Nottingham Dollies prove cloned sheep can live long and healthy lives

July 26 2016

Nottingham Dollies. Credit: The University of Nottingham.

Three weeks after the scientific world marked the 20th anniversary of the birth of Dolly the sheep new research, published by The University of Nottingham, in the academic journal *Nature Communications* has

shown that four clones derived from the same cell line—genomic copies of Dolly—reached their 8th birthdays in good health. The video can be seen here.

Nottingham's Dollies—Debbie, Denise, Dianna and Daisy—have just celebrated their 9th birthdays and along with nine other clones they are part of a unique flock of cloned sheep under the care of Professor Kevin Sinclair, an expert in developmental biology, in the School of Biosciences.

The research—'Healthy ageing of cloned sheep'—is the first detailed and comprehensive assessment of age-related non-communicable disease in cloned offspring. Published today, Tuesday 26 July 2016, it shows that at between seven to nine years of age (60 to 70 in human years) these cloned sheep were showing no long-term detrimental health effects.

Dolly made history as the first animal to be cloned from an adult cell using a technique known as somatic-cell nuclear transfer (SCNT). The late, Professor Keith Campbell was instrumental in this pioneering work. In 1999 he joined The University of Nottingham where he continued his research in reproductive biology until his death in 2012. The flock of clones are his legacy to the University.

This latest study was led by Professor Kevin Sinclair, a close colleague of Professor Campbell's.

Professor Sinclair said: "Despite technological advances in recent years' efficiency of SCNT remains low but there are several groups across the world working on this problem at present and there is reason to be optimistic that there will be significant improvements in future. These improvements will stem from a better understanding of the underlying biology related to the earliest stages of mammalian development. In turn this could lead to the realistic prospect of using SCNT to generate stem

cells for therapeutic purposes in humans as well as generating transgenic animals that are healthy, fertile and productive. However, if these biotechnologies are going to be used in future we need to continue to test their safety."

Nottingham's cloned offspring

Nottingham's oldest clone was born in July 2006. The four Finn-Dorset clones—'the Dollies'—were born in July 2007. A female Lleyn clone was born in August 2007 along with a second clone (breed unknown). In June 2008 six more Lleyn ewes were born.

These animals originated from studies undertaken by Professor Campbell between 2005 and 2007 which sought to improve the efficiency of SCNT. The four Finn Dorsets were derived from the mammary gland cell line that led to the birth of Dolly. The other clones came from fetal fibroblasts.

Detailed health assessments—including x-ray and MRI

Longevity and healthy ageing among SCNT clones have long been contentious issues and much was made of Dolly having to undergo treatment for osteoarthritis some time prior to her death in 2003 at six years old.

During 2015 Nottingham's cloned sheep underwent a series of comprehensive assessments for non-communicable diseases including obesity, hypertension and osteoarthritis—three major comorbidities in aged human populations. The examinations included the use of anaesthesia to carry out x-rays and MRI scans.

The research was carried out under the authority of the United Kingdom Animal (Scientific Procedures) Act 1986 with approval from The University of Nottingham Animal Welfare and Ethical Review Board.

The flock was tested for glucose tolerance and insulin sensitivity. They underwent radio-telemetric assessments to check their heart rate and blood pressure. They had a full musculoskeletal examination carried out by Dr Sandra Corr, a veterinary orthopaedic specialist from the University's School of Veterinary Medicine and Science and a co-author of this research.

Radiological examinations of all main joints were followed by MRI scans of their knees, the joint most affected by osteoarthritis in Dolly. Their health was compared with a group of naturally bred six-year-old sheep living under similar conditions at the University.

No major health issues

Professor Sinclair said: "Healthy ageing of SCNT clones has never been properly investigated. There have been no detailed studies of their health. One of the concerns in the early days was that cloned offspring were ageing prematurely and Dolly was diagnosed with osteoarthritis at the age of around five, so clearly this was a relevant area to investigate. Following our detailed assessments of glucose tolerance, insulin sensitivity, blood pressure and musculoskeletal investigations we found that our clones, considering their age, were at the time of our research healthy."

Despite their advanced age the cloned sheep—including the four Dollies—were showing no signs of diabetes, high blood pressure, or clinical degenerative-joint disease. Although some of the animals were showing radiographic evidence of mild, and in Debbie's case, moderate osteoarthritis none of the animals were lame and none required treatment

for osteoarthritis.

No detrimental long-term adverse effects of SCNT

There is still a long way to go before SCNT is perfected. However, this research has shown that cloned animals can live long and healthy lives.

Professor Sinclair said: "It is well established that prior to conception and in the early stages of pregnancy during natural or assisted reproduction subtle chemical changes can affect the human genome leading to development and late-onset chronic diseases. Given that SCNT requires the use of assisted reproductive procedures it is important to establish if similar diseases or disorders exist in apparently healthy aged cloned offspring."

More information: Healthy ageing of cloned sheep, *Nature Communications*, nature.com/articles/doi:10.1038/ncomms12359

Provided by University of Nottingham