Lasker Clinical Medical Research Award

The bumpy road to human *in vitro* fertilization

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My first ideas of human *in vitro* fertilization (IVF) arose with my PhD in Edinburgh University in the early 1950s. Supervised by Alan Beatty, my research was based on his work on altering chromosomal complements in mouse embryos. All went very well, as haploid, triploid, tetraploid and more-bizarre embryos emerged[1], and I learned about mouse meiosis, fertilization, embryos, blastocysts and chromosomes as well as immense amounts of reproductive physiology. The arrival of Alan Gates in Edinburgh relieved my midnight labors. Also working with Alan Beatty, he brought the Organon preparations of gonadotrophins which induced immature mice to ovulate many eggs and mate with adult males. Transfer of their embryos to adult mice produced fully normal offspring in huge numbers.

Science in Edinburgh was incredibly fruitful. Another PhD student, Ruth Fowler (later my wife), and I decided to test these Organon hormones on adult mice. They again induced estrus and timed oocyte maturation, with erratic numbers of ovulated oocytes, fertilization, cleavage, implantation and fetal growth to full term[2]. Julio Sirlin and I[3] applied radioactive tracers to spermatogenesis, oogenesis and embryology, labeling DNA, RNA and proteins. My professor, Conrad Waddington, discussed ethics and genetics with senior churchmen, which proved invaluable for me, as ethics would feature immensely in my future work on human conception. After a year at the California Institute of Technology with Albert Tyler, a welcome from Alan Parkes and Bunny Austin to the National Institute for Medical Research, London, in 1958, shifted me from pure science to biomedicine. Emphasis on immunology gradually decreased as visiting lecturers described their work. Margaret Jackson ran a sperm donation program in Devon. Only 5 feet tall, her heart was double the normal size as she stoutly defended her work against a barrage of critical ethical questions. What could I do for patients? Literally nothing until human eggs were fertilized *in vitro*. Carl Gemzell in Sweden began treating infertile acyclic women with extracts of human anterior pituitaries[4] but, as in mice, oocyte numbers were erratic, so very high-order multiple pregnancies were established alongside patients with singletons or twins. Later, some of his patients died from Creutzfeldt–Jacob disease transmitted by those pituitaries, showing how clinical matters can go badly wrong. Donini *et al.*[5] extracted follicle-stimulating hormone from human menopausal urine and Bruno Lunenfeld[6] applied it clinically, so pituitary glands were no longer needed.

Increasingly committed to human studies, I sought to collect several immature human oocytes from pieces of excised ovarian tissue, mature and fertilize them *in vitro* and transfer the resulting embryos into infertile women to help them conceive. Some gynecologists approached about this project candidly responded they thought the idea preposterous. Molly Rose, a gynecologist who delivered two of my daughters, offered to send occasional slithers of human ovaries. Now I had a supply of oocytes, albeit very rare and precious. Pincus and Saunders[7] had liberated rabbit and human oocytes from their follicles *in vitro* and shown how both matured spontaneously in less than 12 hours. I hurried to apply these findings, and found that oocytes from mice, rats and hamsters did mature within 12 hours. Whatever I did, sheep, cow, rhesus monkey, baboon and human oocytes did not mature, despite my adding hormones, media constituents and feeder cells, changing gas phases, and even perfusing human ovaries *in vitro* with HCG before aspirating follicles. After 2 disappointing years, a slither of human ovary from Molly Rose provided several oocytes. This time I waited longer, for 18 hours, only to face disappointment—the oocyte nuclei were unchanged[8]. For the next three

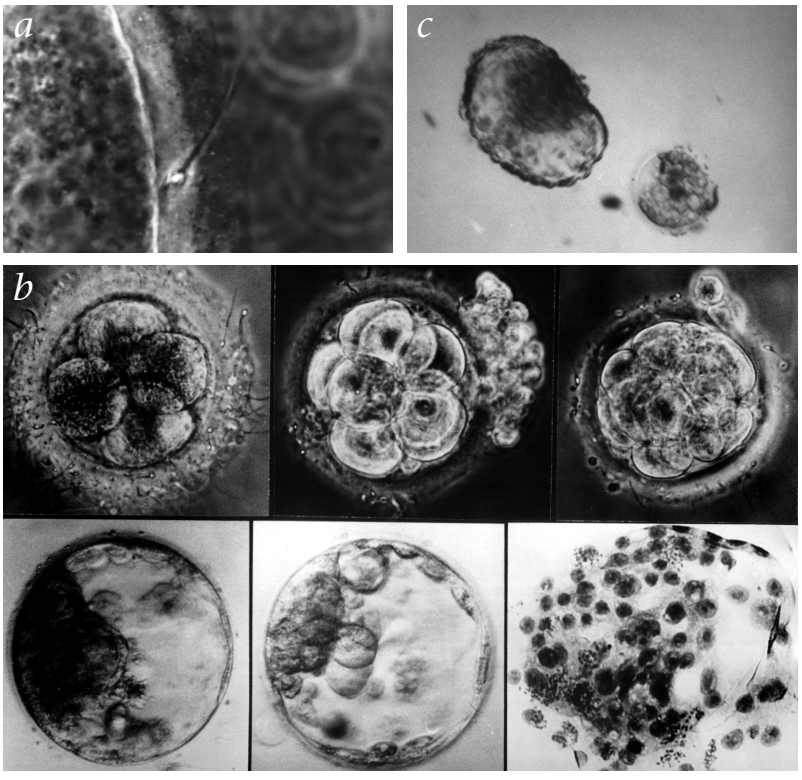

Fig. 1 Initial work in introducing human IVF. ***a***, An early stage of fertilization *in vitro* showing the spermatozoon making contact with the oolemma. ***b***, Living 4- and 8-cell human embryos, a compacting morula, two examples of live blastocysts, and a fixed blastocyst preparation with nuclei and chromosomes. ***c***, A hatched blastocyst at day 9, with a large embryonic disc and bilaminar membrane; the shed zona pellucida contains cells and debris.

oocytes,I waited for 25 hours, and—joy unbounding! A beautiful diakinesis with chiasmata, superb chromatids and nucleoli fading appeared[9]. Pincus's error cost me 2 years. Now, a definite future existed for human IVF. Oocytes had to develop *in vitro* to meiosis-2 arrest and expel a first polar body. These stages were inevitable once diakinesis had begun, and would require an estimated 12 hours.

The rarity of human oocytes left much time available for immunology and for another of my interests: isolating embryo stem cells from mammalian embryos. Stimulated by work with Julio Sirlin, I disaggregated four- and eight-cell rabbit embryos, which produced groups of single cells that persisted briefly in culture. Unexpectedly, John Paul at Glasgow University invited me to work with him and Robin Cole on cytodifferentiation in the early embryo. During that wonderful year, stem cells grew from inner cell mass of rabbit blastocysts to differentiate into blood islands, muscle and connective tissue. Long-lived (immortal) stem cells, stable karyotypically, enzymologically and morphologically, grew rapidly *in vitro* and after cryopreservation[10,11]. I followed these leads as clinical entities 20 years later. While in Glasgow, the first human oocyte matured *in vitro* to metaphase-2 with a polar body in 37 hours[9].

Cambridge beckoned, and I rejoined Parkes and Austin and resumed immunology and oocyte maturation. Cow, sheep, pig and monkey oocytes all matured *in vitro*, each with their own specific intervals[12]. Chris Polge and I found that pig oocytes required 37 hours *in vitro* and *in vivo*, just like human oocytes! Clinical collaboration was essential, with Molly Rose and Howard and Georgeanna Jones at Johns Hopkins, and Victor Lewis in London. Each stage in the maturation of human ovarian oocytes was timed[12]. A memorable 6 weeks at Hopkins included occasional pronuclei forming in inseminated human eggs *in vitro*, as happened again back in Cambridge. Tight controls on pH, osmotic pressure and constituents of medium were probably paying off. Molly Rose sent a piece of human ovary. Barry Bavister had devised a medium with high pH for hamster fertilization *in vitro*, already achieved by Yanagimachi and Chang[13]. Examining that small group of matured and inseminated oocytes was memorable: every stage of fertilization was recorded[14]. We found later that media of lower pH, and other variations, would support human fertilization.

Searching for a clinical partner capable of reaching the ovary using minimal surgery ended as I phoned Patrick Steptoe in 1968, having read of his laparoscopy in the Oldham and District General Hospital. Then the world's master of this method, he could easily aspirate oocytes from their follicles[15]. We teamed up for IVF, and discussed in detail the safety of our proposed procedures, and the underlying ethics. We agreed to work together as equals, pursue our work carefully, and stop if any danger emerged to patients or children, but not for vague religious or political reasons[16]. We stayed together for 20 years, until his death. I reckon he taught me medicine. At the time, he faced immense clinical criticism over his laparoscopy, even being isolated at clinical meetings in London. This disgraceful treatment led me to comment on this shabby treatment of a man opening new concepts in his field as I wrote his biography, just as it had angered many of his clinical colleagues in northern England. He is now regarded as a true pioneer of general endoscopy and, of course, of IVF.

Fig. 2 Reverend Gordon Dunstan talking with Robert Edwards some time in the 1980s. A senior ethicist of the Church of England, Dunstan knew more about the science and medicine of IVF than most scientists and clinicians at the time, having taught himself in detail about new approaches to infertility. His book *Artifice of Ethics*, published in 1974 (ref. 18), devoted several chapters to the science and medicine of IVF and its ethical issues.

Mild ovarian stimulation with human menopausal gonadotropin (HMG) and HCG produced several follicles. Steptoe's aspirations 36 hours after HCG treatment were superb, with the oocytes about to ovulate being surrounded by glistening cumulus cells. Fertilization and embryo growth *in vitro* proceeded excellently. Fascinated, I watched as two-cell, four-cell and eight-cell embryos, morulae, and beautiful blastocysts at 4–6 days grew *in vitro* in various media (Fig. 1). About half the embryos faltered as they approached the blastocyst stage[17]. Most had normal nuclei, even-sized blastomeres and approximately diploid chromosomes, developed to a strict timetable, compacted excellently, secreted blastocoelic fluid, and were obviously vibrant as blastocysts with 100 or more nuclei and many mitoses on day 5. Some blastocysts grew to 9 days, their expanding embryonic discs stuffed full of embryonic stem cells!

Ethicists decried us, forecasting abnormal babies, misleading the infertile and misrepresenting our work as really acquiring human embryos for research. They announced that IVF did not cure infertility, as women remained infertile after having an IVF baby. My response was to put forward spectacles, false teeth and heart transplants. Popes were critical and rigid Protestants were sometimes vicious. A new-found friend, Gordon Dunstan, senior ethicist of the Church of England, wrote his *The Artifice of Ethics*[18] with four chapters on IVF and a penetrating and ethical analysis (Fig. 2). Some years later, the Archbishop in Tiblisi, Georgia, responded identically, instantly making a collection in his cathedral to train Georgian IVF embryologists! It was time to transfer embryos to their mothers. We gained ethical consent from Cambridge and Oldham to open a clinic in Newmarket Hospital, near Cambridge, with a post for Steptoe. The Medical Research Council refused to fund it; at least one member of that committee has since apologized publicly. The Oldham authorities converted the small Kershaw's Hospital into the world's first IVF clinic. With colleagues, I had assessed in detail the teratological risks to babies, with a general consensus that our work was safe. We began transfers in 1972. I assumed human embryo implantation rates matched those of laboratory and farm animals, only realizing some time later that only 20% of them can implant successfully.

Ovarian stimulation with HMG and HCG led to severe endocrine deficiencies in the luteal phase of our patients. Some

patients menstruated 5–6 days after ovulation as their urinary pregnanediol fell abruptly. This disappointing discovery meant that endocrine support was essential until the placenta assumed its endocrine function at 8–10 weeks of gestation[19]. Daily injections of progesterone in oil were needed, but could cause serious scabbing. We substituted Primulot depot, an artificial progestagen given once every 5 days. This ethical decision produced transfer failures for 2 years. Ken Bagshawe in London assayed our patients' blood samples using a new HCG immunoassay, and identified some very short-lived pregnancies, later called 'biochemical pregnancies'. Primulot had acted as an abortifacient (confirmed a few years later), so we mostly abandoned it. To our delight, one clinical pregnancy began after a blastocyst was transferred[20]. Sadly, it was ectopic and had to be removed at 11 weeks or so. Still, my laboratory techniques had sustained a human embryo capable of implantation and early organogenesis.

Luteal-phase weakness had to be overcome. We tested different forms of stimulation: clomiphene and HMG produced excellent luteal phases, bromocryptine and HMG to reduce high prolactin levels in many stimulated patients, and HCG alone to control ovulation in natural menstrual cycles. We did the first gamete intra-Fallopian transfers (in our terms, oocyte recovery with tubal insemination), cryopreserved oocytes and embryos, accomplished oocyte donation to a recipient, and finally moved to natural-menstrual-cycle IVF by closely timing the urinary luteinizing hormone surge in our patients. Lesley Brown was the second natural-cycle patient; her single oocyte was aspirated within minutes, inseminated quickly and transferred exactly as it reached the eight-cell stage. I hoped earlier transfer would benefit from the embryos' spending less time *in vitro*. After an eventful pregnancy (Fig. 3), Louise Brown was born on 26 July 1978 on a momentous evening in Oldham. It is hard to put into words what the occasion of her birth meant to me, and to our wonderful supportive team. It was a purely routine Caesarean section, yes, but with a significance outstripping anything we had done before or were likely to achieve later. Surrounded by hundreds of members of the press, the birth was achieved in secret, to the delight of the parents, staff and ourselves. Details of all our work have been reported elsewhere[21–25]. A success rate of 4, possibly 5, pregnancies of 32 transfers using natural-cycle IVF alerted me to the weakness of human implantation compared with that of other species, which still restricts IVF benefits today.

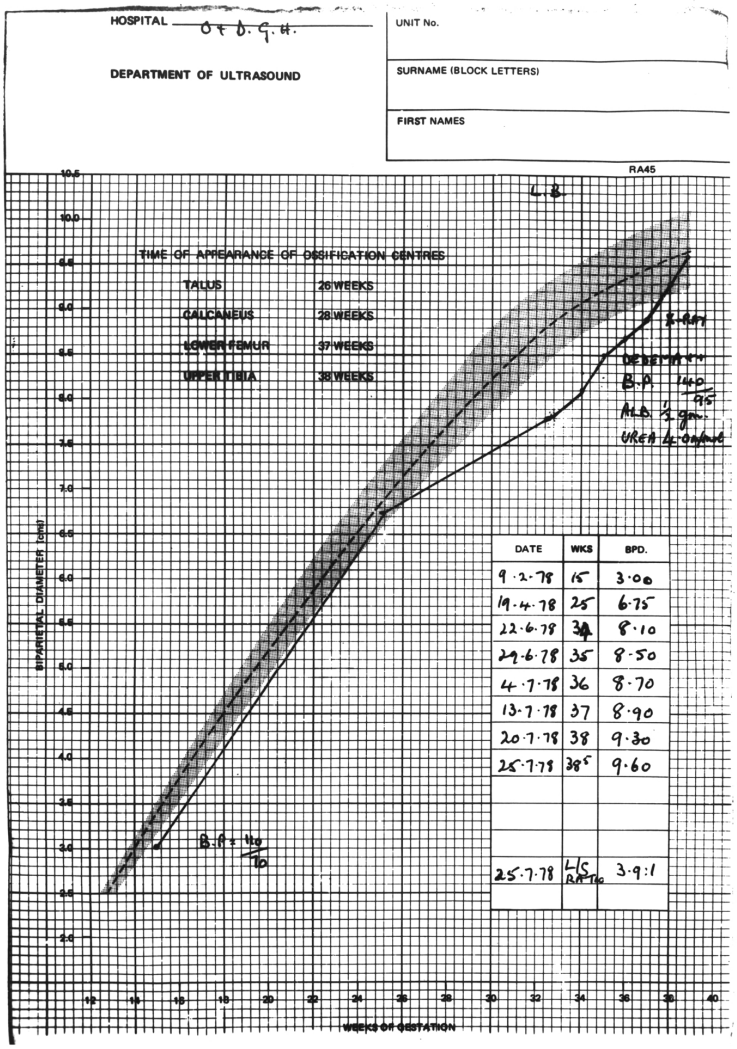

Fig. 3 Growth of the first IVF baby *in utero*, showing low biparietal diameter from week 26 to week 38, when a Caesarean section was done.

Louise Brown's birth marked the end of the beginning of human IVF, acclaimed at the Royal College of Obstetricians and Gynaecologists. This event was snubbed by some clinicians now styled as 'pioneers', who shouted that the test-tube claim was a fake! They did not matter. IVF had to become large-scale, in a center providing the necessary clinical, scientific, consultative, nursing and counseling back-up services, and even providing ward and dining facilities for the immense patient numbers on Steptoe's waiting list. No governmental support was forthcoming, so our work was halted for 2.5 years after Louise Brown's birth. Finally, venture capital was obtained and Bourn Hall opened in September 2000. This Jacobean mansion, with the motto 'Jour de ma Vie', became the world's second (and most beautiful) IVF clinic—and among the largest. So many patients passed through its doors to enable the many clinical trials of IVF success: treatments for infertile men, and for women responding poorly to ovarian stimulation or suffering from endometriosis. We carried out studies on embryos, better endocrine tools and transfer catheters, improved laparoscopy, embryo implantation, biochemical pregnancies, miscarriage, birth and early growth of children. Babies had to be conceived—dozens, fifties, hundreds and thousands—to assess the procedures and safety of IVF (ref. 26). Familiarity with the unexpected was routine, endless small events almost too strange to be true. Staff responded. More than 1,000 children were born by 1989, as normal as children conceived in vivo. Major ethical arguments in the press formed a constant background. I had to issue eight libel actions in the High Court of London on a single day, which is when ethics becomes very practical. I won them all, but the work and worry restricted research for several years.

Many papers were published. Preimplantation genetic diagnosis was resumed, as Jones, Singh and I[27] marked one-half of human spermatozoa and a few available human embryos unsuitable for transfer. This was the first indication of sex

selection of human spermatozoa and embryos *in vitro*. A year or so later, Alan Handyside succeeded with a birth after amplifying Y sequences in human embryos. Still working with PhD students in Cambridge, I supervised Richard Gardner and Peter Hollands, proposing they assessed embryo stem cells for making transgenic mice[28] or to repair damaged bone marrow in lethally irradiated mice[29]. Stem cells from mouse or rat embryos apparently followed fetal pathways through liver to bone marrow in irradiated mice. We moved to clinical development, as Simon Fishel, Chris Evans and I[30] measured HCG output in cultured human blastocysts and reported weak growth of inner cell mass cells. The field of therapeutic stem cells was wide open[30,31], when an ethical decision in Bourn Hall reserved all embryos for their parents, and this research ended[30].

Clinical topics were equally numerous. Male infertility, endometriosis and embryo transfer, cryopreservation of embryos and desperately low human embryo implantation rates were assessed. The essential need for ethicists and counselors to advise patients, and ourselves, was recognized. Gamete donation and surrogate pregnancies were introduced, and immense attention was paid to consent forms and legal aspects. Implantation rates remained stubbornly low despite various forms of ovarian stimulation, indicating embryo quality had been 'decided' long before transfer. The world now joined in IVF, with the introduction of intracytoplasmic sperm injection, improved maturation *in vitro*, sex selection and other items. These years saw the deaths of both Steptoe and Jean Purdy; by then, Steptoe's work had been widely recognized (Fig. 4)

Fig. 4 A happy moment as Robert Edwards and Patrick Steptoe receive an Honorary DSc From Hull University in 1983.

My genetic interests persist in the area of the control of human development. Something must be fundamentally flawed with a reproductive system that allows only 20% of embryos to implant, even in younger couples. Why are so many human spermatozoa immotile or formed abnormally, and why do up to one-half of embryos carry chromosomal anomalies? Such issues hold my interest today as I question our earlier concepts on embryonic differentiation in mammals, or search for functional embryonic homologies between human and *Drosophila, Caenorhabditis elegans* and *Xenopus laevis*[32].

1. Edwards, R.G. The experimental induction of gynogenesis in the mouse. I. Irradiation of the sperm by X rays. *Proc. Royal Soc. Lond. B* **146**, 488–504 (1957).
2. Fowler, R.E. & Edwards, R.G. Induction of superovulation and pregnancy in mature mice treated by gonadotrophins. *J. Endocrinol.* **15**, 374–384 (1957).
3. Sirlin, J.L. & Edwards, R.G. The labelling of mammalian spermatozoa with radioactive tracers. *J. Exp. Zool.* **137**, 363–388 (1958).
4. Gemzell, C. & Johansson, E. B. in *Control of Human Fertility*. Nobel Symposium (eds. Diczfalusy, E & Borell, U.) 15 (Almqvist and Niksell, Stockholm, 1971).
5. Donini, P., Puzzuoli, D. & Montezemolo R. Purification of gonadotrophin from human menopausal urine. *Acta Endocrinol.* **45**, 329 (1964).
6. Lunenfeld, B. Development of gonadotrophins for clinical use. Symposium, Robert Edwards at 75. *Reprod. BioMed. Online* **2**, (in the press).
7. Pincus, G. & Saunders, B. The comparative behaviour of mammalian eggs *in vivo* and *in vitro*. *Anat. Rec.* **75**, 537–545 (1939).
8. Edwards, R.G. Meiosis in ovarian oocytes of adult mammals. *Nature* **196**, 446–450 (1972).
9. Edwards, R.G. Maturation *in vitro* of human ovarian oocytes. *Lancet* **208**, 39–351 (1965).
10. Edwards, R.G. Cleavage of one- and two-celled rabbit eggs *in vitro* after removal of the zona pellucida *J. Reprod. Fertil.* **7**, 413–415 (1964).
11. Cole, R.J., Edwards, R.G. & Paul, J. Cytogenesis and embryogenesis in cell colonies and tissue cultures derived from ova and blastocysts of the rabbit. *Dev. Biol.* **13**, 387–407 (1966).
12. Edwards, R.G. Maturation *in vitro* of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* **208**, 349–351 (1965).
13. Yanagimachi, R. & Chang, M. C. *In vitro* fertilization of golden hamster ova. *J. Exp. Zool.* **156**, 361–376 (1964).
14. Edwards, R.G., Bavister, B.D. & Steptoe, P.C. Early stages of human fertilization *in vitro* of human oocytes matured *in vitro*. *Nature* **221**, 632–635 (1969).
15. Steptoe, P.C. in *Laparoscopy in Gynaecology* (E. & S. Livingstone, Edinburgh, 1967).
16. Edwards, R.G. & Sharpe, D.J. Social values and research in human embryology. *Nature* **231**, 87–91 (1971).
17. Steptoe, P.C., Edwards, R.G. & Purdy, J.M. Human blastocysts grown in culture. *Nature* **229**, 132–133 (1971).
18. Dunstan, G. *The Artifice of Ethics* (SCM Press, Lancaster, 1974).
19. Csapo, A.I., Pulkkinen, M.O. & Kaihola, H.L. The relationship between the timing of luteectomy and the incidence of complete abortions. *Am. J. Obstet. Gynecol.* **118**, 985–989 (1974).
20. Steptoe, P.C. & Edwards, R.G. Reimplantation of a human embryo with subsequent tubal pregnancy. *Lancet* **1**, 880–882 (1976).
21. Edwards, R.G. & Steptoe, P.C. *A Matter of Life* (Hutchinsons, London, 1980).
22. Edwards, R.G., Steptoe, P.C. & Purdy, J.M. Establishing full term pregnancies using cleaving embryos grown *in vitro*. *Brit. J. Obstet. Gynecol.* **87**, 737–756 (1980).
23. Edwards, R.G & Brody, S.A. *Principles and Practice of Assisted Human Reproduction* (W.B. Saunders, Philadelphia, 1995).
25. Steptoe, P.C., Edwards R.G. & Purdy, J.M. Clinical aspects of pregnancies established with cleaving embryos grown *in vitro*. *Br. J. Obstet. Gynecol.* **87**, 757–768 (1980).
26. Steptoe, P.C., Edwards R.G. & Walters, D.E. Observations of 767 clinical pregnancies and 500 births after human conception *in vitro*. *Hum. Reprod.* **1**, 89–94 (1986).
27. Jones, K.W., Singh, L. & Edwards, R.G. The use of probes for the Y chromosome in preimplantation embryo cells. *Hum. Reprod.* **2**, 439–455 (1987).
28. Gardner, R.L. Mouse chimaeras obtained by the injection of cells into the blastocyst. *Nature* **220**, 596–597 (1968).
29. Hollands, P. Embryonic stem cell grafting: the therapy of the future. *Hum. Reprod.* **6**, 79–84 (1991).
30 Fishel, S.B., Edwards, R.G. & Evans, J. Human chorionic gonadotrophin secreted by preimplantation embryos cultured *in vitro*. *Science* **223**, 816–818 (1984).
31. Edwards, R.G. The case for studying human embryos and their constituent tissues *in vitro*. In *Human Conception in Vitro* (eds. Edwards, R.G. & Purdy, J.M.) 371–387 (Academic Press, London, 1982).
32. Edwards, R.G. Ovarian differentiation and human embryo quality. 1. Molecular and morphogenetic homologies between *Drosophila, C. elegans, Xenopus* and mammals. Reprod. BioMed. Online, **3**, Webpaper 2001/156 (2001).

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