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Is there any reproductive future left for men?

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According to Germaine Greer, an Australian academic, journalist and feminist author of 'The Female Eunuch', legendary for her quotes (see at http://en.wikiquote.org/wiki/Greer,_Germaine) men are pointless when it comes to reproduction. 'A society can survive with only one man, no society will survive a shortage of women'. Mrs. Greer also has some ideas about spermatogenesis: 'Because you are a man, you do something as colossally stupid as figuring out a way to generate sperm from cells'.

Probably with intracytoplasmic sperm injection (ICSI) on her mind, she even argued: 'If you are fool enough to produce 40 million sperm at each ejaculation you'd have to be aware that all but one or two of you are surplus to requirements'. And for the researchers interested in male infertility and spermatogenesis she also has an important message: 'With the whole world bathed in gadzillions of dying sperm, a bunch of men have wasted valuable research funding in figuring out a very costly way to whip up a few dozen more'.

But how true are Greer's thoughts? Are men becoming redundant or even obsolete?

Thanks to molecular biology, we know that X- and the Y-chromosomes have evolved from a pair of autosomes (Lahn and Page, 1999). It was first estimated that about 350 million years ago, reptile-like ancestors of today mammals developed an allelic variation causing this organism to develop as a male individual (Graves, 2006).

However, more recent research has put this estimate of divergence 145 million years younger than previously estimated (Veyrunes et al., 2008). This allelic variation, i.e. a male-determining locus, interfered with recombination between the two original autosomes, eventually resulting in the current different gene numbers between the X- and the Y-chromosome.

In contrast to the well-preserved X-linked genes (number about 1.100), the Y-linked genes (number < 200) have been deleted over time. Therefore, the Y-chromosome is in se a pruned X-chromosome.

Before the development of the allelic variation causing the organism to develop a male phenotype, sex was determined by a genetic master-switch with environmental factors interfering with the genetic pathways for sexual determination.

In today's' turtles for example, sex depends on the ambient temperature during the incubation of the eggs (Pieau et al., 2001). During their embryonic development these animals develop indifferent, i.e. bipotential, gonads. The final differentiation of the gonads is thermosensitive, i.e. dependent on the ambient temperature. If the eggs are incubating in a temperature exceeding 28.5 degrees Celsius, ovarian differentiation will take place. When eggs are incubated below this temperature point, then a testis will develop. If a nesting female sea turtle digs a hole on the beach, first she fills the nest with her eggs, then she covers the eggs with sand and eventually returns to the ocean. After an incubation period of about two months, hatching starts and the hatchling's gender depends on the temperature of the sand, with eggs incubated at lower temperature developing into males while eggs incubated at higher temperatures developing into females. As a result of the above-mentioned allelic variation, the control over this differentiation process has been put under genetic control independent of environmental factors such as temperature.

Because of concurrent recombination failures, the human Y-chromosome has been pruned over the course of its existence with an estimated loss of four to five genes per million years. In his book 'Adam's curse – A future without men' Bryan Sikes, an Oxford University professor in genetics, hypothesized that within 5.000 generations, i.e. 125.000 years, reproduction associated to the Y-chromosome will become extinct (Sykes, 2003).

Several mechanisms have been proposed to explain the genetic erosion of the Y-chromosome. An important cause of genetic erosion is the limited recombination between X- and Y-chromosomes.

In addition, the testis is a mutation-prone organ: The adult testis will produce more than a hundred million spermatozoa per day. The cellular metabolism associated to this extensive propagation of cells, either through meiosis or mitosis, generates an overload of free radicals which may have beneficial but also harmful biological actions on the surrounding tissues and cells. Free radicals are known to be a main cause of oxidative damage to DNA, hence they contribute to mutations.

Apart from this mutation-inducing metabolism in the testis, the production of millions of cells per day is associated to error-prone DNA copying. Finally, during spermiogenesis (the process in which the spermatid is rebuilt into a spermatozoon) the spermatogenic cells will lose most of their cytoplasm and will become cells devoid of the major defense mechanisms against oxidative damage.

It is assumed that the testis tries to control the mutation rate to some extent by controlling its own temperature. Higher temperature means higher metabolism while lowering the temperature means a lower metabolic rate. The testis has ingenious mechanisms by which it can lower its temperature to a level lower than the core body temperature. It has a 'temperature muscle', i.e. the cremasteric muscle that can move the testis closer upwards to the body providing an environment with a higher temperature. By relaxing the cremasteric muscle, the testis is lowered away from the body, lowering its temperature.

Apart from this temperature muscle, the testis harbours another ingenious system controlling its temperature: a genuine heat exchanger. The venous plexus pampiniformis enclosing the spermatic artery will exchange heat and as a result cooled arterial blood will eventually enter the organ. In mammals with internal (cryptic) testes, the latter countercurrent cooling mechanism is essential and therefore, the anatomical design of the heat exchanger may be very sophisticated. In dolphins veins from the peripheral surfaces of the fins carry cooled blood via the lumbo-caudal venous plexus which encircles the spermatic artery to the testes (Rommel et al., 1992).

But being mutation-prone may also be advantageous for a reproductive organ. In case of a mutagenic overload, e.g. exposure to radiation potentially inducing massive DNA damage, the Y chromosome is most vulnerable because of limited recombination and DNA repair. Hence, besides autosomal mutations, the Y chromosome will accumulate maximal mutagenic damage in male offspring. Since the Y chromosome's genes are involved in reproduction and survival, the effects of this massive DNA damage will be discontinued at this generation since the paternal overload will incapacitate spermatogenesis in the offspring. This mechanism is referred to as the fuse-box hypothesis of the Y chromosome (Short, 1997).

Will the genetic erosion of the Y chromosome make males to vanish and should we look for strategies to improve our genetic robustness or even find strategies to survive without any genetic contribution of a male?

In nature there are some examples on how to reproduce without any male genetic contribution with parthenogenesis being the best known example. The terminology parthenogenesis refers to the ancient Greek concept of virgin birth. In Greek mythology, Athena, the goddess of wisdom, was born from Zeus' head who was experiencing a bad headache and asked to Prometheus to cleave his head in order to get rid of his pains. This immaculate conception, not involving any sexual partner, is exemplary for the phenomenon of parthenogenesis in nature, i.e. a way of reproducing without fertilization with an oocyte developing into an embryo and an individual without any male contribution.

While parthenogenesis can be observed in many 'lower animals', especially insects, it has also been described in fish and in some reptiles. In the Drosophila manga beinaie (fruit fly) the trick is to use a polar body as a second gamete. In the species Pycnoscelus surinamensis (a cockroach from Suriname) the trick is to have an oogenesis with two mitoses but without meiosis and in the lizard Cnemidophorus uniparens, the oogonia become tetraploid before meiosis takes place.

As can be expected, animals reproducing by natural parthenogenesis only have female offspring.

While natural parthenogenesis has never been described in mammals, in 2004 a Japanese group reported the first mammals created by (non-natural) parthenogenesis. The research team led by Dr. Kono of the University of Agriculture of Tokyo created fatherless mice by fusing an oocyte with an immature oocyte from a newborn female pup which was genetically modified so that the DNA of these eggs had a male imprint. The resulting mouse called Kaguya (the name of the moon princess from a Japanese tale who was created by yet another 'virgin birth') on her turn was able to reproduce in a natural way.

Does this mean that parthenogenesis would be an option for the problem of the disappearing male? Definitely not! Apart from the fact that one of the 'gametes', came from a transgenic animal, Kaguya was the result of more than 400 attempts which

eventually created a genetic make-up allowing normal embryogenesis by luck. Hence this experiment is virtually unreplicable.

Another popular 'plan B' is the generation of male gametes from stem cells. Again in 2004, an American research team led by George Daley reported the first successful derivation of male gametes from embryonic stem cells in a mouse model (Geijsen et al., 2004). This model used green fluorescent protein positive mouse in order to evidence a true derivation from embryonic stem cells.

In their study, they first cultured mouse embryonic stem cells into embryoid bodies. In a medium, containing retinoic acid, some cells within these embryoid bodies differentiated into cells expressing markers for primordial germ cells. Once isolated, these cells could be further cultured in-vitro and expressed meiotic as well as post-meiotic markers. Once injected into eggs derived from a non-green fluorescent protein positive mouse, the fertilized eggs grow into an early-stage mouse embryo expressing green fluorescent protein in all blastomeres.

Is this the way to go? At present, it seems not because again there were few experiments that turned out successful out of a long series of identical experiments failing to produce 'male gametes'. Besides, the embryos obtained did not produce viable offspring after transfer into pseudo pregnant foster mothers.

However, two years later, a team led by Nayernia reported the birth of mice after in-vitro differentiation of embryonic stem cells into 'male gametes' (Nayernia et al., 2006 a). Their publication was rapidly followed-up by a subsequent publication by the same group reporting the derivation of male germ cells in mice from bone marrow stem cells, i.e. adult stem cells instead of embryonic stem cells (Nayernia et al., 2006 b). While all these observations on in-vitro derivation of male gametes from stem cells were obtained from mice models, in 2009 the same research group struck the headlines in newspapers worldwide because of their report in the journal 'Stem Cells and Development' on the production of 'human sperm in a test-tube'. Although many scientists tried to replicate their work in the mouse, this paper reported on the derivation of male gametes from human embryonic stem cells. However, this paper was retracted very quickly as it turned out that plagiarism was involved without, however, any doubts on the validity of the study findings (Nayernia et al., 2009 - retracted).

The dream is there, however, it will remain probably a dream for many years because of methodological uncertainties making replication difficult. In the meantime, we will probably read reports on research, difficult to interpret, difficult to replicate raising more questions than answers. An example of this was a report on the 2008 ESHRE (European Society of Human Reproduction and Embryology) meeting in Barcelona where a Brazilian research group reported on the derivation of human gamete-like cells from adult stem cells of human teeth (Fonseca et al., 2008).

While stem cell work remains fascinating but often difficult to interpret, the key to survival for our species may be hidden in our genes. There are many examples in nature that show that apart from genetic robustness some molecular mechanisms may exist that provide genetic flexibility. The Caucasian vole (a vole is a mouse-like rodent) for example is reproducing by combining a female gamete with a male gamete. Males are phenotypically completely different from females, however, both males and females have a 17,X karyotype (Coskun and Uluturk, 2003). Half of the gametes produced by these animals do not carry any X-chromosome and after fertilization one out of four zygotes does not carry an X-chromosome. One out of four zygotes has two X-chromosomes and will not develop, while half of the fertilized eggs will be 17,X and may develop into either a male or a female animal.

The genetic mechanism behind the primary and the secondary sexual differentiation in these animals remains a mystery. The Caucasian vole or mole vole is only one example of a group of rodent species with this remarkable system of sex determination and gametogenesis. Other members of this family of rodents show different karyotypes including XX or XY but also XX-males (Kolomiets et al., 2010).

But alternative genetic sex-determining mechanisms have been observed in 'higher' mammals too. The black munt jack, a deer living in China, has so-called 'neo-sex chromosomes', which are assumed to be the result of a fusion or translocation between autosomes and sex chromosomes which were subsequently pruned by extensive recombination suppression leaving chromosomes that are similar to the sex chromosomes as encountered in most mammals, including the human (Zhou et al., 2008). Therefore, some evolutionary biologists assume that primates and also the human evolve to new sex-determination systems over less than 20 million years (Graves, 2006).

But maybe the Y-chromosome in itself can be adaptive enough to ensure our survival. Recent studies in chimpanzees show that although the Y-chromosome has been in decay over many millions of years, it may as well survive in its present form because its gene loss has stabilized. The research group led by David Page recently published a very reassuring paper showing that the male Y-chromosome may have lost only one gene in the

past 25 million years (Hughes et al., 2012). Earlier, this research group already showed that in chimpanzees genetic adaptations may introduce extra copies of genes involved in spermatogenesis (Hughes et al., 2005).

If the male reader of this paper is not relieved of his fears that our role may become extinct, the best suggestion comes from Professor Roger Short, a fervent promoter of the survival of the Y-chromosome: 'It's the Y-chromosome that adds spice to life, that keeps men on the 'qui vive' from puberty to old age ever ready to sow their wild oats whenever the occasion arises'.

Male reader do your duty!

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