50 Years Ago

"The great majority of school children are not only robust and healthy, but are taller and heavier than their predecessors", states Sir John Charles, the chief medical officer of the Ministry of Education, in his report for 1958 and 1959 … Only about five per cent of school children contracted a notifiable infectious disease in the years under review. Tuberculosis continued to decline. Poliomyelitis reached its lowest level for thirteen years, and vaccination against it was undertaken vigorously everywhere … The main cause of death among children is through accident … In 1958, 869 children of 5–15 years died from accidents, including 395 from accidents involving motor vehicles, 174 from drowning, and 58 from burns and scalds.

From *Nature* 25 March 1961

100 Years Ago

I have just been told a very interesting story by Mr. James Day of this town. Many years ago he and his father … noticed a fox come searching along the hedgerows … they saw that he was collecting the sheep's wool caught on the thorns and brambles. When he had gathered a large bunch he went down to a pool … and backed slowly brush first into the water, until he was all submerged except his nose and the bunch of wool, which he held in his mouth. He remained thus for a short time, and then let go of the wool, which floated away; then he came out, shook himself, and ran off. Much astonished at this strange proceeding, they took a shepherd's crook … and pulled the wool out. They found that it was full of fleas, which, to save themselves from drowning, had crept up and up the fox's brush and body and head and into the wool, and that thus the wily fox had got rid of them. T. McKenny Hughes

From *Nature* 23 March 1911

under different conditions, the authors used a marker for spermatogenesis[4]: their mice were genetically engineered to express green fluorescent protein (GFP) under the control of regulatory elements for genes that are activated only when germ cells progress into meiosis and beyond. As expected, the tissues that they collected from the testes of newborn mice showed little or no baseline expression of GFP.

The authors had previously optimized[4] various parameters involved in *in vitro* sperm formation, including temperature and the choice of basic ingredients for the medium. Notably, fetal bovine serum (FBS) seemed to be an important component, because its absence resulted in negligible maturation — as evidenced by the lack of the GFP signal.

In their present work, Sato *et al.*[1] confirm the importance of FBS but, borrowing from the field of embryonic-stem-cell biology[5], they find that an alternative to FBS known as knockout serum replacement (KSR) is even more effective. This finding is counter-intuitive, because KSR is commonly used to maintain stem cells in an undifferentiated state. A clue to the mechanism involved comes from the fact that the lipid-rich albumin component of KSR is itself highly effective in boosting differentiation.

The authors used *in vitro* fertilization (IVF) techniques to demonstrate the authenticity of the sperm collected from their cultures: they obtained male and female offspring that were themselves fertile.

The preservation of fertility is a major concern for patients requiring therapy, such as chemotherapy or radiation therapy, that can inadvertently destroy germ cells. In men, this problem can be mitigated by banking sperm before treatment. The solution is less straightforward in pre-pubescent boys. On the basis of pioneering work in animals by Brinster[6] and others, the idea of transplanting cryopreserved spermatogonial stem cells is a reasonable strategy, although it has not yet been rigorously assessed in humans. Furthermore, the technology for the long-term culture and expansion of human spermatogonial stem cells has not been standardized, nor has the safety of the approach been tested.

Sato and colleagues' results suggest a viable alternative. In this scheme, boys would undergo testicular biopsy before chemotherapy or radiation therapy, to obtain tissue for cryopreservation (Fig. 1). If infertility occurs, the testicular fragments could be thawed and sperm obtained from organ culture for IVF. Such a protocol would bypass the need for surgical spermatogonial stem-cell transplantation.

It remains unclear whether the success of this system is due to signalling molecules released by the germ cells themselves, or to molecules released by the surrounding somatic (non-germ) cells, or to both. Nonetheless, the integrity of somatic cells, especially Sertoli cells, seems to be essential. However, this is not surprising, given that germ-cell maturation is known to depend on somatic-cell signals. In fact, even when embryonic stem cells have been used to produce germ cells *in vitro*[7], signals contributed by differentiating testicular somatic cells in the culture seem to be required.

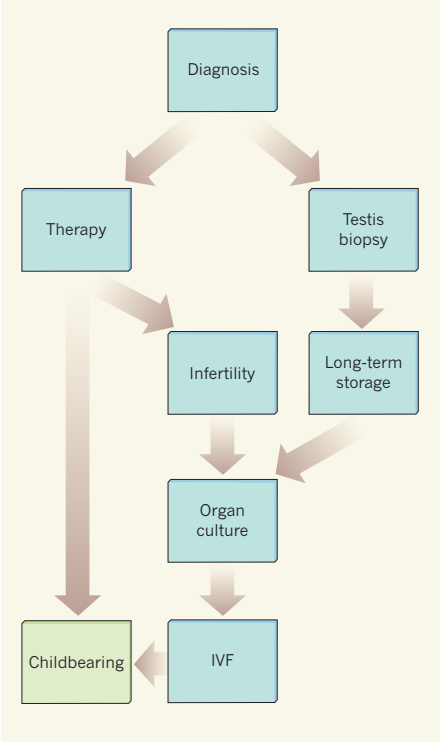

Figure 1 | Possible scenario for preserving fertility, based on the new work[1]. After a boy has been diagnosed with cancer, a biopsy would be performed to obtain testicular fragments for long-term cryopreservation. Later in life, when the individual wants to start a family, fertility would be assessed, and if he cannot produce functional sperm, the stored tissues could be thawed for organ culture. Sperm formed by this *ex vivo* method could then be used for *in vitro* fertilization (IVF).

The exact nature of the external signals that enhance sperm maturation is not the only remaining mystery. It is also not known whether the resulting offspring — especially those produced from cryopreserved tissue — are generally healthy. Indeed, fertility of the offspring is just a crude indicator of whether gametes are 'normal'. Investigations should be made into whether the progeny Sato and co-workers generated by IVF are healthy in other ways (with respect to ageing, immune function, behaviour and so on).

As for the consequences of *in vitro* spermatogenesis at the molecular and cellular levels, previous data have indicated[8] that adverse epigenetic effects occur when cells, especially gametes, are maintained in culture. Whether DNA repair, which is essential for spermatogenesis *in vivo*, functions normally *in vitro* is another concern. Subtle genetic or epigenetic changes could be pivotal for the well-being of subsequent generations. These caveats aside,