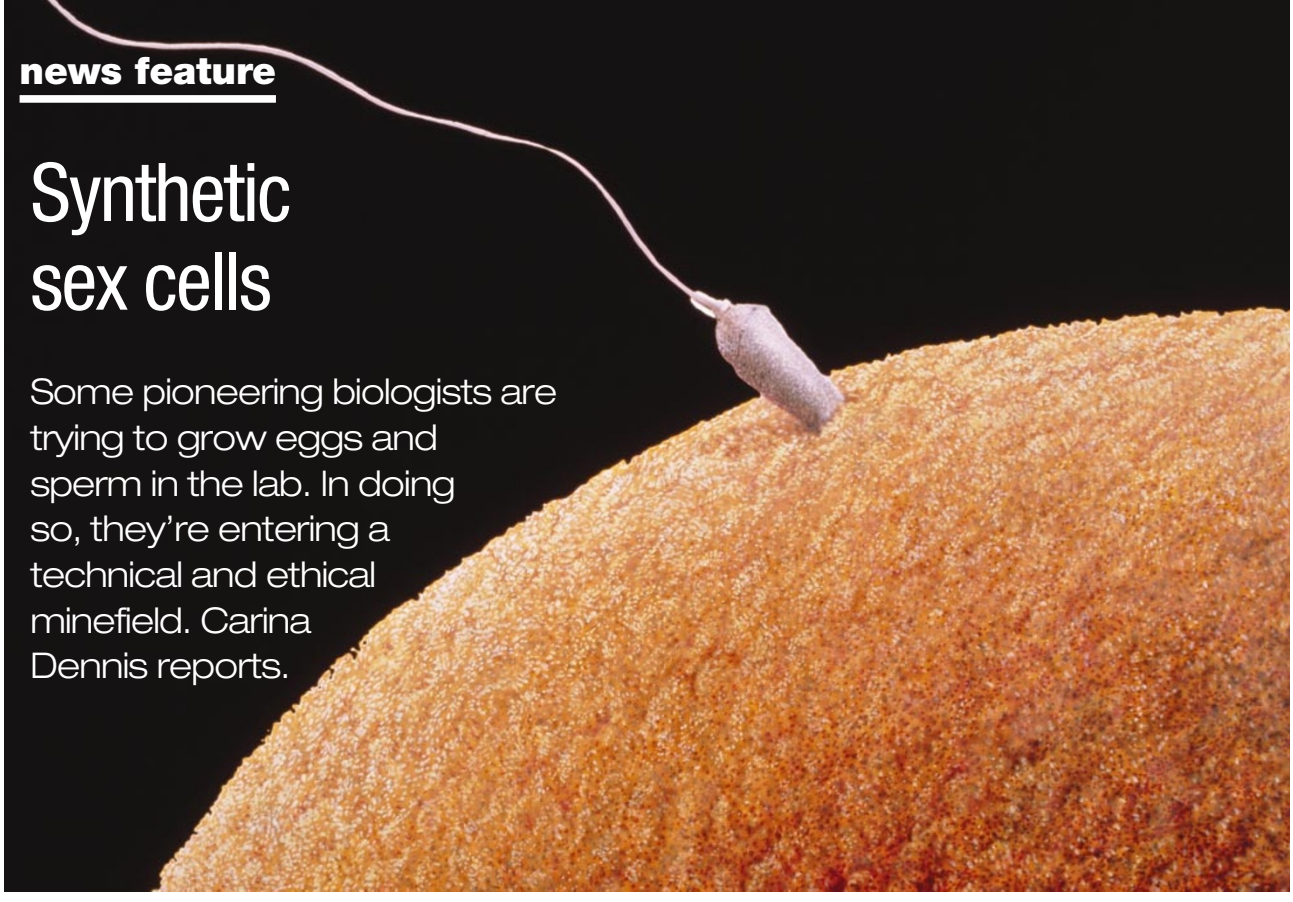
Synthetic sex cells



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Some pioneering biologists are trying to grow eggs and sperm in the lab. In doing so, they're entering a technical and ethical minefield. Carina Dennis reports.

Boy meets girl, sperm meets egg. It's a timeless tale, but one that is increasingly being played out in the culture dish — witness the million-plus children who have so far been conceived by *in vitro* fertilization (IVF), and the widespread application of assisted reproduction to livestock breeding.

IVF may just be the start. In the future, sperm and eggs may not just be brought together in a culture dish — they might also be grown there. Many of the biologists working towards this goal want to understand the developmental processes that give rise to mammalian sex cells. Some also hope that the technology will boost the clinical prospects of 'therapeutic cloning' — which will require a supply of human eggs to turn a patient's own cells into tissues to replace those lost to age, injury or disease. Others see applications for lab-grown sex cells in animal breeding.

For the time being, few researchers are enthusiastic about using lab-grown sperm and eggs for human reproduction — although this prospect is already overshadowing public debate about the work. Just a few weeks ago, many media commentators were outraged at reports from a fertility conference in Spain on attempts to culture human eggs from fetal ovaries. "Your real mother was aborted baby," ran a lurid headline in one British tabloid newspaper.

Even if the ethical questions can be successfully addressed, growing sperm and eggs in the lab poses formidable technical challenges. The production of sex cells is a complex, multi-stage process (see figure, opposite). One major hurdle is coaxing the cells to halve their number of chromosomes, which involves a form of cell division known as meiosis. Another complication is that, under natural circumstances, sex cells do not grow and mature on their own: sperm development is nurtured by helpers known as Sertoli cells; the precursors of eggs are cushioned and nourished by granulosa cells.

Most attention is focusing on eggs, rather than sperm, as these are the scarcer resource. A prize bull, for instance, produces millions of sperm in a single ejaculate, but a prime milking cow will naturally produce only a handful of calves in her lifetime. Yet a female mammal's ovaries contain thousands of cells that have the potential to develop into eggs. Were it possible to culture these cells in the lab, élite cows could yield many more embryos, which would then be implanted into surrogate mothers.

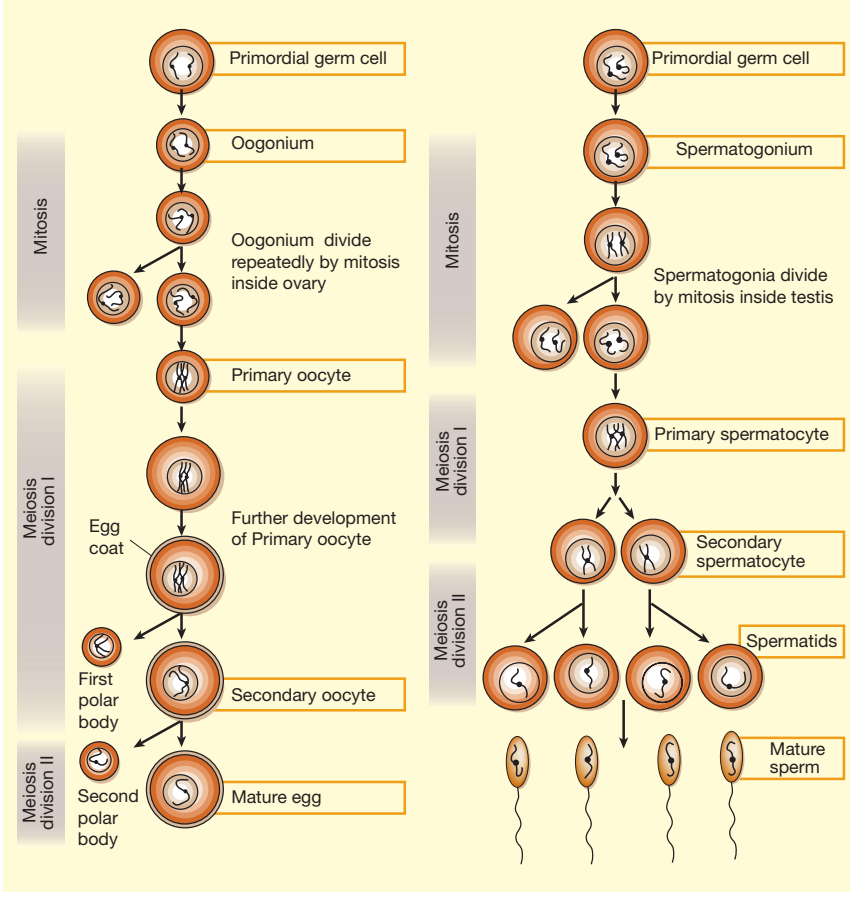
Early developments

John Eppig, a developmental biologist at the Jackson Laboratory in Bar Harbor, Maine, has been trying to grow mammalian eggs in the lab for many years. He reported his first success back in 1996 (ref. 1). Eppig extracted ovaries from newborn mice and cultured them for eight days, before dissecting out precursors of eggs, known as oocytes, and their associated granulosa cells. He then grew these complexes for a further 14 days in a variety of culture media, obtaining the best results with one containing epidermal growth factor — which promotes the growth of granulosa cells and their interaction with oocytes. Many of the resulting eggs began to develop after being fertilized, but the 190 two-celled embryos transferred into female mice generated only one live pup. Nicknamed Eggbert, this mouse was sickly, suffering from obesity and neurological problems in adulthood.

Since then, Eppig has honed his techniques by modifying the nutrient and hormonal supplements that he adds to the cultures. In a paper published online in December last year[2], he reported the birth of 59 live mice from 1,160 two-celled embryos — about one-third of the success rate achieved with IVF embryos generated from normal mouse eggs. The mice are now adults, and so far "look healthy and normal", says Eppig.

A few months earlier, a team led by Izuho Hatada of Gunma University in Japan achieved similar results by culturing oocytes from the ovaries of mouse fetuses about halfway through their gestation[3]. Hatada's team produced 16 live pups, but their lab-grown cells would only undergo the final stages of meiosis if they were first fused with naturally grown mature eggs stripped of their own genetic material. To ease problems with the supply of mammalian eggs, therefore, Hatada's technique needs further work.

Eppig and Hatada's experiments have so far attracted little public comment. But the potential for controversy, should similar techniques be applied to human reproduction, was underlined by the hostile reaction to related work reported on 30 June at a meeting of the European Society for Human Reproduction and Embryology in Madrid. Researchers led by Tal Biron-Shental of Meir



Path to life: the natural steps in egg and sperm creation; experts hope to adapt this process in the lab.

Hospital in Kfar Saba, Israel, had taken slices of ovarian tissue from aborted human fetuses, grown them in culture for a month, and found that the follicles containing immature oocytes had continued to develop. Donated human eggs are in short supply for IVF. But Biron-Shental's suggestion that aborted fetuses might be used to meet this demand was greeted with widespread revulsion — and in some countries, such as Britain, the practice would in any case be banned.

Even if ethical objections to using aborted fetal material could be removed, lab-grown eggs may not be safe for human reproduction. "Culturing methods have improved, but we are a long way from matching Mother Nature," says Eppig. Lab-grown eggs are smaller than normal and seem to be less capable of being fertilized and of developing as embryos.

One concern is that culturing could result in deleterious genetic changes. In unpublished work, Patricia Hunt of Case Western Reserve University in Cleveland, Ohio, has already seen changes in chromosome numbers in cultured mouse oocytes. "The final stages of oocyte growth are critical," she says. "I suspect it isn't the length of culture time, as much as the culture conditions."

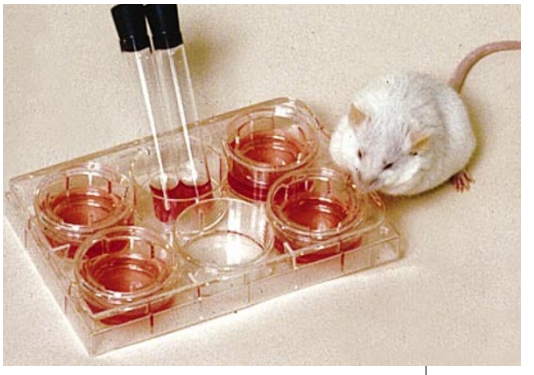
There are also concerns that culturing could disrupt the subtle mechanisms that normally ensure that some genes are activated in an embryo depending on whether they are inherited from the mother or father. These 'epigenetic' marks are laid down in developing sex cells, and their disruption can give rise to developmental abnormalities.

While some biologists refine their techniques for culturing eggs, other researchers are working on the male side of the equation. A team led by John Parks at Cornell University in Ithaca, New York, has taken the precursors of sperm cells from the testes of newborn bulls, and grown them in culture together with Sertoli cells. After several weeks, many cells had developed into spermatids, the round cells from which mature, mobile sperm later develop[4]. More recently, Ryuzo Yanagimachi and his colleagues at the University of Hawaii in Honolulu went one step further. They grew mouse spermatids by a similar method, injected them into mouse eggs, and found that they produced normal, fertile offspring[5].

Cultural mission

Researchers led by Martin Dym, a reproductive biologist at Georgetown University School of Medicine in Washington DC, have managed to generate spermatids in culture without the aid of Sertoli cells[6] — although to do this, they had to engineer the precursor cells to contain a gene for an enzyme called telomerase, which allows cells to divide indefinitely in culture. Dym is now collaborating with Yanagimachi to see if his spermatids can yield live pups. "We have embryos, but no implantation yet," says Dym.

So far, no one has managed to get spermatids to develop into mature sperm in a simple lab culture. "The big hurdle is the final step," says Toshiaki Noce of the Mitsubishi Kagaku Institute of Life Sciences in Tokyo, who is also working on techniques to grow



Fat chance: Eggbert, the first mouse born from a lab-cultured egg, suffered obesity later in life.

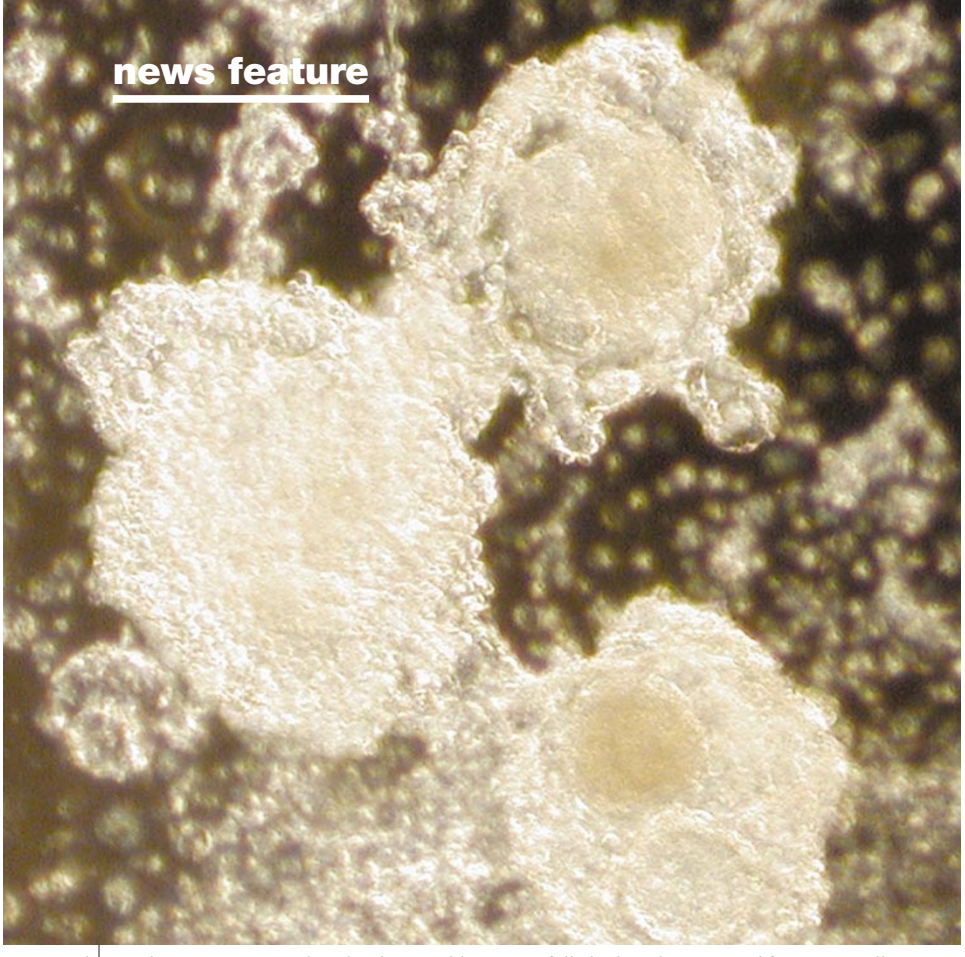
sperm in the lab. It is possible, however, to coax spermatids to mature into fully fledged sperm by transplanting them into animals' testes, using techniques pioneered in the mid-1990s by Ralph Brinster of the University of Pennsylvania in Philadelphia[7–9]. They might even be matured in testis tissue grafted under an animal's skin[10].

Most researchers, when trying to grow mammalian sperm or eggs in the lab, start with precursor cells extracted from ovaries or testes. But in May, a team led by Hans Schöler of the University of Pennsylvania's New Bolton Center in Kennett Square set the field abuzz by reporting the production of egg-like cells from mouse embryonic stem (ES) cells[11]. Schöler and his colleagues allowed the stem cells to grow at high densities until some proliferated into floating clumps, which were then transferred into new cultures.

The researchers found that some of these aggregations developed into structures similar to ovarian follicles — a large, egg-like cell surrounded by smaller ones, reminiscent of granulosa cells. Adding hormones called gonadotropins caused the 'eggs' to be expelled from the follicle, just as in natural ovulation. What's more, the egg-like cells seemed to have entered meiosis — although it remains to be shown whether their chromosomal number was halved. Schöler has also yet to show that his artificial eggs can be fertilized and give rise to mouse pups.

Other researchers are trying to grow sperm from ES cells. In Tokyo, Noce has nudged cultures of mouse ES cells down the developmental pathway that leads to sperm production. This work has yet to be published, but Noce claims that his cultures yielded male primordial germ cells, the earliest precursors of sperm. After being matured into sperm in the testes of adult mice, the cells seemed to be able to fertilize eggs. But the proof will be in the birth of healthy pups. "Even abnormal sperm can activate eggs," warns Noce, "so I don't know whether the sperm are normal or not at this stage."

George Daley, a stem-cell biologist at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts,



K. HÜBNER & H. SCHÖLER

Fresh eggs: structures that closely resemble ovarian follicles have been created from stem cells.

claims to have derived spermatocytes, a later stage in sperm production, from mouse ES cells. Again, the work is unpublished, and Daley warns that it is difficult to confirm his achievement from the cells' molecular characteristics. But he adds: "We have experiments under way to see whether we can isolate sperm that will actually fertilize eggs."

Lab-grown sex cells could readily find applications in animal breeding. In addition to boosting the number of offspring that could be bred from prize female animals, such cells could provide a new means of creating transgenic animals. Mice carrying targeted genetic modifications can be created by altering ES cells, introducing these into pre-implantation embryos and then breeding from the resulting mice to obtain animals with the desired genetic characteristics. For other species, it may be possible to achieve similar results by altering the precursors of sperm, maturing them in the lab, and then using them for IVF. "This would be especially useful for those animals that don't have good ES-cell lines, such as rats and pigs," says Dym.

The prospect of creating a prolific supply of lab-grown eggs would also provide a boost for experiments in therapeutic cloning, which will otherwise by severely limited by the supply of donated human eggs. Here, the idea would be to 'reprogramme' a patient's cells by fusing them with lab-grown eggs that have been stripped of their own chromosomes. After culturing the resulting embryos for a few days, it should be possible to derive human ES cells, perfectly matched to the patient, that could potentially be coaxed into creating a variety of cells and tissues.

For many researchers trying to grow sperm and eggs, the main goal is to unlock the natural secrets of mammalian sex-cell development. Scientists would like to know the cocktail of growth factors and hormones that turn stem cells into precursor sex cells. "This is important because many of the mutations that affect adult fertility have been found to affect genes that act early in germline development," says Peter Donovan, a stem-cell biologist at Thomas Jefferson University in Philadelphia. "It could tell us an awful lot about the parameters that affect human and domestic-animal fertility."

Body doubles

Rather than mimicking the natural processes of sex-cell formation, however, some researchers are trying to trick normal body cells into behaving like sperm or eggs. For instance, Orly Lacham-Kaplan, a reproductive biologist at the Monash Institute of Reproduction and Development in Melbourne, Australia, has injected adult male cells into mouse oocytes that still contained two sets of chromosomes. The oocytes spat out half of the chromosomes from each of their constituent nuclei, and began to develop like fertilized eggs[12]. Whether the resulting embryos are viable remains to be shown, however. "We are in the process of testing whether offspring will be born and whether they will be normal," says Lacham-Kaplan.

Meanwhile, Gianpiero Palermo, an IVF expert at Cornell University's Weill Medical College in New York City, has injected adult cell nuclei into eggs stripped of their chromosomes, and discovered that these nuclei can subsequently be made to expel half of their chromosomes[13,14]. For both mouse and human cells, the resulting 'eggs' can be fertilized — although, again, Palermo has yet to produce any offspring using his method.

Palermo's technique requires eggs, so this will not solve problems with the supply of eggs for either animal or human reproduction. Instead, his eventual goal is to provide fertility treatments for older women whose ovaries are no longer able to produce viable eggs. "One-third of our IVF patients are over 40 years of age," says Palermo. "More than half of their eggs can be abnormal."

Such talk of using artificially produced eggs for human reproduction alarms many researchers in the field — especially as those produced by Palermo's method may be even more prone to abnormalities than those grown by processes that mimic the natural process of sex-cell formation. "It is simply ripping the genetic material asunder with no evidence that normal meiosis has taken place," argues Eppig.

Other researchers point out that the cells that give rise to sperm and eggs possess mechanisms to minimize the accumulation of mutations. Most of the body's cells, however, lack these mechanisms, and so hardly represent ideal genetic stock from which to breed a baby. "It seems risky," says Donovan.

Questions are beginning to be asked about the safety even of existing techniques for assisted reproduction[15,16]. Given our current state of knowledge, most experts believe that any attempt to use lab-derived eggs or sperm for human reproduction — however they are produced — would be an act of extreme folly. ■

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