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'Cloned' Food Animals *Not* True Clones

Commercial release of 'cloned' food animals illegal as well as unethical and unsafe. Prof. Joe Cummins *and* Dr. Mae-Wan Ho

Cloned food animals declared safe by FDA

In 2008 the United States Food and Drug Administration (FDA) completed a 'risk assessment' on the introduction of cloned animals into the food supply. It concluded [1]: "No unique risks for human food consumption were identified in cattle, swine, or goat clones derived via somatic cell nuclear transfer (SCNT). No anomalies have been observed in animals produced by cloning that are not also observed in animals produced by other assisted reproductive technologies (ARTs) and natural mating." FDA defines an animal clone as a genetic copy of a donor animal, similar to identical twins but born at a different time. Most cloning today uses a process called somatic cell nuclear transfer (SCNT). Just as with *in vitro* fertilization, scientists take an immature egg from a female animal (often from ovaries obtained at the slaughterhouse). But instead of combining it with sperm, they remove the nucleus, which contains the egg's genes, leaving behind the other components necessary for an embryo to develop. Scientists then add the nucleus containing the desirable traits from a cell obtained from the animal the farmer wishes to copy. After a few other steps, the donor nucleus and egg fuse, start dividing, and an embryo forms. The embryo is then implanted in the uterus of a surrogate dam. as with in vitro fertilization, which carries it to term [2]. ("Dam" is a term that livestock breeders use to refer to the female parent of an animal).

The truth about SCNT

ISIS has made a substantial submission to the FDA criticizing its misleading stance on cloning, especially for blurring the distinction between ordinary cloning (by subdividing the cells of the early embryo) with SCNT, and stressing that cloned meat and milk are unethical and unsafe [3] (Is FDA Promoting or Regulating Cloned Meat and Milk? *SiS* 33). One key section of the original submission is reproduced below (see Box).

The real story about cloning

At issue is SCNT, the procedure pioneered in creating Dolly the cloned sheep in 1996 [4] Death Sentence on Cloning, *SiS* 19). Cloning from the genetic material of an adult animal means that all the genetic 'elite' qualities of the animal are proven, so the clones in theory will reproduce those 'elite' qualities. More to the point, it allowed the duplication of genetically modified (GM) animals without the normal reproduction process, as GM animals tend to be either sterile or to lose their transgenes or transgene expression in subsequent generations. Dolly was a rehearsal for the cloning of an 'elite' herd of transgenic animals producing valuable pharmaceuticals in their milk. That turned out to be a pipedream. Cloning does not faithfully reproduce the qualities of the adult, elite or otherwise.

The success rate of SCNT is extremely low, and remains so to this day, between 0 and 5 percent across the species: sheep, cattle mouse, pig, goat, rabbit, cat, notwithstanding. Here is how one reviewer among many, Jonathan Hill at the College of Veterinary Medicine, Cornell University, New York described it when he was opposing human reproductive cloning [5].

"In each of the species where somatic cell cloning has been successful, it has also been very inefficient. Early first trimester pregnancy rates are less than ½ that normally expected. Immediately following initial positive diagnosis of pregnancy, extraordinarily high rates of embryonic loss occur, where up to 80% of pregnancies miscarry by the second semester. In late gestation, placental and fetal abnormalities occur at a much higher than normal rate, and finally lowered viability at birth is common."

So hundreds of reconstituted eggs have to be created to get dozens of embryos good enough to be implanted into surrogate mothers just to end up with a few clones born live.

Those few clones that survive after birth are by no means healthy: "Postnatal viability is markedly lower for many cloned animals….Neonatal viability has been shown to be

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compromised due to pulmonary immaturity."

The remaining that seem apparently healthy are not without problems, for "closer investigations have revealed that even some of these apparently normal animals are subtly different from one another and from the naturally produced population… What is of significant concern is that placental development and the intrauterine environment for many clones is suboptimal and this alone may impact on their health in later life."

This was borne out by numerous laboratories involved in cloning. In one experiment [6], 988 SCNT embryos were transferred into cows resulting in 133 calves delivered at term, but only 67 percent survived to weaning at 3 months of age, with an average annual death rate thereafter of more than 8 percent. The offspring of SCNT clones fare better, though they have not been subjected to more discerning tests either.

Dolly had to be put down prematurely at age six on account of severe illnesses, and the company PPL therapeutics that helped to create Dolly failed to find a backer for its GM alpha-1 anti-trypsin produced in cloned transgenic sheep's milk, and had to slaughter its flock of 3 000 transgenic sheep in 2003 [7] (Animal Pharm Folds, *SiS* 19). Thus, SCNT has proven neither technically successful nor economically viable.

Many, including Ian Wilmut, the creator of Dolly, saw that as the end of SCNT cloning for producing animals, and have since concentrated efforts into creating embryonic stem cells for tissue replacement. But that too is misguided and ethically unjustified as many clinical successes in tissue replacement have been documented using the patient's own adult stem cells while embryonic stem cells have yet to prove themselves in a single clinical application so far [8] (No Case for Embryonic Stem Cells Research, *SiS* 25).

The major problem with SCNT clones and with embryonic stem cells made by SCNT cloning is the large numbers of genome-wide epigenetic errors in gene expression associated with the nuclear transfer process, resulting in the high failure rates of clones, and in the eyes of many scientists, precludes the safe use of SCNT-derived embryonic stem cells in tissue replacement [9].

Microarray analysis of more than 10 000 gene in clones found that about 4 percent of the genes in the placenta are different from normal, with a smaller number of genes also affected in the liver [10].

SCNT animals are not true clones

There is a further aspect that distinguishes SCNT from other clones, in that the animals created are not true clones with respect to the mitochondrial (mt) genome. A US law defines assisted reproduction technologies (ARTs) as those that involve the handling of both sperm and eggs. The vast majority of these involve *in vitro* fertilization (IVF), in which oocytes are removed from the mother's body and fertilized with sperm in the laboratory, and returning the embryo to the woman's body. Fertilization of the oocyte is achieved either through incubating sperm and oocytes together (classic IVF) or through direct injection of a single sperm into the oocyte under the microscopic [11].

Generally in mammals, individual animals contain only maternally inherited mtDNA, as paternal (sperm)-derived mitochondria are usually eliminated during early development. Somatic cell nuclear transfer (SCNT) bypasses the normal routes mtDNA inheritance and introduces not only a different nuclear genome into the recipient cytoplast, the enucleated oocyte, but also accompanying mtDNA. This mtDNA 'heteroplasmy' due to persistence and replication of both oocyte mtDNA and somatic cell mtDNA means that offspring generated by SCNT are not true clones. More importantly, the consequences of the heteroplasmy, or possible incompatibility between nuclear and mtDNA genotypes on subsequent development and function of the embryo, foetus and offspring are unknown. Following sexual reproduction, mitochondrial function requires the biparental control of maternally inherited mtDNA. SCNT-associated incompatibility between the recipient cell mt and transplanted nuclear genomes ,may result in energy imbalance and initiate mtDNA disease, or disruption of normal developmental event [12].

Mitochondrial heteroplasmy must not be ignored

True clones would contain both the nuclear and cytoplasmic genotype of the nucleus donor, which is not the case for clones from SCNT. It has been possible to strip most of the mitochondria from the donor cell by treating with ethidium bromide a dye molecule that inserts itself among the stacked bases of mitochondrial DNA. When the nucleus of the somatic cell lacking mitochondria is injected into the egg from which the nucleus has been removed the resulting cloned embryo and maturing animal is homoplasmic (having only egg mitochondria [12-14]. The resulting animal clones are homoplasmic but they are certainly not true clones because they have the nucleus of the cloned animal but the mt genome of the egg. That distinction may seem academic, but the role of the mitochondria in development and disease is profound. The importance of induced dysfunctions related to nuclear reprogramming following SCNT cloning is very consequential (see Box). That impact has been the focus of a great deal of discussion and has not been denied or completely ignored by FDA. But FDA continues to claim that the cloned animals are true clones while they are clearly not.

Mitochondrial heteroplasmy in cloned animals

In humans as in other mammals, mt genome is strictly maternally inherited. Mitochondrial heteroplasmy arises through mutations in the egg mitochondria. Mitochondrial heteroplasmy

may also be found in the tissues of individuals, but that condition is not inherited. In contrast, SCNT gives rise to mitochondrial heteroplasmy. It has been observed that the efficiency of bovine somatic cell nuclear transfer (SCNT) depends on donor-host compatibility. The reprogramming of the donor nucleus is influenced by the donor-host compatibility of the mitochondria [15]. In nuclear transfer-derived embryos, nuclear-encoded mitochondrial DNA transcription and replication factors persist, but not in embryos generated through *in vitro* fertilization. Consequently, nucleo-mitochondrial interaction following nuclear transfer is out of sequence as the onset of mitochondrial replication is a post-implantation event [16].

SCNT using nucleus from fibroblast from the ears of Holstein cattle transferred into the eggs of Lund yellow cows were all heteroplasmic for donor-egg mitochondria [17]. Donor mtDNAs in SCNT pigs could be transmitted to progeny [18]. Moreover, once heteroplasmy was transmitted to progeny of SCNT-derived pigs, it appeared that the introduced mitochondrial populations become fixed and maternally-derived heteroplasmy was more readily maintained in subsequent generations. There are numerous further publications dealing with SCNT-derived mt heteroplasmy, which establish that the phenomenon is a typical consequence of SCNT. It is very clear that FDA's claim that cloned food animals are identical to the donor animal and that the progeny of such animals bear only the genes of the SCNT donor is false and misleading.

Diseases associated with mitochondrial heteroplasmy

The diseases associated with mt heteroplasmy are transmitted through the mother alone. The defects frequently include brain and nerve defects or heart defects. One approach to curing such diseases has been developed using monkey clones to closely mimic human clones. Donor mitotic nuclei from mt-diseased eggs are transferred to eggs from which the nucleus has been removed. The healthy nuclei from the diseased eggs divide in the healthy eggs that provide a full complement of healthy mitochondria in both stem cell lines and in complete embryos. Infant female monkeys developed from the transplanted were free of diseased mitochondria, and capable of giving birth to disease free infants. The method is presented as a way of preventing mitochondrial disease transmission in affected human families [19].

Mutations in mtDNA may cause maternally-inherited cardiomyopathy and heart failure. In homoplasmy, all mtDNA copies contain the mutation. In heteroplasmy there is a mixture of normal and mutant copies of mtDNA. The clinical phenotype of an affected individual depends on the type of genetic defect and the ratios of mutant and normal mtDNA in affected tissues. These included a novel heteroplasmic mutation in tRNA serine in a patient with sudden cardiac death [20]. A well-characterized pathological mutation at a nucleotide position of human mitochondrial DNA was introduced into human teratocarcinoma NT2 cells. In cloned and mixed populations of NT2 cells heteroplasmic for the mutation, there was invariably a tendency toward increasing levels of mutant mtDNA as the cells multiplied. Rapid human teratocarcinoma NT2 cell multiplication was frequently followed by complete loss of mtDNA. These findings support the idea that pathological mt DNA mutations are particularly deleterious in specific cell types, which can explain some of the tissue-specific aspects of mtDNA diseases. Moreover, these findings suggest that mitochondrial DNA depletion may be an important and widespread feature of mtDNA disease [21].

Mitochondrial diseases have been extensively studied and reviewed in recent years [22]. Mitochondrial disorders may be caused by defects of nuclear DNA or mtDNA. Nuclear gene defects may be inherited in an autosomal recessive or autosomal dominant manner. MtDNA defects are transmitted by maternal inheritance. MtDNA deletions generally occur *de novo* and thus cause disease in one family member only, with no significant risk to other family members. MtDNA point mutations and duplications may be transmitted down the maternal line. The father of an ill individual is not at risk of having the disease-causing mtDNA mutation, but the mother of a ill person (usually) has the mitochondrial mutation and may or may not have symptoms. A male does not transmit the mtDNA mutation to his offspring. A female harboring a heteroplasmic mtDNA point mutation may transmit a variable amount of mutant mtDNA to her offspring, resulting in considerable clinical variability among sibs within the same family. Prenatal genetic testing and interpretation of test results for mtDNA disorders are difficult because of mtDNA heteroplasmy. Consuming meat from cloned animals is unlikely to cause mitochondrial disease but consuming meat from heteroplasmic animals is entirely new to human experience worldwide and such animals are bound to have many hidden defects.

Cloning and the law

The law plays a key role in dealing with arbitrary and capricious bureaucratic rulings. The views expressed in articles from law journals show widely different appraisals of FDA in regulating foods from cloned animals, Jennifer Butler, a lawyer and professional molecular biologist, has a clear understanding of the cloning process and heteroplasmy. Her article includes a valuable history of FDA and an excellent proposal that FDA should be replaced with an agency better equipped to deal with technologies involved in genetic modification and animal cloning [23]. A group of lawyers from Proskauer LLP New York reviewed the risks involved in marketing meat from cloned animals or in consuming dairy products from cloned animals, and urged the FDA to institute effective tracking and diagnostics to allow adequate evaluation of the true health risks. But there was no mention of heteroplasmy, and FDA's claim that the animals are true clones implicitly accepted [24]. John Murphy, a lawyer and a professional chemical engineer, commented that there were no valid safety concerns over consuming food from cloned animals and moral concerns were tangential and overboard. He further concluded that labelling of the products of cloned animals is not valid based on unspecified scientific grounds [25]. Butler is the clearly the only lawyer who has made a thorough and comprehensive study of food animal cloning, and hence can speak the most

authoritatively on the issue.

Conclusion

Cloned food animals are not true replicas of the animal donating the nucleus in SCNT. The cloned animals contain heteroplasmic mixtures of mitochondrial genes from both the somatic cell and from the egg receiving the somatic cell nucleus. In nature, only the maternal parent provides mitochondrial genes. SCNT is a process entirely new to nature, and also departs significantly from *in vitro* fertilization. Mitochondrial heteroplasmy, and ensuing mitochondrial depletion, has been implicated in diseases affecting the brain, the central nervous system and the heart. FDA wrongly claims that the heteroplasmic offspring of SCNT are true clones, thereby exposing its pronouncement as public relations propaganda and not science. There is no evident cure for the mitochondrial heteropasmy in SCNT, and for that reason all animals created by SCNT and their offspring are illegal for commercial release, apart from being unethical and unsafe for consumption.

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