*Tenth anniversary issue 1*

www.sciencedirect.com
www.rbmonline.com

FOREWORD

# Historical significance of gonadotrophins in assisted reproduction

R.G. Edwards

*Chief Editor, Reproductive BioMedicine Online, Duck End Farm, Dry Drayton, Cambridge CB3 8DB, UK*

## Introduction

It is a pleasure to open the Proceedings of this Supplement on developments in gonadotrophin treatment and their influence on the oocyte and embryo. Gonadotrophins have been at the heart of assisted human reproduction since the 1950s, and have proved of immense significance to both scientific analyses and clinical practice. They were especially significant for the introduction of IVF and its derivatives and continue to attract intense investigation today. I find distinct comparisons exist between the early days of IVF and those arising today as another revolution in understanding of human endocrinology is promised from the rapid developments in this field.

This meeting provides an example of how this field moved forward in the early twenty-first century. In the following paragraphs, I wish to salute the investigators whose papers are published in this Supplement by stressing two aspects of reproductive endocrinology. The first is a description of work on gonadotrophins, which have influenced my career in initiating IVF since the 1950s. The second theme concentrates on new concepts of oocyte maturation *in vitro* and *in vivo* which were essential aspects of IVF since its beginning. Reference will also be made the close timings of ovarian and embryonic development as the oocyte and embryos are formed in response to gonadotrophic stimulation.

## Early days of human IVF and ovarian stimulation

My experience with gonadotrophins began as a PhD student in Edinburgh in the 1950s when an American visitor named Alan Gates came to work in my laboratory, also as a PhD student. He brought with him samples of Organon's pregnant mare's serum (PMS) as a source of FSH, and human chorionic gonadotrophin (HCG) as a source of LH. Alan had been trained by Mervin Runner, who originated the use of PMS and HCG to induce oestrus, ovulation and coitus in immature mice, many follicles were stimulated, and large numbers of oocytes ovulated and fertilized. Similar treatments were ineffective in adults, as Alan Parkes had shown in rabbits. Alan Gates had come to Edinburgh for his PhD on the transfer of embryos from immature mice to discover if they could sustain normal pregnancies. At the time, I was struggling to collect mouse oocytes and embryos by timing the natural oestrous cycle and then mating oestrous females to obtain blastocysts, a highly tedious proposition. Alan's project intrigued me as a much simpler approach, although I saw no reason why adult mice would not respond to PMS and HCG. I had met Ruth Fowler, who was working on growth hormones in mice, and we decided to test PMS and HCG on adult mice to see if research on fertilization could be made easier for me.

The results were astonishing. Virtually all of the adults entered oestrus at the same moment, copulated, and ovulated immense numbers of oocytes (Fowler and Edwards, 1957). Moreover, all of these events occurred according to a strict timetable. The vast majority of oocytes matured from their germinal vesicle stage to the emission of the second polar body, were fertilized, developed into blastocysts and implanted in their mothers. Superpregnancies were established, which developed to full term. The work on pregnancy had to be cancelled because the mothers were under far too much stress with such enormous

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

pregnancies. Ruth and I worked on every aspect of ovulation, repeated treatments on females, stages of embryonic and fetal death, superfetation, and testing dozens of mouse strains selected for large or small body weight, metabolic deficiencies, and various unusual characteristics. The results were always the same: oestrus, ovulation and implantation could be controlled by the two gonadotrophin preparations. Resulting pregnancies were the first births in adults of any mammalian species primed with gonadotrophins. These findings were to provide the basis for human IVF. The exact timing of successive meiotic stages and the emission of the second polar bodies at ca. 11.5 h in every mouse female was also a pointer to later discoveries in human females treated with gonadotrophins.

Intrigued with my entry into endocrinology, my curiosity was stimulated by concepts of stimulating immature oocytes *in vitro* by exposing them to media containing gonadotrophins. To my astonishment, whatever I did, the oocytes liberated from their follicles matured *in vitro* at exactly the same rate as they had done *in vitro*. Maturation was initiated with the stages of diakinesis after 2 h (see **Figure 1**), succeeded by all the succeeding stages of metaphase 1, then to polar body emission and metaphase 2. Moreover, the oocytes followed this programme spontaneously without any gonadotrophins being added to the media. Diakinesis is a crucial stage of meiosis, and in one sense is the initial step on the preovulatory pathway of the oocyte to full maturity. It is very brief and so provides excellent timing of the exact progress of maturation, and the chromosomes then change gradually as the oocyte enters metaphase 1. It is a stage at which the chromosome pairs are beginning to separate, when chiasmata can be scored very simply, and information can be gained on the origin of some forms of human monosomy and trisomy (**Figure 1**).

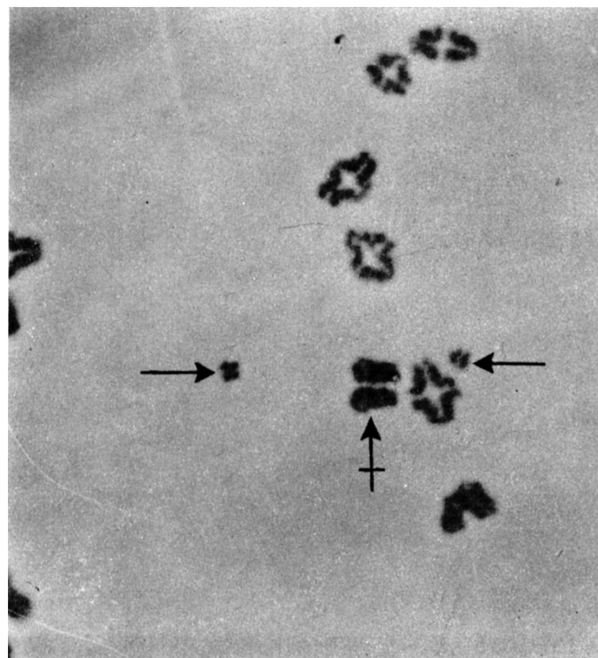

Fig. 1. Part of a chromosomal preparation of a maturing mouse oocyte. Note the structure of the meiotic chromosomes, still arranged in pairs and most displaying a single chiasma. Some of the pairs are anomalous. The arrows indicate two univalents and the crossed arrow a chromosomal pair with no chiasmata (Edwards, 1965b).

The oocytes of rats and hamsters could also be matured *in vitro* and their species-specific durations to polar body emission were very similar to those in mice. However, this discovery of ca. 12 h for maturation could not be repeated with oocytes of agricultural species and those from primates, including humans. As this work was completed, I discovered to my surprise a paper by Gregory Pincus, founder of oral contraception, who had matured rabbit oocytes in this way in the 1940s. In those early days, it was quite difficult to trace papers published 20 years earlier: there were no computerized data bases to help! Pincus also claimed that human oocytes matured after 12 h *in vitro*, yet I simply could not repeat this finding. Human oocytes released from their follicles still possessed a germinal vesicle after 12 h, and whatever I did failed to move them. Almost at my wits end, one day I decided to leave human oocytes *in vitro* for a little longer. To my delight, one of them was identified in 1956 as being in diakinesis after 24 h in culture, and more were found soon afterwards. Suddenly, I knew exactly what was happening: Pincus had been wrong and his data had possibly misled US and Japanese workers who failed to fertilize human oocytes *in vitro*, since they were adding spermatozoa far too soon for fertilization *in vitro*. He had failed to identify diakinesis and misinterpreted the events of meiotic maturation in human oocytes.

It was now a simple task to compare the duration of oocyte maturation *in vitro* and *in vivo* in rhesus monkeys, agricultural species and humans (Edwards, 1965a,b). These studies revealed how each species had its own specific period for oocyte maturation, whether *in vitro* and *in vivo*. Laboratory rodents required ca. 12 h, whereas pig and humans each required 37 h *in vitro*. The route to human IVF was open. It was wonderful to identify the first human oocyte after 37 h *in vitro* as it completed its maturation *in vitro*, reached metaphase 2 and emitted a polar body. This timing was also confirmed in numerous oocytes. This knowledge also indicated that women would ovulate at 37 h post-HCG, and every IVF clinic today accepts these timings as the basis of their work. Moreover, maturing oocytes *in vitro* was so simple that it promised hundreds of mature oocytes for experimental studies. In one study, I was able to store 300 cow oocytes ready for analyses on RNA. All this knowledge led to the first report on the successful maturation and fertilization of human oocytes *in vitro*,

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

as witnessed by Barry Bavister and I. Human IVF was now a certainty.

## Gonadotrophins and ovarian stimulation in women as IVF began

Two significant findings now determined our approach to human IVF as Patrick Steptoe joined the team in 1969. Research by Chang indicated that rabbit oocytes matured *in vitro* and fertilized *in vivo* failed to implant, so it would be necessary to stimulate human oocytes *in vivo* to ensure the resulting embryos would develop normally to term. Donini and Lunenfeld prepared human menopausal gonadotrophin (HMG), just before we entered the clinical phase of human IVF, so we jettisoned PMS in its favour. Their work enabled us to test modest amounts of HMG to stimulate the growth of several follicles in our first patients at the Oldham and District General Hospital. Patients were given with HMG to persuade the growth of several follicles, and then received HCG to stimulate oocyte and follicle maturation *in vivo*. Patrick's laparoscopy confirmed that human follicles were ruptured from 37 h post-HCG, and that oocytes could be aspirated at 36 h, to avoid their shedding from the ovary into the peritoneal cavity where they would be lost. Analyses on eight different steroids in the fluids of follicles collected by laparoscopy which were maturing during the natural cycle or after ovarian stimulation are shown in **Figure 2**. It was clear that the follicles stimulated by HMG were highly variable, indicating they had been at different stages of their growth when the HMG was given (Fowler *et al.*, 1978).

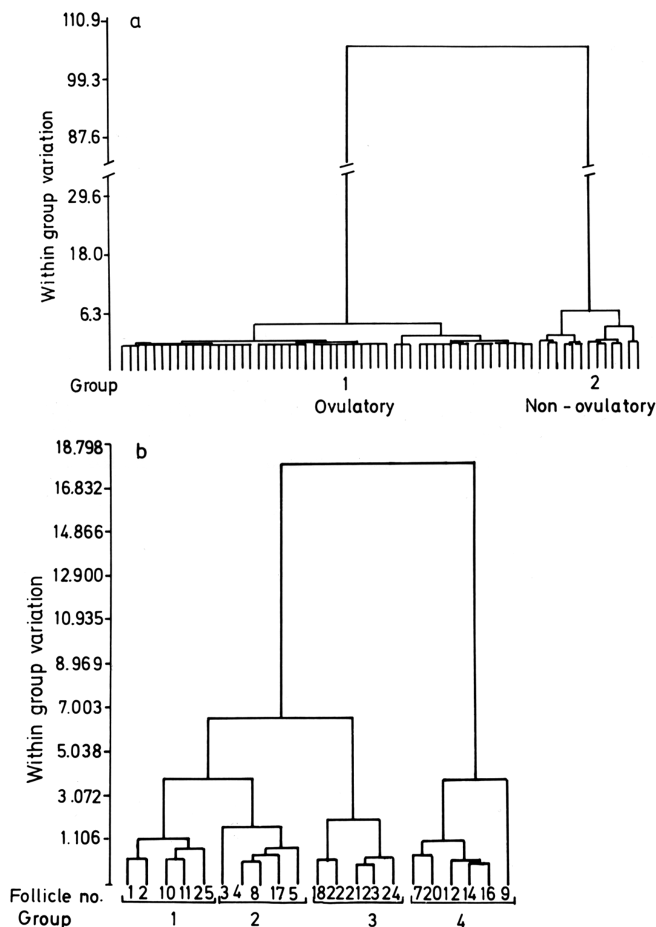


Fig. 2. Cluster analyses on the concentrations of eight steroids in follicles. The upper illustration shows details of the natural cycle, with two distinct classes of follicle, ovulatory and non-ovulatory. The lower illustration reveals the corresponding situation after stimulation with gonadotrophins. Notice the immense variation, with an arbitrary classification into four groups at very different stages of their endocrine maturation (Fowler *et al.*, 1978).

Mature human oocytes looked wonderful *in vitro*, surrounded by delicate masses of cumulus cells. Fertilization *in vitro* now offered no problems as two pronuclei formed in numerous oocytes. Culturing the fertilized eggs *in vitro* led to all the stages of preimplantation development to day 9 *in vitro* (Edwards and Surani, 1978), and the transfer of human blastocysts to their mothers' uteri. We were well on the way to introducing human IVF, and the story of these remarkable years has been narrated elsewhere (Edwards and Steptoe, 1980). A fact that has surprised me ever since those days concerns the huge doses of gonadotrophins currently administered to patients in some IVF clinics. Our first studies revealed that three injections of two 75 IU ampoules of HMG spaced at 2–3 days over the follicular phase were enough to mature several follicles and their oocytes.

Gonadotrophin treatments held a surprise in 1971/2 as we began to transfer human blastocysts conceived *in vitro* to their mothers. Some patients menstruated within 5 or a few more days after oocyte collection. This was not caused by oocyte aspiration, since it occurred in patients treated with HMG and HCG alone. The luteal phase was so brief that any transferred embryos (or those conceived *in vivo*) did not have time to prepare for implantation. We called it the short luteal phase, and it was directly related to concentrations of urinary oestrogens in the follicular phase of our patients. Premature progesterone deficiency was the obvious cause, and would have to be corrected by injecting progesterone-in-oil daily for 9 weeks until the placenta began its endocrine function. This procedure would risk serious scabbing, so we selected the Schering compound named Primulot instead, since it was marketed as a progestagen that saved threatened abortions.

This step nearly ruined our research into IVF. Only later did it become obvious that Primulot was an abortifacient. Without knowing it, we were transferring embryos to their mothers, and then inducing their early abortion. Only when immunoassays for HCG were available to us in the mid-1970s, courtesy of Ken Bagshawe, did we discover that very short-lived pregnancies had occurred in

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

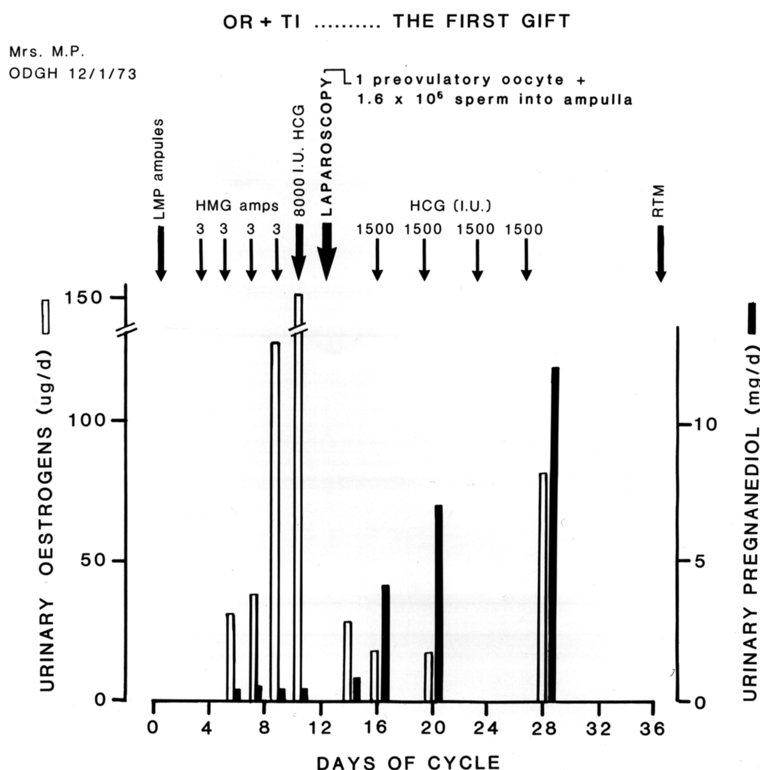


Fig. 3. The endocrine basis of the first GIFT (then called OR + TI) showing the use of HMG for stimulation, of HCG for the induction of ovulation and luteal phase support, and the output of urinary oestrogens and pregnanediol in the patients. Note that a single oocyte was mixed with spermatozoa before its transfer to the oviduct.

several patients after embryo transfer. We called them biochemical pregnancies. Suspicion concerning our lack of success immediately centred on Primulot, and it was jettisoned. Soon after, an IVF patient given progesterone became pregnant after using HMG and HCG – the first clinical pregnancy. Unfortunately, she was found to have an ectopic pregnancy, which had to be removed.

Various hormonal combinations were now tested: clomiphene alone, clomiphene/HMG, bromocriptine/HMG, HCG given at mid-cycle, embryo cryopreservation, oocyte donation and even the first attempts at gamete intra-Fallopian transfer (GIFT) which we called ORTI (**Figure 3**). Clomiphene/HMG produced an impressive luteal phase in our patients. We also tested the natural menstrual cycle via close monitoring of urine samples at mid-cycle using an assay depending on the Japanese compound HiGonavis, which detected small amounts of urinary hormone including the onset of the LH surge (**Figure 4**). Leslie and John Brown were the second patients on natural cycle IVF, and their pregnancy began after the replacement of an 8-cell embryo. We became experienced in natural cycle IVF, and on moving to Bourn Hall we tested the effectiveness of natural cycle IVF and clomiphene/HMG. Each of them is still attractive in being effective and inexpensive, and the use of the latter is described by Dorn and van de Ven in this Supplement. In practical terms, the introduction of the GnRH agonists and recombinant gonadotrophins finally offered the best option.

## Modern ovarian stimulation

Methods of inducing follicle growth and oocyte maturation depend today on highly complex treatments with gonadotrophin-releasing hormone (GnRH) agonists and antagonists, and highly purified preparations of recombinant SH, LH and HCG as described by Engel *et al*. and by Griesinger *et al*. in this Supplement. Combined with advances in our understanding of the events within the maturing follicle, it should soon be possible to equate the actions of these hormones during the preovulatory and ovulatory periods. Fresh research is also revealing small compounds that can bind to FSH and LH receptors to induce similar changes, a highly possible future approach to simpler forms of treatment as described below by Palmer *et al*. Simultaneously, current knowledge now predicts that maturation *in vitro* will challenge maturation *in vivo*, described by von Otte *et al*. in this Symposium. This may well be a huge step, leading to considerable changes in the practice of IVF and its derivatives.

Perhaps closer attention is needed to the doses of gonadotrophins needed for IVF in the earlier years as compared with those used with current methods of ovarian stimulation. A longer period of stimulation with low doses of FSH may be needed in some patients, since small follicles develop FSH receptors. Richard Fleming discusses the nature of follicle recruitment in detail in this conference, a matter still under intensive exploration. Much more information remains to be gained on the exit of follicles from the ovarian pool and the nature of

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

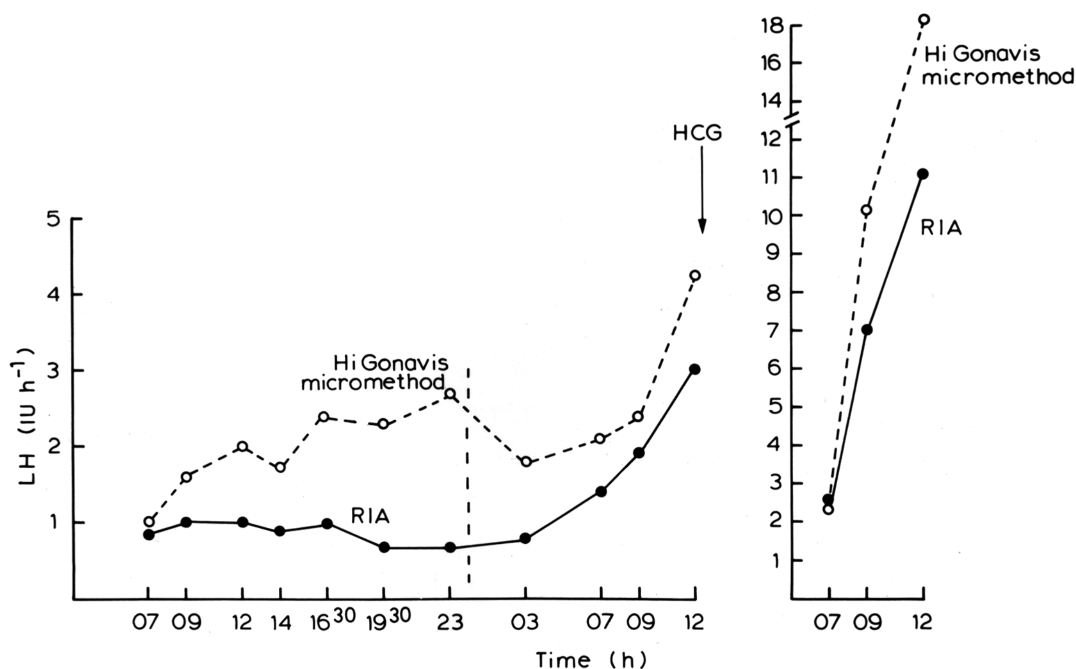


Fig. 4. Comparisons between the use of the HiGonavis assay and radioimmunoassay for identifying the onset of the urinary LH surge. Left: a low rise in LH by both assays starting at 3 a.m. Right: a very sharp surge, starting between 7 a.m. and 9 a.m., and confirmed by both assay methods.

their timed growth through successive stages. Even so, 'overtaking' during the antral period has been reported by several investigators, and it is essential to know if this as happens in earlier phases of growth and during the highly gonadotrophin-sensitive stages.

The availability of recombinant gonadotrophins has revealed fundamental aspects of ovarian stimulation and offers personalized treatments for our patients. The balance between FSH and LH, and the plasma concentrations of these hormones, are matters of intense investigation. In the 1980s, Colin Howles and his colleagues became concerned at the high concentration of LH in stimulation cycles. This situation has been investigated in detail, with the concept of the LH ceiling being described by Macklon and Fauser in this Symposium. Opinions vary, and some investigators stress the need to maintain these concentrations within those of successful cycles or even natural cycles. Some gonadotrophin regimens might also lead to clinical side effects in stimulated patients, as shown by Ledger in a paper presented later in this publication. Even the nature of their packaging, i.e. with vials filled by mass or via bioassays, might lead to divergent properties in the gonadotrophin preparation, depending on their acidic properties as discussed later in this Supplement by Hugues. Much debate has also emerged from the introduction of GnRH agonists, and then antagonists. Once again, considerable research has been undertaken by many clinics on the fascinating opportunities these compounds offer for improved clinical care. Felderbaum has been a leader in this field and presents his opinions in a following manuscript that compares the clinical benefits of these two classes of compounds.

Anomalous menstrual cycles in patients also demand the modification of ovarian stimulation. The polycystic ovary syndrome is a classic case offering problems for stimulation. Responses in these patients may be magnified due to the formation of numerous small antral follicles developing within the ovary. As discussed by Tauchert *et al.* in this symposium, care is needed regarding overstimulation and the effects of hypoandrogenic aspects of the patients' responses. Nevertheless, various protocols on starting and succeeding concentrations of gonadotrophins administered in the follicular phase are now well understood. Finally, and in a sense one of the most difficult points regarding ovarian stimulation and IVF, there is the safety of children conceived in artificial cycles and often via the use of gonadotrophins. Michael Ludwig has considerable experience on this topic, and presents his conclusions below on the greater risk of malformation in children conceived via IVF.

## Maturation of human oocytes *in vitro*: considerable expectations

Data presented at this Symposium, and from the many clinics now deeply involved in in-vitro maturation, offer the prospect of obtaining large numbers of mature human oocytes by undertaking their maturation *in vitro*. This topic was reviewed by von

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

Otta in this Symposium, who outlines the nature of the various treatments including those involving a small dose of FSH to initiate growth in many follicles. Success with this project could change the pace of research into early human preimplantation embryology *in vitro*. Embryos for research have hitherto been very scarce, so that knowledge has improved only gradually. Nevertheless, oocytes from small follicles can be aspirated today and matured *in vitro*, which offers a source of many oocytes during each cycle. Some will be essential for fertilization and embryo transfer to the mothers. Many others may be cryopreserved for later cycles, and many more still may provide material for research on human embryology, since there are many small antral follicles in the follicular phase of the ovarian cycle. The days could be over when a few human embryos considered as too poor to achieve pregnancy were used for research instead.

In this concluding section of my Foreword, this topic holds especial interest to me, since many more embryos are needed for research into better culture methods, embryo diagnosis and selection, and a deeper knowledge on the background genetics to human infertility and embryo growth. Studies on mouse oocytes and those of other laboratory species explode into new directions, and knowledge on human embryos must grow in parallel. Some knowledge has accrued on human embryos, including topics such as the formation of polarities and their significance, responses of embryos to different media, and the role of mitochondria in early development. Preimplantation genetic diagnosis (PGD) detects numerous forms of chromosome anomaly in many human preimplantation embryos, and discarding them reduces the risks of abortion. PGD also removes fear of the effects of familial disease in some human pedigrees. Routine studies in mice are far in advance such the use of gene knock-out and the immense power of transcriptome analyses which provide immense amounts of knowledge on rodent embryos. Similar matters must be studied in human embryos, and have been promised as refinements to these techniques provide basic data on smaller numbers of oocytes or embryos (Bermudez *et al.*, 2004).

Maturing human oocytes *in vitro* could change this scenario by providing many maturing oocytes and embryos. This concept may antagonize some people, yet for others it offers the clinical imperative of helping afflicted human beings. Many embryos will be essential for now to establish human embryonic stem cell lines and gain fundamental information on differentiation and dedifferentiation. Programmes for grafting high quality stem cells to injured people are currently arrested by insufficient knowledge on epigenetic responses of stem cells *in vitro*. Clinical opportunities emerge at every level: from the response of follicles to gonadotrophins, identifying and averting the factors causing chromosomal anomalies. The latter inflict numerous disastrous forms of development on approximately 50% of human embryos *in vitro* and almost certainly *in vivo*. Preimplantation diagnosis helps in their detection, but it will be far more important to clarify understanding of correctable errors in the meiotic cycle in afflicted oocytes. Genetics increasingly dominates such research, providing necessary information on every aspect of embryonic development including the properties of ooplasm, spindle formation and action, how pronuclei form and how timer genes invoke fast or slow cleavage divisions.

Measuring the actions of hundreds of genes in a single analysis has become commonplace, and transcriptomes and other analytical systems promise much greater knowledge on embryonic beginnings. Data are gleaned on the action of particular genes, on genetic pathways, and when they are switched on and off. Gene systems regulate embryonic metabolism, cell division, chromosomal segregation, gene mutations and blastocyst formation. Stem cells receive similar treatment. ES cells derived from the inner cell mass of blastocysts and stem cells can renew particular organs such as the haemopoietic or muscle systems and even brain. These approaches to tissue repair are becoming an accepted proposition, demanding many embryos for research, and the most recent methods of analysis. Taking cell division alone as an example, successive coordinated events include centrosome duplication in late G1, chromosomal DNA replication in the S phase, mitotic spindle assembly a the M phase, segregation of the replicated genome to opposite spindle poles at anaphase, and division to two daughter cells at cytokinesis.

As recently as 2 months ago, a new study revealed even wider approaches to the study of developmental genetics. It utilized short double-stranded (ds)RNAs to interfere specifically with genetic systems in species as far apart as yeast and mammals. The method is called RNAi (interference). Particular genes can be inactivated simply to record subsequent developmental consequences. Larger-scale studies utilize RNAi to inactivate several genes simultaneously or in tandem, and so relate particular genes to their developmental significance. A recent example applied this approach to the embryos of the nematode *Caenorhabditis elegans* between fertilization and the 4-cell stage. Its results could be of immense significance for preimplantation human development.

Its scale was enormous. The investigators (Sönnischen *et al.*, 2005) intended to make a detailed study of 'normal' and various forms of abnormal development. First clarifying normal development

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

**Table 1** Genes involved in specific stages of *C. elegans* development (Sönnischen *et al.*, 2005).

**A. Condition studied and numbers of genes involved**

| Condition | No. of genes |
|---|---|
| Infertility and sterility | 42 |
| Osmotic integrity | 109 |
| Polar body extrusion | 12 |
| Passage through meiosis | 47 |
| Entry into metaphase | 4 |
| Cortical dynamics | 19 |
| Pronuclear, nuclear appearance | 28 |
| Centrosome attachment | 3 |
| Aberrant pronuclear migration or numbers of pronuclei | 38 |
| Spindle assembly | 21 |
| Sister chromatid separation | 64 |
| Nuclear appearance | 5 |
| Chromosome segregation | 23 |
| Cytokinesis | 15 |
| Symmetric divisions (*Par*-like) | 12 |
| Faster timing of embryogenesis | 14 |
| Pace of development | 49 |
| Multiple pronuclei and their severe pleiotropy | 108 |
| Membrane-bounded organelles | 13 |
| Size of the eggs | 5 |
| Aberrant structures including areas devoid of yolk granules | 9 |
| Complex phenotypes | 21 |

**B. Some of the defined characteristics**

| | |
|---|---|
| Spindle characteristics | Its assembly, centation and rotation during pronuclear breakdown, together with meiotic arrest |
| Cleavage characteristics | Furrows, completion and stability |
| Nuclear migration | In the first cleavage division |
| Cortical activity | During cleavage |
| Blastomeres | Numbers of nuclei, their shape, size and rotation |
| Asynchronous division | Tetrapolar spindles |
| Cleavage | Cross-eyed 4-cell stage |
| Yolk granules | Density and size and areas devoid of them |
| Nuclei | Consequential shape, migration and rotation |
| Development | Polar bodies, speed of successive events, numbers, age and stage of embryo development |

in immense detail, their attention turned to the effects of specific genes and their knock-out on the embryonic growth. Hence, initially detailed time-lapse recordings of developing embryos were recorded by means of differential interference contrast (DIC), a powerful technique measuring many characteristics in developing embryos and applied to growth stages from 20 min after fertilization to the 4-cell stage. More than 40,000 DIC recordings were taken in 19,000 individual experiments to clarify normal and variant forms of differentiation. Mothers were then injected with one or several RNAi, each reacting with a known gene sequence, and the effect of single or combinations of several genes were measured. Genes studied covered the whole genome, and included those likely to influence development as RNAi were prepared for 20,256 genes, i.e. 98% of the total genes in *C. elegans*. They concentrated on 2093 genes with the greatest likelihood of functioning in early embryonic stages and scanned each DIC recording for 45 distinct defects, some known to occur in human embryos (**Table 1**), including cortical ruffling, cytoplasmic flow, pseudocleavage, size and shape of pronuclei, numbers and migration of the female and male pronuclei and their apposition and rotation, nuclear breakdown, sister chromatid separation, bipolar spindles, defects

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

in cytokinesis, symmetric cell divisions, pace of development, pleiotropic defects, membrane-bound organelles and the size of the eggs, and eggs with cytoplasmic areas devoid of yolk granules and other organelles (Sönnischen *et al.*, 2005). The number of disorders and the genes involved is more than enough to make a human embryologist green with envy!

Relationships existed between specific phenotypic classes and biochemical systems controlled by individual genes. Specific biochemical pathways involved 16 of the 23 phenotypic classes including 29/49 genes involved in ATP metabolism in the class 'pace of development', 17/28 genes appearance involved in nuclear transport or nuclear pore assembly in 'pronuclear/nuclear appearance, and 16/38 genes related to microtubules including dynein–dynactin assembly, tubulins and their co-factors in 'pronuclear migration'. Genes involved in phenotypic classifications sometimes overlapped. For example, 11/12 genes in the dynein–dynactin complex were also included in 'severe pleiotropic effects', and 13/14 in 'proteasome subunits' were also included in 'passage through meiois'. Previously uncharted genes were identified. Bioinformative gene analyses of phenotypic classes predicted seven novel genes for chromosomal function, four for the assembly of the nuclear envelope, four for cell cycle progression, 11 for ATP metabolism, and seven for protein synthesis.

Fourteen per cent of genes were previously unknown, many with homologies with human, mouse, fly and yeast genes. Subtle defects in chromosomal segregation could not be scored using current methods but be measured later using fluorescent tagging. Other investigators using different approaches identified even more developmental genes than reported by Sönnischen *et al.* (2005).

RNAi is a recent finding, and many other novel forms of investigation are being developed (Edwards, 2005). RNAi has been applied to mammalian embryos, one recent example in mice revealing its relevance. RNAi was used to down-regulate the genes *Par3* and *atypical protein kinase C*, which modified their normal role in imposing polarized divisions in 16-cell stages and morulae (Plusa *et al.*, 2005). Normally, outer cells produce one daughter which migrates inwards and contributes to inner cell mass, and the other remains in an 'outer' position and forms trophectoderm. Exposure to RNAi prevented these polarized divisions, and led to a deficiency of inner cell mass and an overgrowth of trophectoderm. Similar systems occur in other organ systems.

Other forms of modern genetic analyses may soon be related to endocrine systems. Other small RNA forms called microRNAs act after transcription to regulate other genes in muscle, brain, etc. When transfected into human cells, microarrays revealed large numbers of genes shifted their expression patterns (Chen *et al.*, 2004; Lim *et al.*, 2005). Up to 100 genes were modified, and levels in both their transcripts and proteins were affected. The control of tissue-specific gene expression in this manner could be highly significant in studying the responses of cells and tissues to specific hormones.

## Conclusions

It has been a stimulating task to briefly review the historic significance of gonadotrophin stimulation as IVF, ICSI and PGD followed in fairly rapid succession. They have been fundamental to the introduction and development of these techniques. Much remains to be learned, methods simplified, and more effective and less expensive techniques introduced into clinical practice. The search for such knowledge is portrayed in the papers presented in this Symposium.

Endocrine and embryo research may be assisted as oocyte maturation and fertilization *in vitro* become widespread in IVF clinics. They could help to clarify the meiotic causes of trisomy and monsomy in human oocytes, understand the formation of cytoplasmic fragments in so many embryos, and clarify those genes conferring rapid division and high rates of implantation. Transcriptomes and RNAi will clarify those genes causing infertility or disease in parents and children, enabling diagnostic analyses on parents before they attend for IVF. The current pace of research indicates this scenario may be nearer than expected.

## References


Bermúdez MG, Wells D, Malter H *et al.*, 2004, Expression profiles of individual human oocytes using microarray technology. *Reproductive BioMedicine Online* **8**, 325–337.

Chen C-Z, Li L, Lodish HF, Bartel DP, 2004, MicroRNAs modulate haemopoietic lineage differentiation. *Science* **303**, 83.

Edwards RG, 1965a, Maturation in vitro of human ovarian oocytes. *Lancet* **ii**, 926–929.

Edwards RG, 1965b, Maturation in vitro of mouse, sheep, cow, pig rhesus monkey and human ovarian oocytes. *Nature* **208**, 349–351.

Edwards RG, 2005, Genetics of polarity in mammalian embryos. *Reproductive BioMedicine Online* **11**, in press.

Edwards RG, Steptoe PC, 1980, *A Matter of Life*. Hutchinsons, London.

Edwards RG, Surani MAH, 1978, The primate blastocyst and its environment. *Upsala Journal of Medical Science* **22**, 39–50.

Fowler RE, Edwards RG, 1957, Induction of superovulation and pregnancy in mature mice by gonadotrophins. *Journal of Endocrinology* **15**, 374–384.

Fowler RE, Edwards RG, Walters DE *et al.*, 1978, Steroidogenesis in preovulatory follicles of patients given human menopausal and chorionic gonadotrophins as judged by the radioimmunoassay of steroids in follicular fluid. *Journal of Endocrinology* **77**, 161–169.



**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.

Lim LP, Lau NC, Garrett-Engele P *et al.*, 2005, Microarray analyis shows that some microRNAs downregulate larger numbers of target mRNAs. *Nature* **433**, 769–773.

Plusa B, Frankenberg S, Chalmers A *et al.*, 2005, Downregulation of Par3 and aPKC function directs cells towards the ICM in the preimplantation mouse embryo. *Journal of Cell Science* **118**, 505–515.

Sönnischen B, Koski LB, Walsh A *et al.*, 2005, Full-genome RNAi profiling of early embryogenesis in *Caenorhabditis elegans*. *Nature* **434**, 462–469.

**In citing this article please refer to the original:**
Edwards, R.G., 2005. Historical significance of gonadotrophins in assisted reproduction. Reprod. BioMed. Online 10(Suppl 3), 3–10.