

Reproductive ageing and the menopause

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SUMMARY This brief review describes early work initiated by Anne McLaren and John Biggers, in which they repeated on mice a very early experiment carried out by John Hunter on pigs, to test the effect of unilateral ovariectomy on subsequent breeding performance. This and subsequent experiments led to the conclusion that reproductive ageing in the female mouse was largely due to ageing changes in the uterus. As a result of these changes fewer implanted blastocysts are carried to term in the older females, with the result that the size of litters produced gradually drops and ceases altogether well before the expected time of death, thus leading to a period of reproductive inactivity at the end of life. Other organs undergo ageing changes but it appears to be those in the uterus which limit reproductive performance in the female. The somatic organs concerned in bringing the male gametes into the environment are still able to function effectively almost until the time of death so that males have a very short period of reproductive inactivity at the end of their lives. Due to the prenatal onset of meiosis in the germ cells, female mammals and some, but not all, other vertebrates are born with a finite crop of oocytes in the ovary, which cannot be increased after birth. Nevertheless, with the exception of women, female mammals appear to be able to produce ova well into old age, and have them fertilized. When examined after death the ovaries still contain oocytes so this is not a limiting factor in reproduction in old females. In women the situation is completely different. They also have an extended period of reproductive quiescence in middle and old age, the menopause, but, unlike other female mammals, this is not due to failure of the uterus but is caused by the ovary becoming depleted of oocytes in middle age. The reason women run out of oocytes before the end of life, whereas the other mammals which have been studied do not, is associated with the greatly extended lifespan of humans compared to other mammals of equivalent size. There is a linear relationship between longevity and body weight in mammals, small mammals have much shorter lives than large ones. This is probably associated with the increased production of free radical oxygen necessary to maintain body temperature in smaller animals. Heat is lost through the body surface which becomes relatively less as the animal increases in weight, so the smaller animal has to metabolise and thus produces more free radical oxygen to maintain body temperature. For reasons unknown this seems not to apply to humans. The menopause has thus evolved as a consequence of two adaptations: the prenatal onset of meiosis, common to all mammals and many other vertebrates and the greatly increased longevity of all humans, both male and female. In view of this dual origin it is unlikely to have evolved in response to an adaptive need to have grandmothers to help rear the young, as has been suggested!

KEY WORDS: *Age, menopause, uterus.*

In the eighteenth century John Hunter, the famous London surgeon, carried out what must be one of the earliest experiments in reproductive biology. He removed one ovary from a sow and compared its subsequent reproductive performance with that of an entire litter mate. He reported in *Philosophical Transactions of the Royal Society of London* in 1787 (Hunter, 1787) that the sow with only one ovary produced half the number of offspring during the

rest of her life as the number produced by the entire sow. In 1958, not far short of two centuries later, Anne McLaren and John Biggers decided to repeat this experiment on mice. Also working in London, but at the Royal Veterinary College, and using larger numbers of animals, they removed one ovary, either left or right, from two groups of mice and caged them continuously with a male mouse and recorded the size of all the subsequent litters of offspring born.

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The breeding records of these mice were compared with those of a group of mice left entire.

As mice live for up to two years, this was a fairly long term experiment which was the reason for my becoming involved. After the start of the experiment and well before its completion both Anne and John obtained new appointments, Anne in Edinburgh and John in Philadelphia. Needing someone in London to supervise the remainder of the experiment they asked me if I would take over. Thus started for me a very interesting and enjoyable relationship with Anne and John.

I had arrived at the Veterinary College in 1958, soon after Anne and John had been the among the first to transfer embryos grown in the test tube (actually petri dish) to the uteri of mice and get living offspring. This work foreshadowed the use of the techniques of *in vitro* fertilization and embryo transfer in humans and in the excitement of the success in women the early work on experimental animals has largely been forgotten. Anne also, in collaboration with Donald Michie, had carried out pioneering work on the transfer of blastocysts from one mouse to another, which demonstrated the important relationship between preparation of the endometrium and development of the embryo. Discussions with Anne and her colleagues had a very big influence on my subsequent scientific interests, especially my interest in reproductive ageing which stemmed from my involvement with the hemiovariectomy experiment.

The results from this experiment (Biggers, Finn and McLaren, 1962 a) showed that the mice which had been hemiovariectomised gave birth, on average, to half as many offspring as did the entire females. They supported Hunter's original experiment. However careful analysis of the data showed that the situation was quite complicated (Biggers, Finn and McLaren 1962b). As was to be expected there was considerable variation in the age at which the individual females ceased breeding, the number of litters produced by each female and the size of individual litters produced. Plotting the size of litter against age for each pair of mice produced a graph which appeared to be similar in most cases. For all females the second litter was slightly bigger than the first and this was then followed by a period in middle life when the litter size was fairly constant (we called this the plateau period). After middle age there was a period in which the size of litters produced became smaller with each succeeding litter until breeding ceased, well before the expected time of death. There was no difference in the breeding performance of those animals in which the left ovary had been removed and those in which the right was removed, so these groups were combined to give a single hemiovariectomized group. In this experiment the females were given a new male after not producing a litter for 10 weeks and then killed if no litter had been produced after a further six weeks. The uteri were fixed and analysed histologically.

The hemiovariectomised mice during their period of maximum litter size production had litters which were approximately three quarters the size of those of the controls but stopped producing litters sooner. The reduction of fifty percent in the offspring produced was due therefore to the combined effect of smaller litter size and shorter breeding life.

Several interesting facts came from this experiment. One of the most interesting was that at autopsy several of the females, although not having had a litter for several months showed the remains of previously implanted embryos in the uterus. Thus it

appeared that during their post-reproductive period the females were still producing eggs, and these were being fertilized and implanted in the uterus, but the mothers did not carry them to term. To confirm this, I performed a subsequent experiment in which entire females were caged continuously with males and their litter sizes recorded. As before the females showed a plateau period of high litter size which then started to decline. After the start of this decline I autopsied the females and examined their uteri 12 days after the previous litter (mice normally mate at the postpartum oestrus so any embryos should be about twelve days old). I found that the uteri contained a mixture of living offspring and old degenerating implantation sites. However the total number of implantations (living and degenerating) did not differ significantly from the litter sizes during the plateau period (Finn, 1962). This confirmed that it was not ovulation or fertilization which was failing and that the decline in litter size must have been caused by failure of the uterus to bring all the implanted embryos to term.

Another interesting finding was that the male appeared to be having no difficulty in successfully fertilising the eggs, in spite of his increasing age, putting the responsibility for reproductive ageing clearly on the female. To confirm this I carried out another experiment in which I housed male and female mice together and recorded the number and size of litters born. However, every six months, that is before they would have reached the period of declining litter size, I replaced the females with young ones, so that the male, as he got older, was always caged with a female at the height of her reproductive performance. Litters were produced regularly by the females and litter production did not decrease until the male with whom they were housed reached very old age, at which time he had difficulty moving around the cage, let alone mating the female (Finn, 1964). Thus we have a very interesting difference between the male and female mouse with regard to reproductive ageing. Whereas the male is able to continue breeding until well into old age, the female has an extended period of reproductive inactivity at the end of her life. This is of course well known in the human, but, as we shall see later, the reasons are not identical.

From the earlier experiments it appeared that the period of reproductive inactivity at the end of life was due to failure of the fertilized blastocysts to survive to term. This raised several possibilities.

It clearly could not be due to failure of ovulation or fertilisation but maybe there was a shortage of progesterone which caused the decline. To get some information on this point, I carried out a further experiment in which, as well as removing one ovary from a group of mice, I had another group in which I ligated one of their oviducts, thus allowing ovulation and corpora lutea formation to occur on that side but preventing the ova from reaching the uterus (Finn, 1963). This should have the effect of increasing the amount of progesterone available relative to the number of implantations. The life time litter production was compared, as before, between these two groups and a group of un-operated mice. The lifetime production of young was similar in the mice in which the oviduct had been tied as in the hemiovariectomised mice and about half that of the entire mice. However, the pattern of litter output in the tube tied mice was different to that in the hemiovariectomised group. During the plateau period the litter size was very much less in the tube tied group than in the hemiovariectomised group and about half that of the control group. However, unlike in the single ovary mice, the

declining period started about the same time as in the control group and went on for the same period. The presence of excess corpora lutea did not seem to halt the ageing process. However the presence of only a normal complement of implanting blastocysts on the non-ligated side prevented the premature start of the declining period as had happened in the hemiovariectomised animals.

In the original experiment with Anne and John, although we suspected that it was the ageing of the uterus in the middle aged mice that was causing the declining litter size, we could not get much information about the reason for the uterine failure and why it occurred prematurely in the hemiovariectomised mice. Histologically, we showed the presence of significant amounts of a brown pigment (probably lipofuscin), which was well known to appear in aged tissues, including old uteri (Warbrick, 1956, Graham, 1968). There also appeared to be increased amounts of collagen staining material in the wall of the uterus, especially when comparing the barren with the previously pregnant horn in the hemiovariectomized mice. To obtain quantitative data on this, in collaboration with colleagues at University College, London, the individual uteri from a group of postreproductive hemiovariectomised mice were chemically analysed for presence of hydroxyproline (a characteristic protein of collagen) (Finn, Fitch and Harkness, 1963). There was considerably more hydroxyproline, and thus presumably collagen, in the uteri of the old mice. Similar findings have been reported in women (Woessner, 1963), rats (Schaub, 1964) and rabbits (Maurer and Foote, 1972). However the amount of collagen in the horns which had previously been pregnant did not differ from that in the horns from the side on which the ovary had been removed. Clearly it was not the carrying of conceptuses which caused the accumulation of collagen. Nevertheless the presence of large amounts of collagen could be affecting the attainment of pregnancy.

Another possibility was that cellular changes in the endometrium which prepare the uterus for pregnancy and which are controlled by the sequential secretion of ovarian hormones might be affected by ageing of the uterus.

The uterus contains three main cell types in the endometrium which are involved in the preparation for implantation of the blastocysts: epithelial cells which line the lumen and the glands and fibroblasts in the connective tissue stroma. To prepare the uterus for pregnancy, each of these cell types has first to proliferate by mitosis and then to differentiate (Finn and Martin, 1967). These changes are controlled by the sequential secretion of hormones by the ovary (Martin and Finn, 1968, Martin, Finn and Carter, 1970). In the ovariectomized animal there is very little proliferation or differentiation in any of the uterine tissues, the uterus becomes atrophic. Administration of oestrogen alone stimulates division of the luminal and glandular epithelial cells while if progesterone is given in addition to oestrogen then division in the epithelial cells is inhibited and they differentiate whilst the stromal fibroblasts undergo mitosis.

Following the surge in mitosis the three cell types differentiate in preparation for the arrival of the blastocyst. First the luminal epithelial cells differentiate so that the trophoblast layer of the blastocyst can attach to its luminal surface. In areas of the uterus in which there is no blastocyst present the changes on the luminal surface cause opposite surfaces to adhere together, so obliterating the lumen, the so called closure reaction. Following the changes in

the luminal epithelium the endometrial glands differentiate to secrete a nutrient material for the histotrophic nutrition of the early embryo. The stromal cells however do not differentiate unless a blastocyst attaches to the wall of the uterus. This contact of the blastocyst gives a signal which causes the stromal fibroblasts to become very large and polyploid and become joined together by specialised junctions. The cells are now called decidual cells and the uterus is said to undergo the decidual cell reaction. The signal can be mimicked by placing a drop of oil into the uterine lumen (Finn and Keen, 1962).

It is possible to reproduce the proliferative and differentiative changes of early pregnancy in the uteri of ovariectomized mice by administering oestrogen and progesterone and then instilling oil into the uterus (Finn, 1965).

In a series of experiments comparing old and young mice it was shown that cellular proliferation in response to hormones was similar in old and young mice but the luminal closure reaction and the decidual cell reaction was very deficient in the older animals (Finn, 1966, Finn and Martin, 1969). These results suggest that it is failure of differentiation of the endometrium which hinders the attainment of pregnancy in old animals and not failure of mitosis. This was further supported by experiments in which a group of mice were ovariectomized at puberty and then left for 9–15 months before being tested with exogenous ovarian hormones and an artificial decidual stimulus (Pollard and Finn, 1974). Once again the results indicated that there was failure of differentiation but not of proliferation in the old animals.

This was very interesting. In the experiments described earlier, in which animals had been kept virgin, kept pseudopregnant or pregnant for nine months, the uteri must have been subjected to very different patterns of cell division during this time, but this did not affect their subsequent reproductive performance or the time at which they ceased breeding. A prominent theory to explain somatic ageing suggests that cells can only undergo a finite number of mitotic divisions (Hayflick, 1975). Our results do not support this theory.

Thus the experiments devised by Anne McLaren and John Biggers have led to some interesting questions about reproductive ageing. The finding that female mice have an extended period of reproductive inactivity at the end of their lives but males do not is interesting. There is of course a very big difference in the reproductive burden carried by the two sexes. In all multicellular animals the germ cells are dependent on somatic cells to carry out their function - propagation of the species. Both sexes are dependent on the endocrine glands, especially the pituitary and hypothalamus, to control time of delivery of gametes and on tubes to transfer the gametes to the environment, at a time appropriate for fertilization, and provide mechanisms for the care of the embryos produced. However females, especially viviparous ones, clearly have to do far more to produce living offspring.

In all vertebrates the germ cells are dependent on the somatic cells to join up with an oppositely sexed germ cell. The ageing of somatic cells must therefore put a limit on the reproductive potential of multicellular animals. As we have seen, even the male mouse has to stop breeding eventually, when his somatic organs are no longer able to deliver the sperm to the female. However, it is pertinent that in both the male and the female the controlling organs, hypothalamus and pituitary, continue functioning well into old age (as shown by the

continuation of oestrous cycles and spermatogenesis in the older animals), and are clearly not crucial in the timing of reproductive ageing in the female. It appears that of all the somatic organs which are concerned in reproduction, it is the uterus in mammals which ages first and restricts reproduction in the older female.

It is interesting that in evolutionary terms the uterus is one of the newest organs. It started off, in most non-mammalian vertebrates as a simple tube for the transit of ova. It is in fact not found in cyclostomes and first appears in fishes. From being a simple transporting tube it has gradually evolved into an organ capable of sustaining and nurturing the fertilized ovum until the embryo is capable of an independent existence. The physiological processes involved in maintaining the embryo in the uterus are quite complex and it is perhaps not surprising that they should falter in middle age. Even a small defect due to ageing might have an adverse effect on the carrying of the embryo and lead to its death and thus reduction of litter size and eventual termination of breeding. Whilst other somatic organs will at the same time be undergoing ageing changes these have to become quite advanced before the organ ceases to function completely. The hearts and kidneys of most middle aged animals are probably far from perfect but do not cause death of the animal.

It was notable from the earliest experiment, and subsequent ones, that even after she had stopped having litters the female mouse was still having cycles of ovarian activity and producing ova. It has been well shown in mice and other rodents that oestrous cycles although becoming somewhat irregular continue until near the time of death. This is, of course, quite different to the situation in women in whom ovulation and menstrual cycles discontinue well before the end of life resulting in the menopause.

There thus appears to be a fundamental difference between women and other female mammals in reproductive ageing. The reason for the menopause in women is that the ovaries become depleted of ova about two thirds of the way through life, whereas the cessation of reproductive activity in other female mammals is due to failure of the uterus, whilst the ovary still has available ova. Why should women run out of ova in middle age and not other female mammals?

All female mammals, and some, but not all, other vertebrate females, have evolved the very odd situation that before birth or very soon afterwards all the germ cells in the ovaries start undergoing meiosis. Having embarked on this course there is no going back, the cell must complete meiosis, which involves just two further divisions or die. No further mitosis of germ cells can take place. Clearly this puts a finite limit on the number of potential germ cells with which the female is born. This is in sharp contrast to the male, and some lower vertebrate females (Franchi, Mandl and Zuckerman, 1958), where the germ cells do not undergo meiosis until puberty and then not all the germ cells start meiosis at any one time, some being retained to undergo further mitosis. There is thus continual replenishment of the germ line and the production of sperm for the duration of somatic life.

However, in the non-human female mammals studied, the finite store of ova with which she is born appears to make little difference to reproductive potential. Autopsy of very old females reveals the presence of ova into very old age, although obviously the number drops as the female gets older, but the number of ova at birth is sufficient to last a mouse lifetime (Mandl and Shelton, 1959, Jones and Krohn, 1970). What possible evolutionary advantage there can

be for females to commence meiosis of all germ cells before birth is difficult to predict (discussed by Miro 1999).

However, the finite oocyte supply does have a very important effect on reproduction in women. It is the reason for the menopause. Unlike other mammals women use up all their ova by atresia and ovulation by the time they have reached middle age. The reason for this appears to be nothing to do with the ovary but to be a result of the greatly reduced somatic ageing, so that humans live much longer than would be expected.

The longevity of mammals, generally, is related to body size. Species in which the animals are small have much shorter lives than those with large animals. If a graph is drawn of body weight against longevity for various mammalian species it is, more or less, a straight line, with two major exceptions, humans and bats. The possible cause of this relationship is the mathematical relationship between body surface area and body weight. As animals get bigger the ratio of body surface area to body weight becomes less. Small animals have a much greater surface area per unit of mass. Heat is lost from an animal largely through the body surface so that smaller animals will lose more heat relative to their size than large animals and will therefore have to create more energy to keep the body temperature constant. During the breakdown of food into heat there is the production of free radical oxygen. This, it is suggested, is one of the major causes of ageing in mammals (Sohal and Allen 1985, Harman, 1992, Sohal, 1993). Free radicals cause damage to DNA and protein which accumulates as the animal ages and this accumulation is responsible for somatic breakdown with age. Why humans, or indeed bats, are exceptions to this rule is not known. It may have something to do with the fact that humans are less reliant on physiological mechanisms for the control of body temperature, although this is unlikely to be the main cause. There is also the problem of within species variation in longevity. In some species there is a very big difference between the smallest breeds and the largest. This is probably most notable in the dog. Within a species the relationship between size and longevity seems to be reversed with the small breeds living longer than the large. It is difficult to see how this can be explained on the free-radical or any other theory of ageing.

As the menopause in women has evolved as a consequence of two completely separate biological phenomena, the first common to all mammals and many other vertebrates, and the other common to all humans, male and female, it is unlikely to have evolved in response to some adaptive human need, such as providing grandmothers to assist with the rearing of offspring as has been suggested on anthropological grounds (Hawkes *et al.*, 1997).

From this short review it can be seen that the original experiments designed by Anne McLaren and John Biggers led to some interesting work on ageing and reproduction

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