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COMMENTARY

The significance of parthenogenetic virgin mothers in bonnethead sharks and mice

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Abstract Two astonishing virgin births in quick succession have raised interest in parthenogenesis in cartilaginous sharks and mammals. These were believed to be exceptions until numerous female bonnethead (hammerhead) sharks were found to be giving birth at the Henry Doorly Zoo in Nebraska, despite the prolonged absence of male sharks. The birth of a shark pup led to suggestions that spermatozoa from a previous coitus had persisted in the female tract of its mother and fertilized one of her eggs some months later. These proved to be incorrect because the female had been isolated for several years whereas spermatozoa persisted in the female tract for approximately 6 months. Molecular investigations into the pup's DNA failed to find any paternal contribution and proved the pup to be descended from its mother only. Just before this discovery, a study in mice had revealed that parthenogenesis could be induced by overcoming damage to embryonic development that is normally caused by gene imprinting. This was done by fusing two mouse oocytes and then inserting Igf2 into the parthenogenotes, which led to the birth of several parthenogenetic offspring. Modifying epigenesis had thus opened pathways to full-term parthenogenetic development. The birth of these parthenogenotes fulfils the attempts of earlier scientists to invoke parthenogenesis in experimental animals. 

KEYWORDS: bonnethead sharks, mice, parthenogenesis, virgin births

Parthenogenetic (virgin) birth of a bonnethead shark

Conception can take various unusual forms, for example, oocyte meiosis may be averted in various species including snakes, so that offspring are genetically identical to their mother (Groot *et al.*, 2003). Parthenogenesis is well known to be obligate in many vertebrates, e.g. in jawed vertebrate lineages including bony fishes, amphibians, reptiles and birds, and cartilaginous fishes, and occurs occasionally in species normally reproducing sexually. It was thought to be lacking in only two orders, namely cartilaginous fish and mammals. Two recent reports, one on the bonnethead or hammerhead shark and the other in mice, have challenged this interpretation by showing that parthenogenotes can be obtained in each species. It occurred spontaneously in cartilaginous fish and was induced experimentally in a mammal, i.e. the mouse. It had been suspected to be occurring in many species where pregnancies arose despite the long isolation of females from males. These pregnancies were then

attributed to fertilization achieved by the long-term survival of spermatozoa stored in the reproductive tract of females.

Reports of a parthenogenetic birth in a bonnethead shark (*Sphyrna tiburo*) were therefore surprising. They reawakened interest in the significance of parthenogenetic development in vertebrates including mammals. Chapman *et al.* (2007) studied bonnethead sharks at the Henry Doorly Zoo in Nebraska, and observed the birth of a pup whose mother had been isolated from males over several years. Since spermatozoa survive for only 6 months or so in the genital tract of a female bonnethead shark, the pup could not have been conceived from a male parent. It was probably parthenogenetic, and if so, it left mammals as the only exception among major jawed vertebrate lineages to the occurrence of parthenogenesis. This conclusion was reinforced when investigators reported that other female sharks were giving birth despite the long-term absence of male sharks.

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Chapman *et al.* (2007) reported that the bonnethead shark that was mother to the pup had been isolated from males over a very long period. It was first suspected that the pup had arisen from spermatozoa stored in the female reproductive tract after over a period of months after mating. This suggestion was modified when the isolation period was found to have lasted for several years, whereas spermatozoa remain active in the reproductive tract for only a few months. The possibility of a very long delayed fertilization was therefore rejected and the pup was suspected to be parthenogenetic. These investigators therefore applied new techniques of molecular profiling in attempts to discover exactly what had happened inside the reproductive systems of the sharks. Tissues from the pup and from each of three candidate mothers were assessed by measuring four moderately to highly significant polymorphic microsatellite markers, using multi-locus, amplified length polymorphism fingerprinting (AFLP). Resulting insights on the genotypes of several female sharks under investigation provided valuable data that were reinforced by analysing the genotypes of 119 animals in the source population. This use of AFLP had been designed to discover if the pup's genome possessed a paternal contribution, and Chapman *et al.* (2007) found no evidence to prove the existence of a paternal genetic contribution. Another discovery was also highly significant. It showed that the pup was homozygous for an allele inherited from its mother, which offered major evidence of parthenogenesis. This analysis had also determined the identity of the three candidate mothers, and one of them (CM2) was unambiguously shown as being the mother. The chances of conception were clearly very low, yet these data had provided strong evidence of the occurrence of parthenogenesis in a bonnethead shark.

Parthenogenetic (virgin) births in mice

Recognition that cartilaginous fish could undergo parthenogenesis indicated that mammals were apparently the only group among all other known jawed vertebrates that could not undergo parthenogenesis. Yet, amazingly, Kono *et al.* (2004) had already described an experimental approach resulting in parthenogenetic mice. They had examined the factors that made mice so resistant to parthenogenesis, and realised that the cause was due to the expression of epigenetic factors that regulated development in early mammalian embryos. Previous evidence has revealed that maternal and paternal genomes differed epigenetically, and that both had to be active if an embryo was to attain a satisfactory genotype capable of sustaining blastocysts to full term. Imprinting thus required two distinct gametes with their own

sex-specific imprints that together sustain normal embryogenesis. Fusing two eggs would fail to establish this balanced situation and might lead to complex genetic situations as the embryos differentiated.

To a certain degree, this problem was overcome by Kono *et al.* (2004). They were well aware that the genes *H19* and *Igf2* were present in male and female murine gametes respectively, and that *H19* was activated in oocytes whereas *Igf2* was expressed in spermatozoa. It was therefore essential to find a source of *Igf2* to sustain blastocysts. This was achieved by inserting *Igf2* into the nucleus of an oocyte at a genetic site that remained active in embryos to and beyond implantation. The resulting nucleus was isolated and transferred into a recipient oocyte that was expressing *H19* but not *Igf2*. Kono *et al.* (2004) artificially activated 457 out of 598 treated oocytes that had begun their cleavage stages. More than 80% of the activated eggs, i.e. 371, produced blastocysts and these were transferred to 26 recipients. This resulted in 10 live and nine dead offspring, of which one developed to an adult. By now, it has reached 14 months of age and has delivered her own offspring. While this overall result may be disappointing, it is at least worthwhile considering that 80% of the embryos had reached the blastocyst stage and had produced stem cells, which offers a greater degree of success in stem cell biology than attained with standard forms of cloning. It is not known if intergenomic conflicts could arise in hammerhead sharks, nor if gynogenetic embryos in that species would persist for any length of time.

Discussing the evolutionary implications of their findings, Chapman *et al.* (2007) pointed out that parthenogenesis is not easy to detect in sexually reproducing vertebrates. They wished to investigate further the process of asexual reproduction, by extending their knowledge of the relationships between parthenogenesis and the factors that led to female sharks having difficulties in finding suitable mates. According to a commentary on BBC News (2007), over-fishing has indeed led many sharks into intense reproductive pressure and obviously resulted in sharply declining populations. Serious declines in their population numbers may have been avoided by their resort to parthenogenesis and enabled them to overcome this pressure on their numbers. But parthenogenesis has its own problems, including a declining genetic diversity in the species in question and this would in turn restrict the ability of the parthenogenotes to adapt to new environments.

Parthenogenetic and other chromosomal disorders in mammalian eggs

Findings on parthenogenesis in hammerhead sharks will doubtless lead to investigations into other species.

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What is the form of these studies likely to be? In relation to mammals, the implications of these particular findings in the hammerhead sharks and mice obviously concern parthenogenesis and its related situations, namely androgenesis and gynogenesis, which may arise naturally or through the effects of environmental agents. Queries arise about the growth of human embryos *in vivo* and *in vitro* and the consequences for their normal development. Such information is essential for embryologists in IVF clinics who must be made aware of the various forms of parthenogenesis and other anomalies arising spontaneously in mammalian eggs. Haploid parthenogenetic embryos were very rare among >6000 human embryos, of which 20% were aneuploid (Munné *et al.*, 2007). Delayed fertilization is a possibility after human eggs have been inseminated, and can weaken the block to polyspermy and result in dispermy and possibly triploidy. Reports have also appeared of fusions between the polar body and the oocyte. The obvious approach is to gain information on animal species as a prelude to human studies, and it is most interesting that earlier scientific interest in mammals had led to attempts at inducing androgenesis and gynogenesis in mammalian embryos, especially mice.

Mammalian parthenogenesis is synonymous with gynogenesis. It involves the formation of maternal pronucleus only. Spontaneous parthenogenesis in human eggs is usually identified by the presence of a single pronucleus and usually an extruded polar body. External stimuli to the oocyte can also result in the formation of a maternal pronucleus associated with an extruded polar body. Maternal pronuclei can be also be formed if the sperm head in a normally fertilized oocyte is inactivated or extruded (Plachot *et al.*, 1989). Some parthenogenetic mouse embryos can develop to mid-gestation, with a beating heart, 25 somites, normal head formation and a body structure that is nearly normal (Kaufman, 1983). Fertilized eggs may possess one, three or more pronuclei, including some that have a single pronucleus and are possibly parthenogenetic. Damage to the meiotic spindle can also lead to the loss of maternal chromosomes, and tubulin depolymerization during cryopreservation may also induce these anomalies.

Embryos *in vitro* could be mistakenly exposed to factors invoking parthenogenesis. Mammalian ova can be activated by chilling, warming, Ca^{2+} , calcium ionophores, electrical stimuli, pricking, certain anaesthetics, and other means of disturbing the balance between free calcium and the state of the cytoskeletal system (reviewed in Edwards, 1980). Manipulative techniques have also led to the formation of uniparental mice. This was achieved by removing one pronucleus, male or female, from a fertilized egg. In the resulting haploid embryos, diploidy was restored by doubling the chromosomal complement at the first

cleavage division. The resulting embryos perished in cleavage stages or slightly later.

Several investigators have attempted to induce parthenogenesis in rabbit embryos by chilling or warming the ova (Pincus and Shapiro, 1940). Claims were made that some of them developed to blastocysts, e.g. those parthenogenetic mouse embryos that were reported to have developed to the 20–25 somite stages (Kaufman *et al.*, 1977). Human oocytes are no exception to these observations. Some are activated if left too long in culture media whereupon they may be induced to form nuclei. These embryos can easily be mistaken as fertilized eggs.

Other experimental procedures have also been applied to mammalian gametes and embryos *in vitro*. Approaches to the induction of gynogenesis have involved exposing mouse spermatozoa to X-irradiation or to ultraviolet light in order to inactivate the sperm chromosomes if possible. These treatments did not greatly impair the movement of spermatozoa, and they seemed to be fully capable of fertilizing eggs. The treated spermatozoa were then artificially inseminated into female mice in oestrus. Embryos derived from X-irradiated spermatozoa developed through some cleavage divisions, despite the sperm chromosomes having been fragmented or inactivated. Some embryos were gynogenetic haploids and others were aneuploid although the radiation damage seemed to be too great for normal embryonic growth. Ultraviolet exposure damaged the sperm chromosomes, which were often grouped into a large mass of chromatin. Once again, some of the eggs divided but very few developed to blastocysts, the remainder dying during their cleavage stages. It was very clear that different techniques were needed even though similar techniques have been widely tested, e.g. in amphibians (Hertwig, 1911; Fankhauser, 1945), rabbits (Pincus and Enzmann, 1934) and mice (Edwards, 1954). Available evidence indicates that few if any gynogenetic mammalian embryos have developed to term. They might emerge through nuclear transfer techniques which is widely practiced today, notably in farm animals.

Different approaches were needed to produce androgenic embryos in mammals. One approach was to expose oocytes to colchicine before fertilization. This method dissolved the meiotic spindle in the treated oocytes. At fertilization, the chromosomes had detached from their spindle, and were spreading individually through ooplasm. Each chromosome moved to the oolemma and at sperm entry most of them were expelled from the oocyte into small extruded bodies resembling small polar bodies. The single pronucleus derived from the fertilizing spermatozoon developed normally in the oocyte, and was proved to be the male pronucleus by pre-labelling sperm chromatin with ^{14}C -adenine before insemination (Edwards and

Sirlin, 1956). This method was successful in that many embryos were haploid, having shed all the female meiotic chromosomes.

Overall, it is clear that better techniques are required to acquire parthenogenetic embryos in mammals. The best example is probably the human androgenetic hydatidiform mole, which is well known in clinical medicine. The condition has been known for many years, and can be repetitive in some patients.

Disorders at syngamy can lead to human hydatidiform moles with two sets of paternal chromosomes and no maternal chromosomes. They probably arise when dispermic fertilization is succeeded by the expulsion of maternal chromosomes at syngamy. Fertilization involving diploid spermatozoa may be another cause. Perhaps most unusual of all, newborn mice were created by fusing two oocytes, and obtaining an offspring named Kaguya (Kono *et al.*, 2004). This approach has been suggested many times before as a means of enabling two women to have their own child, but caution is needed when fusing two eggs instead of achieving normal fertilization.

Some hydatidiform moles arising in aberrant pregnancies involve swollen cysts of trophoblastic villi and offer another example of androgenesis. Most complete hydatidiform moles are 46, XX, arise from a single spermatozoon and could originate as the maternal chromosomes are shed so that the remaining male chromosomes form XX or YY androgenones, the latter soon dying *in vivo*. Many embryos develop to mid-gestation in women with repeated hydatidiform moles. Risks increase from 1 in ~1000 to 1 in 16 in women who have had two previous hydatidiform moles. Complete hydatidiform moles arising as a consequence of dispermy in eggs that have lost their maternal complement of chromosomes, develop with XX, XY or YY sex chromosomes. Most that survive are XX because embryos carrying a YY complement die very early. Such variances can lead to 92,XXXX or 92,XXYY embryos, which are partial moles. The exact origin of these anomalies requires further analysis, although an unstable second meiotic spindle might have led to a premature first cleavage division. This

could split the egg into equal halves rather than a large oocyte and small first polar body. Often classified as a missed abortion, all their chromosomes are paternal in origin and they can be mono- or dispermic.

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