about it. Last time I checked it had been shown in 24 territories around the world and had been seen by over 10 million people. It also spawned an online game, which is still available and has been played over 30 million times. The project was one of Sheffield’s Impact cases for the last REF.

As you are aware, this issue of The Biochemist is timed to mark the 40th anniversary of the birth of Louise Brown, the first IVF baby. What

’Sperm through the Valley’, taken from The Great Sperm Race, with permission of Blink Films.
would you say were the most important two or three developments in Assisted Reproduction Technology over that time?

Clearly, if I'm allowed, I'd like to choose the original pioneering work of Patrick Steptoe and Bob Edwards. They really didn't get the credit they deserved at the time. Edwards eventually received the Nobel prize in 2010, but Steptoe had died in 1988 and therefore missed out.

As a second choice I'd say the invention of ICSI – intracytoplasmic sperm injection – in which a single sperm is injected directly into the egg. This has been revolutionary for cases of infertility where the problem lies with the male partner.

Are there aspects of fertility treatment that concern you?

There are lots of things that are potentially worrying. Couples seeking help to conceive are incredibly vulnerable emotionally, and there are dangers that they will get exploited by unscrupulous clinics conducting procedures that won't really help, so called add-ons. Take the use of ICSI, which we mentioned earlier. In the UK roughly 50% of IVF treatments involve ICSI and since about half of infertility arises from the male partner, this seems reasonable. But in some parts of the world ICSI can make up over 90% of IVF procedures, and that doesn't seem right.

Better funding of fertility treatments on the NHS in England would help to guard patients against this, because the clinics they'd go to would have to follow the NICE guidelines (funding in Scotland is different, and more generous).

What would you say is the biggest unmet need in current fertility treatments?

At the moment we don't have any treatments for male infertility, only work-arounds such as ICSI and freezing of sperm when a decline in fertility is predictable (e.g. for people undergoing certain chemotherapies or for soldiers involved in front-line combat). In some senses, the success of ICSI has unhelpfully shifted the focus away from understanding and dealing with the underlying biology of the problem. If we knew why some sperm were immotile and how we could revive them, then it might be possible to develop a compound delivered as an ointment or a lubricant, which would allow them to swim. If this then allowed couples to conceive without IVF or ICSI then that could save them a huge emotional and financial burden.

Professor Pacey is an enthusiastic tweeter. To keep up to date with his work, please see www.twitter.com/allanpacey

Further reading, viewing… and gaming

- Spectroscopic Probes of Energy Regulation and Metabolism (SPERM research) http://spermnmr.group.shef.ac.uk
- The Great Sperm Race documentary. A copy is currently available at youtu.be/Fda5rigma14
Feedback cycles are useful concepts in biological systems, our everyday lives, and education. However, Higher Education is finding itself in something of a feedback crisis, with low student satisfaction despite high academic workload in this area. Here we explore how changing our conceptions of feedback in Higher Education could address this, and look at some positive cases of innovative feedback practice within bioscience disciplines.

Feedback cycles: in biology and education

Feedback is the process by which output signals from a given system inform or affect the input - creating a cycle or loop. This is commonplace in our everyday lives and inside our own bodies.

For example, our blood sugar is regulated through feedback (Figure 1A). In most healthy people, insulin, glucagon and other biochemical signals act to keep our blood glucose concentration within tight bounds—whether we’re running a marathon or relaxing after a big meal. In this case, feedback is used homeostatically to stick to the status quo.

Other feedback cycles amplify an outcome rather than maintain it, such as in platelet blood clotting and contractions during childbirth. Either way, feedback is cyclical in nature, rather than a one-way process or pathway.

Feedback has been adopted as a higher educational concept for a few decades now, but it’s become something of a sticking point. Across UK universities, the Assessment and Feedback section of the National Student Survey (NSS) is consistently among the lowest scoring.

Broadly speaking, students claim their feedback is of unsatisfactory quantity or quality. Meanwhile, staff claim the comments they worked hard to produce are largely disregarded.

One potential reason for this disconnect could be a tendency to view teaching as "transmission" from staff to student. In these cases, the educational feedback cycle can become linearized, with an overemphasis on staff rather than student activities (Figure 1B).

If this is true, efforts that solely focus on how feedback is delivered (i.e. by teacher action) rather than on how it is received and used (i.e. by student action) are unlikely to be wholly successful. This is analogous to the disease model of Type II diabetes in blood sugar regulation; there is plenty of insulin (feedback signal) to go around, but the relevant cells aren’t able to respond or utilize that signal effectively.

In these cases, simply adding more insulin (i.e. piling on more feedback signal) isn’t likely to work as a systemic fix, as it does not address the underlying issue. More careful modulation of the signal and how it is received are more likely to work in the long term.

Feedback myths and nostrums

Molloy and Boud encapsulated four feedback “nostrums” or pseudo-medications which may offer some comfort to those tasked with providing feedback. In reality, these myths may preclude addressing the staff–student disconnect (Figure 2).

Redefining feedback: completing the cycle

So how can we generate a healthier, more productive feedback system which benefits both students and staff? There is a current movement to re-conceptualize what “feedback” means in higher education.

Feedback isn’t "done" when information leaves the tutor’s mouth, pen or keyboard. It’s only genuine feedback if the student accepts, internalizes, processes and uses it as an input to improve future work. As it happens, this reconnects feedback in education with its original meaning: completing the cycle.

Another way of looking at it is to move from viewing feedback as transmission to a more active
dialogue between student and educator. In this model, students learn to seek specific judgement from others, becoming more active participants in their learning journeys. (Figure 1C)

**Removing barriers to engagement**

This is not easy. The difference between a student and, say, a pancreatic cell is that the student has the capacity for conscious thought and decision. Students, like most people, find feedback cognitively and emotionally draining.

Nash and Winstone note that “most students in higher education have received little or no prior guidance on how to use feedback effectively”. They advocate shared responsibility as way of removing various barriers to active engagement with feedback.

This starts with genuine conversations raising awareness of the nature and purpose of feedback in its many forms, and discussing rather than dismissing the very real emotional impact feedback can have. It includes training in how to make use of feedback, rather than assuming that cognisance.

Courses can better offer students the agency to make use of feedback with well-connected assessment tasks aligned to programme-level teaching and learning objectives. This allows students to develop their own sense of volition, so they can adopt a greater share of the responsibility, which currently rests heavily on academics’ shoulders. (Figure 3)

**Making it work: positive examples**

For those who claim students don’t engage with feedback, there is plenty of scope for hope. Zimbardi et al used learning analytics to track biomedical students’ access times and clickstream (mouse activities) on audio and typed feedback snippets embedded in situ to scientific report assignments.

The vast majority accessed their feedback (92% of first year, 85% of second year students) and over half interacted with it for more than an hour. Furthermore, students who interacted with their feedback for longer tended to show more improvement in subsequent similar assessments.

**Quality over quantity**

There are ways to provide students with opportunities for complete feedback cycles whilst also reducing staff marking burden. In a research-led bioscience module described by Morrell, students completed eight “News and Views” assignments, of which three were electronically annotated with formative feedback. Students also had access to some marked reports of their peers.

At the end of the module, students could select any two of these assignments for summative submission. The majority chose to submit at least one unmarked assignment. Students who chose to submit unmarked assignments scored significantly higher compared to those students who submitted work which had been previously marked. This could be due to students engaging with and using
dialogue between student and educator. In this model, students learn to seek specific judgement from others, becoming more active participants in their learning journeys. (Figure 1C)

Removing barriers to engagement

This is not easy. The difference between a student and, say, a pancreatic cell is that the student has the capacity for conscious thought and decision. Students, like most people, find feedback cognitively and emotionally draining.

Nash and Winstone note that “most students in higher education have received little or no prior guidance on how to use feedback effectively”. They advocate shared responsibility as a way of removing various barriers to active engagement with feedback.

This starts with genuine conversations raising awareness of the nature and purpose of feedback in its many forms, and discussing rather than dismissing the very real emotional impact feedback can have. It includes training in how to make use of feedback, rather than assuming that cognisance.

Courses can better offer students the agency to make use of feedback with well-connected assessment tasks aligned to programme-level teaching and learning objectives. This allows students to develop their own sense of volition, so they can adopt a greater share of the responsibility, which currently rests heavily on academics’ shoulders. (Figure 3)

Making it work: positive examples

For those who claim students don’t engage with feedback, there is plenty of scope for hope. Zimbardi et al used learning analytics to track biomedical students’ access times and clickstream (mouse activities) on audio and typed feedback snippets embedded in situ to scientific report assignments.

The vast majority accessed their feedback (92% of first year, 85% of second year students) and over half interacted with it for more than an hour. Furthermore, students who interacted with their feedback for longer tended to show more improvement in subsequent similar assessments.

Quality over quantity

There are ways to provide students with opportunities for complete feedback cycles whilst also reducing staff marking burden. In a research-led bioscience module described by Morrell, students completed eight “News and Views” assignments, of which three were electronically annotated with formative feedback. Students also had access to some marked reports of their peers.

At the end of the module, students could select any two of these assignments for summative submission. The majority chose to submit at least one unmarked assignment. Students who chose to submit unmarked assignments scored significantly higher compared to those students who submitted work which had been previously marked. This could be due to students engaging with and using

![Diagram](image-url)
### Figure 2. Feedback myths / nostrums

Feedback myths or nostrums, and the reasons they are problematic versus the reality. Adapted and summarized from Boud and Molloy.

<table>
<thead>
<tr>
<th>Nostrum</th>
<th>Reality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All feedback is good feedback</td>
<td>Overly critical feedback has the power to instil “learned helplessness”. By contrast, purely positive comments may lead to complacency. Both can reduce students’ capacity for development.</td>
</tr>
<tr>
<td>2. The more the merrier</td>
<td>Quantity isn’t everything. Managing too much feedback can lead to cognitive overload, be emotionally draining and harder for students to prioritise the key things to focus on for improvement.</td>
</tr>
<tr>
<td>3. Feedback is telling</td>
<td>Feedback as a monologue from teacher to student is not helpful. It is better centred around what the learner does, not what the teacher does. Students can learn to be the drivers of feedback.</td>
</tr>
<tr>
<td>4. Feedback ends in telling</td>
<td>Descriptive-only comments on a student’s work is just “dangling data”. For students to improve, they need clear support in closing the gap between their performance and their goals.</td>
</tr>
</tbody>
</table>

### Figure 3. Improving active engagement with feedback through shared responsibility

A schematic showing a pathway to improve active engagement with feedback through shared responsibility. Initially, there is a greater educator responsibility to improve student awareness and cognisance. However, responsibility is shared with the students as they learn to develop their own agency and volition. This itself is a positive-feedback cycle. Adapted from Nash and Winstone.
feedback from marked assignments to improve their subsequent tasks.

**Peer feedback**

Students can learn more directly from each other too, in online guided peer feedback activities. Noroozi and Mulder randomly assigned students into threes on a biotechnology module. Students researched and wrote perspectives on a GMO-linked ethics topic, then made feedback comments on their two peers’ assignments, supported by an online guide. Finally students received their peers’ comments and incorporated them into their own essay.

All students completed this activity, encompassing the entire feedback cycle, simultaneously within a four-hour session. Even this brief timeframe was enough to increase domain-specific knowledge and induce attitudinal change towards GMOs, showing critical reflection on the material.

**Broader course design**

There is potential for embedding true feedback cycles within broader course design to increase the likelihood of productive opportunities.

Vanderleile and Alexander put this into practice in a second year metabolic biochemistry course. Teaching content was unchanged, but the assessment and feedback was renovated for better alignment, and used to support learning rather than just testing it.

This included a greater emphasis on online learning environments, more regular, low-stakes formative tasks and a broader variety of tasks including new creative assignments. The interventions coincided with significantly improved final grades, increased lecture attendance and better course engagement overall.

**A new feedback culture**

Developing good feedback practice at multiple levels, from individual comments to whole course redesign, can really pay dividends for students and staff.

These discussions are happening among a background of changes. From increased consumer identity among students, to enhanced technological capabilities, picking out true signals can be challenging.

But over time, it may be possible to generate a new culture of feedback in higher education. With students and staff sharing the responsibility more evenly, completing the feedback cycle could help students become better learners in their academic studies and throughout their lives.

***

Emily Coyte is a digital content creator for Learning Science Ltd, driven to provide engaging and innovative pre-lab and post-lab solutions for higher education. She is a Fellow of the Higher Education Academy and has worked as an Assistant Teacher at the University of Bristol. Her BSc Biochemistry comes from the University of Bristol. Email: emily.coyte@learnsci.co.uk or eccoyte@gmail.com

---

**Further reading**

- Boud, D. and Molloy, E. (2013) Feedback in higher and professional education: understanding it and doing it well. Routledge
- Cann, A. (2014) Engaging students with audio feedback. Bioscience Education 22(1) 31–41
The motivation to experiment — an art and science exchange

As the blindfold was put over my eyes and tied tightly, I began to wonder if maybe I had bitten off more than I could chew. We often talk about being pushed outside of our comfort zone, but I began to realize that to be uncomfortable is a fairly rare occurrence in our everyday lives. But of course, that was the whole point of our art and science exchange with the Masters students of Central Saint Martins at the University of the Arts London.

Back in February 2018, undeterred by "the beast from the east" nine intrepid members of the Biochemical Society battled through the snow to the MA Art and Science studio in North London. This pioneering Masters course investigates the creative relationships between art and science and how to communicate them. Designed in collaboration with Society members and staff alongside CSM course leaders and students, this day-long exchange was an opportunity to explore, compare and contrast the role of experimentation in both science and art. What we hoped to offer participants was the chance to view their own work from a different perspective, or through a different lens and re-think how they approach their work, both in practise and in communicating it to others.

The day started off with introductions and round-table discussions about a series of questions;

1. What is the place of discovery in your work?
2. What role does tradition play in your work?
3. How would your research differ if you had access to unlimited time/resources?
4. What shape is your research?
5. What might be the consequences of misuse of your work?
6. What does a successful failure look like in your field?

The discussions brought up lots of interesting synergies and interpretations. While there were a lot of similarities in their responses, there were definitely different emphases and perspectives on the same questions.

With the ice well and truly broken, the rest of the day took a more active approach, starting off with collaborative poetry writing, group tai chi, sensory sketching (hence the blindfold!), and a chance to explore the students’ work and how they were using science as inspiration and re-interpreting scientific processes. This included activities with organic dyes,
VR, microscopic images and more. There was also the chance to group together and talk about potential future collaborative efforts or various topics of mutual interest. A huge word of thanks must go out to all those who organized and ran activities during the day.

It is very easy, if not inevitable, to spend most of our time within our own little bubble; interacting with colleagues and friends whose day-to-day experience is very similar to ours. The structure of our work places and educational institutions can sometimes make it difficult for scientists and artists to find the time and opportunity to interact, never mind work together. This separation is good for neither field as each has so much to benefit the other. Creativity, experimentation, communication, observation, failure, success, and collaboration are essential features of both art and science and so opportunities to discuss and share different approaches to these are an opportunity to refresh and re-invigorate our working practices.

Further to these intrinsic benefits, there are also substantial gains to be made when engaging different publics with our work. Being able to view what we do from different perspectives and being able to communicate key concepts in a variety of ways to a variety of people can really increase the access to both art and science to groups who would not normally be engaged for various reasons.

As an opportunity to bring people together, events like this hope to light a spark that can result in someone looking at their work in a different light, having a conversation that leads to a collaboration, learning a new skill, or even just feeling re-enthused about their work. Events like this aim to inspire and gently nudged out of our comfort zones to be reminded that there's a big old world out there full of exciting new experiences and different perspectives. And blindfolds, apparently.

The Biochemical Society’s Scientific Outreach Grants and Diversity in Science Grants open in June and September respectively and can be used to help fund art-science collaborations. If you would like to be involved in future art and science activities, please email education@biochemistry.org to find out more. You can read about Central Saint Martins MA Art and Science at http://www.arts.ac.uk/csm/courses/postgraduate/ma-art-and-science/
A day in the life of a bioinformatician

Dr James Campbell studied applied biology at the University of the West of England and went on to receive a PhD for a study of the utilization of glycoprotein substrates by gram-positive pathogenic bacteria. James then joined a proteomics research company focusing on biomarker discovery and the development of quantitative mass spectrometry methods, before moving to The Institute of Cancer Research, London. Since then, James has worked with the next generation sequencing facility and helped set up the bioinformatic analysis pipelines for the Tumour Profiling Unit. He currently leads the Bioinformatics Facility of the ICR’s Cancer Research UK Centre, supporting research groups across the institute. Lorenza Giannella (Training Manager, Biochemical Society) spoke to him about his work.

How did you get into science?
I have always been fascinated by science and took physics, chemistry and biology as A-levels. I went on to study biology as an undergraduate and in particular focused on molecular genetics and biochemistry. I gained my PhD studying microbial proteomics and during this time, needed to learn how to run a webserver and program computers to process the data I was generating. In those days, there weren’t really university courses where you could train as a bioinformatician and there weren’t many biologists who had learned how to program. This combination of skills led me into bioinformatics.

Can you describe a typical day?
Most of the analyses I run use a high performance computing cluster. The first thing I do is to connect to one of our clusters over the network using a software terminal so I can check the status of the analyses running. Much of my work involves writing code to automate data processing or to analyse and visualize data. The other aspects of my work are meeting with other researchers to define the aims and scope of new projects or writing reports to describe the findings of completed projects.

What is your advice for someone who would like to pursue a career as a bioinformatician?
Bioinformatics is a broad term that could mean different things to different people. One thing that is common to most bioinformaticians is the need to program in several computer languages. Perl and Python are both very popular general purpose languages that are used to automate the processing of data sets. R is a specialized language suited to statistical data analysis and data visualization. A lot of people I know have taught themselves to program by reading books and practicing. Aside from a technical
ability, a good knowledge of biology and data analysis approaches is needed so that the right questions can be asked of data sets in order to extract information and make inferences.

What inspires you about your job?
Biology is a fascinating subject to study and with modern technologies we are able to generate vast amounts of data. It’s rewarding to be involved in the analysis of all these available data.

The Biochemical Society runs an online course on R, called ‘R for Biochemists 101’, starting in September.

Register your interest at bit.ly/RforBiochemists101Sept18.

Job profile
A bioinformatician uses their scientific knowledge and information technology expertise to collect and interpret data generated by research. Bioinformaticians create and maintain databases of biological information, develop and use mathematical models for statistical analysis, carry out dynamic simulations and pattern analysis. Sometimes, they are known as biostatisticians or computational biologists, and they can work in different settings, such as the NHS, research institutions or industry.

Qualifications and key skills
Bachelor’s degrees in bioinformatics are not offered by many institutions. The most common route into the profession is a Bachelor’s degree in a life science, medicine or health-related topic, such as biology, biochemistry or biomedical science, and specialization during a postgraduate degree, such as a Master’s degree, MPhil or PhD in bioinformatics. A doctorate is not necessary to pursue this career, but may sometimes be preferred.

Additional competencies include the ability to use complex technology and programming languages, attention to detail and excellent communication skills to collaborate with other researchers and team members.

Responsibilities
Bioinformaticians apply technological resources to answer biological questions in life sciences, medicine and health-related fields. They develop systems, databases and methodologies to collect and analyse data for clinical or research purposes. Bioinformaticians generally work as part of a multidisciplinary team. With further training and/or experience, a bioinformatician can progress into a leadership role.

Salary
Salaries in this area can vary depending on qualifications, experience and work settings. They are typically in the region of £28,000 – £50,000 per year.
Policy Matters

Driving developments in diversity

Emma Sykes
(Science Policy Officer, Biochemical Society)

‘We’ve shifted the deadline - what I hoped to achieve for my daughter I now hope to achieve for my granddaughter’

These were the poignant words spoken by Professor Lesley Yellowlees CBE, at the launch of the Royal Society of Chemistry’s most recent diversity report ‘Diversity landscape of the chemical sciences’ in February 2018.

These words really capture the findings of the report and the wider landscape of diversity and inclusion issues in the STEM community. While there has undoubtedly been progress (and Professor Yellowlees herself, as the first female president of the Royal Society of Chemistry 2012–2014, is an excellent example of changing times), issues in diversity and inclusion persist and progress is slow. Slow enough that goals and objectives to improve diversity within the science community have shifted not by months or years, but by generations. Who knows how many talented potential scientists have forsaken careers in STEM, who knows where our scientific knowledge might be now if they had not.

The arguments for ensuring a diverse community go beyond the scientific or economic. There is a strong moral case for ensuring that the molecular biosciences and wider STEM community are accessible and welcoming to all, regardless of background, personal characteristics, or lifestyle choice. The sciences should be encouraging of everyone, and there needs to be a culture change starting at the very roots of our community. Talent and people should be nurtured, supported and accepted, as only then will research and innovation truly thrive.

One initiative striving to achieve a fundamental culture change and promote diversity, particularly with regards to women in STEM, is the Athena SWAN Charter. Established in 2005 and managed by the Equality Challenge Unit, ECU, the Charter aims to promote gender equality and advance the careers of women in STEM by advocating inclusive and flexible working environments. The Athena SWAN Charter bestows awards to institutions and departments who demonstrate their commitment to supporting and promoting the women in their organizations by meeting a strict set of criteria upheld by the Athena SWAN Charter. The latest figures released by ECU show that over 500 University departments hold an Athena SWAN award, but only 10 university departments hold the highest level of the award, a Gold. Uptake of the award is steadily increasing and reports show that universities are implementing real changes to promote gender equality and improve diversity and inclusivity within their departments.

To promote the Athena SWAN Charter and support bioscience departments in developing their applications, the Biochemical Society, in partnership with several other learned societies*, held an Athena SWAN biosciences best practice workshop on 7 March 2018. To celebrate women in science, the event was designed to coincide with the week of International Women’s day, on 8 March 2018.

The application process for an Athena SWAN award is notoriously tricky as it relies heavily on qualitative data, case studies and testimonials. Data, which many in the biosciences are completely unfamiliar with. The workshop was tailored to address these common pitfalls and speakers
shared crucial insights from assessing applications, to their own experiences of applying to the award. They revealed that SMART action plans are key to application success, and that universities should analyse their qualitative data and use it as the narrative to frame and inform their application.

The Athena SWAN Charter is an excellent initiative that is helping to promote real change in the sector in gender equality, but diversity issues go beyond just gender and different communities within society face unique challenges when pursuing a career or study in STEM. To highlight these issues, the day closed with a lively panel discussion on the topic of ‘broadening diversity beyond gender’ with representatives from MinoritiesinSTEM and LGBT+ advocates.

The Society is dedicated to promoting diversity and inclusion across all of our activities. To practice what we preach, the workshop was livestreamed throughout the day to promote accessibility for those unable to attend in person and individual speaker sessions are free to watch on the Royal Society of Biology’s YouTube page.

The science community has seen significant progress in improving diversity and inclusion, but more is required. It is vital that momentum in this area continues if we are to achieve our goals. For our own part, the Society will continue to support events and activities, like the Athena SWAN workshop, which promote diversity and inclusion and our popular Diversity in Science grants scheme opens again in September 2018.

*Our partners for the Athena SWAN biosciences best practice workshop were the Royal Society of Biology, the British Pharmacological Society, The Physiological Society, the Society for Applied Microbiology and the Equality Challenge Unit.

The Equality Act 2010 identified nine characteristics which are protected by the act. These are age, disability, gender reassignment, marriage and civil partnership, pregnancy and maternity, race, religion or belief, sex and sexual orientation.

Further Reading:

- www.ecu.ac.uk/equality-charters/athena-swan/
- www.rsb.org.uk/policy/policy-issues/equality-diversity
- www.rsc.org/about-us/our-strategy/inclusion-diversity/
- www.youtube.com/user/SocietyofBiology

Protein disulphide bonds—biochemistry, biotechnology and biomedical impact

31 August–1 September 2018
University of Kent, UK

REGISTER AT Bit.ly/Protein_disulphide_bonds
Eleven distinguished scientists and exceptional early career researchers have been honoured in the Biochemical Society’s annual Awards.

The Awards recognize scientists for the excellence of their work and the profound impact their research has had on the scientific community and wider society. They also highlight outstanding work by early career researchers.

Professor Colin Bingle, Chair of the Awards Committee, said: "The Biochemical Society Awards are the perfect way to honour exceptional scientists within the bioscience community. As ever, the entry criteria are tough and the standards high and the awards are a real tribute to the talent within our community. On behalf of the Society, I’d like to congratulate the winners, all of whom have made outstanding contributions in their fields. Well done.”

AstraZeneca Award

The 2019 AstraZeneca Award will be presented to Ervin Fodor of Sir William Dunn School of Pathology, University of Oxford, UK. Ervin’s research focuses on the molecular mechanisms of influenza virus replication, virus–host interactions and host responses to viral infection. His research group played a pivotal role in uncovering the RNA-free high-resolution structure of the influenza virus RNA-dependent RNA polymerase and discovering how influenza virus hijacks the host transcriptional machinery for the transcription of its own genes. A reverse genetics system for influenza virus developed by Fodor and colleagues in the late 90s is used for the preparation of a live attenuated influenza virus vaccine for the UK National Childhood Flu Immunisation Programme.

Of winning the AstraZeneca Award, Ervin said: “I am surprised and delighted to receive the AstraZeneca Award; it is a great honour to join the list of previous eminent winners of this award. I would like to take this opportunity to acknowledge the inspirational scientists who mentored me and thank present and past members of my research group as well as my collaborators whose hard work and dedication underpins all of our research achievements. I am also indebted to the Medical Research Council for generously supporting our research for many years.”

Biochemical Society Award

The 2019 Biochemical Society Award will be presented to Paul Bieniasz from The Rockefeller University, USA. Paul has made major contributions to our understanding of HIV-1 replication. His group helped to elucidate how the ESCRT proteins are recruited by HIV-1 and other viruses to drive particle release, resolved controversies over the location in the cell where HIV-1 virion assembly occurs and illuminated how HIV-1 packages its RNA genome. With collaborators he developed techniques that allowed the first moving images of the genesis of individual virus particles in living cells to be captured. His group has also discovered several host antiviral mechanisms, including proteins that prevent the release of viral particles from cells, inhibit nuclear import of incoming HIV-1 DNA and enable recognition and elimination of non-self RNA molecules based on their nucleotide composition. His group used knowledge of host variation in antiviral proteins to break host range barriers and develop a macaque model of HIV-1 infection. He has also pioneered the field of ‘paleovirology’, showing that extinct retroviruses and their proteins can be resurrected in functional form from molecular fossils that are present in modern genomes.

Paul said “I am surprised and delighted to be receiving the Biochemical Society Award. This honor recognizes the efforts and achievements of colleagues, past and present, as much as it does my own work. I am especially grateful to my wife and scientific partner, Theodora Hatziioannou, who has made me a better man and a better scientist”.

Centenary Award

The 2019 Centenary Award will be presented to R. John Ellis from the University of Warwick, UK. John has made fundamental discoveries through the study of the mechanism of protein folding essential for light harvesting and photosynthesis. His work focused on the pathway by which the key enzyme of photosynthesis (RUBISCO) - the most abundant protein on earth - is made and assembled in plant cells. From this specific case, John developed key concepts which have revolutionized thinking about how proteins assemble and fold in all cells. John termed these other proteins that assist in protein folding and assembly ‘molecular chaperones’ and his key insight showed that these chaperones are not restricted to the protein assembly pathway that he studied, but exist universally in all cells.

John was delighted to learn that he was to be honoured with the Centenary Award.
The 2019 Colworth Medal will be presented to Melina Schuh from the Max-Planck-Institute for Biophysical Chemistry, Germany. Melina investigates how chromosome segregation errors arise during the meiotic divisions of mammalian eggs. Aneuploidy in eggs is a leading cause of pregnancy loss, congenital disorders and the age-related decline in female fertility. During the early stages of her career, Melina Schuh established methods for high-resolution microscopy of live mouse oocytes, the progenitor cells of eggs. This work paved the way for in-depth intracellular studies of meiosis in mouse oocytes, which had only been observed at very low resolution up to this point. Using this technology, she revealed how the spindle, the machinery that separates the chromosomes, assembles. She also uncovered key mechanisms that are involved in the extremely asymmetric divisions of the oocyte, which are essential to preserve the oocyte’s material for the development of the embryo. Moreover, she developed technology for high-content screens in mouse oocytes. This technology overcame multiple obstacles that had precluded high-content screens for meiotic genes in mammals in the past. Her laboratory then went on to establish methods that allowed them to carry out the first studies of meiosis in live human oocytes. Through this work, they could determine the precise sequence of events that is involved in the formation of a fertilizable egg in humans, and identify steps in this process that are particularly error-prone. Unexpectedly, they found that spindles in human oocytes are highly unstable and frequently incorrectly attached to chromosomes. These incorrect attachments lead to problems when the chromosomes of the oocyte need to be separated in preparation for fertilization. Melina’s work also shed light on why aneuploidy in eggs increases as women get older. They found that chromosomes in human eggs disintegrate as women get older. This disintegration causes chromosomes to orient abnormally on the spindle and precludes their accurate separation. More recently, her laboratory developed a new method for protein degradation that is called Trim-Away. This method is the first widely applicable protein degradation method that acts directly on the level of the endogenous protein.

Melina said: “I was absolutely excited when I found out that I will receive the Colworth Medal. This is a great honour, and I would like to thank the Biochemical Society for this prestigious recognition of our work. A significant part of my independent research has been carried out at the MRC Laboratory of Molecular Biology (LMB) in Cambridge, UK. The time that I have spent at the LMB has been a scientifically fruitful and personally very enriching time. I would like to take this opportunity to thank the many wonderful colleagues and mentors at the LMB who have supported me throughout this time. I am also grateful to the fantastic PhD students and postdocs I have had the pleasure to work with. Studying mammalian oocytes is technically very challenging – none of this would have been possible without their curiosity, perseverance and enthusiasm.”

Industry and Academic Collaboration Award

The 2019 Industry and Academic Collaboration Award will be awarded to Maddy Parsons from King’s College London, UK. Maddy’s research is focused on using a range of live-cell advanced imaging approaches to enable precise spatio-temporal dissection of receptor and cytoskeletal signalling. A key focus of this research is in the development and implementation of novel microscopy approaches and FRET-based biosensors to study cell adhesion and migration signalling in 2D and 3D environments. Maddy has established collaborations with clinicians to uncover novel molecular mechanisms underpinning diseases such as skin blistering, cancer, wound healing and inflammation. She also heads a number of multi-disciplinary projects with physicists and biophysicists to develop new methods to define spatio-temporal signalling events in living cells, tissues and organs. As a result of her interest and applications of advanced microscopy, Maddy has developed strong working partnerships with a broad range of industrial collaborators, including Nikon, which led to the establishment of the state-of-the-art, world-class Nikon Imaging Centre at King’s College London of which she is Director. She also currently works alongside a number of other biotech and pharmaceutical companies to develop and apply advanced imaging approaches to understand basic mechanisms that underpin drug discovery and therapeutic development.

Of winning the Industry and Academic Collaboration Award, Maddy said: “I am absolutely delighted to receive the 2019 Biochemical Society Industry and Academic Collaboration Award. I am really passionate about interdisciplinary collaboration, which makes this honour particularly special for me. This award is really shared with all the highly talented and innovative academic and industrial collaborators that I have been privileged enough to work with”.

Melina Schuh

Colworth Medal

Maddy Parsons
The Novartis Medal and Prize

The 2019 Novartis Medal and Prize will be awarded to Caroline Dean from the John Innes Centre, UK. Caroline initiated her independent group in 1988 choosing to study the molecular controls used by plants to judge when to flower, focusing on the response to prolonged cold. This work has led into the detailed mechanistic dissection of conserved epigenetic switching and co-transcriptional pathways at a specific locus, which has become the evolutionary node for variation in flowering time in Arabidopsis and its relatives. The work also addresses fundamental questions about how cells sense, register and remember noisy temperature signals and how those mechanisms have evolved during adaptation of Arabidopsis to its wide habitat range.

Of winning the Novartis Medal and Prize, Caroline said: “I am really delighted to have been selected for the Novartis Medal and Prize. This honour recognizes the excellent work of many post-doctoral scientists and research students who have been in the lab over the last 30 years – an ever expanding Dean lab ‘family’, and a very fruitful collaboration with Prof. Martin Howard. I am also exceedingly grateful to the European Research Council and the BBSRC for funding.”

Sir Philip Randle Lecture

The 2019 Sir Philip Randle Lecture will be awarded to Antonio Vidal-Puig from the Wellcome Trust-MRC Institute of Metabolic Science, Cambridge, UK. Antonio’s research focuses on elucidating the molecular mechanisms linking obesity with insulin resistance, diabetes and cardiometabolic complications and on the development of related therapeutic strategies. His research strategies include a combination of hypothesis driven and non-biased systems approaches that make extensive use of animal models, stem cell biology, human biological samples (including induced pluripotent stem cells), sophisticated omics technologies and bioinformatics integration. Vidal-Puig’s lab creativity is reflected in the “adipose tissue expandability hypothesis” and the concept of “lipotoxicity” to explain the association between obesity and cardiometabolic complications. His specific scientific contributions to four lines of research are: 1. defining the adipose tissue expandability and lipotoxicity hypothesis and the relevance of adipose tissue macrophages mediating adipose tissue inflammation; 2. proposing the use of energy dissipating strategies to reverse lipotoxicity by promoting mitochondrial biogenesis, mitochondrial uncoupling and brown fat differentiation and activation; 3. establishing the relevance of hypothalamic lipid metabolism in controlling energy homeostasis and thyroid hormone and BMP8b controlling brown fat activation and 4. the pioneering use of systems biology and stem cell approaches to elucidate the role of specific lipids and networks in the development the cardiometabolic syndrome.

Of winning the Sir Philip Randle Lecture, Antonio said “I am delighted with this award that recognises the excellent work of the members of my laboratory over the years”.

Teaching Excellence Award

The 2019 Teaching Excellence Award will be presented to Luciane Vieira de Mello from the University of Liverpool, UK. Lu has published over 40 research articles in biochemistry and bioinformatics but in 2012 she decided to direct her career towards teaching and learning. Lu’s broad educational research interest focuses on student–staff partnership, internationalization, and employability. Lu’s contribution has been to devise, test and disseminate a range of novel methods that enable such students to understand and use bioinformatics effectively. Students in science struggle to recognize and reflect on transferable skills gained during their undergraduate life. Lu designed an online reflective log and a skills audit in a placement module to help students recognize and reflect on the transferable skills gained during their undergraduate degree. The positive impact on student experience has been evaluated, and some of the assessment procedures have since been adopted across the Faculty.

Luciane said “I am delighted and honoured to receive the Teaching Excellent Award. I believe it is important to recognise teaching innovations alongside research excellence and applaud the Biochemical Society’s initiative. I am grateful to my colleagues for their support; and to my students for engaging with my teaching innovations. As Paulo Freire said ‘what the educator does in teaching is to make it possible for the students to become themselves’.”
Early Career Research Awards

**Biotechnology**
The 2019 Early Career Research Award for Biotechnology will be awarded to Michael Booth from the University of Oxford, UK. Michael developed novel DNA sequencing techniques for the detection of two newly discovered modified DNA bases. These newly discovered modified bases had been implicated in human development and disease progression; however, there were no sequencing techniques to precisely map them in the genome to uncover their functional relevance. Furthermore, these sequencing techniques have been patented and spun-out into a company, Cambridge Epigenetix. Cambridge Epigenetix now markets the technique as a kit under the name “TrueMethyl®”. Michael is currently based in the group of Hagan Bayley at the University of Oxford as a postdoctoral researcher, and was previously awarded a Junior Research Fellowship at Merton College Oxford, which was held from 2014 to 2017. Here Michael synthesized light-activated DNA and demonstrated its use to stringently control protein expression in synthetic tissues. These synthetic tissues act as functional mimics of neuronal transmission that can be controlled in a precise way.

Michael said: “I was absolutely delighted to find out I have been awarded the Early Career Research Award in Biotechnology by the Biochemical Society. I would very much like to thank two important mentors from my research career, Sir Shankar Balasubramanian and Hagan Bayley, and their respective research groups.”

**Genes**
The 2019 Early Career Research Award for Genes will be presented to Ana Casañal from the MRC Laboratory of Molecular Biology, University of Cambridge, UK. Ana has been working on the determination of the structure and function of the cleavage and polyadenylation factor (CPF) from yeast. In eukaryotes, mRNA 3’-end processing is a key step in gene expression regulation that involves both cleavage and polyadenylation of the nascent RNA transcripts. CPF is a 1 MDa complex that cleaves the nascent mRNA, adds a poly(A) tail and dephosphorylates RNA polymerase II to coordinate transcription. mRNA polyadenylation is deregulated in human diseases and is hijacked by viruses. Therefore it is critical to understand how the mRNA 3’-end processing machinery functions and how its activity can be regulated. Ana’s research represents a breakthrough towards the understanding of 3’-end processing. She played a key role in the determination of the first cryo-EM structure of the CPF poly(A) polymerase module, providing an understanding of its function in RNA processing. Ana’s work provides detailed insight into polyadenylation, and also has broad relevance in gene expression, RNA biology, and multi-protein assemblies.

Of winning the Early Career Research Award for Genes, Ana said: “I am delighted and honoured to have been selected for the Early Career Research award of the Biochemical Society. This would not have been possible without the support from my PhD and postdoc supervisors, Prof. Valpuesta, Dr. J. A. Marquez and Dr. Lori Passmore. I am extremely grateful to Dr Lori Passmore and Dr David Barford for their encouragement through my challenging research and my colleagues for their contributions to my work and great team effort.”

**Signalling**
The 2019 Early Career Research Award for Signalling will be presented to Qian Wu from the University of Cambridge, UK. Qian’s research focuses on understanding the functional mechanisms of human DNA damage response and repair signalling networks and identifying potential targets for future drug discovery. By combining different methods, her research explores three major properties of this complicated signalling network: 1. Spatial architecture of individual proteins and protein complexes; 2. Temporal arrangement of these protein complexes; 3. Regulation of protein-protein interactions through post-translational modification. She has made significant contribution to the field by characterizing the structure, function and assembly of various key protein complexes in the human Non-Homologous End Joining (NHEJ) pathway for repairing DNA double-strand breaks, the most toxic DNA damage form in our cells. She also expanded her research on understanding the structure and regulation of the assembly of BRCA1 protein complexes in DNA damage response and repair signalling.

Qian said: “I was absolutely thrilled and extremely happy when I found out that I got this award! It is a great honour as an early career researcher to have my research recognized by the award committee. I couldn’t have achieved this without the strong support from Professor Sir Tom Blundell, my mentor since my Ph.D and all my wonderful colleagues in the Department of Biochemistry, University of Cambridge. More excitingly, I am really looking forward to taking the encouragement from this award to initiate my own independent research in the Astbury Centre for Structural and Molecular Biology, University of Leeds.”
Upcoming Events

Translation UK 2018
5–6 July 2018, Manchester, UK

Proteomics and Related Metabolomics of Oxidative Damage and Glycation: a Technical Workshop
9–11 July 2018, Coventry, UK

Small G Proteins in Cellular Signalling and Disease
9–12 July 2018, Cambridge, UK

Experimental Techniques for Studying Proteins and Lipids in Biological Membranes
31 July–1 August 2018, Birmingham, UK

Structure and Mechanism of Membrane Proteins
2–3 August 2018, Birmingham, UK

Protein Disulphide Bonds: Biochemistry, Biotechnology and Biomedical Impact
31 August–1 September 2018, Kent, UK

Biennial International LRRK2 Meeting
2–4 September 2018, Padua, Italy

84th Harden Conference: Single-molecule Bacteriology
9–12 September 2018, Oxford, UK

Acylation of Intracellular and Secreted Proteins: Mechanisms and Functional Outcomes
10–12 September 2018, Brighton, UK

R for Biochemists 101
10 September 2018
online course

The Changing Landscape of Research on Ageing: Models, Mechanisms and Therapies
7 November 2018, Glasgow, UK

Synthetic Biology UK 2018
19–20 November 2018, Bristol, UK

Meeting Reports

Bioenergetics Christmas Meeting 2017

19 December 2017, Birkbeck College, University of London

The meeting started with a keynote lecture by Professor Bill Rutherford FRS, from Imperial College, entitled ‘Photosynthetic reaction centres: relating light, oxygen, survival and the energy limits’ followed by 10 short talks from post-docs and PhD students.

Professor Rutherford gave a brilliant account of the state of the research in photosynthetic reaction centres, presenting some very exciting ideas and new perspectives to the field. The presentations that followed were equally exciting and all speakers were thankful for the opportunity to present their work to an informed audience. Dr Andrew Hitchcock from the University of Sheffield was awarded a certificate for the best presentation.

The meeting was very well attended with people coming from across the world. People resurrected plans of collaborations, started new ones and there was a real sense of enthusiasm and optimism. It was an excellent networking event for the bioenergetics community, with many very senior researchers present, discussing the meeting with junior colleagues.

Amandine Maréchal
(Birkbeck College, University of London, UK)
Local Ambassador – Isabel Pires

Dr Isabel Pires is a cancer research scientist and lecturer in biomedical science at the University of Hull, where she has been since 2012. Isabel’s research focuses on understanding how low oxygen (hypoxia) regulates the biology of tumours, particularly the increased aggressiveness in cancer associated with resistance to therapy and metastatic potential. She is the Programme Director for the MSc in Translational Oncology at the University of Hull. Isabel is also an enthusiastic science communicator, who can be found at events such as festivals and science cafes chatting about cancer biology, as well as a keen advocate for improving the visibility of women in science.

What motivated you to become a scientist?
I have always been interested in knowing and learning. My first love was history and art, but as a teenager I became interested in science for three main reasons: 1) I had a fantastic biology techniques teacher in high school who taught me the scientific process, even in simple school lab practical sessions; 2) I read the works of great science communicators such as Carl Sagan and Hubert Reeves at a formative time in my life, which fuelled my interest in science; 3) Dinosaurs – Jurassic Park has a lot to answer for in terms of inspiring a generation of biologists!

What inspires you about molecular bioscience?
During my 1st degree, I became fascinated in understanding signalling pathways at the molecular level. I used to draw detailed diagrams to help me with revision, and became very interested in how molecular pathways are regulated. In particular, I love to look at my data and try and puzzle out the molecular mechanics of how a particular cellular response occurs. That is why I love to study tumour hypoxia, as it represents a fascinating context in which physiological signalling changes to allow cells to survive and become more invasive.

What are you reading at the moment?
At work, I have been reading a lot of papers on the DNA damage response and gene expression regulation in hypoxia. Personally, I am more than halfway through “The Left Hand of Darkness” by Ursula K Le Guin, and I am looking forward to reading “Handywoman” by Kate Davies, which is coming out soon.

What's on your desk right now?
Desk: Papers, note books, marking, student drafts, my keep-cup (empty). Bench: qPCR notes and my lab coat.

What’s been the greatest challenge in your career so far?
Juggling a healthy work–life balance, and keeping focused.

What is your advice for someone who would like to pursue a career in molecular bioscience?
Work hard, keep focused, plan ahead, use a calendar and to-do lists. Get a mentor you feel comfortable with. Be kind to others, remember to take time to be kind to yourself. Most of all, be patient. As my PhD supervisor told me right at the start, you cannot run without learning how to walk.

What do you do in your spare time?
When I have spare time, I collect hobbies… I love to knit, garden, draw, sew, you name it. I probably have a WIP (Work-in-Progress) for it. I love to read (which I have been trying to do more to get away from computer screens), art house cinema, and going for walks with my husband. I am also training for a 10k race later in July, so I like to be busy!

What are you reading at the moment?
Personally, I am more than halfway through “The Left Hand of Darkness” by Ursula K Le Guin, and I am looking forward to reading “Handywoman” by Kate Davies, which is coming out soon.

What are you reading at the moment?
At work, I have been reading a lot of papers on the DNA damage response and gene expression regulation in hypoxia.

What is your advice for someone who would like to pursue a career in molecular bioscience?
Work hard, keep focused, plan ahead, use a calendar and to-do lists. Get a mentor you feel comfortable with. Be kind to others, remember to take time to be kind to yourself. Most of all, be patient. As my PhD supervisor told me right at the start, you cannot run without learning how to walk.

What do you do in your spare time?
When I have spare time, I collect hobbies… I love to knit, garden, draw, sew, you name it, I probably have a WIP (Work-in-Progress) for it. I love to read (which I have been trying to do more to get away from computer screens), art house cinema, and going for walks with my husband. I am also training for a 10k race later in July, so I like to be busy!

The 19th International Symposium on Chromaffin Cell Biology 2017

22–26 August, 2017, University of Sheffield, UK

With 90 presentations spread over 5 days, the ISCCB meeting once again delivered a unique and holistic view into how discoveries made at the molecular level link to a deeper understanding of mammalian biology and the pathophysiology of complex conditions.

The conference started with a focus on the molecules that regulate exocytosis and endocytosis. Andrew Peden (Sheffield) discussed his latest research findings on the role of non-neuronal SNAREs, SNAP29 and STX19 in post-Golgi trafficking. Further insight into SNAREs’ function was provided by Jakob Sorensen (Copenhagen) using mutagenesis to show that the charge on the outside of neuronal SNARE-bundles lowers the electrostatic energy barrier to fusion. Using high-resolution optical tweezers, the Biochemical Society sponsored speaker, Yongli Zhang (Yale), measured the energy generated by SNARE zippering, demonstrating it was sufficient to overcome the energy barrier. The early career researchers’ symposium, focussed on the use of super-resolution imaging techniques, providing a new look in to the molecular organization of the exocytotic machinery, while Shigeki Watanabe’s (John Hopkins) pioneering use of “flash-and-freeze” and electron microscopy brought new clarity to the role of clathrin in synaptic vesicle endocytosis.

Prizes for student presentations went to Misty Marshall (Uppsala), Joannalyn Delacruz (Cornell), Bassam Tawfik and Ayoze Santana (La Laguna).

Elizabeth Seward (University of Sheffield, UK)
Secure successes for the future of bioscience

It is always good to look ahead. Next year will be our tenth anniversary as an organization, and development of the next RSB strategy is now underway – we will use this to listen to our members and Member Organizations including The Biochemical Society in order to find the best way to deliver a bright future for the bioscience community.

We recently reached an exciting milestone, with the RSB exceeding 18,000 members. Every addition to our biosciences network makes us stronger, and we are more likely to overcome challenges when we work together.

We also saw the handover to our new President – Professor Dame Julia Goodfellow succeeded Professor Dame Jean Thomas after four years of service, and we look forward to working closely with Professor Goodfellow to further build on the incredible foundation of work laid by her predecessors.

With Brexit approaching, there remain many unknowns about the future. Questions about how trade and transport will be regulated have raised key questions about our national biosecurity. Alongside the risk of allowing harmful organisms into the UK is the vital need to efficiently move and trade the products and elements of biotechnology and research.

Capacity to detect and deal with all potential biosecurity threats at our borders would require increased resources, almost certainly beyond our available skills base.

I recently wrote to the House of Lords EU Energy and Environment Subcommittee, in relation to its Brexit: Plant and Animal Biosecurity inquiry to highlight how vital continued collaboration, and movement of information and expertise is for supporting international biosecurity standards and maintaining high levels of biosafety in the UK.

Continued engagement with the bioscience community on this issue will be important and we value input from the Biochemical Society membership.

As an organization we also aim to ensure a better future for bioscientists; providing them with the greatest support possible during their professional life, training and education.

In partnership with the University Bioscience Managers Association, we awarded the HE Biosciences Technician of the Year award to Gill Scott, a technician manager at the University of Warwick who achieves the best for her students.

Through the Heads of University Biosciences, a RSB Special Interest Group, we awarded this year’s HE Biosciences Teacher of the Year to Dr Dominic Henri from the University of Hull, for his terrific enthusiasm and drive for pedagogical development.

These awards allow us to celebrate those who are exceptional in their fields, and showcase the work done by HE practitioners in training the next generation of bioscientists.

Similar values underpin our university degree accreditation scheme, and at the end of April we saw the annual Degree accreditation Award Ceremony, held at the Houses of Parliament. This year, an additional 17 institutions have seen degrees receive accreditation, bringing the total of number of institutions recognized to 52, including four outside of the UK.

As well as recognizing high quality bioscience education to degree level, the accreditation scheme highlights academic excellence, drives up standards, and shows that universities are equipping their students with the skillsets that employers value.

It is essential that learning environments are inclusive and supportive. With this in mind, we partnered with several organizations, including The Biochemical Society, to deliver a workshop for academics wishing to pursue an Athena SWAN award. The day saw a number of informative and fascinating sessions on the diversity scheme, and we hope all who attended or viewed online took food for thought back to their institutions.

The continued support and engagement of The Biochemical Society and its membership means we can look forward with confidence to working together to deliver a brighter and more secure future for the biosciences.
CEO Viewpoint

The 107th Biochemical Society Annual General Meeting will take place this year on 26 July, at our headquarters, Charles Darwin House. The AGM is always a great opportunity to update members on another busy year of activities, and this year we will also announce the Biochemical Society’s next Chair, who will take over from Professor Anne Dell who is stepping down in July next year.

Last April, the Society announced the winner of the inaugural International Award, which recognises research conducted outside the UK that illustrates the importance of the molecular biosciences in the advancement of life sciences research. This month, the award will be presented to Job Dekker (from the Howard Hughes Medical Institute and the University of Massachusetts Medical School) by the Society’s President, Professor Sir Pete Downes, at the 24th IUBMB and 15th FAOBMB Congress (4-8 June) in Seoul, South Korea where Job will deliver his award lecture.

The eleven winners of our 2019 awards are also now public. The awards honour talented scientists at every stage of their careers, recognizing the outstanding work of both distinguished researchers and early career scientists. You can read more about the winners and their contributions to the molecular biosciences here: https://bit.ly/2v6AGkG.

At the time of writing, a year since the triggering of Article 50 has just passed, a significant milestone marking precisely one year until the UK is expected to leave the European Union. Leading up to this, the Society continues to work closely with the Campaign for Science and Engineering (CaSE) (which published a report on Brexit to mark this important anniversary: https://bit.ly/2v8SH1L) and also the Royal Society of Biology. Partnering with organizations such as CaSE and the RSB lends the Society a strong voice with which to ensure the views of our community are heard by the UK government during this critical period of negotiations.

As part of our work with the Royal Society of Biology, later this month the Society will attend Parliamentary Links Day (26 June). Organized annually by RSB on behalf of the STEM community, Links Day is the largest science event on the annual Parliamentary events calendar, and aims to strengthen dialogue between the scientific community and parliament, by bringing together scientists, learned societies, MPs, and Peers. Each year, the event assumes a different theme; at the time of writing, the proposed theme for this year is “Science and the Industrial Strategy”.

Finally, by the time you are reading this we will have wished farewell to two valued colleagues, Niamh O’Connor (Director of Publishing) and Helen Albert (Community and Press Editor) whom I would like to thank here in recognition of their hard work and dedication to the organization. Niamh, who left us in May to take up an exciting new role as Journals Publishing Director at PLOS, first joined the organization in late 2013. During the past four and a half years she played a leading role in transforming our publishing and commercial activities. She led the modernization of our business, with the introduction of new editorial and production systems, which have helped to give Portland Press a competitive edge within the industry, and she was also instrumental in brokering the new sales partnership with the Royal Society of Chemistry which will help to broaden the reach of our publications while strengthening our revenue streams. Additionally during her tenure Niamh has overseen considerable changes to our communications function – including the re-branding exercise in 2015, an innovative approach to social media, and the launch of The Biochemist Blog. Through her expert knowledge of publishing policy, Niamh has helped to raise the profile of the Society and Portland Press, which will also continue to be an asset to the sector more generally through her work with ALPSP.

Helen, who also left in May, has taken on the position of Editor-in-Chief of Labiotech.eu, based in Berlin. Helen played a vital role as Editor of this magazine since joining the organization in 2015. She helped to reinvigorate the publication, having worked closely with the Editorial Board to develop a new and ambitious strategy for the magazine. Through her work with the Editorial Board, Helen updated the content of the magazine, with input from our Designer and Brand Guardian, to make it more accessible while not compromising its scientific value, as well as introducing new regular features such as interviews, Local Ambassador focus articles, and careers articles in collaboration with our Training Manager. She also developed and launched The Biochemist Blog, an online forum for news, views and opinions.

It has been a pleasure to work with both Niamh and Helen and, on behalf of everyone here at the Society and Portland Press, I would like to wish them all the best in their new endeavours. ■
State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization: Volume 3. Defining the Next Generation of Analytical and Biophysical Techniques Edited by John E. Schiel, Darryl L. Davis, and Oleg V. Borisov

This volume of review/research articles is the third part of a comprehensive series produced by biopharmaceutical professionals that describes the state of the art in emerging technologies for characterization of monoclonal antibodies (mAb). Analyses of NISTmAb IgG1 (developed as part of the Biomanufacturing program at the National Institute of Standards and Technology: NIST) alongside detailed methodology for use of equipment augments the value of this book as it can be used as the basis for experimental design.

Nuclear magnetic resonance (NMR) and hydrogen-deuterium exchange mass spectrometry (HDX-MS) were identified as potentially the most powerful for high-resolution assessment of mAb higher-order structure. The irreversible covalent labelling techniques for characterization of mAb structure follow on well from those initial chapters, but it would be useful for the reader if related mass spectrometry chapters (chapters four, seven, ten, eleven, and twelve) were grouped. The authors acknowledge that protein aggregation is a key challenge in mAb product manufacture. Certainly, the simultaneous multiple sample light scattering technique may allow prediction and therefore control of protein aggregation by continuous monitoring of process parameters that cause this phenomenon.

Chapters describing removal of adventitious agents and host cell proteins highlight the scale of product contamination in biopharmaceutical manufacturing as well as the various strategies employed to define and limit the influence of those critical quality attributes on mAb drug safety and efficacy. One of the high points of this book is the description of microfluidic platforms with capabilities to significantly enhance the efficiency and, importantly, reduce the cost of mAb manufacture. The Polaris-HR HPLC-MS chip that was used to analyse the NISTmAb model, can be developed into a high-resolution technique that complements the NMR and HDX-MS techniques. It has not escaped the notice of the authors that bioinformatics analysis tools are necessary for the majority of the techniques that they described. Standardized bioinformatics interrogation of large datasets generated from platforms of orthogonal techniques may be achieved with new software (Byologic, Byomap, Byonic).

This collection of reviews will be of use to industrialists working in the biopharmaceutical fields and scientists in academia who would like to improve and update workflows for their biologics research. Although analytical techniques tend to evolve rapidly, this book will likely be relevant for the next five years. It should be part of the analytical scientist’s toolbox together with the previous two volumes.

Mollicutes: Biology and Pathogenesis Edited by Glenn Browning and Christine Citti

This book is a must for anyone working with or thinking of working with mollicutes and also other fields such as horizontal gene transfer (HGT), evolution and taxonomy. It covers most areas of mollicute biology ranging from the different “omics” of single mollicutes up to biofilms and mollicute interactions with its host and other microorganisms. Throughout the book the details of HGT, mobile genetic elements and their importance in terms of plasticity they give to mollicutes is almost comprehensively described with useful explanations of the different elements and mention of online databases and tools for anyone wanting to make use of them, making this book of interest to anyone looking to learn more about HGT, genome structures and how they influence biology.

To its credit “Mollicutes” has input from 32 experienced authors and this workforce is evident in the tidiness and structure of the sections as well as the flow of the text which is in most chapters well written and engaging with lovely descriptions of methodologies capable of inspiring readers to think about creative scientific exploration methodologies. In parts (especially the taxonomy chapter) the style of the text could even be described as a humorous narration of historical and scientific facts.

Some of the basics are repeated in many chapters but not to the extent that this becomes tedious. This has the benefit of allowing for readers to find only the information they need using the comprehensive index, which also serves as an abbreviation index, and still get all the information they need. A list of tables would have given the finishing touches to this well-structured mollicute background collection and readers are encouraged to bookmark the tables which are wonderfully comprehensive and useful lists of mollicute characteristics.

Overall I can recommend that this is an excellent reference manual for anyone working with mollicutes and it also makes for some fascinating reading for anyone with a biology background.

Diane Hatzioanou (Public Health England, UK)

Acylation of intracellular and secreted proteins: mechanisms and functional outcomes

10–12 September 2018
Old Ship Hotel, Brighton, UK

Find out more: bit.ly/Protein_acylation

Bernice Wright (Imperial College London, UK)
Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials By Paul Bouis

I don’t know how I came across the book “Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials” but I am very glad that I noticed it. This book has helped me to clarify some of the conceptual things in chemistry that I learned at the university level but slowly erased during transition of my research career into other fields. This book is an enjoyable read.

The brand-new edition of this book provides a comprehensive review of basic and applied aspects of chemical reagents used in a chemistry laboratory. Readers can easily learn about the definitions of specification, validation, and detection in chemical analysis from the introduction chapter, which is considered to be one of the most essential parts of any laboratory setting. The following chapter is devoted to the fundamentals of analytical procedures, e.g. gravimetry, titrimetry, colorimetry, potentiometry, atomic absorption, chromatography etc. These techniques are essential for complete understanding of the chemistry of a substance, i.e. its chemical composition quantitatively as well as qualitatively. In other words, these methods can be used to check the efficacy, identity, purity, and safety of the sample. The next chapter focuses on common solvents, buffers and indicators used in the course of laboratory experiments. A detailed description has been given on the methodology of the preparation of the standard/stock solutions and the mixtures from various elements and compounds. In the monograph sections, authors have set new requirements, specifications introduced new test methods for determining the purity/quality of around 500 chemicals. In addition to standard-grade reagents, I was delighted to see the new section of some unknown materials, e.g. explosives and organophosphorous insecticides. In summary, this book will indeed be useful not only to teachers, undergraduates, graduates, researchers, and instructors, but also to those who are interested in learning about chemistry in general.

Saptaswa Sen (Royal Institute of Technology, Sweden)

People in white coats
By Benoît Leblanc
(http://peopleinwhitecoats.blogspot.co.uk)

**Crossword Competition**

Win

This month’s crossword prize is a Science Museum Set of 4 Chemical Compound Coasters. Simply email the missing word, made up from letters in the highlighted boxes to biochemist@biochemistry.org, by Friday 6 July 2018.

Please include the words ‘June crossword competition’ in the email subject line.

**Congratulations to the winner of the April competition:**

Thomas Walpole

The missing word from last issue’s competition was MAGNETORECEPTION.

Thomas Walpole received a UGears 70009 - Engine, 3D Wood Kit.

Terms and conditions: only one entry per person, entrant must be a current Biochemical Society member; closing date Friday 6 July 2018. The winner will be drawn independently at random from the correct entries received. The winner will receive a Science Museum Set of 4 Chemical Compound Coasters. No cash alternative available. No employee, agent, affiliate, officer or director of Portland Press Limited or the Biochemical Society is eligible to enter. The winner will be notified by email within 7 days of the draw. The name of the winner will be announced in the next issue of The Biochemist. The promoter accepts no responsibility for lost or delayed entries. Promoter: Biochemical Society, Charles Darwin House, 12 Roger Street, London WC1N 2JU; do not send entries to this address.
FREE ONLINE COURSE
Biochemistry: the Molecules of Life

Starts 1 October 2018

Targeted at students aged 15–19, this three week MOOC (Massive Open Online Course) will provide an introduction to biochemistry, ideal for those thinking of pursuing studies in molecular bioscience.

JOIN NOW
www.futurelearn.com/courses/biochemistry
#FLbiochem

Developed by:
Submit your work bit.ly/portlandpress-submit