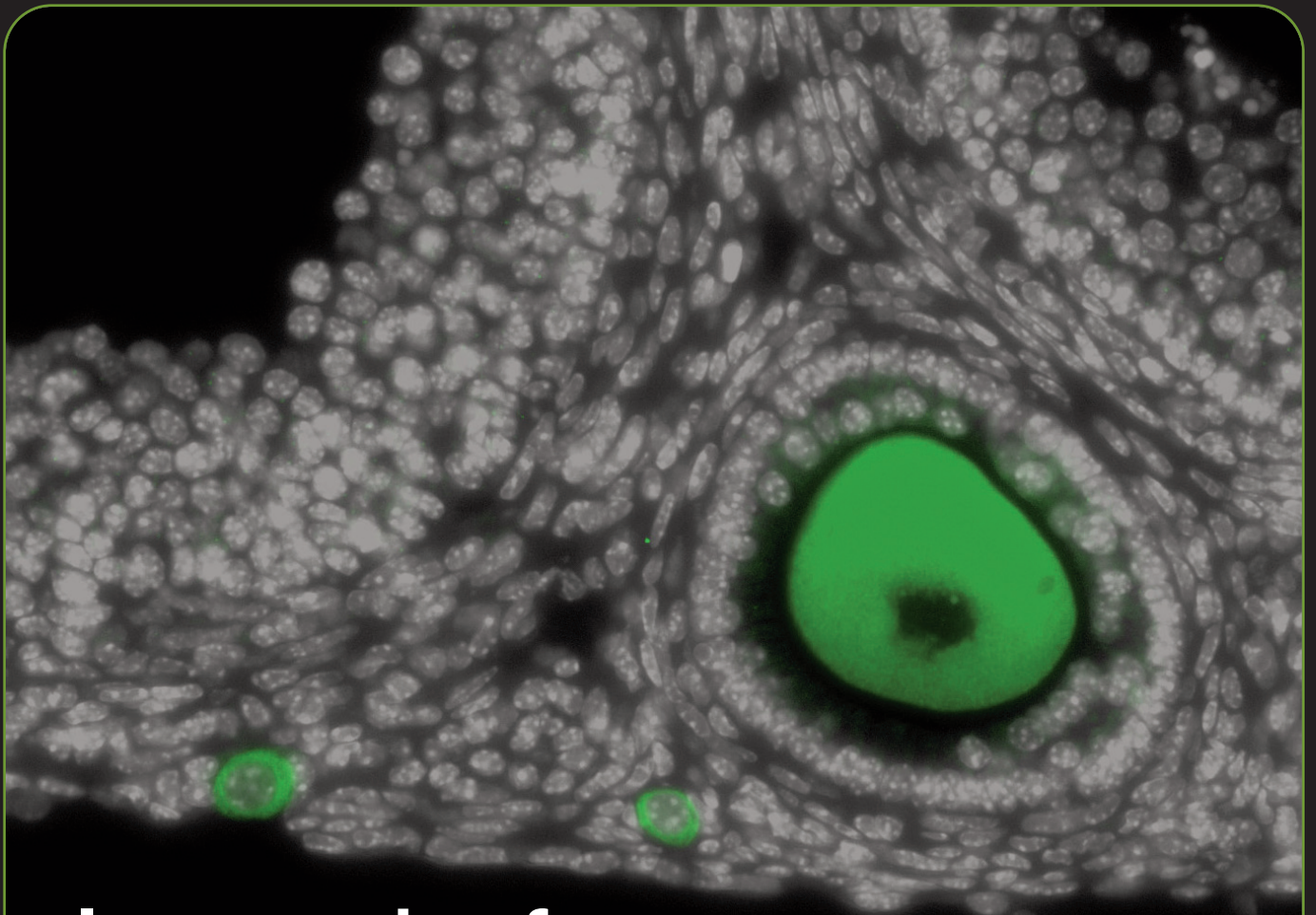


# focus on REPRODUCTION



## In search of oogonial stem cells

- Best of ESHRE & ASRM 2017
- Switzerland: 35 years of IVF

// MAY 2017



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MAY 2017

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## CHAIRMAN'S INTRODUCTION

As I will shortly complete my two-year term as Chairman of ESHRE, this will be my last editorial for *Focus on Reproduction*. At our Annual Meeting in Geneva I will become Past Chairman, and Roy Farquharson will take over.

ESHRE's chairmen's cycle of serving two years as Chair Elect (to gain understanding of the Society's structure, processes and tasks), two as Chairman, and two as Past Chair is a well functioning system which ensures continuity and 'memory' within the system - and a smooth turnover of chairmen. It was also established from the very beginning that the chairman's position should alternate between a clinician and a scientist. This too is in my mind a wise decision, which has enhanced the perspective and diversity of the Society and its Executive Committee.

Two years ago I was asked what I wanted to achieve as a Chairman. My immediate reaction was to look at the structure of the society and improve communication. I had seen how difficult it is to engage our members within the Society. Indeed, looking back at my own views of ESHRE as a young embryologist I only knew that there was an annual meeting each summer. Unfortunately, I think this is still the way we are seen by a majority of our members, as a society that has an annual meeting (and some Campus workshops).

We are sometimes accused of not being a democratic society. But, when we send out surveys or election forms for SIG membership or applications for national representatives, we receive a very low response, if any.

So, in order to try and find out what our members want and how they see us, we last year commissioned a membership survey to be carried out as an exam project by a business management school. In addition, the three chairmen plus the Chairman of our SIG Committee met with our young SIG deputies to discuss how we can involve and stimulate young professionals in reproductive medicine. It was very interesting to see that both of these initiatives raised several similar findings: we need to be much more active on social media, have more hands-on courses and workshops, arrange more network activities, and show more clearly the advantages of being a member of ESHRE. These thoughts will now be incorporated into our forward planning for the Society.

With this I wish to thank all of you who have supported and worked with me during my two-years term - my ExCo colleagues, the fantastic team at ESHRE Central Office, the SIGs, and all others in the ESHRE structure who work so hard to maintain and develop our Society.

And last but not least, I wish to remind you of this year's Annual Meeting. It takes place in the beautiful city of Geneva, situated in a wonderful location between the lake and the Alps. We invite you all to come and share four great days of science in the form of pre-congress courses, posters, plenary lectures, free communications, and encounters with old and new friends from all over the world.

Kersti Lundin  
ESHRE Chairman 2015-2017

// MAY 2017

# Abstracts aim high

- Open programme selected from more than 1700 abstracts
- China now the most prolific nation

MORE THAN 230 abstracts of original studies - from a fountain-high total of 1725 submissions - have been selected for oral presentation in Geneva. A further 800 abstracts have been selected for poster presentation.

'The number of abstracts submitted for Geneva continues at a consistently high level,' said ESHRE Chairman Kersti Lundin. 'No other meeting in reproductive medicine can now command this sort of support year after year.'

The Geneva abstract total not only marks a near-record entry (slightly down on last year's 1764 abstracts) but also reflects the very high standards now required for oral selection. The acceptance rate for oral presentation is now around 13%, making the acceptance rate not far removed from that of ESHRE's flagship journal *Human Reproduction*.

As ever, submissions were refereed blind by a selection committee, which included, among others, the co-ordinators of ESHRE's 12 Special Interest Groups. Selection for the oral or poster programme

*The second of day 1's opening keynote lectures will be presented by Professor Dennis Lo, whose discovery of cell-free fetal DNA in maternal plasma paved the way for non-invasive prenatal testing.*



was dependent entirely on the committee's score.

As ever, the greatest number of abstracts were in clinical science, of which embryology (343 total abstracts) is now the most prolific. Female fertility (246 abstracts), andrology (203), reproductive endocrinology (198), reproductive genetics (129) and endometriosis (117) were also popular.

All abstracts, which were submitted in the *Human Reproduction* format, were reviewed according to ESHRE's standard procedure of screening and scoring. Screening aims to ensure that abstracts are designated to the correct topic category, while selection for oral and poster presentation is done solely on the basis of scores awarded by reviewers. The International Scientific Committee finally selected 235 abstracts for oral presentation from the 1725 submitted.

For the first time ever, the highest number of abstracts came from China (146 submissions), with Spain (143), Japan (122), UK (116) and USA (101) close behind. The ever-growing presence of China and Japan in the scientific programme of an ESHRE Annual Meeting continues, a trend also reflected in submissions to the ESHRE journals.

And just for the record, another noteworthy trend is the number of abstracts now submitted with female first authors - 985 against 740 male.

## GENERAL ASSEMBLY TO RATIFY SELECTION OF FIVE NEW ExCo MEMBERS



Thomas Ebner is an embryologist and IVF specialist recently made Professor at the University of Graz, Austria, with research interests in vitrification and culture media. He was certified by ESHRE as a senior clinical embryologist in 2008, and recertified in 2012, is a Board Member of ALPHA-Scientists, and a representative of Austria in ESHRE's CNR.



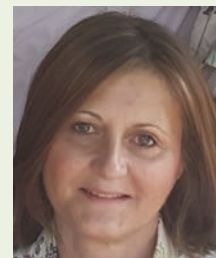
Anja Pinborg is Professor in Obstetrics & Gynecology at Hvidovre Hospital, University of Copenhagen, and specialist in reproductive medicine. She has published more than 90 original papers with focus on ART safety and reproductive epidemiology. She is a member of the steering committee for ESHRE certification of nurses/midwives.



Professor Karen Sermon is Chair of the research groups in genetics and regenerative medicine at the Vrije Universiteit Brussel (VUB) in Belgium. Karen was Chair of the PGD Consortium from 1998-2006 and later Co-ordinator of the SIGs Reproductive Genetics and Stem Cells. She is Chair of the steering group for the ESTEEM study.



Professor Thomas Strowitzki is Medical Director of the Department of Gynecologic Endocrinology and Fertility Disorders at Heidelberg University Women's Hospital, Germany. He has research interests in PGD, PCOS and endometriosis, and has been a representative of Germany in ESHRE's advisory CNR.



Vidaković Snežana Head of Department for ART and Minimally Invasive Surgery for ObGyn, at the Clinical Centre of Serbia and Chair of ObGyn at the University of Belgrade. Her interests are in reproductive endoscopic surgery and she was a co-author of the ESHRE/ESGE new classification of female genital tract congenital anomalies.

### Agenda of the 2017 General Assembly of Members

To be held on Tuesday 4 July 2017, from 18.00 to 19.00, at the Palexpo, Geneva, venue of the 33rd Annual Meeting.

1. Minutes of the last meeting (held in Helsinki and published in *Focus on Reproduction*, September 2016)
2. Matters arising
3. Membership of the Society
4. Society activities
  - Annual meetings - Campus meetings
  - Studies and data collection - Accreditation and certification
  - Special Interest Groups
5. *Human Reproduction* journals
6. Paramedical Group
7. Financial report
8. Ratification of the new Executive Committee
  - Cristina Magli as Chairman Elect and retirement of Juha Tapanainen as immediate Past Chairman
  - Petra De Sutter (BE), Georg Griesinger (DE), Grigoris Grimbizis (GR), Tatjana Motrenko (ME), and Andres Salumets (EE) to step down as members having served two two-year terms
  - Thomas Ebner (AT), Anja Pinborg (DK), Karen Sermon (BE), Thomas Strowitzki (DE), and Snežana Vidaković (RS) as new members
  - Mariette Goddijn (NL), Nick Macklon (GB), Basak Balaban (TR), Borut Kovacic (SI), and Rita Vassena (ES) to serve a second two-year term as members
  - Estratios Kolibianakis (GR) to become an ex officio member as Chair of the SIG Committee
9. Retirement of the Chairman, Kersti Lundin (SE), and installation of the new Chairman, Roy Farquharson (GB)
10. Election of the Honorary Members for 2018
11. Any other business
12. Date of the next Annual General Assembly

### Scientific programme

The main scientific programme is now in place and its high quality begins in the very opening two keynote lectures. The subject and presenter of the Human Reproduction lecture are derived from papers with the highest number of full-text downloads during their first six months of publication in the journal, for this

year between January 2015 and June 2016. This year's lecture will be given by Carlos Simon from Spain based on his paper on uterine stem cell therapy. You can find more details of this winning study on page 8.

This lecture is followed immediately by a keynote presentation from chemical pathologist Dennis Lo from the Chinese University of Hong Kong, whose

discovery of cell-free fetal DNA in maternal plasma in 1997 led to the rapid development of non-invasive prenatal testing. In Geneva Lo will present an update on NIPT, which, he will argue, looks set to play an increasingly important role in future obstetric care.

This year's invited programme continues with a choice of reviews on topics of huge current interest - notably, ovarian rejuvenation, germline gene editing, endocrine disruptors, cryopreservation, artificial gametes and pregnancy failure. Of great interest to many will be presentation of first results from ESHRE's ESTEEM trial, a preimplantation genetic testing study of polar body analysis by array CGH.

### Posters

As ever, around 800 abstracts have been selected for poster presentation. As before, and in line with the congress's paper-free credentials, there will be no paper posters or poster boards. However, discussions will be arranged for those selected posters considered for the two poster awards (in basic and clinical science).

### Precongress courses

Fifteen precongress courses will be staged on the Sunday preceding the Opening Ceremony. The majority are organised by ESHRE's Special Interest Groups, but there are additional courses run by the editors of the ESHRE journals on academic authorship, by the ASRM on the techniques of embryo transfer, and on providing realistic information to patients organised by the Paramedical Group.

For the first time there will also be a precongress course organised by the Cochrane Gynecology Group on how to prepare a systematic review in reproductive medicine. And one course likely to generate special interest is that organised as an exchange course by the Middle East Fertility Society on the oocyte as the main determinant of embryo quality. This MEFS course examines a scientific oocyte 'hypothesis' under the direction of an expert to explain its possible contribution to embryo quality - thus, the mitochondria hypothesis with Dagan Wells, mitochondrial supplementation (Kutluk Oktay), hypoxia (Jeremy Thompson), oocyte rejuvenation (Johnny Awwad), aneuploidy (Elpida Fragouli), polar body (Alan Handyside), and cumulus cells (Samir Hamamah).

Precongress course attendance has been growing rapidly in recent years, with some courses now attracting well over 500 registrations. Those interested in attending in Geneva should be sure to book in good time.

### Social programme

The Opening Ceremony, to be held on Sunday 3 July at 19.00, is the first of the meeting's social events and will be followed by a welcome reception in the exhibition area. Admission to the Opening Ceremony, which will take place in the main hall of the congress centre, and welcome reception are complimentary. All registered participants are warmly invited to both



**Don't forget ESHRE's charity run, which this year will take place on Monday evening at 6.30 pm on a course adjacent to the congress centre. The run gives ESHRE congress participants the chance to do a little good for their own health and help raise funds for patient groups throughout Europe.**

events. At the Opening Ceremony ESHRE will pay tribute to this year's two Honorary Members for their outstanding contribution to reproductive medicine and science (see facing page).

ESHRE's charity run will start near the congress centre on Monday 3 July at 18.30. The run, now in its fourth year, gives ESHRE members a chance to team up with Fertility Europe, ESHRE's partner patient organisation. You can register for the run (and/or make a donation as an extra) online on the registration form.

An ESHRE evening networking event will take place on Tuesday 4 July at 20.00. Venue for this relaxed evening - with fingerfood, drinks and entertainment - will be the Bâtiment des Forces Motrices, pictured below, a 19th century industrial building now restored as a magnificent arts and entertaining space. This is an optional event but will give everyone the chance to say hello with friends and colleagues. The entrance fee is just €30 per person, and registration details are on the ESHRE website.



# This year's honorary members represent achievements in Switzerland and Australia

As ever, the two selected for honorary membership of ESHRE in 2017 are nominally 'retired' and are either a distinguished representative of the host country or one of international reputation. The former is the Swiss IVF specialist Marc Germond, while the latter is the Australian reproductive endocrinologist Rob Norman. They will each receive their honorary memberships of ESHRE at the Annual Meeting in Geneva.



Marc Germond, involved in ESHRE guidelines from the very beginning.

Marc Germond was head of the Centre for Medically Assisted Procreation (CPMA) in Lausanne, Switzerland, from 2005 to 2016 and was President of the Foundation for Andrology, Biology and Endocrinology of Reproduction (FABER) created in 2005 to support research in ART and andrology. Germond's special interests include the use of lasers in reproductive medicine, andrology, and the ethics in ART. Indeed, as early as 1989 he was a member of the ESHRE Ethics Committee which would eventually produce the first 'European guidelines regarding medical practice related to assisted reproduction and prenatal diagnosis', published in *Focus on Reproduction* in 1991.

It was also Germond's great contribution to ESHRE to organise the 2001 Annual Meeting in Lausanne, the Society's first in Switzerland and the last to be organised in the 'smaller' congress cities. Germond set up the Swiss national ART register in 1992 (based largely on the French FIVNAT model) and was also a founding member of the Swiss Society of Reproductive Medicine.

- Read Marc Germond's brief history of IVF in Switzerland on page 20.



Rob Norman, distinguished Australian endocrinologist.

It's no small coincidence that Marc Germond trained in IVF in the early 1980s with Colin Matthews in Western Australia, who received his own honorary membership of ESHRE in Lausanne in 2001. And it's in Western Australia that this year's international recipient, Rob Norman, is based, until recently as Professor of Reproductive and Periconceptual Medicine at the University of Adelaide. He also led the Robinson Research Institute, Australia's most prestigious research group in reproductive and child health, comprising more than 450 researchers and many students working in basic reproductive science, cell biology and stem cells. However, Norman will be best known to ESHRE for his work in preconceptional care and PCOS. He was a leading member of the 2003 Rotterdam ESHRE/ASRM consensus group on PCOS, whose 2004 report remains to this day *Human Reproduction's* most cited publication.

Norman is a specialist in endocrine biochemistry and is Australia's leading clinical reproductive endocrinologist, with an interest in the role of weight in female fertility and other periconceptional influences. More recently he was a member of the group reporting to the WHO on global guidance for the management of women with anovulatory infertility and PCOS (see page 12).

# Stem cell therapy in the restoration of endometrial function

## ● Carlos Simón to deliver this year's opening Human Reproduction keynote lecture

This year's Human Reproduction keynote lecture, which will open ESHRE's Annual Meeting in Geneva, will be given by the distinguished and prolific Spanish clinical researcher Carlos Simón. His paper - 'Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study' - had more full-text downloads than any other original reports in *Human Reproduction* during the first six months following online publication between January 2015 and June 2016.

This will be the eighth renewal of the Human Reproduction lecture and its annual presentation has in these few years set a record-breaking precedent of bumper crowds and maximum attendance to fire the ESHRE congress into life. Last year in Helsinki around 4000 packed the auditorium for the two opening keynote lectures, and tradition now determines that the speaker will address one of the biggest audiences ever in reproductive medicine.

All of which should be no concern to Carlos Simón, whose record as a speaker, author and investigator is one of the world's most prolific. He will be best known to ESHRE members for his work in endometrial receptivity and implantation, which most recently led to a customised array for its molecular assessment. He is presently Professor at the University of Valencia in Spain, and Scientific Director of Igenomix, a company founded by Simón to translate basic research in reproductive medicine to clinical practice.

For the past decade, however, Simón has extended his research into the field of stem cells, developing embryonic stem cell lines for the Spanish National Stem Cell Bank and obtaining two stem cell lines from blastomere biopsy. The paper behind the lecture in Geneva is a pilot study of bone marrow-derived stem cells used as therapy in the treatment of Asherman's syndrome and endometrial atrophy.<sup>1</sup>

Simón's group in Valencia had already shown that CD133+ bone marrow-derived stem cells could be effective in the reconstitution of the human endometrium in these two incurable pathologies. An experimental animal study reported in 2015 showed that the injection of CD133 bone marrow-derived stem cells engraft around endometrial vessels and induce proliferation of surrounding cells through paracrine molecular activity.<sup>2</sup>

Now, the same application of cell therapy in a cohort of 16 patients has been shown to increase the volume and duration of menses over three months, as well as the thickness and angiogenic processes of the endometrium while decreasing



*Carlos Simón will deliver this year's Human Reproduction keynote lecture - on stem cell therapy.*

intrauterine adhesion scores. The resumption of menstruation occurred in 15 of the 16 patients, and several pregnancies were achieved spontaneously and through ART.

Asherman's syndrome, says Simón, is an uncommon disorder in which the uterine cavity is obliterated by intrauterine adhesions - leading to amenorrhea, infertility, and recurrent pregnancy loss. This pilot study thus suggests that advanced therapy using autologous blood CD133+ bone marrow-derived stem cells is an effective fertility treatment for women with these incurable uterine pathologies.

Since publication in 2016, the study has progressed further to a phase II clinical trial authorised by the Spanish drug agency and performed at Valle de Hebron Hospital and IVI Barcelona. 'Next,' says Simón, 'we must move to a phase III randomised international study with around 100 patients before it can be adopted as routine clinical practice.' If successful, he adds, 'the study will imply that we can treat with cell therapy what we cannot now improve with drugs or surgery, which will open new therapeutic possibilities for these pathologies.' Patients treated in the *Human Reproduction* study had had reparative hysteroscopies, with no significant improvement, and none had achieved pregnancy.

1. Santamaria X, Cabanillas S, Cervello I, et al. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study, *Hum Reprod* 2016; 31: 1087-1096.
2. Cervello I, Gil-Sanchis C, Santamaria X, et al. Human CD133+ bone marrow-derived stem cells promote endometrial proliferation in a model of Asherman syndrome. *Fertil Steril* 2015; 104: 1552-1560.



# MEPs urge action to address infertility in EU

## ● ESHRE audit of fertility in Europe presented to European Parliament

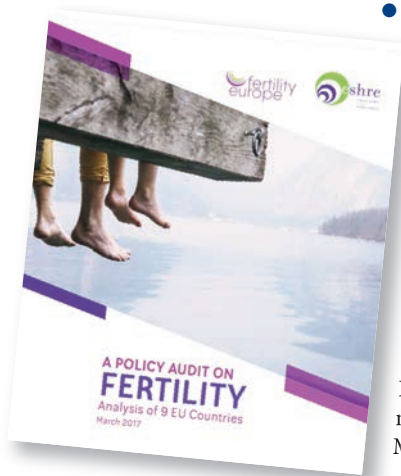
An audit of infertility in Europe performed by ESHRE and Fertility Europe has exposed numerous barriers to access infertility services across the European Union, prompting a call to action on EU infertility policy. In attendance at the European Parliament - and to give patients a voice - was the most famous IVF patient of all, Louise Brown, the world's first 'test-tube' baby. 'My birth gave real hope,' Louise told the meeting. 'A way of beating infertility that really worked.'

Speaking as host of the policy launch Norica Nicolai (MEP Romania) said: 'As policymakers, our duty is to develop and implement policies that serve the people we are entrusted to represent. The findings from this study cannot be ignored. It is our obligation to further analyse the results and change how we view and prioritise infertility.'

The call to action listed five key recommendations as the principal steps to improve infertility policies in Europe:

- Remove barriers to access infertility treatment by making it a priority on the public health agenda

- Include fertility policies in the national demographic plans to address EU's low fertility rate (1.58, which is now well below the stabilising rate of 2.1).
- To highlight infertility as one in the promotion of gender equality
- Accept infertility as a medical condition, to be reflected in the workplace in the same way as any other
- Encourage best practice among EU member states by developing a new Comparative Analysis of Medically Assisted Reproduction in



*Louise Brown, who will celebrate her 40th birthday next year, introduced the ESHRE audit report to the European Parliament.*

the EU; the 2008 study (SANCO/2008/C6/051) is outdated

The audit covered the patchwork of regulation, practice and policy in most member states, but concentrated in detail on nine: Czech Republic, France, Germany, Italy, Poland, Romania, Spain, Sweden and the UK. For example, the report found that regulatory variation is especially seen in treatment availability, most notably in embryo selection via PGS, anonymity of donors (gametes and embryo) and surrogacy. While the UK is the only country where surrogacy is legalised, legal vacuums in the Czech Republic and Romania allow for its practice.

The report was presented within the framework of 'differing' fertility rates in Europe, ranging from Spain and Poland with a rate of 1.32 to the UK (1.81), Sweden (1.88) and France (2.01). There remains a lack of updated comparative information on infertility rates in Europe, with calculations 'based on different methodologies'.

## ESHRE in stakeholder representation to EU directive evaluation

It is now more than a decade since the EU Tissue & Cell Directives lay down standards of quality and safety for the 'donation, procurement, testing, processing, preservation, storage and distribution' of human tissues and cells. Evaluation of the legislation on procedures and safety has been ongoing by the European Commission under the responsibility of DG SANTE/B4, and one of the steps defined in its roadmap is consultation with identified stakeholders. ESHRE is a recognised stakeholder in reproductive cells.

The latest in a series of ad hoc meetings between DG SANTE members, stakeholders and representatives of the Competent Authorities on Substances of Human Origin Expert Group took place in Brussels in February, with two main topics on the agenda: 'donor safety and vigilance' and 'clinical outcome monitoring and demonstration of efficacy'.

ESHRE as a stakeholder was represented by Carlos Calhaz-Jorge, chair of the EIM Consortium, to present the Society's perspectives. These included a strong call for attention to the 'non-logical' features of legislation on partner donations and to the need for a reliable and mandatory registry system. These two issues raised many questions from Commission staff and representatives of some competent authorities.

Other stakeholders participating in the meeting came from the blood and stem cell fields.

The meeting was billed as the prelude to a 12-week open public consultation due to start April. The evaluation roadmap will be completed with another stakeholder meeting at which further information gaps will be filled in.

*Carlos Calhaz-Jorge  
Chair, EIM Consortium*

# *Transatlantic harmony*



**The fifth Best Of ASRM and ESHRE meeting took place in Paris in February. Almost 1000 took part in what is becoming a rapidly growing event in the reproduction calendar.**

The first Best Of ESHRE and ASRM took place in 2010 with the aim of bringing together world authorities in the science of reproductive medicine, with updates on the latest concepts and developments presented in a framework of lectures, debates and back-to-back sessions. But there was always a secondary endpoint to this meeting, to present these developments from the sometimes divergent transatlantic perspectives of North America and Europe. And this year, nowhere was this divergence of approach more evident than in two presentations describing the barriers to treatment faced by those living on these two transatlantic continents.

For Eli Adashi, from Brown University, Rhode

Island, the explanation for such markedly limited public or insurance-funded options in the US lay with the country's social and political culture. For despite the fact that the ASRM had designated infertility 'a disease' in 1993 (and reaffirmed in 2008 and 2013), no other public or private body had been prepared to accept this definition. Culturally, politically and legally the US has taken a 'hands-off' approach to infertility, what Adashi described as a 'quintessentially American' libertarian response. And it's this same laissez-faire attitude, he added, which explains why there is no formal responsibility for the 'underwriting' of infertility care in the US. What care there is dominated by out-of-pocket expense, self-insurance

A bumper audience of almost 1000 registrants attended this fifth Best Of meeting, this time hosted in Paris in late February. The increasingly popular event is now on a biannual track, alternating venues between Europe and North America. The sixth renewal - in 2019 - will be held once again in New York.



and just 15 state mandates for infertility insurance, many of which will not provide full IVF coverage. 'It's dismal and socially unjust,' said Adashi, adding that 'in the final analysis it's all about cost'.

Cost too, said Jackie Boivin from the University of Cardiff is an 'important' consideration in Europe, although the 'non-economic' barriers to full care here seem much greater than in the US. Her estimate was that around 44% of infertile couples do not seek treatment for all sorts of reasons, a figure apparent even in Nordic countries despite generous financial support. Among the non-financial barriers cited by Boivin were low levels of education, the female partner in full employment, 'reproductive choice' ('decisional avoidance', voluntary childlessness), limited access and of course legal constraints. Boivin's proposal to control such causes was equitable funding, adequate education in fertility, advocacy and research - but, as in the presentation of Adashi, there was a sense that cost raised the largest barrier.

#### PGS, yet again

The debate which raised the greatest clinical interest

was on the role of PGD and PGS in the diagnosis and prevention of recurrent pregnancy loss (RPL). Although this was not necessarily a reflection of transatlantic disharmony, the two protagonists - William Kutteh from Vanderbilt University Medical Center and Mariette Goddijn from the Amsterdam Medical Center - did represent views with a certain continental drift. Goddijn's case for an expectant management approach to RPL lay in three pieces of strong evidence: that reported pregnancy rates after PGS are no higher than after natural conception; that pregnancy rates anyway after natural conception are 'relatively good', and that ICSI following PGS is associated with complications and high cost. Goddijn emphasised that the case for expectant management in RPL - or at least the case against PGS - was recently made in a *Human Reproduction* report in which a retrospective cohort study of 300 RPL patients found similar live birth and miscarriage rates in both the expectant management and PGS groups. PGS for RPL? Forget it, said the editor in an HR Alert.

However, this same HR study was criticised by Kutteh (retrospective, selection bias, different patient



*Eli Adashi, left, and Jacki Boivin, centre, found cost a universal barrier to comprehensive fertility treatment on each side of the Atlantic, while Nick Macklon, right, reviewed recent developments in understanding the endometrium's own embryo selection function.*



*The perennial debates on PGS were left largely in the hands of William Kutteh and Mariette Goddijn, left, on the efficacy of PGS in recurrent pregnancy loss, and of Richard Scott on the issues raised by PGS in broader patient populations.*



group ages), whose case for PGS in RPL lay largely in the growing incidence of aneuploidy with female age and the possibility that comprehensive chromosome screening following blastocyst biopsy in good prognosis patients improves embryo selection and implantation rates. This potential, Kutteh emphasised, would be greater in centres 'with significant experience' in biopsy and testing. However, despite the strong evidence of the US trials, Kutteh did concede that the overall value of PGS in unselected patients is still 'unclear', but recognised its potential value ('promising') in RPL.

The case for PGS in the larger patient population lay with Richard Scott from Reproductive Medicine Associates of New Jersey (whose association with IVI of Valencia was announced just before this meeting) and the substantial body of evidence assembled by his group. Despite the strength of that evidence, Scott recognised 'real questions' hovering over comprehensive chromosome screening: the safety of taking the embryonic sample; predictive value; mosaicism; and cost. In answer to the first, Scott was emphatic that 'day 3 biopsy will soon be of historic interest only' and that blastocyst biopsy will increase implantation rate (while cleavage stage biopsy will not). On predictive value, a prospective study performed by Scott's own group showed that CCS is indeed 'highly predictive' of reproductive potential - which in this study was around 98% for negative

predictive value at all ages from 32 to 42. His arguments in cases of mosaicism (lower predictive value) and on cost were less persuasive. Scott also presented data reflecting the precision with which PGS can identify sub-chromosomal defects (duplications and defects) and the impact they are likely to have on outcome if not identified.

Scott was taking part in a debate on whether aneuploid embryos should ever be transferred; his answer was never, provided they are truly aneuploid, which of course requires PGS to verify. His opponent in the debate, Siobhan Quenby from the University of Warwick, UK, entertainingly proposed that 'diversity' in the gene pool provided the spice of life - and anyway, she added, the maternal decidua can make embryo selection far better than any laboratory test.

All of which proved a perfect introduction to a cutting-edge presentation from Nick Macklon from the University of Southampton, UK, on the emerging concept of embryo selectivity and the function of the endometrium in early embryo development. Macklon's theme, based on his own work with colleagues at the University of Warwick, was that the endometrium is more than merely 'receptive' - that it allows implantation of the healthy embryo but also prevents implantation of the unhealthy embryo and goes on to nourish and incubate the preimplantation embryo. The decidualised stromal cells of the endometrium recognise the incompetent embryo, such that those stromal cells do not migrate to poor quality embryos. This activity was visualised in remarkable film of the



*Herman Tournaye, left, found little strong evidence in support of DNA fragmentation testing in sperm; Robin-Lovell Badge, centre, urged caution (and guidelines) in the progress of genome editing; Human Fatemi reviewed luteal coasting in cycles with agonist triggering.*

## Belgium's 'Walking Egg' project steps into the developing world

It is now ten years since Willem Ombelet and colleagues founded the Walking Egg project to bring effective fertility treatment to developing countries. Despite continued objections to funding from outside sources (other priorities, overpopulation . . . ) the project has so far developed a treatment package which includes a modified stimulation protocol (based on clomiphene), a one-day one-stop diagnostic system, and a simplified IVF lab with home-made incubator. The programme has been tested in Ombelet's own clinic in Genk and has so far produced results not inconsistent with those elsewhere in Belgium. In 2016 the Walking Egg system, applied in 163 patients, achieved an ongoing pregnancy rate of 32%. The next stage, said Ombelet, is to further develop the hub-and-satellite model around selected centres in Africa, and develop too a relatively inexpensive self-contained laboratory system. Funding, added Ombelet, remains the ongoing problem.



endometrium actually interacting with viable and less viable embryos. Hence the concept of the 'choosy' endometrium and the mother making her own embryo selection (as Siobhan Quenby had earlier suggested). This was somewhat explained, said Macklon, by the endometrium's transcriptional response to developmentally competent and incompetent embryos. Recurrent implantation loss, he suggested, might occur when the stromal cells do migrate to non-competent embryos and fail to decidualise normally. In such cases, the less choosy 'superfertile' mothers will experience more miscarriages. So what does this mean clinically? First, said Macklon, citing neutral results from the recent trial of progesterone in RPL, the established models of implantation have not led to effective tests of intervention. 'New concepts are required,' he said, which recognise the active - and not passive - role of the endometrium. And one marker of this activity might be an endometrial gene expression signature which accurately predicts implantation failure. This expression of endometrial genes, said Macklon, might yet provide the basis of a new - and finally accurate - test of uterine receptivity.

### What works - and doesn't work

Among the emerging concepts reviewed in Paris were sperm DNA fragmentation testing, 'hippo' signalling as a tool for preventing POI, human germline editing, and luteal 'coasting'. On sperm DNA fragmentation tests Herman Tournaye from the VUB Brussels was only lukewarm. DNA fragmentation, he pointed out, happens normally during spermatogenesis and any reproductive consequences are merely associations, not causations. It thus remains unclear who might benefit, even though the tests seem recommended in couples with repeated ART failure or miscarriage. Tournaye also emphasised that the range of present tests all offer a 'proxy' result in that the tested sperm cells are thereafter unsuitable for clinical use. Moreover, despite the hype of antioxidants, there are no proven therapies to remedy subfertility associated with high levels of DNA fragmentation in sperm.

The concept of luteal phase support after GnRH agonist triggering for oocyte maturation - as described by Human Fatemi from IVI Abu Dhabi - seems a much surer prospect, built on the well established 'segmentation' principle for the prevention of OHSS in IVF. Yet, as Fatemi noted, OHSS cases do - albeit rarely - occur with agonist triggering and the likely explanation is severe luteolysis. Luteal support with hCG following the agonist trigger results in good pregnancy rates and low risk of OHSS.

The meeting's opening presentation proved the perfect demonstration of translational research - by which prolonged investigation of the architecture and vasculature of the ovary, and of its 'hippo' signalling pathway, proved the basis of an experimental restoration of fertility in patients with POI. Aaron Hsueh from Stanford University recalled how genomic and genetic studies implicating hippo signalling genes in POI, PCOS and ovarian reserve had led to the possibility of in vitro activation (IVA) and childbirth in women previously diagnosed with POI. Hsueh and his Japanese colleagues' work on ovarian physiology and signalling pathways had identified 'residual follicles' which could be activated and grown into embryos for implantation and pregnancy. Hsueh found that blocking a protein known as PTEN (in mouse and human ovaries) stirred dormant follicles into life. 'Our treatment was able to activate or awaken some of the remaining primordial follicles and cause them to release eggs,' said Hsueh, who reported that so far three babies had been born in women previously diagnosed with POI.

The genome editing technique of CRISPR Cas9 is a long way from clinical use, but it was described by Robin Lovell-Badge of the Francis Crick Institute in London as 'a revolution over the past five years'. He devoted his presentation to the report of the US National Academies of Science, Engineering, and Medicine (see page 15), whose conclusions reflect a degree of scientific and clinical consensus evident throughout most of this ESHRE/ASRM meeting. Lovell-Badge, a member of the report's writing group, said progress should not go ahead without guidelines, nor beyond the treatment of disease. 'Caution is needed,' he said, 'but that does not mean prohibition.'

*Simon Brown  
Focus on Reproduction*

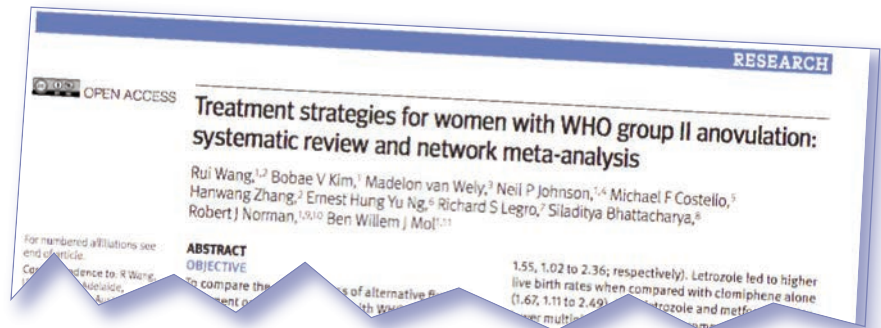
# Ovulation induction in WHO group II anovulation

## ● Despite its outlaw status, letrozole is found most effective in terms of live birth

Letrozole, an aromatase inhibitor developed for the treatment of hormonally-responsive breast cancer and not indicated for induction of ovulation, has proved the most effective treatment for WHO group II anovulation in terms of live birth. However, a combination of clomiphene citrate and metformin was the most effective treatment in terms of pregnancy - though not live birth.

The findings emerged from a huge systematic review of data available in the Cochrane register on eight ovulation induction treatments included in 57 trials.<sup>1</sup> All methods tested proved superior to placebo or no treatment in terms of ovulation and pregnancy in women with WHO group II anovulation (OR 2.43-6.11).

As a result of the findings the authors conclude that expectant management is not recommended, and similarly discount gonadotrophins because of the 'probability' of multiple pregnancy. They add that clomiphene alone was not competitive in the analysis in terms of effectiveness (pregnancy, live birth and ovulation) or safety (multiple



pregnancy).

Which leaves letrozole and a clomiphene/metformin combination as first-line choice - and neither letrozole nor metformin are approved for the treatment of anovulation in most countries; in some countries, following a scare in 2005 about congenital abnormalities, letrozole is explicitly forbidden in fertility indications.

Presently, with data such as these showing that letrozole is at least as effective as (or better than) clomiphene for ovulation and pregnancy in WHO group II anovulation, and that babies born are at no higher risk for congenital anomalies, letrozole remains unapproved for ovulation induction.

Letrozole was not included in the latest (2013) PCOS guidelines from NICE, nor in the ESHRE 2008 Thessaloniki treatment consensus on PCOS, which recommended clomiphene as first-line treatment for ovulation induction and either exogenous gonadotrophins or laparoscopic ovarian surgery as second-line. The use of exogenous gonadotrophins was associated with an increased risk of multiple pregnancy.

1. Wang R, Kim BV, Van Wely M, et al. Treatment strategies for women with WHO group II anovulation: systematic review and network meta-analysis. *BMJ* 2017; 356: j138.

## WHO assembles evidence for its global guideline in PCOS management

A summary of evidence assembled to support development of the WHO's global guidance on the management of PCOS also suggests that letrozole - as well as clomiphene citrate - can be used as first-line therapy for the induction of ovulation, with gonadotrophins and laparoscopic surgery recommended as second-line treatment.<sup>1</sup> Metformin alone, say the guidelines, 'has limited benefits in improving live birth rates'.

For those women with PCOS failing to become pregnant with ovulation induction therapy or having additional infertility factors, the guidelines will recommend that IVF 'can be used, with protocols to minimize the risk of OHSS'. The guidelines add that 'IVM offers a promising alternative to conventional IVF' but, when compared with conventional IVE, yields significantly fewer mature oocytes with significantly lower implantation rates.

However, this far-reaching review, which covers lifestyle measures and bariatric

surgery, only recommends weight loss measures in obese women 'largely on the basis of general health benefits', and considers bariatric surgery in cases where BMI is  $\geq 35$  kg/m<sup>2</sup> and lifestyle therapy has failed.

The authors report that this huge WHO exercise followed the WHO handbook for guideline development but built too on the work of the PCOS Australia Alliance. However, in terms of definition the guidelines 'strongly recommend' the Rotterdam diagnostic criteria as developed by ESHRE and the ASRM in 2004.

Next step, say the authors, is for WHO to assess the evidence among stakeholders and finally publish the guideline.



Adam Balen, from Leeds, UK, first author of the WHO guideline for PCOS.

1. Balen AH, Morley LC, Misso M, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance, *Hum Reprod Update* 2016; 6: 687-708.

# 'IVG to supplant IVF': Horizon scanning in reproductive medicine

## ● Claim that gametes more likely to be of stem cell than gonadal origin

There is a strong possibility that IVF will be supplanted by IVG - in vitro gametogenesis - as science contemplates the possibility of replacing gametes of gonadal origin with those derived from stem cells. The prediction comes in a much reviewed commentary in *Science Translational Medicine*, whose authors claim that the feasibility of IVG has already been demonstrated in mice.<sup>1</sup> Hikabe and colleagues in Japan, for example, produced oocytes capable of supporting fertilisation and parentage from murine ESCs entirely in vitro.<sup>2</sup>

The commentary proposes that IVG is likely to transform reproductive medicine in several ways: that eggs need no longer be collected for IVF, that gametes could be grown for patients who have become iatrogenically infertile, and genome editing could be used to correct mutations in the gametes of patients with genetic infertility disorders. IVG could also be used to produce new embryonic stem cell lines, personalised to individuals, without the need for donor eggs.

However, such distant possibilities raise the inevitable hand of regulation and ethical concern. In the US, say the authors, IVG-derived eggs and sperm will likely be regulated as a 'cellular and gene therapy product' and will thus require extensive preclinical safety trials in mammalian species. There will also be likely objection to the generation of ESCs for research purposes - and to the prospect of enhanced embryo selection from a huge potential pool of embryos. This prospect, add the authors, would be exacerbated if IVG were combined with genome editing such as CRISPR Cas9, allowing not only selection but also alteration. However, IVG's 'most

disruptive' effect might be in society's concept of parentage, which already, in the experiments of mitochondrial replacement therapies, has given us 'three-parent' IVF.

The authors offer no time-line to their forecasts but note that 'in the near future' the impact of IVG will likely be limited to the science of germ cell biology. However, 'with science and medicine hurtling forward at breakneck speed', the rapid transformation of

reproductive medicine may well come sooner than we think, even if it is still not yet technically or legally feasible to produce a human baby via IVG.

1. Cohen IG, Daley GQ, Adashi E. Disruptive reproductive technologies. *Sci Transl Med* 2017; 9: doi: 10.1126/scitranslmed.aag2959.
2. Hikabe O, Hamazaki N, Nagamatsu G, et al. Reconstitution in vitro of the entire cycle of the mouse female germ line. *Nature* 2016; 539: 299-303.

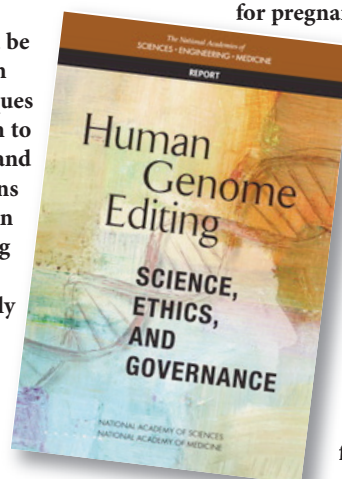
## Cautious support for germline editing

**A long-awaited report following the 2015 summit on gene editing from the US National Academies of Science, Engineering and Medicine has recommended that scientists should in future be allowed to make genetic modifications to human embryos destined for transfer and implantation. Such moves, says the report, could eliminate genetic diseases such as sickle-cell anaemia or cystic fibrosis.**

However, the 261-page report concludes that these developments would be acceptable only when gene-editing techniques are advanced enough to be used in humans, and once strict restrictions and regulations are in place. **But by opening the door to the creation of genetically modified babies, the US National Academies have taken a massive step forward; indeed, a**

report in *Nature* in 2015 said that 'in our view, genome editing in human embryos using current technologies could have unpredictable effects on future generations'.

Now, following the US summit and its report, and given the many scientific, ethical and legal questions which still surround gene editing, the National Academies have concluded that scientists shouldn't yet perform germline editing on embryos intended for pregnancy. But they did decide that altering human embryos as part of basic research was acceptable. And further ahead still the report recommends restricting the technique to severe medical conditions for which no other treatment exists. It also calls for international co-operation, a strict regulatory and oversight framework, public input into decisions and long-term follow-up of children.



# Still little strong evidence to recommend a freeze-all protocol

## ● New study finds no reason to delay FET beyond one cycle with freeze-all approach

Evidence in the does-it/don't-it debate on freeze-all embryos is slowly accumulating, but still seems stuck at the definite-maybe stage. A Cochrane review just published, which was based on only four eligible trials, found the evidence 'of moderate to low quality due to serious risk of bias and (for some outcomes) serious imprecision'.<sup>1</sup> The review thus found no clear evidence of a difference in cumulative live birth rate

between the freeze-all and the conventional IVF strategies. As expected and evident in 'segmentation' studies, the prevalence of OHSS was lower after the freeze-all strategy. However, in terms of outcome, a final verdict will surely need the results of a large RCT- such as the E-Freeze trial now under way in 13 UK centres,

Of course, as the editor of *Human Reproduction* has pointed out recently, not every intervention in IVF requires an RCT to prove its worth. But freeze-all, like PGS or endometrial scratch, seems one of those logical but sensitive interventions for which only a final-word RCT will do.

And that responsibility may now rest on the E-Freeze trial now moving into its second year in the UK. The trial, a collaboration of the National Perinatal Epidemiology Unit and the University of Aberdeen, aims to recruit 1086 couples in a live birth outcome comparison between fresh and elective frozen embryo transfers. The £1.4 million trial will also consider the health of the mother and costs to the health services.

Principal Investigator Abha Maheshwari led a systematic review in 2012 suggesting that FET pregnancies have better obstetric and perinatal outcomes, but a 2016 analysis of more than 100,000 pregnancies in the HFEA database suggested that 'controlled trials are needed before elective cryopreservation of all embryos is practised in preference to the current practice of fresh embryos'.<sup>2,3</sup>

Maheshwari told *Focus on Reproduction* that 572 couples had agreed to take part in the trial (though not all had had egg

collection). At the end of March 181 had been randomised, with a target still set at 1086. 'We are expecting the trial to recruit until March 2019,' said Maheshwari.

'Since our 2012 paper,' she said in a press statement last year, 'support for our view that frozen embryos can lead to better, or at least equal results to using fresh embryos has gained more support and it is generally accepted that the quality of the embryo is not compromised via the freezing process.'

It was anticipated that a Dutch randomised trial ('Freeze All Progesterone') would also test the hypothesis that a freeze-all approach results in higher ongoing pregnancy rates than fresh transfer. Serum levels of progesterone were to be measured on the day of ovulation trigger as a marker of endometrial receptivity. However, following the completion of a pilot study (in 205 patients) the larger planned RCT has apparently been suspended, with results from the pilot study now due for publication later this year.

The Cochrane review also noted 12 'ongoing studies', including two registered in Australia.

The latest brick in this flimsy wall has come from a cohort of 512 freeze-all IVF patients in Barcelona whose treatments were analysed retrospectively to find no statistical difference in outcome between transfer in the first or second cycle after fertilisation and freezing.<sup>4</sup> 'According to these results,' wrote the editor of *Human Reproduction*, 'there is no need to



wait one or more cycles after freezing all embryos before performing FET.'

In their introduction to their study report the authors reiterated the often repeated endometrial arguments in favour of a freeze-all strategy, but add that no studies so far have suggested how long it takes for the pattern of endometrial gene expression and the immune environment of the uterus to return to their pre-stimulation state. These results suggest that LBRs will be the same whether FET is performed during the first or subsequent menstrual cycles following pick-up.

1. Wong KM, van Wely M, Mol F, Repping S, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database of Systematic Reviews 2017, Issue 3. Art. No.: CD011184. DOI: 10.1002/14651858.CD011184.pub2.

2. Maheshwari A, Pandey S, Shetty A, et al. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2012; 98: 368-377.

3. Maheshwari A, Raja EA, Bhattacharya S. Obstetric and perinatal outcomes after either fresh or thawed frozen embryo transfer: an analysis of 112,432 singleton pregnancies recorded in the Human Fertilisation and Embryology Authority anonymized dataset. *Fertil Steril* 2016; 106: 1703-1708.

4. Lattes K, Checa MA, Vassena R, et al. There is no evidence that the time from egg retrieval to embryo transfer affects live birth rates in a freeze-all strategy. *Hum Reprod* 2017; 32: 368-374.



# Results from ESTEEM trial for presentation in Geneva

## ● Polar body analysis as test of aneuploidy

The ESHRE Study into the Evaluation of oocyte Euploidy by Microarray analysis (ESTEEM) stopped recruiting at the end of last year, and the results will be presented at the next Annual Meeting in Geneva. The number of patients enrolled just fell short of 400 (396 in total), which makes ESTEEM the largest randomised controlled trial on preimplantation genetic testing for aneuploidy (PGT-A) carried out so far.

The ESTEEM trial will provide an answer to the question of whether comprehensive chromosome analysis of the first and second polar body of zygotes improves pregnancy rates in patients of advanced maternal age (between 36 and 41 years).

The trial compares cumulative pregnancy rates in this well-defined study population from frozen-thawed embryo transfer cycles, and is being analysed according to the intention-to-treat principle. This is in contrast to currently available RCTs on PGT-A, in which patients were only enrolled when they had a sufficient number of embryos for analysis, with only fresh transfer outcomes evaluated.

Although biopsy has now moved on to the blastocyst stage, with microarray analysis increasingly superseded by next generation sequencing, ESTEEM still has the power to provide clear answers on the validity and efficiency of PGT-A in this patient population. The rationale for choosing polar body biopsy was based on avoiding complications from mosaicism at the cleavage stage and later - moreover most aneuploidies are maternal in origin.

Microarray analysis has been widely validated, and, although it is not as sensitive as NGS to detect mosaicism, this is not an issue in the ESTEEM study, which analyses single cell samples.

The Steering Committee plans to report the results in *Human Reproduction*, but for an early look at the ESTEEM outcome, please join us at our meeting in Geneva (Monday 3 July at 12:15).

*Karen Sermon (Co-ordinator), Joep Geraedts, Patrick Bossuyt, Veerle Goossens for the ESTEEM Steering Committee*

# ESHRE and ESHG in measured position paper on germline gene editing

ESHRE has teamed up with the European Society of Human Genetics to produce a substantial position paper on germline gene editing. The text has been made available for review (on the ESHRE and ESHG websites) and will be published later this year.

The paper makes recommendations against a background which recognises the 'promising' application of germline gene editing in a range of serious genetic disorders (especially Mendelian) but with 'serious' ethical and societal concerns.

The recommendations have been divided as reproductive and non-reproductive, mainly in research functions, with the latter allowed as basic research 'subject to societal oversight and taking account of relevant ethical guidelines and (inter-)national legal regulations'.

A subsequent 'step to the clinic' may be considered, says the report, provided it is 'embedded in a formal and rigid research trajectory'. However, noting the EU Clinical Trials Regulation No.536/2014 that 'no gene therapy clinical trials may be carried out which result in modifications to the subject's germ line genetic identity', the authors note that currently, on strict interpretation, it may well be that clinical germline gene editing research will be 'impossible' in the EU.

## A new ESHRE Special Interest Group in fertility preservation

A Special Interest Group dedicated to fertility preservation has been added to ESHRE's roster of 12 SIGs. The steering committee will be led by Edinburgh reproductive endocrinologist Richard Anderson, with support from Kirsten Louise Tryde Macklon (Deputy), Michael von Wolff (Deputy), Jan-Bernd Stukenborg (Basic Science) and Clara Gonzalez Llagostera (Junior Deputy).

As other ESHRE SIGs, the SIG Fertility Preservation will provide education through pre-congress courses and Campus meetings, often in collaboration with other SIGs. The SIG will also



*Richard Anderson, Co-ordinator of ESHRE's new SIG in fertility preservation*

contribute to the programme of the ESHRE Annual Meeting, and review abstracts for that meeting.

'With the development of new techniques, young men and women - and even children - with cancer or other life-threatening diseases now have an option to preserve their potential fertility,' said Co-ordinator Richard Anderson. 'The scope of the SIG will thus include fertility preservation in women, men and children with malignant and other serious diseases, in transgender individuals, and for non-medical reasons.' The greater success of cancer treatments today means that more and more patients are surviving the disease.

The SIG will create a forum within ESHRE for professionals interested in fertility preservation to support and promote research within the field.



*‘A journal for everyone’*

## Q&A with Siladitya Bhattacharya, Editor-in-Chief of *Human Reproduction Open*, on the new open access journal from ESHRE

**FoR: What prompted ESHRE to launch an open access journal?**

**SB:** *HROpen*, the new open access journal from ESHRE, is an exciting project because it addresses particular unmet needs within the sector. In response to the question ‘Is there a need for yet another fertility journal?’ I would like to argue very strongly that there is - for three reasons:

- *Immediacy and access.* Today we need to deliver scientific literature as soon as it’s available and make it available to everyone, not just to those who can afford to pay at the point of delivery.
- *The value of incremental contributions in science and discovery.* Increasingly, the focus of many journals is almost exclusively on novelty. While disruptive technology and massive leaps in discovery are critical to scientific progress, there is also merit in incremental developments in science. *HROpen* is a journal that values both kinds of contributions.
- *Connecting patients to the science.* Most medical journals have ignored the needs of our ultimate consumers - our patients. What we see now is that our patients, who are often very well informed in reproductive medicine, do their own research with

standard search engines and access information which is often inconsistent with data from the scientific literature. What we offer in *HROpen*, for the first time in our specialty, is an amalgamation of science with an understanding of what consumers need, and a means and determination to make the content available to them. We do this by providing a lay summary of every article, which is rooted in its context and presented in everyday language, making it totally intelligible for all patients who wish to understand what this science and new discovery means to them.

**What would you say to those who say this will dilute the journal’s scientific content?**

I disagree! This is about making good quality science accessible, not about dumbing it down. All articles in *HROpen* will be rigorously peer reviewed to the highest standards. We want articles that will have maximum impact on people and society. In the 21st century quality scientific publishing can be evaluated in a number of dimensions; certainly, it is still about citations but there are other considerations - the impact of the research, how it affects the way we perform clinical techniques or provide patient care,

the way our clinical practice is viewed by our peers, and even by society as a whole. Ultimately, it is about whether we can make lives better for people with reproductive problems.

#### **And was that behind composition of the editorial board for *HROpen*?**

We are lucky to have a skilled and diverse group of clinical and non-clinical scientists covering a wide range of specialities. The associate editors represent a truly international perspective, located geographically across all the major continents.

In addition we have a very experienced lay editor whose role is to make the science intelligible to patients through summaries. This approach will make it absolutely clear how the research is relevant to the condition and what it might mean in terms of any planned or future treatment.

#### **So where are we now with publication?**

The journal platform for article submission was launched in November last year. The first content was published at the end of March this year.

So now's the time to invite researchers to consider *HROpen* as a vehicle to bring their research to a wider audience. Being part of the ESHRE family of journals ensures that published articles and their authors will benefit from the quality stamp associated with such a prestigious Society. The open access model ensures the widest and quickest dissemination of their research, making it accessible to their colleagues, peers, the wider community, and of course, patients.

The journal publishes on continuous publication model, so authors and readers needn't wait for papers to be collated into an issue before access to content. As soon as a paper is accepted, it's out in the public domain and available for everyone to find.

ESHRE has agreed to waive all article processing charges (APCs) of papers accepted by the editorial board at the present time; this provides a unique opportunity for authors across the world to contribute to this journal in its early years.

#### **Where do you expect submissions to come from?**

I'd expect articles to come from the ESHRE community and from around the world – with a healthy balance of commissioned and submitted papers. We want to showcase the best research without any geographical constraints. We wish to publish the best science we can and to bring the most relevant research to the full population of patients we serve.

The overall aim is to highlight every phase of scientific thinking from inception to delivery.

#### **What impact would you like *HROpen* to have?**

I'd like the community to read and talk about *HROpen*, to understand that this is a journal for everyone. I want *HROpen* to go beyond the narrow boundaries of who we are as clinicians, as scientists, and indeed as



patients. I want the journal to speak to everybody who has an interest in reproductive medicine and for everybody to feel that it has something in it of interest, relevance and significance to them.

#### **Why should authors publish with *HROpen*?**

Three reasons; access, access, and access. To give their research maximum exposure, both in a geographical sense and to reach out beyond the narrow boundaries of the scientific community.

#### **And how do they do it?**

If they have views or opinions of interest to the community, or have research findings they wish to share, they should submit by

following the *HROpen* link at <https://academic.oup.com/hropen/>.

#### **And talking of authors, what's the best piece of advice given to you throughout your career?**

To question everything and challenge dogma.

To engage actively with all stakeholders and listen to the diversity of views in framing a research question. And once we get an answer, to make it accessible to everyone. Science hidden away is not good science.

## First publication in March

Issue 1 of *HROpen*, published in March this year, carried an editorial, a review of ovarian tissue cryopreservation (from Richard Anderson and colleagues in Edinburgh), an original validation study of three SNPs for testosterone levels (from Japan), and a report on the practice of ovarian tissue and oocyte storage in Europe from an ESHRE working group.

In his editorial Bhattacharya described *HROpen* as a 'journal for the future'. The traditional paper-based scientific journal, he wrote, is limited by the technology which created it over 600 years ago, adding that the internet has raised expectations in all aspects of life. 'Science and medicine are no different,' he said. 'No longer are people prepared to wait for knowledge to be collated and released in the neat packages of journal issues. *HROpen* will ensure that they do not have to.' This new journal, says Bhattacharya, 'will harness the full potential of the internet to deliver scientific knowledge which is current, personalised and fit for purpose.'

The journal is open for well-conducted studies in reproductive medicine 'in its wider sense', clinical research reports including phase III trials, protocols and pilot results, editorials, reviews or commentaries, and of course correspondence. Readers and possible authors can register to receive table of contents e-mail alerts as soon as new issues of *HROpen* are published online.

# IVF in Switzerland: One step back, two steps forward

● Marc Germond on a multiplicity of regulation and referendum which has seen Swiss IVF limited by legislation but finally in 2017 looking ahead to a new law allowing a full range of treatments including PGD

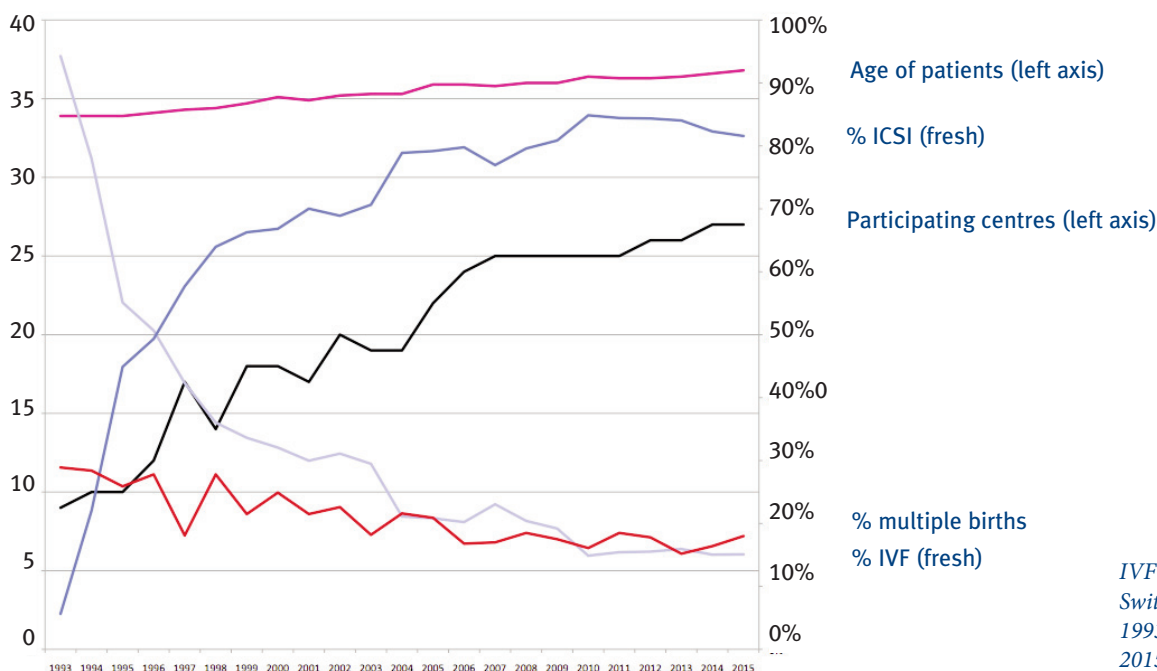


*Marc Germond: 'The 2017 revised legislation will hopefully allow clinicians to treat infertile couples with the latest evidence-based methods.'*

For various reasons IVF has never been either a political nor a social health priority in Switzerland. The first IVF delivery here took place in 1982 (a stillbirth), and four more years were needed for the birth of a second. This protracted situation reflects the multiplicity of opinion, religious or obedient minority intervention, direct democracy (including the right of initiative and referendum) and, since 2001, a restrictive legislation determined to prevent unethical conduct. Indeed, Swiss regulatory and legislative process necessarily had to follow a very slow evolution between 1984 and 2017 (see the table opposite).

In addition, IVF/ICSI has never been reimbursed by the Swiss authorities or health insurance companies. This legal, financial and social framework has so far induced many 'side effects': notably, a tendency to

transfer more than two embryos, great difficulty in introducing single embryo transfer (eSET), low efficiency of transfer at the full blastocyst stage, no advantage from time lapse technology, a high rate of multiple pregnancies, ineffective embryo selection, difficulty in comparing results from different centres in an objective manner, low efficiency of the freezing process, no access to PGD/PGS, short duration of 2PN zygote freezing, low efficiency of social freezing,



*IVF and ICSI in Switzerland: trends from 1993 (2279 cycles) to 2015 (10,038 cycles).*

## A multitude of regulatory and legislative developments

1984	Swiss Academy for Medical Sciences	Guidelines: not compulsory
1987	First popular initiative	Aims to prohibit IVF and DI
1992	First national vote (74% yes)	Inclusion in the Swiss Constitution: Article 24 allowing IVF and DI
1994	Second popular initiative ('protection of human dignity')	Aims to prohibit IVF and DI
2000	Second national vote (79% yes)	Rejection of second popular initiative and acceptance of the Federal Council's indirect counter-proposal: Loi sur la Procréation Médicalement Assistée (LPMA)
2001	LPMA Act	Allows IVF and DI, freezing of three supranumerary zygotes (2PN stage), a maximum of three embryos developed per cycle, set up of a national ethic commission.
2015	Third national vote (62% yes)	Acceptance of DPI/PGS: modification of article 199 of the Swiss Constitution and of the LPMA (accepted)
2005	Referendum	Aims to refuse the new LPMA
2016	Fourth national vote (62% yes)	New LPMA accepted: allows creation of up to 12 embryos, to freeze embryos up to 5 or 10 years, PGD/PGS, mandatory IVF and genetic laboratory accreditations
2017?	Implementation of adapted LPMA Act	Final text not yet available

encouragement of IVF tourism . . . and so on.

IVF/ICSI data have been collected since 1993, when an interest group of the Swiss Society for Reproductive Medicine was formed ([www.sgrm.org](http://www.sgrm.org)) based on the model of the French registry the FIVNAT: FIVNAT-CH. This allowed for anonymised data from all centres (except one which joined the register in 2015) to be collected on a voluntary and self-financed basis. Since 1997, the quality of the collected data has been audited by a neutral international auditor and transmitted to the Ministries of Justice. Since 2005, data collection has been completed, controlled and published by the Federal Statistical Office. A more recent review of assisted reproductive medicine in Switzerland has been published, pointing out the benefits and drawbacks of the current Loi sur la Procréation Médicalement Assistée (LPMA).

The data described in Figure 1 represent 22 years of summarised IVF activity in Switzerland. For the reasons mentioned above, embryo selection, eSET, and the evaluation of cumulated pregnancy rate have rarely been applied in our country. Figure 1 thus focuses on variables which allow an objective assessment of the Swiss IVF/ICSI results. Implantation and pregnancy rates obtained before 2001 have not been evaluated in the same way as later and are therefore not presented here. The yearly pregnancy rates and more details can be found at

<https://www.bfs.admin.ch/bfs/fr/home/statistiques/sante.assetdetail.40834.html>

The Swiss results are routinely transmitted to the European IVF Monitoring consortium (EIM) of ESHRE and to the International Committee Monitoring Assisted Reproductive Technologies (ICMART).

The 2017 revised LPMA will hopefully be implemented this year. It will allow clinicians to treat infertile couples with effective tools. Counselling and individualised treatments will be more efficient and will allow Swiss patients to have access to the latest evidence-based methods for treatment at home.

Find out more at:

Germond M, Senn A. A law affecting assisted procreation is on the way in Switzerland. *J Assist Reprod Genet* 1999; 16: 341-343.

Van den Berg M, Hohl M.K, De Geyter Ch, et al. Ten years of Swiss National IVF Register FIVNAT-CH. Are we making progress? *Reprod Biomed Online* 2005; 5: 632-640.

De Geyter C. Assisted reproductive medicine in Switzerland, *Swiss Med Wkly* 2012; 142: w13569.

Germond M, Urner F, Chanson A, et al. What is the most relevant standard of success in assisted reproduction?: The cumulated singleton/twin delivery rates per oocyte pick-up: the CUSIDERA and CUTWIDERA. *Hum Reprod* 2004; 19: 2442-2444.

The Italian scientist Rita Vassena is a member of ESHRE's Executive Committee, a former Co-ordinator of the SIG Stem Cells, and a co-author of two recent ESHRE position papers - on genome editing with CRISPR-Cas9 technology and on stem cells in clinical medicine. She talks to Focus on Reproduction about the growing overlap between basic science and reproductive medicine.



## Keeping pace with the speed of science

**'Regulation works, provided it's well thought through and relevant.'**

**FoR: You live in Barcelona, work in Barcelona. Has it always been home?**

**RV:** No, not at all. I moved to Barcelona from the US ten years ago, but I was born and raised in Italy. So I trained in Italy and then in Canada, and after I got my PhD I moved to the US. It was from the US that I was recruited to Barcelona.

**So what was your training?**

I guess that by training I'm a veterinarian. But I never really practised because when I got to my first year of university - at the veterinary college in Milan - in the anatomy laboratory I

encountered the ovary. To graduate at the time in Italy we had to write an experimental thesis and mine was on oocyte competence - in cows! From Milan I went to Canada for two years to train and from there back to Milan for my PhD in biotechnology applied to reproduction.

**And still in animals?**

At that time, yes. However, the work then was always done with an eye on translation, using domestic animals as models. The goal was not to improve animal production, but more to understand physiology and improve

assisted reproduction. In Canada, for instance, I was studying the relationship between the follicles as seen on ultrasound and the developmental competence of the oocyte inside the follicle. The idea was to have predictive imaging parameters.

**So how did you make the move from animals to humans?**

It was gradual. When I was in the US I was working on embryo quality in mice using nuclear transfer. I was doing lots of in vitro culture of embryos and at that time - in the early 2000s - there was growing interest in pluripotency and stem cells. So my next step was working on stem cells. But at the time, working on pluripotent stem cells meant working on human embryos donated from clinics - and that's where I crossed paths with the human. And that's also when I moved to Barcelona, to work with the group of Anna Veiga at the Barcelona Stem Cell Bank on human embryonic genome activation. When my tenure there was almost over after about five years I was recruited by the clinic where I work now, Clinica EUGIN. They were looking to establish a formal research department and thought my background made sense. I'd always been a scientist but by training I also had an eye on the world, on the organism as

a whole and the efficiency of a biological process.

**We know that Spain is by far Europe's most prolific country on ART. Is it all run in a private system?**

No, IVF can be done in public hospitals. but most cycles are performed in private clinics.

**So given your scientific background, you're quite happy to be working in this private environment?**

Yes. EUGIN is in fact a group of 11 clinics in five countries. It's large enough - and forward-thinking enough - to recognise the importance of research for improving patient care. They have invested in research with a basic research laboratory with nine full-time scientists - PhD students, post-docs, technicians - which I direct. So it's not much different from an academic environment.

**And your main areas of research?**

We work in four macro areas - clinical embryology, stimulation protocols, psychology and counselling, and basic research. Our basic science lab is housed deliberately outside the clinic in a research park, where the milieu for basic research is much better. And here we are working in three areas - the genetics of fertilisation failure, oocyte quality and especially the epigenetic aspects, and third, which is actually just starting up, embryonic-endometrial interactions.

**How does this translational aspect work and how relevant is it?**

It should be done from solid basic science - what happens, why it happens, the biochemical background, how it might work in humans, and so on. That's how translation should be done. But what we're seeing is that some aspects which might be useful clinically are not being fully investigated before translation. As soon as any potential is seen, there is a rush to the clinic. And because in IVF the barrier for entry for experimental technologies is relatively low, this happens quite often. This to me means that translation moves too fast. In fact, it's not really translation.

**But isn't this the fault of the scientists? As soon as they have this hint of progress, they want everyone to know . . . press releases, public statements, Twitter . . . In publicising these developments they're setting up false expectations.**

I disagree. Press releases from basic scientists are quite detailed and usually worded quite cautiously.

**But why issue a press release in the first place. What's the point if you don't want publicity?**

It's not about publicity, it's about an appropriate message and letting people know about your discovery. And every funding agency will ask you to do it. It's not even a choice. It's the researchers' responsibility to ensure the details are accurate, but responsibility for public expectations belongs to the press.

**The press have been saying in the past few weeks that IVG - in vitro gametogenesis - will eventually supersede IVF. It's a big proposition that an artificial gamete may one day be just as functional as a real one. What's your view?**

We had a fantastic paper in November last year from the group of Hayashi in Japan in which the whole cycle of germline development was reconstituted in vitro. So they took stem cells and drove them to become primordial germ cells, then meiotically competent oocytes and finally fully mature oocytes which were fertilised and generated normal offspring.

**So it was this experiment which got the whole discussion going?**

Yes. It was a truly amazing achievement. It was in mice, and this now opens the door to be repeated in the human. Scientifically speaking, it's a very logical step. But then regulation will be necessary, with a very difficult ethical discussion. Having said that, I think that having an almost unlimited amount of in vitro gametes - oocytes especially - that are meiotically competent will be very useful for drug testing, toxicology, drug safety in the germline, before they are available as treatments in patients. This kind of testing is impossible right now.

**I'm assuming, however, that you do believe this must be regulated in some kind of way?**

Yes. IVG will be a very important step in understanding basic biology. But if we were to consider its use as assisted reproduction it would have to undergo a long and involved discussion and regulatory approval.

**And this is what you were saying in the two ESHRE position papers you were involved in, on in vitro gametogenesis and genome editing?**

In general, the idea that something should not be studied because it might be dangerous or unpleasant or unexpected doesn't usually work. You might ban something, but then it

*Continued over page*

usually goes below the radar. So it's much

## PROUST QUESTIONNAIRE\*

● **Your idea of total happiness?**  
Summer in the mountains, a good book, and my family close by

● **Your greatest fear?**  
Being inconsequential

● **What do you most deplore in others?**  
Idleness

● **And in yourself?**  
Impatience

● **Your greatest extravagance?**  
Stiletto heels - lots of them.

● **What quality do most admire in a man?**  
His cleverness

● **And in a woman?**  
Her cleverness

● **Which talent would you most like to have?**  
Being able to play a musical instrument

● **Your greatest achievement?**  
That would have to be my two daughters

● **Your favourite writers?**  
Mikhail Bulgakov above all, but also Tim Robbins, Kurt Vonnegut, Jonathan Franzen, Paul Auster

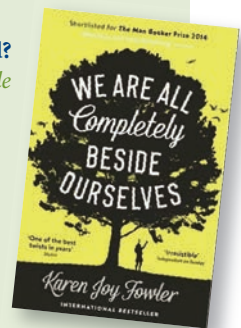
● **The last book you read?**  
*We are all completely beside ourselves* by Karen Joy Fowler

● **When not working, what's your favourite occupation?**  
Reading and dancing tango

● **Where did you spend your latest vacation?**  
On the Italian island of Sardegna

● **If not Spain, where would you most like to live?**  
Enthusiastically, anywhere in Canada

\* A personal questionnaire celebrated and originally made popular by the French writer Marcel Proust



# ESHRE survey supports development of a new guideline in ultrasound for ART, gynaecological pathology and early pregnancy

Ultrasound plays an essential and frequent role in ART. However, there seems to be great national variance in the provision of ultrasound in terms of practice and technique. Between November 2016 and February 2017, ESHRE carried out a survey to explore these differences.

This survey was conducted online among ESHRE members, with 335 responding. Its main aim was to explore a role for ESHRE in the collation of recommendations given by other societies or in published guidelines, and in the development of standards for ultrasound in ART and early pregnancy which might be implemented in Europe and beyond.

The majority of respondents were medically qualified, with 7% of the sample having 'mixed' background as nurses, midwives, counsellors or managers. Participants generally had an appropriate level of knowledge and experience to answer the questions.

Two-thirds of participants reported that there is a national body regulating fertility services in their country of residence (mainly European). However, there were no national guidelines for infertility treatment in one-third of countries (predominantly Eastern European). Furthermore, 50% of participants from Asia, and 80% of from South America and Africa, also replied that they had no guidelines for ART. In these countries, the guidelines of ESHRE, ASRM and NICE were most frequently used.

Only 25% of respondents had a national ultrasound



guideline for ART, gynaecological pathology and early pregnancy. Some had 'partial' ultrasound guidelines - as for example in Australia, Bulgaria (not for ART), Canada, France, India, Israel, Netherlands (not for ART), UK (not for ART) and USA. Respondents in countries where there was no guideline available reported that they used the ESHRE, RCOG, NICE, ASRM and ISUOG guidelines instead.

Importantly, 85% of responders recognised a need for separate standardised guideline regulating USS in ART, gynaecological pathology and early pregnancy.

Notably, 181 respondents suggested the topics opposite for coverage in a potential guideline:

Two-thirds of respondents said they would like to be

## *In Profile: continued from previous page*

better to acquire knowledge and regulate it as you acquire it. This has always been done. Look at PGD. When it was first introduced there were concerns about designer babies, blue eyes . . . but now PGD is well regulated. The whole idea of designer babies never materialised. So regulation works, provided it's well thought through and relevant.

**Give the speed of progress in reproductive science, do you think a society like ESHRE has the vision to keep pace and provide relevant guidance?**

ESHRE is already a very successful society, and we mustn't forget that. We're doing a lot that's right, and we have to move forward against the background of those

achievements. But I think keeping pace with advances in reproductive science will be a challenge. The global nature of research in reproduction means that what's going on in Japan might have immediate repercussions in Europe. Around 30% of ESHRE's membership is non-European, so thinking globally is even more necessary if ESHRE is to remain an authority in reproduction.

**So how can ESHRE keep up if scientific progress is moving so fast? Guidelines,**

**position statements, journal editorials . . . how can they always be up-to-date?**

The challenge here is to maintain relevance and I think this can be done in several ways. For instance, I think we should develop our relationships with other specialist societies, and I think this needs a structural approach. I also think we should be strategic in our approach to new membership, so that we can offer what a new younger member might want to see in terms of scientific interest and support.





### Technical aspects

- Standards on transvaginal ultrasound for early pregnancy examination
- BASIC TVS & 3D USG, TVHSS
- Transabdominal US
- Doppler US in Infertility: embryo and uterine artery
- Diagnostic ultrasound (SIS, GIS, HyCoSy)
- Endometrium: zoom / magnifying / and doppler for evaluation
- USS calipers position
- Interior diameter vs exterior diameter
- Machines?

### ART

- USS in ART
- ART definitions and methods
- Basic ultrasound scan at first clinic visit (uterine cavity prior to IVF; assessment/treatment of adnexal pathology in ART; endometrial-myometrial pathology in ART; uterine blood flow)

### Ovarian reserve

- Antral follicular count (AFC) criteria
- Ovarian evaluation
- Place of baseline USS for ovarian reserve (AFC)

### Ovulation and cycle monitoring

- Follicle monitoring, follicular tracking
- Luteal support monitoring and follicular measurements during ovarian stimulation
- Folliculogram and folliculometry
- Follicular growth follow up
- Follicle maturity assessment
- Standardised/automated follicular monitoring in IVF
- Triggering criteria
- Cycle monitoring including USS guidelines for OHSS
- Ultrasound diagnostic criteria in severe OHSS

### Oocyte retrieval

- Egg collection definition and technical aspects
- Empty egg syndrome
- Transabdominal egg retrieval (laparoscopy)
- Management of complications of egg collection

### Embryo transfer

- Abdominal ultrasound during embryo transfer
- Transmyometrial embryo transfer

### Interventional

- USS aspiration of endometriomas
- Ultrasound guided procedures
- Cysts management and aspiration
- Reproductive surgery before ART
- USS guided fetal reduction vs embryo reduction

### Early pregnancy

- Normal and abnormal early pregnancy
- Follow up of early pregnancy
- Early ultrasound in recognising malformations
- In pregnancy after IVF/ET and early pregnancy scans
- Diagnosing a failed pregnancy
- Clear definition for a non-viable early pregnancy
- Diagnosis and management of miscarriage
- Ectopic pregnancy
- Recurrent miscarriages
- Aneuploidy screening, preterm birth prevention, pre-eclampsia
- Multiple pregnancy

### Gynaecological pathology

- Assessment of uterine anomalies
- Diagnosis of uterine and ovarian pathologies
- PCOS assessment
- Assessment of endometrial cavity and myometrial anomalies (endometrial polyps size and location; fibroma in relation to the endometrium)
- Cysts (size and pattern) - ovarian cysts
- Adnexal pathology / corpus luteum
- Primary ovarian insufficiency
- Hydrosalpinx diagnosis
- PID
- Endometriosis (deep endometriosis; grading; endometrioma)
- Adenomyosis diagnosis

### Endometrium

- Standards for measuring endometrium and myometrium.
- Endometrial thickness
- Endometrial receptivity
- Endometrial function evaluation
- Description of appearance of endometrial stripe
- Endometrial biophysical score

### Quality management

- Diagnostic sonographic documentation of specific procedures (recorded, systematic US examinations)
- Standard operating procedures
- Quality of interventional ultrasound procedures
- Infection control, clinical governance/KPIs
- Patient's preparation

### Certification/training

- Certification and training requirement for new doctors and nurse practitioners
- Competence in both examination and intervention
- Developing steps to 'certify' individuals with the skill of follicle assessment and embryo transfer.
- Training in USS for infertility and early pregnancy
- Training, equipment, accreditation needs

included in any potential guideline development group and 50% gave their e-mail address for contact.

In conclusion, the strength of our survey lies in the number of responses, its targeted users and broad views on different clinical topics. Limitations are an absence of expert interviews and different answers from the same country on national guidelines. However, we believe that the results of this survey support the development of a new ESHRE guideline on the use of ultrasound in ART, for gynaecological pathology and for early pregnancy.

The SIGs Safety & Quality in ART, Endometriosis & Endometrial Disorders, and Implantation & Early Pregnancy would like to thank all who took part in the survey.

*Arianna D'Angelo*  
Co-ordinator SIG Safety & Quality in ART  
*Andrew Horne*, Co-ordinator SIG Endometriosis  
& Endometrial Disorders  
*Emma Kirk*,  
Co-ordinator SIG Implantation & Early Pregnancy  
*Adiya Urazbayeva*, Cardiff University, UK

# In search of oogonial stem cells

Jonathan Tilly's claims of 'oocyte renewal' from germline stem cells caused a sensation when reported in 2004. Would postmenopausal conceptions now be possible?

Would reproductive biology need a rewrite?

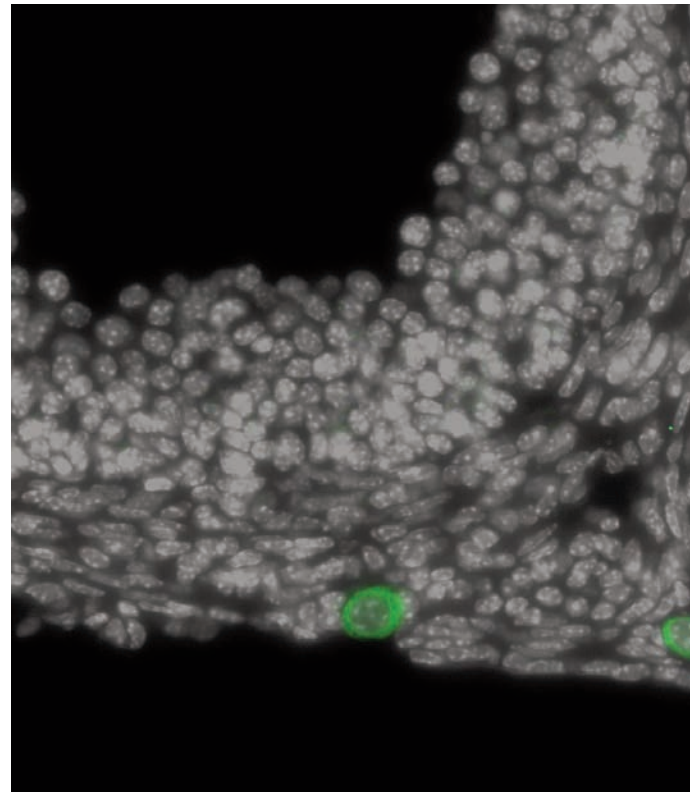
Susana Chuva de Sousa Lopes goes on the trail of oogonial stem cells and asks if new technologies can finally prove (or disprove) their existence.

Well over a decade has passed since the group of Jonathan Tilly in Chicago caused uproar and disbelief with their claim in *Nature* of 'oocyte renewal' from germline stem cells in the postnatal mammalian ovary. Today, 13 years later, the controversy over Tilly's conclusions remains as hot as ever. Intellect tells us to face the facts, yet imagination keeps the sparkle alive. Just what exactly are these cells? Jonathan Tilly remains the enfant terrible of the field, always up for a fight. Will a combination of modern single cell technology and oocyte-differentiation protocols finally bring this controversy to an end?

## A crash course on (human) oogenesis: from specification to menopause

One of the dogmas of reproductive biology is the 'fact' that, in contrast to men (who have a resident population of germ stem cells in their testes, the spermatogonia), women are born with a finite number of germ cells or oocytes in their ovaries (around one million). This pool of oocytes diminishes exponentially with age through atresia and as result of ovulation during the reproductive years. By the age of 45, a woman's chance of natural pregnancy is low and she will usually enter menopause at around 50 years of age.

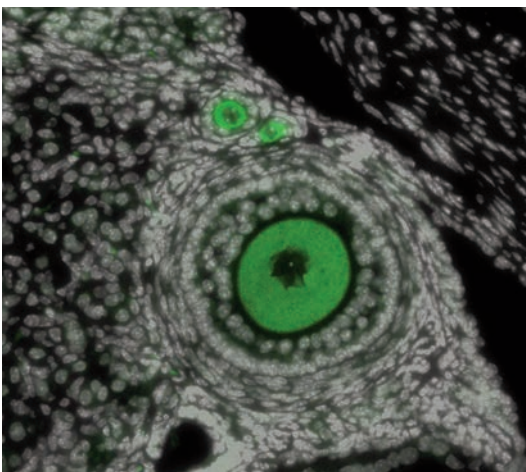
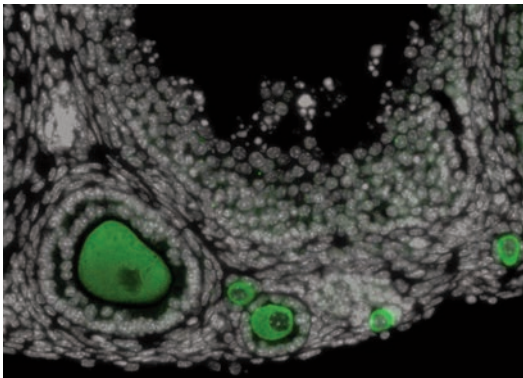
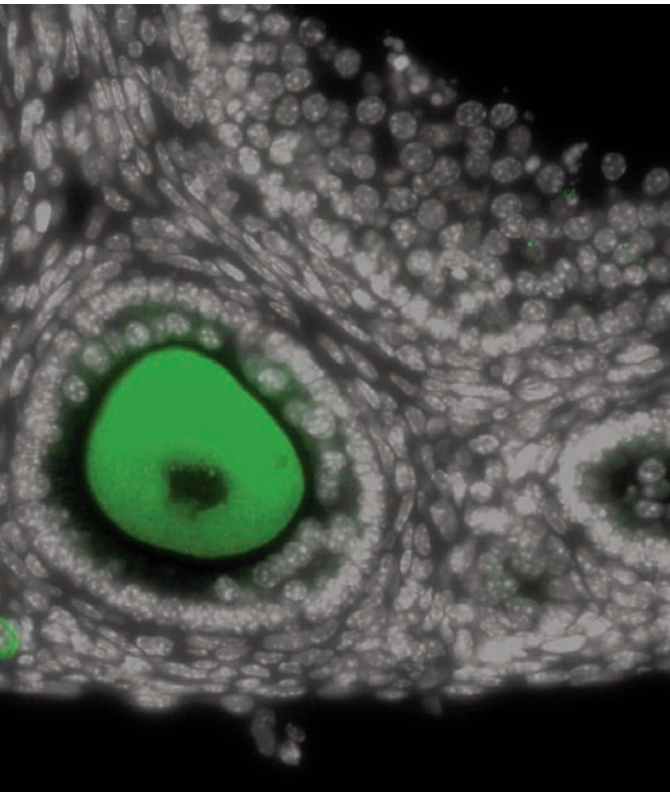
The timing of germ cell specification in humans is unknown. However, germ cells have been reported in the human embryo after



3-4 weeks of development, or after 5-6 weeks of gestation in the dorsal part of the yolk sac at the junction with the embryo (as reviewed recently by Tang et al).<sup>1</sup> In theory, the window of specification is the only time during development when germ cells can be induced; if, due to a mutation, germ cells are not formed, the resulting embryo will be sterile.

Not surprisingly, mouse and human gametogenesis differ considerably, particularly in oogenesis. Female germ cells in mice develop rather synchronously, except for the short-lived meiotic wave.<sup>2,3</sup> In humans, female germ cells develop in a radial fashion: the cells located more interiorly transit sooner from early germ cells (Pou5F1 positive; Ddx4 negative) to pre-meiotic late germ cells (Pou5F1 negative; Ddx4 positive) to meiotic cells to primary oocytes in primordial follicles.<sup>4,5</sup> The early germ cells in the outer part of the cortex become fewer as development progresses, until at birth all oocytes have initiated meiosis and have been incorporated into primordial follicles.

Moreover, it has been known for some time that human and mice germ cells express different sets of markers. One of the most relevant is Sox2, one of the four Yamanaka-factors necessary to reprogram somatic cells into (induced) pluripotent stem (iPS) cells. Interestingly, mouse early germ cells express Sox2, but human germ cells don't.<sup>6</sup> To date it is unclear whether a different Sox family member replaces the function of Sox2 in human germ cells. One study has suggested that Sox17 might perhaps be important in human specification.<sup>7</sup> However, other Sox family members are present in early human germ cells, whereas Ifitm3 and Prdm14 are not/less expressed in human germ cells.<sup>8</sup>



*Will the real OSC please stand up? Mouse adult ovary immunostained for Ddx4 (green), counterstained with DAPI (white), showing different stages of oocyte maturation. Photos from Josephine van der Klaauw.*

### What was (and is) the controversy about?

In 2004 Tilly claimed that there are still mitotically active 'germ cells' in adult mouse ovaries and that they could somehow still sustain both oocyte and follicle production.<sup>9</sup> These cells were coined 'oogonial stem cells' (OSCs) or germline stem cells. They expressed Ddx4 (a marker of late germ cells) and appeared to incorporate bromodeoxyuridine (BrdU), suggesting a proliferative state. The paper landed like a bomb, but Tilly resolutely withstood the criticism and produced a follow-up study.

Even more spectacular findings followed a year later, with Tilly now suggesting that mouse bone marrow (and peripheral blood) could be a source of circulating germline progenitor cells, able to repopulate oocytes in the ovary.<sup>10</sup> This was a troubled scenario for women with bone marrow transplantations wondering whether their children conceived after the transplantation were their own genetic children or were genetically linked to the bone marrow donor. The findings also presented a puzzling scenario for cancer patients who had become infertile as a result of chemotherapy and had afterwards received bone marrow transplantation. Why was their fertility not restored?

In 2006 Eggen and colleagues performed a parabiosis-study, physically joining two mice, one wild-type and one transgenic (b-actin:GFP), to allow blood circulation between the two, but found no evidence that circulating cells could repopulate the oocyte pool.<sup>11</sup> The issue was never raised again.

It was not until 2009, when Wu published a paper showing that oogonial stem cells (OSCs) could be purified from neonatal as well as from adult mouse ovaries (by immunomagnetic-cell sorting using a Ddx4 antibody to isolate OSCs) that the OSCs were once again back under the spotlight - but for all the wrong reasons.<sup>12</sup> Scepticism now was because Ddx4 is a cytoplasmic protein without membrane-bound domains, and as such the usefulness or specificity of using a Ddx4-antibody as surface marker to successfully isolate living OSCs was heavily questioned.

Nevertheless, Wu et al reported that Ddx4-purified OSCs could be maintained in culture for months and showed that, after transplantation to the ovary, these same OSCs were able to undergo/resume oogenesis, producing mature oocytes which were then successfully fertilised and gave rise to progeny.<sup>12</sup> These extraordinary findings have not been replicated and published by an independent lab, but I do wonder how many students in labs around the world have actually tried to repeat the experiments.

Based on Wu's purification technique, Tilly developed it to work on fluorescence-activated cell sorting (FACS) using Ddx4-antibodies to purify live OSCs.<sup>13</sup> This time, OSCs from both human and mouse ovarian tissue were isolated using Ddx4-antibodies as surface marker by FACS and cultured for

## Turning back the clock:

a period of months. Mouse and human OSCs expressed an unusual mixture of early markers (Prdm1, Dppa3, Ifitm3), late markers (Tert, Dazl, Ddx4), meiotic markers (Dmc1 and Sycp3) and even zona pellucida markers (Zp3), all characteristic of follicular oocytes.

To test the functionality of OSCs, mouse OSCs were transplanted back into female ovaries and reported to develop into mature oocytes that were fertilised and to give rise to preimplantation embryos. In parallel, human OSCs were reaggregated with dissociated human ovarian cortical strips, with Tilly reporting the presence of OSC-derived oocytes encapsulated in primordial/primary follicles in the co-culture after a couple of days. Going a step further, Tilly xenografted human ovarian cortical strips that received an injection of OSCs into female mice and the grafts were retrieved and analysed after seven and 14 days. As in the co-culture, OSCs were observed encapsulated in structures resembling primordial/primary follicles, but no follicular growth was reported.

More recently, three back-to-back manuscripts have been published on the nature of the Ddx4-positive OSCs, each reporting some similar and some contradictory results.<sup>14,15,16</sup> Apparently, all the groups were able to sort by FACS a population of cells from the ovary using a Ddx4-antibody and to culture-expand it.

Interestingly, Liu and Hovatta showed that organs other than the ovary seem to have cells that can also be purified by FACS using the same Ddx4-antibody.<sup>16</sup> This is exciting, suggesting that similar (Ddx4-sorted) cell populations may exist in other organs. Moreover, analysis by single cell transcriptomics showed that the OSCs sorted with the Ddx4-antibody did not themselves express Ddx4 RNA. On the same lines, Wolff suggests that the Ddx4-antibody could be cross-reacting with an unknown protein, and that they were unable to detect Ddx4 expression in the FACS-sorted cells.<sup>14</sup>

It would be very interesting to compare the identity of the Ddx4-sorted cells from these different organs and include in the comparison bone marrow and circulating blood cells. Previously, Tilly observed similarities between OSCs and bone marrow/circulating blood. It may be that the Ddx4-sorted cells are indeed some blood-related (rare and proliferative) cell type resident in the ovary, but also in other organs - and thus not an ovarian-specific cell type. Tilly, however, replied that the isolation method used by Liu and Hovatta was different from the one his group used. Could they indeed be different cells?

We await a systematic comparison at the transcriptomics level of the Ddx4-sorted cells from many organs, bone marrow and circulating blood cells to grasp the unique nature and the identity of OSCs. This is a key first point in the conundrum that can now be rigorously addressed.

### Can we finally prove or disprove OSCs?

Until recently, the protocols used to generate mouse mature oocytes in vitro have been met with great hope

The 'Autologous Germline Mitochondrial Energy Transfer' (AUGMENT) treatment is currently being offered in conjunction with ICSI as an approach to enhance pregnancy outcomes. Marketed as a means of improving egg health, it is aimed at patients with poor embryo development and multiple failed cycles. AUGMENT involves transfer of mitochondria harvested from autologous egg precursor cells (EggPCs) into the patient's oocytes together with the sperm during ICSI.

The premise is that the production of cellular energy by mitochondria is compromised in mature eggs. Boosting their energy levels by transferring "young", precursor cell mitochondria may improve their quality, in turn supporting better embryo development. Theoretically, EggPCs have not undergone an extended period of meiotic arrest and as such may harbour fewer mitochondrial DNA mutations.<sup>1</sup>

However, AUGMENT therapy has undoubtedly been met with a great deal of scepticism. Although it has been applied in several infertile couples, with benefits of the treatment reported, the small sample size and suboptimal study design warrant careful interpretation.<sup>2,3</sup> While promising in principle, the commercial launch and rapid clinical implementation prompted intense debate, particularly regarding the efficacy of the technique and its potential risks. Many urged caution and appropriate scientific scrutiny.

The foremost argument against AUGMENT has been safety. As mitochondrial transfer is an invasive procedure requiring laparoscopic surgery before IVF, the lack of quality controls prior to clinical translation have raised many concerns. The effects of adding extra mitochondria to oocytes from cells at different

but disappointing outcome.<sup>17,18,19</sup> Although the resulting cells are oocyte-like either morphologically (large round cells) or because they express a handful of oocyte markers, their functionality remains poorly characterised. It is extremely challenging to get to the mature oocyte-stage in vitro starting from stem cells - and to my knowledge we have not yet achieved it. The bottleneck is undoubtedly our failure to instruct stem cells to undergo normal meiosis.

Although Wu has shown that OSCs could be transplanted to the ovaries of sterilized female mice and offspring could be generated after natural mating, Tilly never went further than using the OSC-derived mature oocyte retrieved from the ovaries to produce blastocysts to show functionality.<sup>12</sup>

However, both Tilly and Wolff showed differentiation to oocyte-like cells from OSCs in vitro.<sup>14,15</sup> These are shown as large round nude cells, expressing a handful of germ cell/oocyte markers.

# Egg precursor cells as a source of oocyte invigoration

stages of development have not been investigated, while the threshold of mitochondrial DNA copies required for the efficient support of embryonic development remains undefined. Excessively high mitochondrial DNA copy numbers have been linked to detrimental effects in mice.<sup>4</sup> As such, the impact of altering energy levels within oocytes requires further consideration. At present, studies have determined extensive variability in the number of mitochondrial DNA copies in oocytes and embryos.

Epigenetic changes propagated by micromanipulation or resulting from nuclear mitochondrial incompatibility also pose a relevant concern, particularly as potential consequences may only manifest later in life.

Although experimental evidence surrounding mitochondrial replacement from egg precursors into mature oocytes is scarce, several animal studies have shown potential advantages of mitochondrial supplementation from somatic cells. Moreover, reports in humans suggest improved oocyte and embryo quality following autologous mitochondrial transfer from granulosa cells.<sup>5</sup> However, favourable effects have only been verified during early preimplantation development and further scientific evidence is necessary when considering broader infertility indications. Certainly, cytoplasmic transfer has shown promise for treating cases of repeated implantation failure in IVF, resulting in the birth of over 20 babies. However, as patient numbers are also limited, it is still unclear whether successful outcomes can in fact be directly attributed to the technique itself. As models predominately use mitochondria harvested from sources other than putative egg cells, controversy surrounding the existence of EggPCs still remains.

What we need, however, is a good working protocol that clearly ends with the production of healthy offspring from these derived oocyte-like cells. And now, we may finally have this protocol.

At the close of 2016 Hayashi et al described a protocol to differentiate mature mouse oocytes from pluripotent stem cells.<sup>20</sup> This is strictly speaking not entirely *in vitro*, as the differentiating germ cells had to be co-cultured with mouse fetal gonads. These fetal gonadal cells do what they do best - they create the necessary niche so that the differentiating cells undergo meiosis and become encapsulated in follicles. These follicles were then separated manually and set to grow individually, and the resulting mature oocytes isolated, fertilised and progeny obtained ( $\pm 3.5\%$  of the 2-cell stage embryos transferred initially).

This assay to test differentiation of stem cells to mature oocytes has the potential to become extremely useful. For we are now able to study those important

Like many other innovations in the world of infertility, AUGMENT has attracted much attention and interest in both the scientific and broader community. Although its clinical implementation has challenged ethical, legal and social norms, further efforts in validation will undoubtedly open a plethora of opportunities in both clinical and basic research. Substantiating the significance of its outcomes may provide great promise; however researchers, clinicians and their patients must maintain heightened awareness of all the potential implications of this new technique.

● An opinion statement on mitochondrial transfer to improve IVF outcome was published by the ESHRE SIG Stem Cells in 2016 (see <https://www.eshre.eu/Specialty-groups/Special-Interest-Groups/Stem-Cells.aspx>)

*Björn Heindryckx and Mina Popovic  
for the ESHRE SIG Stem Cells*

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factors which the fetal gonadal cells provide. Moreover, with some modifications and, if this assay proves robust enough, it could become the gold standard in the field to test the potential of stem cells, including OSCs, to differentiate into mature oocytes.

We thus now have the technological tools (1) to characterise and compare at the single cell level OSCs and determine their precise identity, and (2) to differentiate side-by-side stem cells into (large quantities) of mature oocytes that can be used for molecular characterisation and tested for functionality. This may at last provide the opportunity to resolve this controversial matter once and for all.

## What about human OSCs?

Human OSCs, or what Tilly now calls 'egg precursor cells', could also be compared by single cell transcriptomics to other (Ddx4-sorted) cells in the body, bone marrow and blood. This could be done

**SUSANA CHUVA DE SOUSA LOPES:  
'WE NOW HAVE THE  
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PRECISE IDENTITY.'**



between labs, so that we are all on the same page regarding the identity (and isolation) of the egg precursor cells.

According to Tilly, these cells are found in the protective lining of the ovaries and their mitochondria can be harvested and used to rejuvenate oocytes using Augment technology (see box on previous page). Tilly is one of the scientific founders of OvaScience, a company using rejuvenated oocytes to treat patients, with the first successful live birth reported in 2015. It remains unclear whether other (Ddx4-sorted) cells with similar properties can be harvested from different human organs/tissues and whether their mitochondria could also be used for rejuvenation. The use of such technology for oocyte rejuvenation is still a matter of huge debate.

Finally, the methods used by Tilly to differentiate egg precursor cells into oocytes are unclear, as is the applicability of the Hayashi-protocol to human stem cells at present. Gametogenesis in humans and mice is different, as is the timing of meiosis initiation and the expression of several markers. Hence, translation of the mouse protocol to the human may prove challenging. Time and perseverance will tell.

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Dr Susana Chuva de Sousa Lopes is Associate Professor in the Department of Anatomy and Embryology at the Leiden University Medical Center, Leiden, the Netherlands, and member of ESHRE's SIG Stem Cells

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# Aneuploidy, mosaicism, self-correction and preimplantation genetic testing

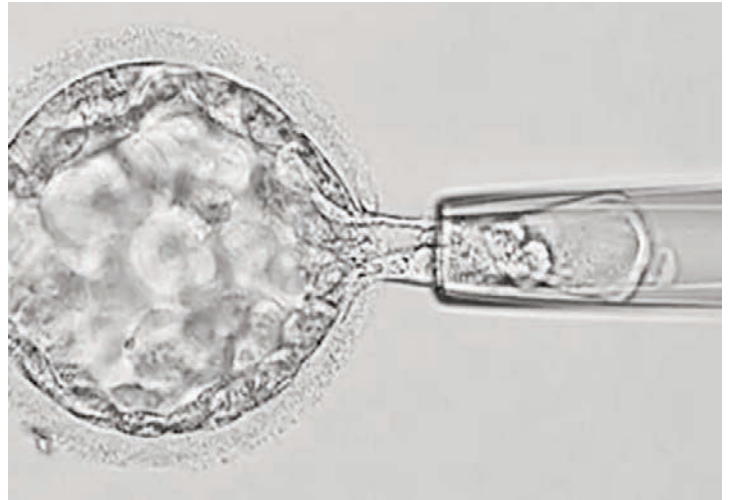
## ● What's new in reproductive genetics

It is now well established that human cleavage stage embryos display a significant rate of chromosomal abnormalities of meiotic and postzygotic origin, often with several cell lineages coexisting in one embryo. Furthermore, the development of methods of comprehensive chromosome screening, by array-CGH or massive parallel sequencing, has shown that, along with whole chromosome abnormalities, human embryos frequently carry segmental gains and losses.

There is not much known on the origin of these abnormalities, and, from the perspective of the fertility clinic, there is still some debate on their extent - on whether embryo mosaicism poses a problem for preimplantation genetic testing for aneuploidy (PGT-A), and even on whether PGT-A should be subject to randomised controlled trials to prove its effectiveness. Indeed, few topics generate more heated discussions in the field of reproductive genetics than that of chromosomal abnormalities in embryos and the sense or nonsense of PGT-A. A short summary of some of the work done in recent years in this field, from basic science studies up to papers on clinical outcomes, thus seems appropriate.

### The origins of aneuploidy

Research on the origin of these abnormalities is scarce, mainly because of the difficulties in obtaining sufficient human embryos for research. On the gamete front, a very interesting recent study on crossover frequencies in human male and female meiosis has uncovered a novel factor in the high susceptibility of human oocytes to meiotic error.<sup>1</sup> It has been known for a long time that the presence and localisation of chiasmata as a result of crossover in prophase I of meiosis correlate to the susceptibility of chromosomes to meiotic missegregation. In this work, the authors prove that



recombination is higher in female than in male meiosis and that in female (but not male) meiosis about 25% of the intermediates fail to mature into crossover products. Wang et al termed this 'female-specific crossover maturation inefficiency', and postulate that it contributes to the general high susceptibility of human oocytes to meiotic errors and to the age-related rise in aneuploidy.

On the susceptibility to mitotic errors in early human development, a favourite hypothesis is that the levels of components of the chromosome passenger are complex and the spindle attachment checkpoint have a significant impact on aneuploidy. For instance, Avo Santos et al. found that the meiotic kinase Aurora C persists in early preimplantation embryos, while Aurora kinase B, expressed in somatic cells and also in the oocyte, undergoes degradation and only reappears after the morula stage leaving a window of comparatively reduced protection.<sup>2</sup> Aurora C is therefore a maternal factor required for correction of abnormal chromosome attachments in the first mitotic divisions - and critical reductions in abundance of Aurora C/B may contribute to relaxed control of chromosome segregation in early embryogenesis. Furthermore, chromosome passenger complex localisation in the prometaphase of zygotes is less confined to the inner centromeres than at later stages of embryogenesis, suggesting that a 'sloppy' localisation of the complex in the zygote contributes to an increase in chromosome mis-segregation, especially of the paternal chromosomes.<sup>3</sup>

Consistent with the idea of permissive checkpoints in the early embryo, Vera-Rodriguez and colleagues identified a subset of mitotic checkpoint genes that were differentially expressed in aneuploid embryos than in euploid, including BUB1, BUB3 and PTTG1 and cell cycle regulator TP53.<sup>4</sup> While overall human embryos appear to possess a functional spindle assembly checkpoint in all stages of preimplantation development, a prolonged arrest in metaphase does not result in apoptosis as in somatic cells. Instead, blastomeres of cleavage stage embryos overcome transient arrests by

initiating a new S-phase, thereby becoming polyploid, and then likely progress to a multipolar cell division in the next cycle, leading to cells with chaotic chromosome complements until day 5 of development when apoptosis may occur.<sup>5</sup> All in all, it appears that early human embryos have a combination of factors that result in a susceptibility to aneuploidy: they may have an 'atypical' control of mitosis, they have cellular diversity in terms of their transcript levels and they do not activate apoptosis when things go wrong until they reach the blastocyst stage.

### Self correction

Another very intriguing fact is that blastocysts contain relatively lower proportions of aneuploid cells than cleavage-stage embryos, and that mosaic aneuploid/euploid blastocysts are able to implant in the uterus and lead to normal pregnancies and newborns.<sup>6,7</sup> A variety of mechanisms have been proposed for this loss of aneuploid cells in the embryo, a process often termed as 'self-correction'. These include allocation of the aneuploid cells to the trophectoderm, cell growth advantage of diploid cells in mosaic embryos or extrusion or duplication of an aneuploid chromosome. There is evidence that this 'self-correction' happens, but little is known of its inner workings. For instance, a recent study investigating embryos with morphokinetic abnormalities, which were expected to produce chromosomally abnormal cells, showed that embryos are able to exclude aneuploid cells during compaction.<sup>8</sup> Embryos even appear to be able to develop normally when starting with a triprounuclear zygote or multinucleated state at the 2-cell stage.<sup>9</sup> Apparently, the ability of a triprounuclear embryo to develop is determined by the parental origin of the extra pronucleus.<sup>10</sup> Furthermore, Bolton et al showed that mosaic mouse embryos have full developmental potential provided they contain sufficient euploid cells - the aneuploidy cells preferentially undergo apoptosis in the inner cell mass.<sup>11</sup>

Finally, a recent study in somatic trisomic cells may help resolve the mystery of how self-correction in early embryos may work. Amano et al showed that expression of the human zinc finger and SCAN-domain-containing-4 gene (ZSCAN4) in trisomy 21 or trisomy 18 human fibroblasts leads to a significant reduction of aneuploid cells, with elimination of the chromosome involved in the trisomy and without affecting other chromosomes.<sup>12</sup> Interestingly, in the mouse ZSCAN4 is expressed at the 2-cell stage and has been shown to be required for maintaining a normal karyotype in mouse embryonic stem cells, providing the link to a potential role in genome stability during early development.

### Blastocyst stage biopsy

With all this knowledge on cleavage-stage mosaicism, and the apparent inefficiency of PGT-A on day 3, the clinics have now moved to the blastocyst stage as the preferred moment for testing. This has led to the

question of how well a trophectoderm biopsy represents the inner cell mass. Although several studies have shown that the abnormalities found in the trophectoderm do indeed match with those found in the inner cell mass of the blastocyst,<sup>13,14,15,16,17</sup> others have not confirmed this.<sup>18,19,20</sup> These discrepancies may be due to a sampling effect in the trophectoderm, and to the fact that aCGH, mostly used in these studies, is not always capable of detecting mosaicism. Massive parallel sequencing appears to be more sensitive, and is able to detect mosaicism as low as 5% at the genomic DNA level and correctly quantify trisomy and monosomy in single blastomeres.<sup>21</sup> On the other hand, it also seems to be more prone to error(s). A recent study found that there was a very poor correspondence between aCGH and MPS results for the same embryo sample, with only 11% of results matching.<sup>20</sup> Whatever the source of the inconsistencies between parts of the same embryo, it is clear that we are not yet in the optimal setting.

### Dealing with mosaic embryos

In any case, assuming that blastocyst stage biopsy accurately represents embryo ploidy, and given the fact that pregnancies can be achieved from the transfer of mosaic embryos, a new topic of debate has arisen in the field of PGT-A: how do we deal with mosaic embryos? To add guidance, the Preimplantation Genetic Diagnosis International Society recently issued a position statement on chromosome mosaicism and PGT-A. They make recommendations on how to treat different levels of mosaicism, and on how to prioritise embryos for transfer, with special emphasis on avoiding aneuploidies involving chromosomes associated with uniparental disomy, intrauterine growth restriction and liveborn viability.<sup>22</sup>

### The ongoing need for RCTs

Last but not least, after years of attempts and following multiple studies providing contradictory results on the clinical utility of PGT-A, it seems that performing an adequate well-designed RCT providing strong evidence on the efficacy of this procedure is indeed a very difficult task. Several PGT-A studies have so far been accused of favourable patient selection (patients whose embryos reach the blastocyst stage), of small patient numbers, of biased conclusions based only on the first embryo transfer in a fresh ART cycle (and excluding subsequent frozen/thawed transfers and therefore not investigating the total reproductive potential of each initiated cycle), false assessment of pregnancy outcome with reference to embryo transfer rather than to intention to treat (cycle started), and - similarly - inappropriate reporting of live birth rates.<sup>23,24</sup>

Such apparent failings led the authors of a recent study to perform a 'hypothetical' RCT analysis, which considered success rates from published studies with respect to the chance of an embryo developing to the blastocyst stage, the chance of the blastocyst being



aneuploid, and the chance of implanting and live birth. The authors concluded that patients having PGS and blastocyst transfer achieved a lower live birth rate than patients with non-PGS day 3 or non-PGS blastocyst transfer.<sup>25</sup>

There is no doubt that the decision to offer PGT-A cannot be based on hypothetical estimate, biased studies or the gut feeling that it should work. The truth is there is still a very strong need for well-designed RCTs, the gold standard of evidence-based medicine.<sup>26</sup> In a recently conducted questionnaire most experts offering PGT-A agreed on this and called for studies on separate subsets of patients, but also agreed on the difficulties of performing them. It is hoped that the continuous assembly of data will finally identify (or not) those patients who truly benefit from this treatment. At the moment, and while waiting for the results of those trials, we should consider the well-designed studies (whether RCTs, retrospective analyses or case reports) and appreciate their contribution to current knowledge. With this in mind, it is essential that the current level of evidence is adequately presented to the patients in support of their decision to complement their fertility with PGT-A or not.<sup>27,28</sup>

*Claudia Spits, Georgia Kakourou, Tania Milachich, Signe Altmäe, Ursula Eichenlaub-Ritter for the SIG Reproductive Genetics and PGD Consortium*

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Claudia Spits (BE), Co-ordinator  
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Ursula Eichenlaub-Ritter (DE), Past Co-ordinator



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# A snapshot of oocyte cryopreservation

As I am about to hand the baton of Co-ordinator to Willem Ombelet at the Annual Meeting in Geneva, it is a pleasure to announce publication of our paper on the statutory background, storage and use of oocyte cryopreservation (OoC), and ovarian tissue cryopreservation (OtC) in Europe. The report is published in the first issue of *HROpen*, achieved thanks to a two-year collaboration with members of the EIM Consortium and Committee of National Representatives.

The subject was chosen because non-medical auto-cryopreservation of oocytes has been the subject of intense social debate and the new freedom it may offer some women to achieve pregnancy as insurance against age-related infertility. Proper information is needed for valid consent, and information starts with data, which so far have only come from a few centres of excellence. We also felt it was time to place the non-medical indication within in the bigger picture of oocyte cryopreservation; medical indications have been much less in the news, even though it is now part of good care for women with serious disease which threatens their ovarian reserve.

In the study we found that a specific statutory framework for OoC and OtC varies - from absent to strict in 24 European countries - and that we could analyse a total of 34,705 OoC cycles reported during the five-year study period 2010-2015 from 17 countries with existing data. A continuous increase in yearly numbers was observed during the study period.

In a detailed analysis of activity for 2013, we found that a total of 9126 aspirations for OoC were reported from 16 countries. Amongst the 8885 oocyte aspirations with fully available data, the majority (5323 cycles, 59.9%) were performed for egg donation, resulting in the highest yield per cycle, with an average of 10.4 oocytes frozen.

We defined other indications for OoC in the following groups: 10.9% of cycles were performed for 'serious disease' such as cancer, 16.1% for 'other medical indications' defined as 'part of an ART cycle', and 13.1% for non-medical reasons. Thus, this latest most debated indication in societal terms actually forms a clear minority of all indications.

In spite of this, our discussion and several other papers published before and since our study show how this has captured the interest of many informed



*Our report on ovarian tissue cryopreservation among the first to be published in HROpen.*

women, as well as society at large. While our current European data can only represent part of the picture, we hope our study prompts a better understanding of the reality of the chances of having a child born after OoC (and /or OtC).

## SIG educational activities

We are continuing our collaboration with colleagues in the SIGs Ethics & Law and Safety & Quality in ART with a pregress course in Geneva on the fertility and parental intentions of transsexuals, whose legal rights are increasingly recognised in Europe, although patchily.

A workshop next September in Helsinki entitled **What can we learn from ART disparities in Europe?** will highlight and analyse the social and economic factors associated with poor access and availability of reproductive technologies - including discrepancies in monitoring and reporting results. This is a collaboration with the EIM Consortium and SIG SQART in which we hope to develop a model for improved patient care.

Finally, by the Annual Meeting in Geneva our members will have elected a new Deputy and the Steering Committee a new Junior Deputy. They should be fully involved in activities in 2018, which already include a meeting at the Council of Europe, a Campus meeting on egg donation, and a pregress course on surrogacy at the 2018 Annual Meeting. We look forward to seeing many of you at these events, and to discussing further ideas at our business meeting following this year's pregress course.

*Françoise Shenfield, Co-ordinator  
SIG Global & Socio-cultural Aspects of Infertility*

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# Continued support for WERF EPHect project

## Another milestone reached in research collaboration

The World Endometriosis Research Foundation's (WERF) EPHect tools are moving towards their next milestone in providing the first three-yearly update based on feedback received by its users, currently 17 centres in 11 countries. A workshop led by EPHect principal investigators Professors Stacey Missmer and Krina Zondervan was held in March at the Society of Reproductive Investigation's annual meeting, in which 28 investigators - with representation from the ESHRE SIGEED - discussed potential changes to the WERF EPHect tools, as well as the optimal way to utilise these.

It was unanimously agreed that the tools need to be available on electronic platforms to ensure ease of completion and uptake, which WERF is now working towards. Furthermore, it was announced that the WERF EPHect tools will be available in the following languages later this year: Danish, Dutch, English, French, German, Italian, Polish, Spanish, and Turkish, allowing a considerable amount of new centres to join this global collaboration of investigators in endometriosis.

'The workshop was a great opportunity to provide user-relevant feedback on the excellent protocols that have resulted from this initiative following their implementation so far in 17 centres across the world,' said SIGEED Co-ordinator Andrew Horne. 'It was encouraging to hear that the majority of the feedback was positive, and that WERF EPHect plans to move towards an electronic platform to facilitate data collection.'

● For more information on WERF EPHect, email: [ephect@endometriosisfoundation.org](mailto:ephect@endometriosisfoundation.org). To register your centre as a WERF EPHect user, please go to: [www.endometriosisfoundation.org/ephect](http://www.endometriosisfoundation.org/ephect)

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disseminate and implement a core outcome set for endometriosis, engaging with healthcare professionals, researchers and women with endometriosis.<sup>1</sup> It is hoped that the implementation of a core outcome set within future clinical trials, systematic reviews and clinical guidelines will enhance the availability of comparable data to facilitate evidence-based care.

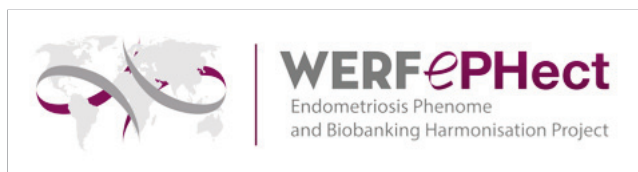
The World Endometriosis Society has recently published an international consensus statement on the classification of endometriosis through systematic appraisal of evidence and a process that included representatives of national and international, medical and non-medical societies, patient groups, and companies with an interest in endometriosis (from 19 countries).<sup>2</sup> This is the first time that a large, global consortium has convened to evaluate the best available evidence on the classification of endometriosis and reach consensus.

### Upcoming activities

On 17 May we are running a joint ESHRE/ASRM pre-congress course ahead of the 13th World Congress on Endometriosis ([www.endometriosis.ca/wce2017](http://www.endometriosis.ca/wce2017)) in Vancouver, Canada. This is a half-day course titled **Unravelling the mystery of infertility and endometriosis**. On 2 July we are holding our annual pre-congress course at the ESHRE Annual Meeting in Geneva on **Endometrial receptivity**. From 18-19 we are running a Campus workshop on **Methodological approaches for investigating endometrial function and endometriosis** in Edinburgh (also a joint venture of ASRM and SIGEED). Finally, we are joining the SIG Early Pregnancy and SIG Safety & Quality in ART to run a hands-on practical Campus workshop on **Ultrasound in assisted reproduction technologies (ART) and early pregnancy: blended training approach** on 16-17 November 2017 in Cardiff.

Andrew Horne  
 Co-ordinator

*SIG Endometriosis & Endometrial Disorders*



### Highlights of other work from SIGEED membership

We would also like to highlight some of the excellent work that a number of our members have been involved in.

The International Collaboration to Harmonize Outcomes and Measures for Endometriosis (iHOME) has recently published a protocol to develop,

1. Hirsch M, Duffy JM, Barker C, et al. International Collaboration to Harmonize Outcomes and Measures for Endometriosis (iHOME). Protocol for developing, disseminating and implementing a core outcome set for endometriosis. *BMJ Open* 2016; 6: e013998.

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# Developments in reproductive transgender care

## EU 'work package' on tissue and cells in ART moves forward

This year's pregress course in Geneva will be run in collaboration with the SIGs Psychology & Counselling, Ethics & Law and Global and Socio-cultural aspects of infertility on **Transgenderism and reproduction: State of the art in fertility options for transgender and people with sex reassignment.**

Hormonal and surgical treatments for transgender people have a devastating effect on their chance to reproduce. Transgender people also tend to start sex reassignment treatment at a young age, when reproductive wishes are not yet clearly defined or fulfilled. They are therefore a growing patient population at our fertility clinics, seeking advice on how to fulfil their child wish or fertility preservation treatments.

While genital reconstructive surgery definitely results in sterility, hormone therapy has an important, but partially reversible impact on fertility. Despite pervasive discrimination and invisibility, in recent years transgender people have experienced significant advances in social acceptance, and the media attention given to the transition of certain celebrities has undoubtedly had an effect. Reproductive transgender care is a true niche in the ART community. However, it is clear that more and more trans people are 'coming out as trans' and many will present at ART centres for advice and treatment.

Our course in Geneva will not only review what the possibilities and options for fertility preservation are for transgender people, but will also develop an awareness around transgenderism and fertility. Experience from centres of excellence will be shared; knowledge on treatments, counselling and the trans' perspective on fertility will be discussed.

### Campus courses

Book your diary for the following exciting Campus courses:

- 28-29 September in Helsinki in collaboration with the SIG Global and Socio-cultural aspects on (in)fertility and the IVF Monitoring (EIM)

Consortium on **What can we learn from ART disparities in Europe? Safety, quality and socio-cultural factors.** This course will provide an up-to-date overview of fertility treatment patterns and trends in Europe. Are there differences in treatment protocols and reimbursement policies? Do they have an impact on the outcome of the treatment? How does

### STEERING COMMITTEE

Arianna D'Angelo (GB), Co-ordinator  
 Kelly Tilleman (BE), Deputy  
 Ioana Rugescu (RO), Deputy  
 Zdravka Veleva (FI), Junior Deputy  
 Willianne Nelen (NL), Past Co-ordinator  
 Augusto Semprini (IT), International Advisor  
 Daniela Nogueira (FR), Basic Science Officer



this affect cross-border fertility care and when does this become infertility tourism? National registries can deliver the data to answer these questions - which is the objective of this Campus course: learn the data, find trends in the analysed results and develop evidence-based policies in fertility treatment.

- 16-17 November in Cardiff, UK, **Ultrasound in assisted reproduction technologies (ART) and early**

**pregnancy: blended training approach.** This course is organised in collaboration with the SIGs Endometriosis & Endometrial Disorders, Implantation & Early Pregnancy, the Paramedical Group and the British Society of Gynaecological Imaging (BSGI). This is not just a theoretical course, but will also provide a practical hands-on training in ultrasound. The course will focus on controlled ovarian stimulation and hyperstimulation, endometrium and implantation, ovarian and adnexal pathologies, interventional ultrasound, and the quality and safety aspects of ultrasound. Because of the practical aspects of the course, only 70 delegates will be accepted on a first come first served basis. So book your place now!

- 30 November- 2 December in Ljubljana, Slovenia, **Reproductive medicine between science and commercialization**, in collaboration with the SIG Embryology. This Campus course will be quite provocative, aiming to draw a line where science in ART ends and commercialisation starts.

### Quality in ART: Guideline development

Thank you to all for helping us with our preliminary survey on guideline development for ultrasound in ART. It was very successful, and has been reported in full by the three SIGs involved on page 24 of this issue.

An application for the clinical guideline on female fertility preservation was successfully submitted and accepted by ESHRE's Executive Committee. A new working group is now being established, and more updates will be made available in the next issue of *Focus on Reproduction*.

We are now in the process of shaping our activities for 2018. We appreciate any input from our members, so contact us.

Arianna D'Angelo  
 Co-ordinator SIG Safety & Quality in ART

# EURO-GTPII working group on the specific risk assessment of innovations in ART

Euro-GTPII ([www.goodtissuepractices.eu](http://www.goodtissuepractices.eu)) aims to establish good practice as applied to tissues and cells preparation and patient follow-up procedures to ensure their safe and effective application and evaluation. This Euro-GTPII project gives continuity to the first Euro-GTP initiative, which developed good practices for activities carried out in tissue establishments. The third guide on safety and quality of tissues and cells, which also includes ART, will be published by the end of this year and be available through the ESHRE website via the SIG SQART web page.

The kick-off meeting for the ART work package in the Euro-GTPII project took place in Ghent in March. Headed by Rita Piteira (Euro-GTPII co-ordinator) and Kelly Tilleman (leader of ART work package), the group of ten experts discussed the applicability of the proposed methodology, a generic tool able to assess novelty and risks.

ART is a field where technologies rapidly change and the introduction of a new technique or process depends on the investigation of both its efficacy and safety, with follow-up programmes in patients and the resulting children. Several studies have concluded that developments in ART procedures are often implemented too quickly, that the level of novelty is sometimes very high and the procedure considered highly experimental. For example, the designation of 'experimental' for oocyte vitrification was a source of debate in ESHRE a few years ago; the SIGs Ethics & Law and SQART were appointed by ESHRE's Executive Committee to produce a tool which might help decide when a technique could be considered experimental or not. A proposed framework reflects the continuous progression of new procedures from



experimental through innovative to established.<sup>1</sup> It was this model that was actually used as a basis for the Euro-GTPII generic tool used to assess the novelty level of procedures and processes. This Euro-GTP II generic tool is based on risk factors identified in every novel procedure. The result of this exercise is an assessment of overall risk and alongside the novelty of the procedure, which is then correlated with the extent of follow-up studies needed.

The ART team is now working on developing this Euro-GTPII generic tool as an ART-applicable instrument, which will be circulated among ESHRE members. It is very important that results from this project are supported by the ART community and we are therefore

planning on feedback of all stakeholders. The ART Euro-GTPII team will have its second meeting during the ESHRE Annual Meeting in Geneva, where the timing of circulation and consultation will be determined. If you have any questions about this project please contact us through Kelly Tilleman ([Kelly.Tilleman@UZGent.be](mailto:Kelly.Tilleman@UZGent.be))

1. Provoost V, Tilleman K, D'Angelo A, et al. Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014; 29: 413-417.



Members of the ART work group at its first meeting in March.

## EuroGTP II Outcomes

### ● EuroGTP II Guide:

Definition of the methodology to assess the novelty grade (risk value):

- Definition of minimum safety and efficacy data that should be provided prior to use in routine - risk based approach methodology
- Definition of the validation studies, clinical studies, and follow up programs

### ● Interactive assessment tool

Will provide information related to

- Procedures, protocols and clinical data required to ensure quality and safety
- Practical assessment of extended studies and follow-up programs needed to implement, evaluate and authorise a novel T&C product, process or therapy.

# Adjuvant treatments under the Campus spotlight

The SIG Reproductive Endocrinology has already completed its first educational activity of the year with its Campus meeting, organised by Bulent Urman and Stratis Kolibianakis on **The multifaceted challenge of female reproductive ageing**. This well attended event took place in Athens, offering in depth review and discussion on the physiology of the ovarian ageing process and the management of couples with early age-related fertility decline. The 360-degree analysis of reproductive issues across lifespan included the obstetrical complications associated with advanced maternal age, premature ovarian failure and novel aspects of physiological menopause.

## Ovarian stimulation guideline

This year will also see further steps in our process of building the ESHRE guideline on ovarian stimulation for IVF/ICSI. A complete set of relevant PICO questions has now been agreed on by the guideline development group, first literature searches have been completed, relevant paper selection is in full action, and first data extractions have been made. On such topics as FSH dosage, protocol, response monitoring and OHSS prevention the GDG group aims to provide strong answers on the questions raised. We hope that a first draft of the guideline will be available for peer review early in 2018.

### STEERING COMMITTEE

Frank Broekmans (NL), Co-ordinator  
 Peter Humaidan (DK), Deputy  
 Daniela Romualdi (IT), Deputy  
 Georgios Lainas (GB), Junior Deputy  
 Roy Homburg (GB), International Advisor  
 Jenny Visser (NL), Basic Science Officer  
 Stratis Kolibianakis (GR), Past Co-ordinator



SIG RE Deputy Daniela Romualdi.



### Activities in 2017

Our pregress course in Geneva considers how ovarian stimulation can be optimised by an individualised approach, the pharmacodynamics of stimulation drugs, and the physiology of folliculogenesis.

Later in the year we will host a Campus meeting in Vienna on the impact of adjuvant treatments on pregnancy potential in IVF. This symposium on a currently very hot topic will offer update information on the rationale for adjuvant treatments at the level of ovary, oocyte, spermatozoa, embryo and endometrium.

Taken together, this is a year full of activities, and we looking forward to meeting you all in Geneva in July to share how we are moving ahead. So, mark your agenda for the SIG RE Business Meeting, to take place on Tuesday 4 July at 13.00 hrs.

*Frank Broekmans, Daniela Romualdi  
 Co-ordinator, Deputy, SIG Reproductive Endocrinology*

## SIG STEM CELLS

# The overlap between human embryology and stem cells

Our pregress course in Geneva will focus on the molecular crosstalk and overlap between 'human embryology' and 'human embryonic stem cells'. The influence of patient parameters, embryo quality and culture conditions on both human embryonic development and embryonic stem cell derivation will be presented. Next, the technology of genome-wide transcriptomics by RNA sequencing has made dramatic progress in the last few years, allowing transcriptomics at the single cell level in human embryos, and giving new insight into how embryonic development is regulated. Epigenetic resetting in early development has also attracted much interest but until recently it has been difficult

### STEERING COMMITTEE

Björn Heindryckx (BE), Co-ordinator  
 Cristina Eguizabal (ES), Deputy  
 Susana de Sousa Lopes (NL), Deputy  
 Mieke Geens (BE), Junior Deputy  
 Rita Vassena (ES), Past Coordinator

to obtain epigenetic profiles at the single embryo level, especially in human. Finally, different pluripotent stem cell types exist, which could be due to the stage of embryonic origin, and there is huge overlap in the chromosomal abnormalities frequently observed in both human embryos and human embryonic stem cells.



Finally we are planning a new symposium in early 2018 on **In vitro modelling: from embryo to gametes**, which will be held in Bilbao, Spain. This Campus course will describe and discuss highly innovative techniques for in vitro modeling, progressing from the embryo right up to the derivation of gametes.

# Time for change in Steering Committee composition

In July the composition of the SIG Embryology Steering Committee will undergo major changes. After a two-year term, Giovanni Coticchio will step down as Co-ordinator, to be replaced by Susanna Apter. Susanna is an exceptional scientist and undoubtedly will lead the group to new and important achievements. Maria José de los Santos Molina and Sophie Debrock will step down from their positions as Past Coordinator and Deputy, respectively. Both have been crucial in our major achievements, notably in the *Revised Guidelines for Good Practice in IVF Laboratories*, the *ESHRE/Alpha Consensus on ART Laboratory Performance Indicators* (expected to be published later this year) and many scientific and educational events. Debbie Montjean will also step down from the position of Junior Deputy. Over the last two years, Debbie has brought a note of fresh energy to the group and, for this reason, we hope she will continue to collaborate. The only unchanged player in this phase of renovation is Roger Sturmey, our Basic Science Officer. Roger is a first-class scientist and his contribution has already made an impact in the quality of our educational events. So we now encourage ESHRE members who follow and support the activities of the SIG Embryology to apply for the three positions that soon will become available in the Steering Committee.

## Precongress course Geneva

Our precongress course this year - **Cellular and molecular biology for clinical embryologists** - will meet many educational needs. With the increasing importance of technology in the IVF laboratory, there is a growing demand from clinical embryologists for a

## STEERING COMMITTEE

Giovanni Coticchio (IT), Co-ordinator  
 Sophie Debrock (BE), Deputy  
 Susanna Apter (SE), Deputy  
 Debbie Montjean (FR), Junior Deputy  
 Roger Sturmey (GB), Science Officer  
 Maria José De los Santos (ES), Past Co-ordinator



deeper understanding of the fundamental biology of gametes and embryos. The panel of speakers is a 'dream team' of expertise, with some of the speakers well known to an ESHRE audience. The subjects included in the programme are among the most topical for IVF embryologists and scientists: oocyte-cumulus cell communication, oocyte

cell cycle and cytoskeleton regulation during maturation, sperm function and gamete fusion, fertilisation and fertilisation failure, chromosome and DNA integrity in reproductive cells, mitochondrial function and epigenetic regulation in oocytes and embryos.

## Campus events

Later this year, two major events will be hosted by the SIG Embryology. In collaboration with the SIG Reproductive Genetics and with local input from Chris Barratt, Scott Nelson and Siladitya Bhattacharya, our Campus **From gametes to blastocyst** will be held in Edinburgh on 12-14 October. We are also pleased to announce the meeting on **Reproductive medicine between science and commercialization**, organised in Ljubljana on 30 November-2 December and promoted locally by Borut Kovačič, Veljko Vlaisavljević and other Slovenian colleagues. The purpose of the workshop is the correct route for developing and introducing new laboratory and clinical ART methods. Particular emphasis will be given to the safety and effectiveness of such methods.

*Giovanni Coticchio*  
 Co-ordinator SIG Embryology

## Steering committee changes

During the Annual meeting in Geneva, Björn Heindryckx will step down as Co-ordinator, and become immediate Past Coordinator. Cristina Eguizabal will become the new Co-ordinator of the Steering Committee. Cristina is currently Group Leader in Cell Therapy and Stem Cells and Chief of Research Unit at the Basque Center for Blood Transfusion and Human Tissues in Galdakao (Bizkaia) Spain. She has a great experience in pluripotent stem cells and primordial germ cells, especially in differentiation protocols towards germ cells amongst other cell types. This change means that a new election for the position of a Deputy will take place, so a warm call for all the stem cell

researchers to join the SIG Stem Cells to promote pluripotent stem cell research and its overlap with embryology in Europe.

The other remaining Deputy, Susana Chuva de Sousa Lopes, will remain in post for another term of two years. Finally, a new Junior Deputy has to be elected by the Steering Committee, as Mieke Geens already served a term of two years. We would like to thank Mieke for her valuable contributions over the past two years, especially in her efforts to update and modernise our SIG webpage. Sarita Panula, our current Basic Science officer, continues for another year.

*Björn Heindryckx*  
 Co-ordinator SIG Stem Cells

# Adding up the cost of IVF adjuvants

- Consensus appeals for honest, clear patient information
- Reliance on RCTs as gold standard evidence



It began as a storm in a small English tea-cup, an ‘investigation’ by the BBC into the use of adjuvant therapies (‘add-ons’) by UK IVF clinics. In support of the BBC probe was a new study reported in the *BMJ* in which the Centre for Evidence-Based Medicine in Oxford claimed there was little evidence of benefit in most of 27 add-ons identified and examined.<sup>1</sup> These included ovarian reserve testing, assisted hatching, hysteroscopy, PGS V1 and V2 and sperm DNA testing. ‘There is an urgent need for randomised controlled trials for many interventions that are currently being offered,’ the report urged.

The popular press had a field day, accusing clinics of exploiting patients and charging for useless procedures.

It made little difference that in the small print of the study it was made clear that the BBC had actually commissioned the study and that the BBC reporter fronting the programme ‘is a freelance editor at the *BMJ*’. Conflict of interest, or what? At best it was shoddy journalism, and yet another example of the BBC manufacturing the news it would later report.<sup>2</sup> The study’s lead researcher, Carl Heneghan, told the BBC reporter/*BMJ* editor: ‘Some of these treatments are of no benefit to you whatsoever and some of them are harmful’ - the latter a reference no doubt to the Mastenbroek PGS study of 2007 and a Cochrane review the year before.

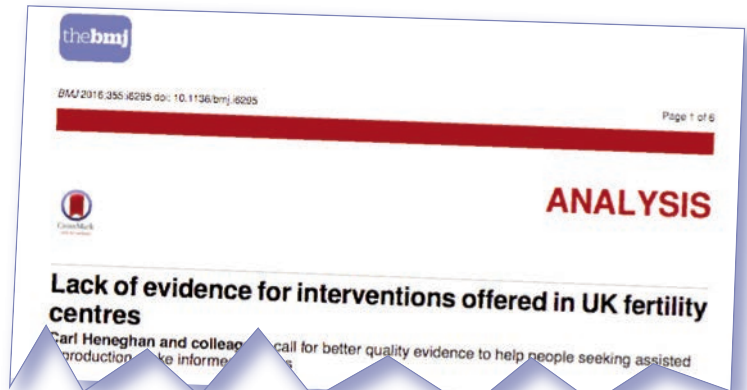
A letter criticising the study was sent to the *BMJ* from Adam Balen, chair of the British Fertility Society, and 60 other specialists, including David Adamson as Chairman of ICMART and Bart Fauser as Chair of the WHO Global Guidelines Taskforce for the Management of Infertility. The letter’s

main objection was that the study had obscured the real definition of add-ons and that many of them - such as ovarian reserve testing or testicular sperm extraction - had a ‘clearly defined role in specific situations’. The study was thus ‘inherently flawed’, its scientific basis ‘clinically and scientifically unsound’. The letter also pointed out that objections made by one of the peer reviewers were not addressed, and that using live birth rate as the only indicator of evidence ‘oversimplifies a hugely complex process and fails to recognise the significant scientific research underlying decisions to bring treatments into clinical practice’.

This domestic tiff took on a wider perspective when 11 internationally recognised experts, including the Chairman of ESHRE, offered their ‘opinion’ in *Human Reproduction* that there are ‘numerous examples where adjunct treatments are used in the

absence of evidence-based medicine and often at an additional fee’.<sup>3</sup> The paper examined six add-ons - embryo glue and adherence compounds (evidence ‘suggestive’ of benefit), sperm DNA fragmentation testing (limited evidence), time-lapse imaging (limited evidence), PGS (limited evidence), mitochondrial DNA load measurement (no evidence), and assisted hatching (no evidence) - and repeated the advice that new introductions should always depend on preliminary work on animal models, human embryo research and well designed RCTs with a follow-up of all children born from the procedure.

More recently still, the question of add-ons (‘do they add up?’) was the subject of an oversubscribed public meeting at the RCOG in London at which Adam Balen repeated his claims that the *BMJ* paper was ‘flawed’ and the chair of the HFEA admitted there had been a ‘step change’ in the use of adjunctive therapies,







notably endometrial scratch, assisted hatching and time-lapse imaging.

There were two common denominators to the presentations in London: the need for clear information to patients and the quality of evidence behind that information. It was clear from many in the audience that, despite its gold-standard pedigree, RCT evidence was proving increasingly difficult to generate. How many recent Cochrane reviews (as in the latest on freeze-all protocols) have concluded that evidence was low quality and insufficient to draw strong conclusions?

In London the barriers to the introduction and completion of meaningful RCTs were identified as funding, bureaucracy and recruitment. Important studies, it was said, have been running more slowly than anticipated because of slow recruitment (and not necessarily just because of funding).

So how will the relatively small world



even need an RCT for every procedure. For some, as Hans Evers, the editor of *Human Reproduction*, made clear in his March editorial, it is clearly unnecessary, but these, he emphasised, are not the situations where RCTs are urgently needed.

Consensus, if any, seems to be on the side of honest and accurate patient information and the randomised trial as the best source of that information. The *HR* editorial acknowledged that 'appropriately powered, well-designed, peer-reviewed RCTs, with an LBR outcome measure' are the gold standard of evidence-based medicine, even though they are not easy to run, especially against 'high noise' backgrounds.

For its part, the HFEA in Britain has included 'safe, ethical, effective proven treatment' in its 2017-2020 strategy, which it hopes to achieve through 'effective evidence-based treatment and add-ons, and science that is well explained'. This shifts much

The quality of evidence as a source of patient information was a recurring theme at the RCOG meeting in London, with veteran IVF specialist Simon Fishel, left, questioning the feasibility of providing strong RCT evidence for every single procedure. He said - as he has done in the past - that, if practice always waits for the RCTs, innovation and the introduction of new techniques will inevitably slow overall progress and the uptake of more accurate technologies. Adam Balen too, while committed to accurate patient information, acknowledged that RCTs can be 'difficult' to perform and may not always be necessary.

Nevertheless, an HFEA review of the websites of UK IVF clinics found that most offered at least one add-on, with little evidence of efficacy. The HFEA chairman admitted that most of such claims were outside the scope of the Authority's legislative power, so she too appeared to accept the criteria of clear information and patient choice. The HFEA, she said, will use a traffic light system on its new website to inform patients about the quality of evidence on specific add-ons.

● The meeting, staged at the RCOG, was organised by the Progress Educational Trust (publisher of *BioNews*) and supported by the British Fertility Society.

of reproductive medicine provide the data that everyone seems to demand? Do we need a more realistic definition of 'evidence', as Darren Griffin controversially suggested in *Focus on Reproduction* in January, or do we

responsibility back to the individual clinic (which is where we came in at the start) and their acceptance of a culture of 'responsible innovation'.

Treatment add-ons, says the HFEA, is not a straightforward issue, adding: 'We do not want to create a situation in which innovation in fertility treatment is stifled. There may well be a place for treatment add-ons in the clinic. However, we want patients to have access to good quality, reasonably-priced treatments which maximise their chance of a pregnancy and birth.' And most protagonists in what is now becoming an ideological as well as scientific debate would surely not disagree with that.

● ESHRE's SIG Reproductive Endocrinology will run a Campus course on 'the impact of adjuvant treatments on pregnancy potential in IVF' in September. The course will take place in Vienna on 15-16 September.

Simon Brown  
*Focus on Reproduction*

1. Heneghan C, Spencer E, Bobrovitz N, et al. Lack of evidence for interventions offered in UK fertility centres. *BMJ* 2016; 355: i6295. doi:10.1136/bmj.i6295.
2. <http://www.bbc.co.uk/news/health-38094618>
3. Harper J, Jackson E, Sermon K, et al. Adjuncts in the IVF laboratory: where is the evidence for 'add-on' interventions? *Hum Reprod* 2017; 3: 485-491.

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