

Scientific contributions of Twink Allen

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On 6 June 2021, we lost a colleague and friend and without a doubt one of reproductive biology's giants. Professor William Richard Allen CBE, FRCVS, ScD, universally known as Twink, will not only be remembered as a pre-eminent pioneering scientist in equine reproduction but also for practical advances that significantly influenced the clinical management of breeding mares and stallions. These scientific achievements were matched by his enthusiastic, charismatic, determined and urgent approach to science consistently applied in his endeavour to search for new knowledge. Twink was a captivating speaker and educator who had a unique ability to convey the excitement and relevance of his science to a wide audience. As a consequence of these qualities, combined with his openness to welcome a constant stream of visitors to his laboratory, he inspired and influenced students and scientists from across the globe. Twink was also a regular attendee at Society for Reproduction and Fertility (SRF) conferences, held SRF and later honorary membership for over 50 years and was the recipient of SRF's most prestigious award in 2009, the Marshall Medal.

Under the mentorship of Professor Roger Short, FRS, from the early days, Twink's first-class scientific investigations tackled fundamental research questions of the highest calibre. During his early years in Cambridge, he developed an assay for equine chorionic gonadotrophin (eCG) that became the international standard for many years (Allen 1969a). He used this assay to ascertain the levels of eCG in the blood of horse mares and donkey jennies carrying intra- and interspecies conceptuses (horse, donkey, mule and hinny). This work was published as a single-author paper in *Nature* (Allen 1969b) – quite extraordinary for a young candidate. The observations of eCG levels in these pregnancies were best explained by the theory of genomic imprinting with paternal genotype determining the size of the chorionic girdle and ultimately eCG production. Decades later, the phenomenon of imprinting is widely studied as a key regulatory mechanism of placental development and function with over 100 imprinted genes identified in the equine placenta (Wang *et al.* 2013, Dini *et al.* 2021).

Continuing his research in Cambridge, Twink then made the discovery that prostaglandin F2 alpha (PGF2A) analogues induce luteolysis in mares (Allen & Rosedale 1973). Alongside this work, he concurrently developed a

rapid ELISA for the measurement of serum progesterone (Allen & Sanderson 1987) allowing him to show that the majority of mares who failed to return to oestrous had a persistent corpus luteum. The subsequent application of this knowledge has increased the overall fertility rate of mares at commercial stud farms by allowing more matings in fertile oestrus cycles in a single breeding season. Nearly 50 years on, PGF2A analogues are in daily use by equine reproductive specialists across the globe. For most scientists, the magnitude of this clinical discovery would be enough for one decade but not for Twink. Following the initial reports by Eric Palmer and colleagues on the use of ultrasonography to visualise the reproductive tract of large animals, Twink was quick to embrace the technology demonstrating its value to monitor oestrous, ovulation and early pregnancy in mares. Working alongside veterinary surgeons in Newmarket, he showed its diagnostic value for the early detection of twin pregnancies and the subsequent reduction of twins to a singleton (Simpson *et al.* 1982), essentially eliminating in a single study a common cause for a mare to abort her pregnancy in mid to late gestation.

Twink also made significant contributions to the development of artificial reproductive technologies in mares. He produced the first equine monozygotic twins achieved by micromanipulator-assisted bisection of morula stage embryos (Allen & Pashen 1984, Skidmore *et al.* 1989). Other 'firsts' included the production of the first pregnancy by intracytoplasmic sperm injection of an *in vitro* matured oocyte and transfer of the resultant blastocyst back into the uterus of a mare (Li *et al.* 2001) and the first successful birth of a horse following international transport of a frozen-thawed embryo (Allen *et al.* 1976). Twink's enthusiasm for these techniques also popularised the commercial use of equine embryo transfer, and it is now in widespread use globally in the horse industry.

While Twink's curiosity, imagination and drive meant that he addressed a great breadth of research questions, he maintained his fascination with the biology of early equine pregnancy throughout his scientific career. In his very early work following his PhD, he made the seminal discovery that the equine endometrial cups are comprised of trophoblast cells of fetal origin (Allen *et al.* 1973), and therefore were not a decidual reaction of the maternal endometrium as had been thought for the previous half-century (Amoroso

1955). In a number of extensively cited reviews, he repeatedly and meticulously imaged and described the chorionic girdle and chorioallantois of the equine placenta (Allen 2001, Allen & Wilsher 2009, Antczak et al. 2013).

In this issue, two review articles are featured that discuss the key genes involved in normal equine placental development (Loux et al. 2022) and how these genes are disrupted when the placenta is exposed to environmental constraints or pathological processes (Robles et al. 2022). Both articles extensively reference Twink's many contributions to the study of the equine placenta. Metanalysis by Loux et al. (2022) identified insulin growth factor (IGF) 2 as the most abundantly expressed growth factor in the early equine placenta, with the exact function of IGF pathways in equine placentation yet to be determined. IGF2 is a paternally imprinted gene (Wang et al. 2013) and interestingly was localised to the trophoblast cells of the chorionic girdle and allantochorion in early studies by Twink (Lennard et al. 1995). Yet another reminder that Twink had an uncanny ability to perform pertinent experiments well ahead of his time.

At the turn of the century, and well before Developmental Origins of Health and Disease (DOHaD) was a prominent field of research, Twink became fascinated with the Barker Hypothesis, the concept that the maternal uterine environment can influence not only fetal growth but also adult health and disease. In the early 2000s in experiments that echoed the Shetland pony × Shire crosses of Walton and Hammond (Walter & Hammond 1938), he utilised the significant differences in uterine size observed between Welsh and thoroughbred breeds to create a growth-restricted (thoroughbred embryo in Welsh uterus) and nutrient-rich model (Welsh embryo in a thoroughbred uterus) of equine pregnancy. These important studies, discussed in more detail in this issue (Robles et al. 2022), showed an association between maternal size and placental exchange surface area and fetal growth (Allen et al. 2002) as well as postnatal organ function (Allen et al. 2004). A final example of Twink pushing the boundaries of science well in advance of the expansion of this field.

Twink was a pioneer of heroic, insightful experiments. His scientific achievements will undoubtedly continue to sow the seeds for new ideas for decades to come. His generosity in sharing ideas and expertise earned him countless friends across the globe in a life fully lived.

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