CSIRO PUBLISHING

www.publish.csiro.au/journals/hras *Historical Records of Australian Science*, 2011, **22**, 291–303

Wesley Kingston Whitten 1918–2010

J. N. Shelton[A,C] *and P. J. McCullagh*[B]

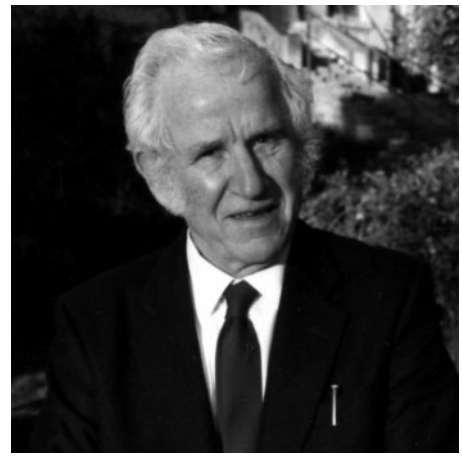
[A]1/185 Landsborough Ave, Scarborough, Qld 4020, Australia.
[B]520 Mac's Reef Rd, Bungendore, NSW 26214, Australia.
[C]Corresponding author. Email: jsh65492@bigpond.net.au

Wesley Kingston Whitten (1918–2010) was recognized as one of Australia's most innovative biological scientists. His studies were the precursor of the science of preimplantation embryology and the technology of assisted reproduction. He pioneered the study of mammalian pheromones and their receptor, the vomeronasal organ. He elucidated the genetic basis of hermaphroditism and mosaicism, and the timing and mechanism of X chromosome inactivation. Several of his recombinant mouse strains continue to provide models for a number of diseases.

Family Background and Education[1]

Wesley Kingston ('Wes') Whitten was born at Macksville on the north coast of New South Wales on 1 August 1918. He was the third of five children of Alfred Giles Whitten, a Methodist minister, and Ethel Annie Whitten (née Cock). He married Enid Elsbeth Cay Meredith ('Beth') in December 1941 and they had four children (Gregory, Mark, Jane and Penelope). After Beth's death in 1999, he married Mary Taylor, a long-standing friend and colleague. He passed away in Canberra on 24 May 2010.

Because of his father's occupation, Wes lived in a number of country areas during his upbringing and frequently holidayed at his uncle's farm at Quirindi, New South Wales. It is easy to assume that these early experiences aroused an interest in nature and biology, which brought him much enjoyment during his lifetime and possibly led to his decision to study Veterinary Science. Wes' early education was at East Maitland Boys' High School and from there he won a scholarship to the University of Sydney where he graduated BVSc in 1939 and BSc in 1941. In 1962 he was awarded a DSc by the University of Sydney for his research at the Australian National University described below.



Early Career

Wes' initial professional appointment (1940–1) was as Veterinary Science Fellow at the Walter and Eliza Hall Institute in Melbourne. Like many of his contemporaries, Wes joined the Army in 1941 and served until 1946, first as a Captain in the Veterinary Corps providing veterinary care to a large contingent of horses in the Hunter Valley in New South Wales, and later as Officer-in-Charge of the Land Head Quarters Food Laboratory.

On discharge from the Army, Wes joined the Division of Animal Health of the Australian Government's Council for Scientific and Industrial Research (CSIR) to work on the effect of nutrition on fertility of sheep. At the time, Australia did ride on the sheep's back economically and it was becoming evident that ewes grazing highly nutritious clover-dominant pastures were showing significantly reduced fertility. Wes studied the effect of oestrogens on the food intake of

[1]The photograph is of Wes Whitten at Indiana University in 1984.

 10.1071/HR11009 0727-3061/11/020291

sheep (8) and also the possibility that inactivation of gonadotrophin may play a role in reduced fertility of sheep grazing clover-dominant pastures. This problem was eventually solved and it was discovered that the strain of clover that had come to Australia, initially as packing material, synthesized a variety of phytooestrogens. Wes later lamented (90) 'we missed the opportunity to identify and market that first oral phyto-contraceptive'.

During his time with the Division of Animal Health, 1946–9, Wes maintained a colony of mice in a small balcony room on the fifth floor of the University of Sydney's Medical School. Here he conducted a number of studies on the inactivation and modification of gonadotrophins by enzymes, viruses and periodate ions (6, 7, 9, 10, 13, 14, 15). This would have been seen as a possible tool in the control of fertility in animal and human populations.

John Curtin School of Medical Research and the Culture of Embryos

The establishment of a new and different university—the Australian National University (ANU) in Canberra—to provide opportunities for undertaking long-term research of the highest internationally recognized standard—provided the circumstances for Wes Whitten to begin an outstanding career in reproductive biology and behaviour. In 1949 Wes was appointed to set up an Animal Breeding Establishment (ABE) at the John Curtin School of Medical Research (JCSMR) within ANU. In 1950–1 he studied facilities for laboratory animals in Europe and the USA and on his return played a major role in organizing the construction of a building for the ABE capable of providing large numbers of rabbits, guinea pigs, rats and mice to the School.

In addition to his role in managing the breeding and maintenance of animals for researchers in JCSMR Wes was funded to carry out cognate research. The large mouse breeding colonies in the ABE provided a great opportunity to his enquiring mind to investigate the culture of embryos. The finding early in the twentieth century that tissues could be maintained in nutrient-rich aqueous solution led to attempts to grow embryos in such solutions composed mainly of fluids of biological origin. Biggers (1987) and Nielsen and Ali (2010) summarized the numerous attempts to culture embryos in the first half of the twentieth century and noted that none of these experiments were successful in obtaining continuous growth of embryos. Attempts to use artificial media were also unsuccessful (Waterman 1934).

Rock and Menkin (1944) achieved very limited success with human oocytes using Locke's solution and serum while Hammond (1949) also had some success with 8-cell mouse embryos using physiological saline supplemented with hen-egg white and yolk. Thus there was at this time no method of culturing mammalian embryos *in vitro* that was reliable enough to allow the systematic study of preimplantation embryos. Working in the John Curtin School of Medical Research, Wes used a modified Ringer's bicarbonate solution to culture mouse embryos from the 8-cell stage to the blastocyst stage. This was the first demonstration of a reliable medium for the culture of mammalian embryos (18, 22). It had been assumed by earlier workers that the reason for the failure of embryos to survive and develop outside the maternal environment was that the Fallopian tubes provided some intrinsic factor that was essential for the welfare of embryos. Whitten's observations and experiments led him to conclude that in addition to appropriate osmolarity of the medium, essential for the growth of embryos were purity of water, a source of carbon dioxide and an appropriate carbohydrate source of energy. This was long prior to the ready availability in laboratories of apparatus to provide water purified by multiple distillation and reverse osmosis.

Wes used water from the Molonglo River that flows through Canberra and that had none of the added chlorine common in most city water supplies. It was supposed that embryos were anaerobic, an hypothesis that Wes tested by the addition to the medium of lactate, which the embryos would not be able to metabolize if they were anaerobic. In Wes' words (90), he found that 'they love the stuff '. He went on to say: 'I was awed to be the first to witness the hatching of mammalian embryos *in vitro*'. In 1956 he published a note in *Nature* (18) describing the first chemically-defined medium for the culture of embryos, which became known as Whitten's medium. He published a full paper in *Nature* in 1957 (22) and further updated the composition and preparation of the medium in 1971 (41).

Whitten (22) noted that mouse morulae degenerated when cultured in an atmosphere of 5% carbon dioxide and 95% oxygen but normal development occurred in 5% carbon dioxide in air. Subsequently, use of 5% carbon dioxide in air or with 5% oxygen and 90% nitrogen became standard procedure for embryo culture. Many years later, with Ali and Shelton, Whitten (88) reported that the two systems are equally effective in generating quality embryos. Whitten's papers (18, 22, 41) have been cited many hundreds of times. The formula for Whitten's medium has been used as a basis for many of the media formulated in the following years and used in embryo laboratories and Assisted Reproductive Technologies around the world.

Whitten's work and subsequent refinements permitted a massive upsurge in research into the development of preimplantation mammalian embryos. Despite the success of Whitten's medium, it was significant that Wes' experiments utilized multi-cellular embryos. Zygotes did not develop beyond the 2-cell stage. This became known as the 2-cell block and the cause of this phenomenon has been the subject of much research and conjecture. Whitten in 1957 (22) had shown that late 2-cell embryos developed into blastocysts if lactate was added to the medium but earlier stages did not cleave under these conditions. Whitten and Biggers (36) demonstrated that the 2-cell block is strain-dependent and that hybrid zygotes produced a large proportion of blastocysts whereas many inbred strains did very poorly. These findings supported evidence (Biggers, Whittingham and Donahue 1967) that the cleaving zygote requires specific exogenous sources of energy at different stages of development and these may be supplied by secretions of the Fallopian tubes. The reason(s) for the 2-cell block have not been fully elucidated although many mechanisms have been incriminated. Biggers (1998) in reviewing the culture of preimplantation embryos concluded that 'even the best available media inevitably cause imbalances in the environment in which the embryos are forced to develop because they consist of only a small subset of the compounds present in the natural environments.'

After his move to the Jackson Laboratory in Maine in the USA, Wes continued to report major findings in the fertilization of oocytes, the culture of embryos and the cryopreservation of embryos. To facilitate advancement of assisted reproductive technologies in all mammalian species, it seemed obvious that it was necessary to know and understand the optimal conditions required for the fertilization of oocytes *in vitro*. Hoppe and Whitten (58) found that preincubation of sperm for three hours before mixing with ova increased the number of ova fertilized and concluded that sperm maturation occurs *in vitro*. They went on to establish the level of albumin needed in the medium to achieve optimal fertilization rates of mouse oocytes (59) and suggested that this might be affected by the concentration of oxygen in the gas phase of the culture. Whilst they speculated on the possible function of albumin during fertilization, they admitted that attempts to achieve fertilization *in vitro* using a chemically defined medium had not yet been successful.

The necessity of protein in the *in vitro* culture of mammalian embryos was the subject of considerable conjecture and experimentation in many laboratories and became more urgent following the development of human ART and the realization that important viral and prion diseases (eg. HIV, CJD and vCJD) may be transmitted by the use of blood products in the processes of *in vitro* fertilization and embryo culture. In Whitten's original medium, glycine was the only source of fixed nitrogen. Bunim (1960), also at the Jackson Laboratory, suggested that the function of glycine was not for protein synthesis but to chelate ions of copper or zinc that might have contaminated the medium.

Following the demonstration by Brinster (1967) that the embryo draws heavily on its own stores of protein during the first three days of development, Cholewa and Whitten (40) in 1970 substituted polyvinylpyrrolidone, a high-molecular-weight colloid, for albumin in the culture medium. They obtained good growth of outbred 2-cell mouse embryos and claimed that there was no absolute requirement for exogenous amino acids in the culture of embryos at this stage. In 1971, Wes published with Biggers and Whittingham (46) a complete review of the culture of mouse embryos *in vitro*. This established a basis for the consolidation and development of mammalian embryo culture systems world-wide.

Despite the realization of the risks, most commercially available media for *in vitro* fertilization

and culture of human embryos continued to contain serum albumin. However Wes' influence inspired a younger researcher at ANU, Jaffar Ali, to examine thoroughly the requirements for amino acids and other nutrients in media used for the whole gamut of *in vitro* fertilization and culture. This resulted in the production of a protein-free medium (Ali, Shahata and Al-Natsha 2000).

Wes Whitten made an enormous contribution to the science of embryology and to the technology of all aspects of human assisted reproduction. Ironically, his major motivation was the possibility of using scientific knowledge to control the burgeoning world population, which he saw as a looming problem. He recorded that while he was still at JCSMR he was offered support from the World Population Council but found that neither JCSMR nor any other Australian institution was prepared to act as host for such a grant.

Cryopreservation of Embryos

Following the successful cryopreservation of spermatozoa (Polge, Smith and Parkes 1949) major impetus was given to the objective of embryo cryopreservation. Wes and his colleagues saw this technology as an important adjunct to cryopreservation of sperm in the preservation of genetic material, since it provided the opportunity to preserve the complete genome rather than half the genome as in the cryopreservation of sperm. However the cryopreservation of multi-cellular embryos presented problems not encountered with single-cell spermatozoa. Following a closer examination of the cryobiological factors that influence survival (suspending medium, cryoprotective agents, cooling rate, final storage temperature and warming rate) Whittingham, Leibo and Mazur (1972) reported that full-term foetuses and live young were obtained from 1-cell, 2-cell and 8-cell embryos stored at −196°C for up to eight days. Whittingham and Whitten (57) realized the importance of this development in the transport of genetic material and in 1974 obtained live young from 8-cell embryos stored at −196°C for eight months and transported from the USA for transfer to recipients in England. This report initiated a great deal of international interest in the cryopreservation of mammalian embryos, particularly farm animals and humans. Subsequently, cryopreservation procedures, cryoprotectants, cooling and warming regimes, slow-freezing and vitrification have been the subject of many hundreds of reports, and embryo storage and transport both locally and internationally have become commonplace.

Delayed Implantation

The JCSMR animal house also provided the opportunity for the observant scientist to study aspects of reproduction in mice and to contribute to the endocrinology of mammalian reproduction. The mouse is one of about a hundred species of mammal that exhibit delayed implantation during lactation. The female exhibits fertile oestrus soon after birth of a litter but the embryos do not implant in the endometrium until lactation wanes. As a result, the gestation is prolonged from a normal of around 21 days to about 40 days. Whitten conducted a series of elegant experiments (17, 25) in 1955 and 1958 to show that the absence of implantation was due to reduced secretion of oestrogen from the ovaries, which in turn was caused by reduced secretion of gonadotrophin by the pituitary. These conclusions were reached without the assistance of modern-day techniques of assay of steroid and pituitary hormones but by using a sound knowledge of the physiological effects of reproductive hormones.

Pheromones

Another of Wes Whitten's major scientific interests was animal behaviour, particularly reproductive behaviour. The circumstances leading to Wes' interest in pheromones, which generated a series of reports over a decade, provide an excellent illustration of Pasteur's aphorism that chance favours the prepared mind. The Whitten mind was very well prepared, something that was to be demonstrated repeatedly throughout his subsequent career. His service responsibilities to the John Curtin School required him to provide 1,000 newborn mice each week for use in virus isolation by the research group of Frank Fenner, Ian Marshall and Gwen Woodroofe. Their research necessitated provision of these newborns at a steady rate. In search of an explanation for the major obstacle to accomplishing this, namely the availability of an excess of newborn

mice on one day with inadequate numbers on following days, Wes launched into an investigation that was to define the influence of pheromones on reproduction.

Whitten's initial observation that the sexual cycles of female mice vary with the conditions under which they are caged was reported in 1956 (19). He observed that mating of mice does not occur with equal frequency during the first four nights after pairing, with the number of matings on the third night being much greater than expected. If the male was in a separate basket within the females cage for two days prior to pairing, there was more frequent mating on the first night. He defined the duration of the male effect and the stage of the female cycle at which the male effect operated (26). When female mice were caged in groups they became anoestrous (27) and when exposed to a male they resumed normal sexual cycles. He concluded that the oestrous cycle of the mouse is modified by the presence of a male or his excreta, and that an exteroceptive stimulus functioning through one of the chemical senses was probably involved.

When he removed the olfactory bulbs of mice, he found that the ovaries and uteri of mature female mice were significantly smaller than those of control animals, but there was no effect on the testes of mature males (20). This was clear evidence that the olfactory sense is involved in the effect of the male on the female. This effect of the male is independent of visual, auditory or tactile stimulation and was postulated as being produced by an olfactory-mediated pheromone (27, 33).

In 1963 Wes presented a paper suggesting that the vomeronasal organ is a sex receptor in mice (30). On moving to the Jackson Laboratory in Maine, USA, in 1966 he continued his interest in the role of exteroceptive factors in animal behaviour and, utilizing a bioassay he devised with Wilson and Beamer (78), he and colleagues produced conclusive evidence that the vomeronasal organ is a receptor for the prime pheromone produced by male mice (79). With Bronson, (34) he showed that the pheromone is present in the urine of males and androgenized spayed females but not in the urine from castrate males; and, using the bioassay to examine the pattern of response to serial, 10-fold dilutions of male urine, he found a dose response with maximum response at 10^{-2} dilution and no response at dilutions greater than 10^{-3} (78). They also showed that it is transported by movement of air (35) suggesting that the pheromone is volatile.

Another exteroception–mediated effect of the male on reproduction in mice is the Bruce Effect (Bruce 1959, 1960), whereby pregnancy is blocked in mated females when exposed to strange males. This effect is mediated via the olfactory sense and the responsible pheromone is in male urine and dependent on androgens. Whitten with Chapman and Desjardins (39) showed that the effect was associated with a decrease in pituitary luteinizing hormone in recently-mated females. This was confirmed when Whitten with Hoppe (50) demonstrated that pregnancy block could be initiated by administration of gonadotrophin.

Whitten's original observations in the animal house in Canberra, reported in 1956 (19, 20), aroused a great deal of scientific interest in the role of pheromones in mammalian behaviour and the sensitivity of the vomeronasal organ to chemical stimuli (30). Subsequently Whitten published a number of reviews of the role of mammalian pheromones and olfaction in reproduction (33, 37, 38, 54, 62, 64). Many constituents of male mouse urine have been identified and some have been synthesised. With colleagues, Whitten identified dihydrothiazoles in the urine of male mice (65) and subsequently Jemiolo, Harvey and Novotny (1986) synthesised two of the volatile constituents of male mouse urine and showed their ability to induce the Whitten effect in female mice. In addition to the effects of male mouse urine pheromones on the oestrous cycle and pregnancy block in females, it is now clear that pheromones are important in communicating not only reproductive readiness but also social status and recognition. It is also clear that many factors including background genetic variation and interaction with MHC genes (55) influence the concentration of mouse pheromones.

Whitten and colleagues also showed that the genetic background of female mice influences mating patterns and postulated a dominant mode of inheritance for the third-night mating tendency and a recessive mode of inheritance for the first-night tendency (77). Volatile compounds and non-volatile peptides have been

suggested as the pheromones responsible for the chemosignals between individual mice (Brennan 2004). In humans, information received from the nose is rarely of great significance because most of our olfactory receptor genes are non-functional pseudogenes. However, rodents not only have a vast repertoire of olfactory genes, they also possess two additional sets of receptors in the vomeronasal organ that is specialized for pheromone reception (Keverne 2000).

Studies of the vomeronasal organ show that it is a chemoreceptive organ that is thought to transduce pheromones into electrical responses that regulate sexual, hormonal and reproductive function in mammals. The threshold of detection by neurons of the vomeronasal organ for some chemicals is said to be near 10^{-11} M, which is extremely low (Leinders-Zufall, Lane, Puche, Ma, Novotny, Shipley and Zufall 2000).

At the Jackson Laboratory, Whitten recognised the opportunity to investigate the role of pheromones in marking behaviour in another species, the red fox (*Vulpes vulpes* L.), found in north-eastern Maine. With colleagues at the Jackson Laboratory and Indiana University, Wes identified eight odorous volatile compounds from frozen samples of red fox urine recovered from areas marked by foxes during winter (68). One of these was Δ^3-isopentenyl methyl sulphide, a major compound in the urine and previously unknown (69). They set up a test situation in the snow of a Maine winter to examine the effect of a solution containing the eight synthetic volatile compounds on the marking activity of wild red foxes (74).

It had been observed that both male and female foxes, when travelling, may mark with urine small raised objects every 30–40 metres. Following significant snowfall, Whitten and colleagues made small mounds of snow in two areas frequented by foxes and placed test solutions of the compounds on half the mounds. From the number of treated mounds marked by the foxes they concluded that one or more of the volatile compounds induced marking behaviour in the foxes. This was evidence of a pheromone influencing behaviour in a wild species. It is evident that Whitten's observations in the animal house in Canberra (19, 20, 23, 26, 27) played a major progenitive role in the science of chemosensory communication.

The Jackson Laboratory and X Chromosome Inactivation

In 1960–1, Whitten spent a year, by invitation, at the Jackson Laboratory in Bar Harbor, Maine, and he later remarked that he was greatly influenced by the attitude of the research scientists there to the use of genetics as an additional dimension in experimental biology. He added that he was unsuccessful in his attempt to apply it to the problem of reproductive pheromones. On his return to JCSMR he became dissatisfied with his role, which was increasingly interpreted by the School administration as being to ensure a supply of experimental animals rather than to pursue 'cognate research'. In retrospect, failure to appreciate the quality and originality of his research probably reflected the unfamiliarity of senior members of the School with reproductive biology. Recognising this situation, Wes resigned to become Assistant Director (Endocrine Products) at the National Biological Standards Laboratory, where he concentrated on improving the standard of endocrine products on sale in Australia. Having failed to give up the idea of doing research, he resigned from NBSL in 1966 and joined the Jackson Laboratory. He recorded that the next fourteen years were the most exciting of his scientific career. He had adequate funds from NIH, excellent animal facilities, a good crew and a flow of graduate students. He also had access to the breeding records of the Jackson, which breeds several million mice per year. The Jackson Laboratory was founded by C. C. Little in 1929 and has long been the Mecca for mouse genetics. The laboratory mouse plays a key role in mammalian genetics and biomedical research because it is strikingly similar to humans in physiology, anatomy and genetics. Research in mouse genetics is applicable to humans because over 95% of the mouse genome is similar to that of humans, while the development of inbred lines makes the mouse a powerful tool in identifying the genetic basis of both normal and disease traits. The discovery of polymorphic genes enabled rapid genetic mapping and in the 1960s and 1970s biochemical genetic marker systems based on isoenzymes were developed. These markers proved very useful in studying the development and activation of the embryonic genome. Much of this innovation occurred at the Jackson Laboratory and Whitten

found this an immensely stimulating environment. With Chapman and Ruddle (44) he used the electrophoretic variants of glucose phosphate isomerase to study the time of paternal gene activation in hybrid mouse embryos. His results, using the timing of the presence of the sire's variant of glucose phosphate isomerase, indicated that the embryonic genome is functional at Day 5 of the preimplantation embryo. His interest in embryonic development led him to investigate the time of X chromosome inactivation in the mouse embryo (49, 51, 53).

X chromosome inactivation is the mechanism used in mammals for dosage compensation of X-linked genes between chromosomally XX females and XY males. One of the two X chromosomes in females becomes transcriptionally inactive in every cell of the early embryo and remains so in all somatic cells throughout life. Studies of X chromosome inactivation have led to an understanding of a number of sex linked genetic diseases. The X inactivation process in female placental mammals is random in affecting one or other of the X chromosomes and like all random events can be skewed. This skewing can result in disease when the predominant active X chromosome carries a mutant form of an important gene so that there is inadequate production of a protein. Diseases shown to be caused by skewed X chromosome inactivation include Duchenne muscular dystrophy, haemophilia, and a form of mental retardation. Thus understanding of the time and mechanism of X inactivation is important. Whitten and Deol (49, 53) examined the possibility that X inactivation did not occur at the same time in all tissues by comparing clone numbers in the retinal epithelium of mosaic and chimeric mice at 12 days *post coitum*. Their results were later considered to be inconclusive (Nesbitt 1974), but their work stimulated further investigations into the timing and mechanism of X inactivation and the role of genes on the X chromosome in genetic diseases. During his studies of the early development of mouse embryos, Whitten noticed that the rate of development seemed to vary between different genotypes, and subsequently with Shire (80, 81) he examined the time of first cleavage for embryos sired by eight genotypes of males and from eleven genotypes of females. They concluded that both paternal and maternal genotype have important effects on the timing of first cleavage and that this be kept in mind when comparing data from matings involving different genotypes.

Sex Determination and Y Chromosome Non-disjunction

Wes Whitten realised the potential of the many mouse strains and the knowledge of their genetic background at the Jackson Laboratory for the study of sex determination and gonadal development. This was particularly so with the BALB/cWt strain of mice, which showed a particularly high incidence (3%) of true hermaphroditism among day-15 foetuses (66). With colleagues he showed that the hermaphrodites were chromosomal mosaics (i.e. XO/XY or XO/XY/XYY) and that the cause of the hermaphroditism was Y chromosome nondisjunction (66, 76). In a subsequent series of observations he found that by weaning age the percentage of hermaphrodites had declined to 0.4%. He suggested that subsequent to Day 15 of foetal life, some of the gonadal abnormalities are sequestered with development towards the male phenotype more common, and that the proportions of the male and female elements determine whether a particular foetus will continue to develop as a hermaphrodite. His studies included a detailed morphological description of the gonads in hermaphrodites (71, 72, 83), and he postulated that when the proportion of XY cells in a sex mosaic is greater than one-third, the individual will develop into a male phenotype (72). Observations on these mice were consistent also with the earlier view (45) that most sex chimeras (XX↔XY) develop as phenotypic males. He suggested that H-Y antigen may have a role in protecting germ cells from a meiosis-inducing substance and speculated that his observations might support the conclusion of Evans, Ford and Lyon (1977) that 'the sex of the germ cell is not an autonomous property, but is determined by the nature of the gonad in which it finds itself'. Whitten's studies of the BALB/cWt mice and their propensity to produce hermaphrodites led him to conclude that they were good subjects for studies on the development of gonads, accessory structures and endocrine function, and that they provide a model for chromosome nondisjunction and perhaps also for clone selection. There are many human conditions

that are caused by nondisjunction of the sex chromosomes.

Another sub-strain of BALB/c mice (BALB/cBm) was the subject of intensive study by Whitten, Carter and Beamer because sex-reversing non-disjunction of the Y chromosome in this strain produced a low sex ratio and hermaphrodites in the progeny of male BALB/cBm mice (86). They concluded that the Y chromosome of these mice is unable to interact normally with the mitotic spindle of some genotypes, and that it was likely that the primary non-disjunction occurs at first cleavage. They suggested that the high rate of non-disjunction that occurs in some matings may provide a model for Turner's syndrome and hermaphroditism in humans.

Spontaneous Ovarian Tumors – Potential as a Model

Whitten worked with a number of colleagues at the Jackson Laboratory and these collaborative projects continued after he left the laboratory in 1978. After a visit to the laboratory in 1993, Wes remarked that the recombinant strains that he had helped set up were paying off, particularly strains from the hybrid in which he found granulosa cell tumours. With Beamer and Hoppe (84) he had investigated these tumours in young SWR mice and SWR-derived inbred mice and they developed a working hypothesis that the occurrence of tumours in SWR and SWR-derived mice results from the interaction of a unique SWR factor, perhaps cytoplasmic, with nuclear genomic material common to Swiss mouse stocks.

Beamer and his colleagues (1993, 1998) continued these investigations and located susceptibility genes at four loci in SWR-derived inbred mice and proposed that androgens synthesized in response to normal gonadotrophic stimulation initiate tumourigenesis in the genetically susceptible ovary. They also observed a similarity of the tumours to the ovarian granulosa cell tumours that occur in young girls. These recombinant strains set up by Whitten and colleagues assumed even more significance when a report was published by Willemsen *et al.* (1993) suggesting a causal relationship between ovarian stimulation with gonadotrophin and granulosa cell tumours in twelve women. Although an increased incidence of ovarian granulosa cell tumours has been observed in women that have had ovarian stimulation for IVF, it appears that possible genetic susceptibility of some women to ovarian tumourigenesis following gonadotrophic stimulation has not been thoroughly investigated.

Return to Australia

In 1978 a newly appointed Director of the Jackson Laboratory initiated a change in research direction from genetics to tumour immunology and, to enable the recruitment of new staff, Wes was offered early retirement which he accepted and returned to Australia. A few years later, Wes remarked that with a subsequent change of director, 'the Jackson is back on course'. After returning to Australia, Wes resided for a few years in Tasmania where he was an Honorary Research Associate in the Department of Zoology at the University of Tasmania and bred cattle and sheep on a small hobby property. In 1980 he moved to Canberra, where he held honorary positions in the John Curtin School, CSIRO Wildlife and Ecology, and the Cooperative Research Centre for Biological Control of Vertebrate Pest Populations.

Wes was often in my (JNS) laboratory at JCSMR—sometimes after several kilometres in the swimming pool—and in addition to promoting interesting discussions on a wide variety of subjects, he provided valuable advice to research scholars including Andras Szell and Jaffar Ali who were conducting doctoral research on embryo culture and cryopreservation.

Whitten had a wide curiosity and an interest in nature that not only motivated his original research projects but also lured him into numerous less formal investigations. One of these was to investigate with a colleague from CSIRO Wildlife whether the cue to nest-building by the female magpie was provided by the male's display. When the display was delayed by an implant of oestrogen, the males began to build nests within 48 hours of the implant. We still do not know the importance or otherwise of the male's display.

Wes also spent a period at Macquarie Island studying reproduction in southern elephant seals. No doubt it was the same intense interest in nature that inspired him to visit Alaska and the Galapagos Islands late in his life.

Parallelling his aptitude for discerning associations between apparently disparate

observations, Wes maintained a sensitivity for the wider implications of his subjects of scientific interest. His scientific achievements equipped him very adequately to become involved in arguments about issues which, while outside the laboratory, could be explicated using the skills he had acquired during four decades in laboratories. He was uniquely qualified to provide an international perspective on Australian reproductive physiological issues, uninfluenced by domestic biases and conflicting interests. Such a qualification was based on a close familiarity with the Australian scene in the 1950s and 1960s, on which an international perspective had been superimposed over the following decades. Three episodes during the 1990s serve to illustrate this point. All three involved conflict with local medical clinicians. In all three, Wes was ultimately unsuccessful.

In 1994, the report of an inquiry commissioned by the Australian Minister for Human Resources and Health dealing with 'the use of pituitary derived hormones in Australia and Creutzfeldt-Jakob disease' was published (Allars 1994). This inquiry examined a programme initiated in the early 1960s to supply human gonadotrophin and growth hormone, prepared from cadaveric pituitary glands, to a small group of clinicians with the objective of treating patients affected by infertility and growth retardation respectively. While not recognized at the time, and only grudgingly acknowledged by the time of the inquiry, the programme constituted little more than an experiment undertaken, notwithstanding available evidence of potential risks, on uninformed and unconsenting subjects. Following release of the report, Wes and several of his contemporaries who had been associated with CSIRO or the National Biological Standards Laboratory in the 1960s went in to bat on behalf of those subjects. Wes was fully conversant with the state of scientific knowledge before the commencement of the programme—knowledge that had persuaded the American authorities not to administer material from cadaveric sources to patients. He and his colleagues drew attention to warnings expressed before the programme commenced as well as multiple glaring deficiencies in the application of protocols for pituitary gland collection at autopsy. The issue that motivated Wes in the 1990s was the refusal of the Australian Government to consider compensation for individuals whose lives, and in some cases sanity, had been affected by uncertainty about them possibly developing vCJD. Wes and his colleagues participated in a parliamentary inquiry as well as in legal actions against the Government that sought to rectify this, but without success. An attempt to have his scientific assessment of what had been known about the potential dangers of therapeutic use of cadaveric human pituitary extracts at the time the programme began accepted for publication in a journal was also unsuccessful.

Another cause to which Wes was committed in the 1990s was that of the 'Tall Girls'. This group of women had been submitted to early induction of menarche in order to curtail their growth. As Wes succinctly expressed it, there had been concern that, unless their growth in height was checked, they would be unable to find suitable partners as debutantes. The group of women who had been treated as teenagers suspected that the treatment had reduced their subsequent fertility. When agitation for an examination of their concerns was in full swing, Wes arrived at the laboratory one day to announce proudly that he had been appointed as an honorary Tall Girl. A scientific investigation subsequently failed to support the existence of a link between earlier treatment and infertility.

Following the production of Dolly the sheep in the late 1990s, Wes was co-opted to a working party preparing advice to the Australian Health Minister on cloning. He brought a much-needed international perspective to the subject. On reading the reference list that had been compiled to accompany the draft scientific chapter, he announced that there was only one Australian co-author among the forty listed papers, adding as an afterthought that this co-author had been born in Kenya and had undertaken the published research in Wisconsin. This observation was rather at variance with the assessment by the Australian media of the Australian contribution to the science. Wes' suggestion, incorporated in the draft advice to the Minister, was for the establishment of a national primate centre similar to the ones with which he was familiar in the USA. This was rejected by the Australian practitioners who saw no need for a non-human primate experimental animal, and was relegated to an appendix in the revised document.

Awards and Affiliations

Wes was a member of many scientific associations including:

American Association for Advancement of Science
Society of Endocrinology
Society for the Study of Fertility
Society for the Study of Reproduction
Society for Developmental Biology

Wes was elected to the Australian Academy of Science in 1982. He was awarded the Marshall Medal of the Society for the Study of Fertility in 1993 for his outstanding contribution to the study of fertility and reproduction, and in 1996 he was presented with the Pioneer Award of the International Society for Embryo Transfer.

In 2001 Wes was awarded a DSc (*honoris causa*) by the Memorial University of Newfoundland.

In 2009 the Australian National University named its new animal breeding building the Wes Whitten Building.

References

Ali, J., Al-Natsha, S. D., and Shahata, M. A. M. (2000). Formulation of a protein-free medium for human assisted reproduction. *Hum.Reprod.* **15**, 145–156.

Allars, M. (1994). Report of the inquiry into the use of pituitary derived hormones in Australia and Creutzfeldt-Jakob Disease. Australian Government Publishing Service.

Beamer, W. G., Shultz, K. L., Tennent, B. J., and Shultz, L. D. (1993). Granulosa cell tumorigenesis in genetically hypogonadal-immunodeficient mice grafted with ovaries from tumor-susceptible donors. *Cancer Res*. **53**, 3741–3746.

Beamer, W. G., Shultz, K.L., Tennent, B .J., Nadeau, J. H., Churchill, J. A., and Eicher, E. M. (1998). Multigenic and imprinting control of ovarian granulosa cell tumorigenesis in mice. *Cancer Res*. **58**, 3694–3699.

Biggers, J. D. (1987). Pioneering mammalian embryo culture. In: *The Mammalian Preimplantation Embryo.* (Ed. B. D. Bavister) pp. 1–22; Plenum Press: New York, USA.

Biggers, J. D. (1998). Reflections on the culture of the preimplantation embryo. *Int. J. Dev. Biol.* **42**, 879–884.

Biggers, J. D., Whittingham, D. G., and Donahue, R. P. (1967). The pattern of energy metabolism in the mouse oocyte and zygote. *Proc. Natl. Acad. Sci. USA* **58**, 560–567.

Brennan, P. A. (2004). The nose knows who's who: chemosensory individuality and mate recognition in mice. *Horm. Behav*. **46**, 231–240.

Brinster, R. L. (1967). Protein content of the mouse embryo during the first five days of development. *J. Reprod. Fertil.* **13**, 413–420.

Bruce, H. M. (1959). An exteroceptve block to pregnancy in the mouse. *Nature (London)*. **184**, 105.

Bruce, H. M. (1960). A block to pregnancy in the mouse caused by proximity of strange males. *J. Reprod. Fertil*. **1**, 96–103.

Bunim, L. (1960). *In vitro* development of mouse blastulae in Versene-Ringer solution. In: *Summer Student Report, Jackson Laboratory*.

Evans, E. P., Ford, C. E., and Lyon, M., F. (1977). Direct evidence of the capacity of the XY germ cell in the mouse to become an oocyte. *Nature (London*). **267**, 430–431.

Hammond, J., Jr. (1949) Recovery and culture of tubal mouse ova. *Nature (London).* **163**, 28–29.

Jemiolo, B., Harvey, S., and Novotny, M. (1986). Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. *Proc. Natl. Acad. Sci. USA* **28**, 4576–4579.

Keverne, E. B. (2000). Neuroendocrinology briefings. 11: Pheromones and reproduction. *J. Neuroendocrinol*. **12**, 1045–1046.

Leinders-Zufall, T., Lane, A. P., Puche, A. C., Ma, W., Novotny, M. V., Shipley, M. T., and Zufall, F. (2000). Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature (London).* **405**, 792–796.

Nesbitt, M. N. (1974). Chimeras vs inactivation mosaics: Significance of differences in pigment distribution. *Dev. Biol*. **38**, 202–207.

Nielsen, H. I., and Ali, J. (2010). Embryo culture media, culture techniques and embryo selection. A tribute to Wesley Kingston Whitten. *J. Reprod. Stem Cell Biotechnol.* **1**, 1–29. Available from http://www.ivf-hub.net/images/journals/June2010/ecm_tribute.pdf [accessed 2 January 2011]

Polge, C., Smith, A. U., and Parkes, A. S. (1949). Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature (London)* **164**, 666.

Rock, J., and Menkin, M. F. (1944). *In vitro* fertilization and cleavage of human ovarian eggs. *Science* **100**, 105–107.

Waterman, A. J. (1934). Survival of young rabbit embryos on artificial media. *Proc. Natl. Acad. Sci. USA.* **20**, 145–146.

Whittingham, D. G., Leibo, S. P., and Mazur, P. (1972). Survival of mouse embryos frozen to −196°C and −269°C. *Science* **187**, 411–414.

Willemsen, W., Kruitwagen, R., Bastiaans, B., Hanselaar, T., and Rolland, R. (1993). Ovarian stimulation and granulosa-cell tumour. *Lancet* **341**, 986–988.

Bibliography

1. Bolliger, A., and Whitten, W. K. (1940). Observations on the urine of *Trichosurus vulpecula*. *Aust. J. Sci.* **2**, 178.
2. Whitten, W. K. (1943). Some observations on the excretion of 17-ketosteroids and oestrogens in the urine of sheep. *Aust. J. Exp. Biol. Med.* **21**, 187.
3. Whitten, W. K. (1944). Treatment of corneal ulcers by tincston. *Aust. Vet. J.* **20**, 344–345.
4. Whitten, W. K. (1945). The cyanogenetic properties of linseed nuts. *Aust. Vet. J.* **21**, 79–80.
5. Carne, H. R., Wicham, N., Whitten, W. K., and Lockley, R. P. (1946). Infection of man by the virus of contagious pustular dermatitis of sheep. *Aust. J. Sci.* **9**, 73.
6. Whitten, W. K. (1947). Enzymatic inactivation of mare serum gonadotrophin. *Aust. J. Sci.* **10**, 83.
7. Whitten, W. K. (1947). Antigonadotrophic action of human saliva *in vivo. Aust. J. Sci.* **10**, 49–51.
8. Austin, C. R. Whitten W. K., Franklin, M. C., and Reid, R. L. (1947). The effect of hexoestrol on the food intake of sheep. *Aust. J. Exp. Biol. Med. Sci.* **25**, 343–346.
9. Whitten, W. K. (1948). Inactivation of serum gonadotrophin by influenza virus and receptor destroying enzyme of *vibrio cholera. Aust. J. Sci. Res.* B. **1**, 271.
10. Whitten, W. K. (1948). Inactivation of pituitary and chorionic gonadotrophin by influenza virus and R.D.E. *Aust. J. Sci. Res.* B. **1**, 388.
11. Bolliger, A., and Whitten, W. K. (1948). The paracloacal glands of *Trichosurus vulpecula*. *J. Proc. Royal Soc.* NSW LXXXII, 36.
12. Ferguson, K. A., and Whitten, W. K. (1948). The occurrence of identical twins in sheep. *Aust. Vet. J.* **24**, 156.
13. Whitten, W. K. (1949). Enzymatic inactivation of serum gonadotrophin. *Nature (London)* **63**, 534.
14. Whitten, W. K. (1950). Inactivation and modification of serum gonadotrophin by periodate ions. *Aust. J. Sci. Res.* B. **3**, 346.
15. Whitten, W. K. (1953). Inactivation of gonadotrophins IV. The effect of periodate ions and blood-group enzyme on the biological activity of gonadotrophin. *Aust. J. Biol. Sci.* **6**, 300–304.
16. Wright, F. B., and Whitten, W. K. (1953). Factors affecting adrenaline pulmonary oedema. *J. Pathol. Bacteriol.* **66**, 63–77.
17. Whitten, W. K. (1955). Endocrine studies on delayed implantation in lactating mice. *J Endocr.* **13**, 1–6.
18. Whitten, W. K. (1956). Culture of tubal mouse ova. *Nature (London)* **177**, 96.
19. Whitten, W. K. (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J. Endocr.* **13**, 399–404.
20. Whitten, W. K. (1956). The effect of removal of the olfactory bulbs on the gonads of mice. *J. Endocr.* **14**, 160–163.
21. Whitten, W. K. (1956). Physiological control of population growth. *Nature (London)* **178**, 992.
22. Whitten, W. K. (1957). Culture of tubal ova. *Nature (London)* **179**, 1081–1082.
23. Whitten, W.K. (1957). Effect of exteroceptive fac- tors on the oestrous cycle in mice. *Nature (London)* **180**, 1436.
24. Whitten, W. K. (1957). The effect of progesterone on the development of mouse eggs *in vitro. J Endocr.* **16**, 80–85.
25. Whitten, W. K. (1958). Endocrine studies on delayed implantation in lactating mice; role of the pituitary in implantation. *J. Endocr.* **16**, 435–440.
26. Whitten, W. K. (1958). Modification of the oestrous cycle of the mouse by external stimuli associated with the male; changes in the oestrous cycle determined by vaginal smears. *J. Endocr.* **17**, 307–313.
27. Whitten, W. K. (1959). Occurrence of anoestrus in mice caged in groups. *J. Endocr.* **18**, 102–107.
28. Gottschalk, A., Whitten, W. K., and Graham, E. R. (1960). Inactivation of follicle-stimulating hormone by enzymic release of sialic acid. *Biochim. Biophy. Acta*. **38**, 183–184.
29. Whitten, W. K., and Dagg, C. P. (1961). Influence of spermatozoa on the cleavage of mouse eggs. *J. Exp. Zool.* **148**, 173–183.
30. Whitten, W. K. (1963). Is the vomeronasal organ a sex chemoreceptor in mice? *Proceeding of the 2nd Asia and Oceania Congress in Endocrinology.* **11**, 86.
31. Whitten, W. K. (1964). Contraceptives. *A Canberra Consumer Special Report*. 63 p.
32. Whitten, W. K. (Ed.), 1965. The control of human fertility in Australia. *Med. J. Aust.* **1**, 63–79.
33. Whitten, W. K. (1966). Pheromones and mammalian reproduction. *Advances in Reproductive Physiology.* (ed. A. McLaren) Academic Press, London. **1**, 155–177.
34. Bronson, F. H., and Whitten, W. K. (1968). Oestrus-accelerating pheromone of mice; assay, androgen-dependency and presence in bladder urine. *J. Reprod. Fertil.* **15**, 131–134.
35. Whitten, W. K., Bronson, F. H., and Greenstein, J. A. (1968). Estrus-inducing pheromone of male mice: transport by movement of air. *Science* **161**, 584–585.
36. Whitten, W. K., and Biggers, J. D. (1968). Complete development *in vitro* of the preimplantation stages of the mouse in a simple chemically defined medium. *J. Reprod. Fertil.* **17**, 399–401.

37. Whitten, W. K. (1969). Mammalian pheromones. In: *Olfaction and Taste, Proceedings of the Third International Symposium.* (Ed. C. Plaffmann) Rockefeller University Press, New York. pp. 252–257.
38. Whitten, W. K., and Bronson , F. H. (1970). Role of pheromones in mammalian reproduction. In: *Advances in Chemoreception* (ed. J. W. Johnston Jr., D. C. Moulton, and A. Turk) Appleton – Century – Crofts, New York. **1**, 309 – 325.
39. Chapman, V. M., Desjardins, C., and Whitten, W. K. (1970). Pregnancy block in mice; changes in pituitary LH and LTH and plasma progestin levels. *J. Reprod. Fertil.* **21**, 333–337.
40. Cholewa, J. A., and Whitten, W. K. (1970). Development of two-cell mouse embryos in the absence of fixed nitrogen source. *J. Reprod. Fertil.* **22**, 553–555.
41. Whitten, W. K. (1971). Nutrient requirements for the culture of preimplantation embryos *in vitro*. *Advances in the Biosciences*, **6**, 129–141.
42. Crispens, C. G., Jr., and Whitten, W. K. (1971). Strain difference in sex ratio response of mice to lactate dehydrogenase virus infection. *Experientia*. **27**, 41–42.
43. Whitten, W. K. (1971). Parthenogenisis: does it occur spontaneously in mice? *Science*. **171**, 406–407.
44. Chapman, V. M., Whitten, W. K., and Ruddle, F. H. (1971). Expression of paternal glucose phosphate isomerase-1(Gpi-1) in preimplantation stages of mouse embryos. *Dev. Biol.* **26**, 153–158.
45. Mullen, R. J., and Whitten, W. K. (1971). Relationship of genotype and degree of chimerism in coat color to sex ratios and gametogenesis in chimeric mice. *J. Exp. Zool.* **178**, 165–176.
46. Biggers, J. D., Whitten, W. K., and Whittingham D. G. (1971). The culture of mouse embryos *in vitro.* In *Methods of Mammalian Embryology.* (Ed. J. C. Daniel Jr.), pp. 86–116. W. H. Freeman & Co., San Francisco.
47. Whitten, W. K., and Champlin, A. K. (1972). Endocrinology In *Handbook of Physiology* vol. 2, Am. Physiol.Soc. Washington D C, (Ed. R. O. Greep) pp. 109–123.
48. Epstein, C. J., Weston J. A., Whitten, W. K., and Russell, E. S. (1972). The expression of the isocitrate dehydrogenase locus (ld-1) during mouse embryogenesis. *Dev Biol.* **27**, 430–433.
49. Deol, M. S., and Whitten, W. K. (1972). Time of X chromosome inactivation in retinal melanocytes of the mouse. *Nature (London)* **238**, 159–160.
50. Hoppe, P. C., and Whitten, W. K. (1972). Pregnancy block: initiation by administered gonadotrophin. *Biol. Reprod.* **7**, 254–259.
51. Hoppe, P. C., and Whitten, W. K. (1972). Does X chromosome inactivation occur during mitosis of first cleavage. *Nature (London)* **239**, 520.
52. Milet, R. G., Mukherjee, B. B., and Whitten, W. K. (1972). Cellular distribution and possible mechanism of sex differentiation in XX–XY chimeric mice. *Can. J. Genet. Cytol.* **15**, 933–941.
53. Deol, M. S., and Whitten, W. K. (1972). X-chromosome inactivation: does it occur at the same time in all cells of the embryo? *Nature (London)* **240**, 277–279.
54. Whitten, W. K., and Champlin, A. K. (1972). Bibliography (with review) on pheromones and olfaction in mammalian reproduction. *Bibliography of Reproduction* **19**, 149–156; 297–303.
55. Whitten, W. K. (1973). Genetic variation of olfactory function in reproduction. *J. Reprod. Fertil. Suppl.* **19**, 405–410.
56. Whitten, W. K., and Champlin, A. K. (1973). The role of olfaction in mammalian reproduction. In: *Handbook of Physiology* (ed. R. O. Greep) Vol.II Part 1, pp. 109–124. Physiological Society, Washington.
57. Whittingham, D. G., and Whitten, W. K. (1974). Long-term storage and aerial transport of frozen mouse embryos. *J. Reprod. Fertil.* **36**, 433–435.
58. Hoppe, P. C., and Whitten, W. K. (1974). Maturation of mouse sperm *in vitro. J. Exp. Zool.* **188**, 133–136.
59. Hoppe, P. C., and Whitten, W. K. (1974). An albumin requirement for fertilization of mouse eggs *in vitro. J. Reprod. Fertil.* **39**, 433–436.
60. Anderson, E., Hoppe, P. C., Whitten, W. K., and Lee, G. S. (1975). *In vitro* fertilization and early embryogenesis: a cytological analysis. *J. Ultrastruct. Res.* **50**, 231–252.
61. Whitten, W. K. (1975). Chromosomal basis for hermaphrodism in mice. In: *The Developmental Biology of Reproduction* (Ed. C. L. Markert and J. Papaconstantinou) pp. 189–205. Academic Press, New York.
62. Whitten, W. K. (1975). Responses to pheromones by mammals. *Proceedings of the Fifth International Symposium on Olfaction and Taste.* (Ed. D. A. Denton and J. P. Coghlan) pp. 389–395. Academic Press, New York.
63. Whitten, W. K. (1977). Composites. *Science* **195**, 1323.
64. Whitten, W. K. (1977). A review of mammalian olfaction. In: Reproductive Processes and Behaviour. (Ed. R. Doty). *American Scientist* **65**, 632.
65. Liebich, H. M., Zlatkis, A., Bertsch, W., Van Dahm, R., and Whitten, W. K. (1977). Identification of dihydrothiazoles in urine of male mice. *Biomed. Mass Spectrom.* **4**, 69–72.

66. Beamer, W. G., Whitten, W. K., and Eicher, E. M. (1978). Spontaneous sex mosaicism in BALB/cWt mice. *Basic Life Sci*. **12**, 195–208.
67. Whitten, W. K. (1978). Combinatorial and computer analysis of random mosaics. *Basic Life Sci*. **12**, 445–463.
68. Jorgenson, J. W., Novotny, M., Carmack, M., Copland, G. B., Wilson, S. R., Katona, S., and Whitten, W. K. (1978). Chemical scent constituents in the urine of the red fox (*Vulpes vulpes*, L.) during the winter season. *Science* **199**, 796–798.
69. Wilson, S. R., Carmack, M., Novotny, M., Jorgenson, J. W., and Whitten, W. K. (1978). Δ^3-Isopentenyl Methyl Sulphide: A new terpenoid in the scent mark of the red fox. *J. Org. Chem*. **43**, 4675–4676.
70. Whitten, W. K., and Champlin, A. K. (1978). Pheromones, estrus, ovulation and mating. In: *Methods in Mammalian Reproduction* (Ed. J. C. Daniel) pp. 403–417. Academic Press, New York.
71. Whitten, W. K. (1979). Gonadal development in hermaphrodite mice. *In Vitro* **15**, 19–22.
72. Whitten, W. K., Beamer, W.G., and Byskov, A. G. (1979). The morphology of fetal gonads of spontaneous mouse hermaphrodites. *J. Embryol. Exp. Morphol*. **52**, 63–78.
73. Wilson, M. C., Whitten, W. K., Wilson, S. R., Jorgenson, J. W., Novotny, M., and Carmack, M. (1979). Marking behavior in wild red foxes in response to synthetic volatile urinary compounds. In: *Chemical Signals* (Ed. D. Muller-Schwarze and R.M. Silverstein) pp. 29–38. Plenum Press, New York.
74. Whitten, W. K., Wilson, M. C., Wilson, S. R., Jorgenson, J. W., Novotny, M., and Carmack, M. (1980). Induction of marking behavior in wild red foxes (*Vulpes vulpes* L.) by synthetic urinary constituents. *J. Chem. Ecol.* **6**, 49–55.
75. Novotony, M., Jorgenson, J. W., Carmack, M., Wilson, S. R., Beamer, W., and Whitten, W. K. (1980). Chemical studies of the Primer Mouse pheromones. In: *Chemical Signals – Vertebrates and aquatic invertebrates* (Ed. D. Muller-Schwarze and R. M. Silverstein). Plenum Press, New York. pp. 377–380.
76. Eicher, E. M., Beamer, W. G., Washburn, L. L., and Whitten, W. K. (1980). A cytogenetic investigation of inherited true hermaphroditism in BALB/cWt mice. *Cytogenet. Cell Genet*. **28**, 104–115.
77. Champlin, A. K., Beamer, W. G., Carter, S. C., Shire, J. G., and Whitten, W. K. (1980). Genetic and social modifications of mating patterns of mice. *Biol. Reprod*. **22**, 164–172.
78. Wilson, M. C., Beamer, W. G., and Whitten, W. K. (1980). Puberty acceleration in mice. I. Dose-response effects and lack of critical time following exposure to male mouse urine. *Biol. Reprod*. **22**, 864–872.
79. Kaneko, N., Debski, E. A., Wilson, M. C., and Whitten, W. K. (1980). Puberty acceleration in mice. II. Evidence that the vomeronasal organ is a receptor for the primer pheromone in male mouse urine. *Biol. Reprod*. **22**, 873–878.
80. Shire, J. G., and Whitten, W. K. (1980). Genetic variation in the timing of first cleavage in mice: effect of paternal genotype. *Biol. Reprod*. **23**, 363–368.
81. Shire, J. G., and Whitten, W. K. (1980). Genetic variation in the timing of first cleavage in mice: effect of maternal genotype. *Biol. Reprod*. **23**, 369–376.
82. Rose, R. W., Swain, R., and Whitten, W. K. (1981). Cadmium toxicity and its effect on the testes of an Australian marsupial (*Trichosurus vulpecula*). *Bull. Environ. Contam. Toxicol* **26**, 233–236.
83. Whitten, W. K. (1981). The anatomy of fetal mouse hermaphrodites. *Proc. Anat. Soc. Aust. & NZ*. 1981: 1–4.
84. Beamer, W. G., Hoppe, P. C, and Whitten, W. K. (1985). Spontaneous malignant granulosa cell tumors in ovaries of young SWR mice. *Cancer Res*. **45**, 5575–5581.
85. Whitten, W. K. (1989). The moon and menses. *Am. J. Obstet. Gynecol*. **160**, 519–523.
86. Whitten, W. K., Carter, S. C., and Beamer, W. G. (1991). Sex reversing non-disjunction of the Y chromosome produces exceptionally low sex ratio (% males) and hermaphrodites in the progeny of male BALB/cBm mice: the roles of the maternal genotype and the Y chromosome. *Reprod. Fertil. Dev*. **3**, 255–265.
87. Beamer, W. G., and Whitten, W. K. (1991). Do histocompatibility genes influence sex ratio (% males)? *Reprod. Fertil. Dev*. **3**, 267–276.
88. Ali, J., Whitten, W. K., and Shelton, J. N. (1993). Effect of culture systems on mouse early embryo development. *Hum. Reprod*. **8**, 1110–1114.
89. Burruel, V. R., Yanagimachi, R., and Whitten, W. K. (1996). Normal mice develop from oocytes injected with spermatozoa with grossly misshapen heads. *Biol. Reprod*. **55**, 709–714.
90. Whitten, W. K. (2001). Address to Convocation, In President's Report, 2000–2001 honour roll. Memorial University of Newfoundland. Available from http://www.mun.ca/2001report/index.php?includefile=menu/honour.php&section=10&includefile1=content/honour/whitten.php [accessed 2 January 2011].