

A study on current risk assessments and guidelines on the use of food animal products derived from cloned animals



Sun Jin Hur*

Department of Animal Science and Technology, Chung-Ang University, 4726 Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi-do, 17546, Republic of Korea

ARTICLE INFO

Article history:

Received 27 March 2017

Received in revised form

14 July 2017

Accepted 23 July 2017

Available online 24 July 2017

ABSTRACT

The author widely surveyed and analyzed the food safety issues, ethical issues, permits, and approval of animal products from animals cloned by somatic cell nuclear transfer worldwide. As a result of a 2-year survey, the author found that there is no evidence that meat and milk derived from cloned animals or their progeny pose a risk to food safety in terms of genotoxicity, adverse reproductive effects, or allergic reactions. Most countries have not approved meat and milk derived from cloned animals, and their progeny are entering the food supply. To establish the guidelines, the author suggests four principles of safety assessment for meat and milk derived from cloned animals. The four main principles for safety assessment are similarities of chemical composition, adverse reproductive effects, genotoxicity, and allergic reactions under the influence of meat and milk from cloned animals and noncloned counterparts. This principle means that meat and milk derived from a cloned animal are safe if there are no differences in the four safety assessments of meat and milk between cloned animal's progeny and noncloned counterparts.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Animal cloning is a biological process of producing genetically similar animals, and somatic cell nuclear transfer (SCNT) is the most common animal cloning technology because somatic cells can be easily obtained and cultured in vitro. Cloned animals are highly attractive to livestock breeders because cloning essentially produces an identical copy of an animal with superior traits (Butler, 2009). Nonetheless, safety of meat and milk derived from cloned animals and ethical issues in animal cloning are still controversial topics. In 2002, the National Academy of Sciences' National Research Council (NAS/NRC) report commissioned by the FDA concluded that there is no evidence that food products derived from adult somatic cell clones or their progeny pose a food safety risk. The US government has released a draft proposal declaring that food from cloned cattle, pigs, and goats is "likely to be as safe as" food from their noncloned counterparts. FDA has found no science-based reason to require labels to distinguish between animal products from cloned animals and commercial animal breeds.

In January 2008, FDA released a final assessment in which it concluded that meat and milk from cow, pig, and goat clones and the offspring of any animal clones are as safe as food we eat every day (FDA, 2008). Not only has animal cloning been widely done, but also the FDA has approved the use of meat and milk derived from clones; consumers and the government are seriously concerned about allowing cloned animals and their derived products into food chains (Panarace et al., 2007). Moreover, some of the questions are still unresolved, e.g., ethical and moral issues and the low success rates of SCNT. For these reasons, many developed countries except the United States will not allow the meat and milk derived from cloned animals or their offspring to enter the food supply in the near future. For instance, food derived from animal clones falls currently under the jurisdiction of the Novel Food Regulation of the EU. Although most countries have not issued a risk assessment guidance regarding meat and milk from cloned animals, a guideline study must address unforeseen situations in the future. Indeed, some Korean scientists asked and made SCNT a requirement for producing meat and milk derived from cloned animals. Therefore, the purpose of this study was to provide information on the risk of animal products derived from cloned animals and to establish guidelines for the use of meat and milk derived from animals cloned by SCNT. This study should be helpful for the establishment of other guidelines for novel foods derived from animals.

* Department of Animal Science and Technology, Chung-Ang University, 4726 Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi, 17546, Republic of Korea.
E-mail address: hursj@cau.ac.kr.

2. Materials and methods

I surveyed more than 100 studies published in the past 10 years related to the link between the animal products derived from cloned animals and safety. This study includes electronic searches of the PubMed, Medline, Scopus, Wiley Online Library, Springer Link, AGRIS, CAB, and Google Scholar databases by means of the following search terms: SCNT, cloned animals, meat and milk derived from cloned animals, safety of animal products derived from cloned animals, guidance, guideline and regulation (in various combinations). Numerous reports released from the government and research institutes of the USA, EU, Japan, New Zealand, Australia, or Korea were analyzed in this study. Risk assessment tests for this study are cited from reports mentioned below, which extensively determined chemical composition, quality parameters, similarity of reproduction and adverse breeding effects, similarity of genotoxicity, and similarity of allergic reactions to consumed meat and milk between the progeny of cloned-animal origin and its noncloned counterparts in rodent models. These reports compared chemical composition and quality parameters of meat and milk between progeny of a cloned animal and noncloned counterpart, and they compared reproduction and adverse breeding effects, genotoxicity, and the allergic reaction in experimental animals that consumed meat and milk derived from the progeny of a cloned animal and from a noncloned counterpart. After analysis of differences or similarities, these previous researchers drew a conclusion whether meat and milk derived from the progeny of a cloned animal are safe or not. In the present study, risk assessment and tests for guideline establishment were cross-referenced from these other studies.

3. Risk assessment studies on animal products derived from cloned animals

This paper extensively surveyed the risk assessment studies on animal products derived from cloned animals and the summarized results are presented in Table 1. Most investigators found that chemical composition of meat and milk was similar between animals derived from clones and noncloned counterparts. The US FDA report in 2008 and Japan NARO Institute of Livestock and Grassland Science (NILGS) report in 2011a,b contain extensive analysis of the chemical composition of meat and milk derived from clones and noncloned counterparts, and then these reports concluded that chemical composition is similar between clones and noncloned counterparts. Although several studies [Takahashi and Ito (2004), Rudenko et al. (2004), and Tian et al. (2005)] found some differences in parameters, they reported that most parameters were within the normal range in terms of chemical composition of meat, milk, carcass, and blood between clones and noncloned counterparts. In terms of quality of meat and milk, the Japanese NARO NILGS report, an FDA report in 2008, and unpublished data from Korean National Institute of Animal Science concluded that quality parameters were similar in meat and milk between clones and noncloned counterparts. In terms of animal health and adverse effects, most studies found that consumption of clone-derived meat and milk did not affect the health and did not have toxic effects in experimental rodent animal models. Moreover, dietary meat and milk derived from clones did not cause genotoxicity, adverse reproductive effects, and allergic reactions in various experimental rodent animal models. As result of these studies, this author also concluded that meat and milk derived from cloned animals are as safe as food from their noncloned counterparts. According to these studies, meat and milk derived from cloned animals can be used as a novel food. Nevertheless, social consultation must be a prerequisite in terms of ethical and moral issues of animal cloning,

improving the success rate and standardization of SCNT; and alleviation of anxiety among consumers.

4. Definition of meat and milk derived from animals cloned by SCNT for the guidelines

A cloned animal is a creature representing a genetically identical copy of an animal because of SCNT, and the definition of cloned animals includes the progeny, which is children and grandchildren of the cloned animal. The definition of animal products derived from cloned animals are meat and milk derived from progenies (2nd and 3rd generation) of a cloned animal, not the cloned animal's lifetime (1st generation). The definition of progenies of cloned animals is second and third generation from the cloned animals, i.e., children and grandchildren of cloned animals. Due to a genetic dilution and mutation, it is hard to determine whether the fourth generation (great-grandchildren) are progenies of a cloned animal or not. In this regard, cloned animals (1st generation) should be represented only by parent animals (whether both parents or half-blooded) and by progenies (2nd and 3rd generation) of cloned animals; meat and milk should be produced by natural mating or artificial insemination not by SCNT in terms of the proposed guidelines.

5. Risk assessment procedures for meat and milk derived from a cloned animal

The risk assessment procedures are subdivided into two steps (before and after slaughtering), the first step is analysis and management of the animals and the farm before slaughtering, and the second step is analysis of differences in meat and milk between the progeny of cloned animals and their noncloned counterparts.

The first step for analysis and management of all animals and the farm before slaughtering can also be subdivided into four steps mentioned below.

The first step is establishment of a tracking system for all the animals and farm of origin.

The second step is standardization of SCNT and SCNT stability for meat and milk production. This step must be consider genome reprogramming or abnormally expressed.

The third step is to determine the health of cell donor animals and surrogate dams.

The forth step is to determine the health of cloned animals and their progeny.

Currently, there are no accurate ways to identify where animal products come from (cloned animals produced by SCNT or noncloned animals). For that reason, the use of meat and milk derived from cloned animals may be hard to control by the government in terms of enforcement of regulations against misuse or violation of labeling. Therefore, a tracking system of animals and farms of origin should be provided prior to establishing the guidelines. Moreover, the health condition of all the animals (cell donor, surrogate dams, cloned animals, and their progeny) must be consider in terms of ethical and moral issues. If we pass the first step (before slaughtering), then risk assessment moves on to the second step mentioned below.

The second step to determine the safety is subdivided into six steps as follows.

The first step is a survey of the experience of human consumption of meat and milk derived from cloned animals throughout the world.

The second step is analysis of similarity of chemical composition of meat and milk between the progeny of cloned animals and noncloned counterparts.

Table 1

A summary of risk assessment studies in animal products derived from cloned animals.

Species	Samples	Test parameters	Clones vs. normal	References
Compositions				
Cattle	Meat	Crude Fat, moisture, protein combustion, protein- Kjeldahl, ash, balance (protein + moist + ash + fat), tryptophan, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, hydroxyproline, myristic acid, myristoleic acid, pentadecanoic acid	Similar	FDA, 2008, Cyagra Dataset
Cattle	Meat	Water, protein, lipids, carbohydrates, ash, cholesterol, amino acid composition, fatty acid composition, vitamin concentration, mineral concentration, digestion rate	Similar	NARO ILGS, 2011a
Cattle	Meat	Protein, fat, carbohydrate, fiber, ash, water, vitamin A, vitamin B groups, vitamin D ₃ , vitamin E, vitamin K ₂ , niacin, pantothenic acid, folic acid, biotin, choline, minerals (Ca, P, K, Na, Mg, Fe, Zn, Cu, S, Mn, I, Se, Mo)	Similar	NARO ILGS, 2011b
Cattle	Milk	Body weight, total intake, milk yield, milk fat, milk protein, milk lactose, lipolysis, fatty acid compositions	Similar	Bernard et al., 2015
Cattle	Milk and dairy products	Fatty acid composition, amino acid composition, mineral composition, vitamin composition, IgG concentration in colostrum, chemical composition of TG3, color of milk	Similar	Laible et al., 2007
Cattle	Milk	Fat, protein, lactose, α -casein, β -casein, κ -casein, α -lactalbumin, β -lactalbumin, BSA, IgG, fatty acids, SFC, magnesium, calcium, sodium, potassium	Similar	Wells et al., 2004
Cattle	Milk	Total solids, fat, protein and lactose, pH, acid degree value, somatic cell count, protein profiles, fatty acid profiles, mineral profiles	Similar	Walsh et al., 2003
Cattle	Milk	Total protein, fat, solids, lactose, milk urea nitrogen and SCS; protein profiles	Similar	Tian et al., 2005
Cattle	Milk	Total fat, protein and solids not fat	Differences in quantity; otherwise similar	Takahashi and Ito, 2004
Cattle	Milk	Milk fat, protein, lactose, solids not fat, total solids	Similar	Aoki et al., 2003
Cattle	Milk	Ash, carbohydrate, lipid, protein, water, calcium, cholesterol, amino acid composition, fatty acid composition, vitamin concentration, mineral concentration, digestion rate	Similar	NARO ILGS, 2011a
Swine	Blood	Creatinine, alkaline, phosphatase, BUN ² , ALT, albumin, phosphorus, calcium, serum, protein, glucose, globulins, A/G ratio, total T3, cortisol	Similar	Archer et al., 2003
Cattle	Blood	Blood chemistry parameters	6 out of 592 parameters different	Rudenko et al., 2004
Cattle	Blood and Carcass	Blood chemistry, hematology parameters, composition of fatty acids, composition of amino acids, vitamins and proximate	Similar	FDA, 2008
Swine	Carcass	Hot carcass weight, carcass length, dressing percentage, back fat thickness, first rib, tenth rib, last rib, last lumber, loin eye area	Similar	FDA, 2008
Cattle	Carcass	Organ or body part weights: % total meat and fat, % moisture, crude protein and crude fat in six muscles; profiles of fatty acids; amino acids composition; histopathology of all organs	12 out of over 100 parameters different, but all within normal industry range	ViaGen dataset Tian et al., 2005
Cattle	Carcass	Total water, protein, lipids, carbohydrates, ash, cholesterol, amino acids, fatty acid	Similar	Takahashi and Ito, 2004
Cattle	Carcass	Body composition and fasting glycemia of rats fed by milk from cloned cows	Similar	Tome et al., 2004
Swine	Carcass	Amino acids, fatty acids, cholesterol	Similar	FDA, 2008
Cattle	Live body	Changes of body weight gain	Similar	ViaGen dataset, Walker et al., 2007
Cattle	Live body	Red blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, PT, APTT	Similar	Yamaguchi et al., 2007
Cattle	Hormone	Changes of hormone (cortisol, norepinephrine, epinephrine)	Similar	NARO ILGS, 2011a
Cattle	Hormone	Changes of hormone (cortisol, norepinephrine, epinephrine)	Similar	Heyman et al., 2007
Quality of meat and milk				
Cattle	Meat and carcass quality after slaughter	Carcass characteristics: live body weight, hot carcass weight, cold carcass weight, dressed meat weight, body fat content, bone weight Carcass grade: area of loin, marbling, meat color, texture, aging, back fat thickness, body length, carcass length, meat grade Meat quality and chemical composition: water, protein, fat, ash, cooking loss, shear force, water-holding capacity, CIE L*, a*, b*	Similar	Korean National Institute of Animal Science, unpublished data
Cattle	Meat quality during storage	CIE L*, a*, b*, shear force, cooking loss, water-holding capacity, hardness, tenderness, cohesiveness, chewiness, protein degradation, lipid oxidation, pH	No trends within normal range	Korean National Institute of Animal Science, unpublished data
Cattle	Milk quality	Alcohol test, specific gravity, acidity, pH, somatic cell counts, fat, protein, sugar, total solids, solids not fat, amino acid composition, mineral contents	Not significantly different	Korean National Institute of Animal Science, unpublished data
Cattle	Meat Quality	Carcass weight, meat grade, rib eye area, rib thickness, subcutaneous fat thickness, moisture, ether extract, crude protein, cooking loss, shear force, water holding capacity	Similar	NARO ILGS, 2011b
Swine	Meat quality	Longissimus pH at 24 h, carcass muscle score, NPPC quality scores, color, marbling, firmness, Hunter L*, a*, b*	Similar	FDA, 2008 ViaGen dataset

(continued on next page)

Table 1 (continued)

Species	Samples	Test parameters	Clones vs. normal	References
Animal body health and adverse effects				
Cattle	Meat fed rat	Hemoglobin, hematocrit, Retics, red blood cell index; mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red blood cells, white blood cell differential counting: lymph, mono, eosino, baso, white blood cells; platelet coagulation tests: prothrombin time, activated partial thromboplastin time	Similar	Lee et al., 2013
Cattle	Meat fed rat	Morphological characteristics, body weight, feed intake, water intake, blood tests, urine tests, eyeball tests, organ weight, histopathological interpretation	Similar	Kang, 2003
Cattle	Meat powder fed rat	Mortality, body weight, food consumption, sensory/reflex function, grip strength, motor activity, estrus cycle, urinalysis, hematology, calcium, inorganic phosphorus, γ -GTP, organ weight, pathology	Similar	NARO ILGS, 2011a
Cattle	Milk powder fed rat	Mortality, body weight, food consumption, sensory/reflex function, grip strength, motor activity, estrus cycle, urinalysis, hematology, calcium, inorganic phosphorus, γ -GTP, organ weight, pathology	Similar	NARO ILGS, 2011a
Cattle	Milk powder fed	Erythrocytes, leukocytes, crystals, epithelial cells, casts, fat globules, body weight, brain, salivary gland, heart, lung, liver, kidney, adrenal gland, spleen, pituitary gland, thyroid gland, prostate, seminal vesicle, testis, epididymis, ovary, uterus	Similar	NARO ILGS, 2011a
Genotoxic				
Cattle	Meat	<i>S. typhimurium</i> : TA 100, TA 1535, TA 98, TA 1537, <i>E. coli</i> : WP2uvrA, Positive control (+) S9 mix: TA100, TA1535, TA98, TA1537, WT2uvrA, Positive control (–) S9 mix: TA100, TA1535, TA98, TA1537, WP2uvrA	None	Lee et al., 2011
Cattle	Meat fed mouse	Morphology, body weight, feed intake, frequency of micronuclei induction, bone marrow pathology	None	Kang, 2006
Mammalian cells	Meat	Chromosome aberration test, Tetrazolium-based colorimetric (MTT) assay	None	Kang, 2006
Cattle	Meat	<i>S. typhimurium</i> : TA98, TA100, TA1535, TA1537, <i>E. coli</i> : WP2uvrA, Positive control (+) S9 mix: TA98, TA100, TA1535, TA1537, WT2uvrA, Positive control (–) S9 mix: TA98, TA100, TA1535, TA1537, WP2uvrA	None	Kang, 2006
Cattle	Meat fed mouse	Body weight, incidence of micronucleus appearance, polychromatic erythrocyte rate	Negative	NARO ILGS, 2011a
Cattle	Milk fed Mouse	Body weight, incidence of micronucleus appearance, polychromatic erythrocyte rate	Negative	NARO ILGS, 2011a
Reproduction toxic				
Cattle	Meat fed rat	Weight of cauda epididymis, weight of testis, epididymal sperm, deformity, motility, per 10 squares, per gram cauda, testicular sperm per 10 squares, per gram of testis	None	Hwang et al., 2010
Cattle	Meat fed pregnant rabbit	Corpus luteum implantation, number of live fetuses, sex ratio, number of fetal death, fetal mortality	None	Lee et al., 2010
Cattle	Meat fed rat	Mating results: pairs mated, coitus confirmed, pregnant, male fertility, female fertility, Cesarean section analysis: implantation, resorption	None	Yang et al., 2011
Cattle	Meat fed rat and rabbit	Mortality, body weight, feed intake, organ weight, histopathological interpretation, pregnant, cesarean section, birth weight, placenta weight, gender ration, organ morphology, skeletal examination	None	Kang, 2006
Cattle	Meat fed rat	Morphological characteristics, mortality, body weight, feed intake, weaning period test, autopsy, organ weight, histopathological interpretation	Similar	Kang, 2006
Cattle	Meat powder fed rat	Mortality, body weight, food consumption, sensory/reflex function, grip strength, motor activity	Mostly similar	NARO ILGS, 2011a
Cattle	Milk powder fed rat	Reproductive functions: estrus cycle, copulation index, fertility index, gestation length and gestation index	Mostly similar	NARO ILGS, 2011a
Cattle	Meat powder fed rat	Ophthalmology, urinalysis, hematology, red blood cells, hemoglobin, hematocrit, white blood cells, clinical chemistry, organ weight, pathology	Similar	NARO ILGS, 2011a
Cattle	Milk powder fed rat	Mortality, body weight, food consumption, sensory/reflex function, grip strength, motor activity	Similar	NARO ILGS, 2011a
Cattle	Milk fed mouse	Reproductive functions: estrus cycle, copulation index, fertility index, gestation length and gestation index	Similar	NARO ILGS, 2011a
Cattle	Meat powder fed rat	Ophthalmology, urinalysis, hematology, red blood cells, hemoglobin, hematocrit, white blood cells, clinical chemistry, organ weight, pathology	Similar	NARO ILGS, 2011a
Cattle	Milk powder fed rat	Litter size, live birth index, sex ratio, lactation index, body weight, external abnormalities, organ abnormalities, visceral malformation, sensory/reflex function	Similar	NARO ILGS, 2011a
Cattle	Milk fed mouse	Litter size, live birth index, sex ratio, lactation index, body weight, external abnormalities, organ abnormalities, visceral malformation, sensory/reflex function	Similar	NARO ILGS, 2011a
Cattle	Milk fed mouse	Estrous cycle, copulation, fertility index, gestation length, gestation index	Similar	NARO ILGS, 2011a
Allergic reactions				
Cattle	Meat	Meat digestibility, allergenicity and mutagenicity after feeding to mice and rats	None	Takahashi and Ito, 2004
Cattle	Milk	Allergic reaction (diameter of dye leakage)	Different	NARO ILGS, 2011a
Cattle	Meat fed rat	Passive cutaneous anaphylaxis test	None	Kang, 2006

The third step is analysis of similarities of reproduction and adverse breeding effects between rodent models who consumed meat and milk from the progeny of cloned animals and noncloned counterparts.

The fourth step is analysis of similarity of genotoxicity in in vitro and rodent animal models who consumed meat and milk from the progeny of cloned animals and noncloned counterparts.

The fifth step is analysis of similarities of allergic reactions in in vitro and rodent animal models that consumed meat and milk from the progeny of cloned animals and noncloned counterparts. After all the steps of risk assessment were finished, the question was whether to allow meat and milk derived from cloned animals into the food supply.

5.1. Four major principles of risk assessment of meat and milk derived from cloned animals

As result of the extensive survey, this author suggests four major principles of risk assessment of meat and milk derived from cloned animals for the guidelines (Table 2).

If cloned animals and their progenies are healthy, then the meat and milk derived from them are safe.

If chemical composition is similar between cloned animals and their noncloned counterparts, then their meat and milk are safe. If meat and milk quality is similar between cloned animals and their noncloned counterparts, then their meat and milk are safe. If genotoxicity, adverse reproductive effects, and allergic reactions under the influence of meat and milk are similar between cloned animals and their noncloned counterparts, then their meat and milk are safe.

For risk assessment, comparison conditions (breed, gender, age, body weight, diet, or experiments) between cloned animals and their noncloned counterparts must be the same.

6. The procedure of risk assessment before slaughtering

6.1. Establishment of a tracking system from the farm to market

As mentioned above, determining where animal products come from, i.e., from cloned animals produced by SCNT or from noncloned animals, is difficult because clones and cell donor animals share exactly the same genetic information, and cloning (SCNT) does not involve any genetic modification. Therefore, enforcement of regulations or control by the government against violations of labeling and misuse have been difficult to date. Therefore, record management for the background of all the cloned animals, cell donors, surrogate dams, and the animal farm are a prerequisite for guideline establishment. Every individual animal should be fitted with a radio-frequency identification (RFID) tag from birth to market, and the government or a related organization should build this tracking system.

Table 2

Four principles for risk assessment of meat and milk derived from cloned animals for guidelines.

No	Principles for risk assessment	Remarks
1	If cloned animal and their progenies are healthy, the derived meat and milk are safe	Comparison conditions (breed, gender, age, body weight, diet and experiments) must be the same
2	If chemical composition is similar between cloned animals and their noncloned counterparts, then their meat and milk are safe	
3	If meat and milk quality are similar between cloned animals and their noncloned counterpart, then their meat and milk are safe	
4	If genotoxicity, reproduction toxicity, and allergenicity of meat and milk are similar between cloned animals and their noncloned counterparts, then their meat and milk are safe	

6.2. Standardization of SCNT

Animal cloning by SCNT has been widely done, but success rates with SCNT are still lower, and the procedures of SCNT are a little different although the main principles of SCNT are the same. Therefore, standard procedures of SCNT, i.e., chemicals, equipment settings, or laboratory conditions are prerequisites for animal cloning.

6.3. Determining the health of all animals (cell donor and surrogate dams)

To increase the success and stability of animal cloning, all animals such as cell donors and surrogate dams need to be healthy. The main monitoring items for the health of cell donor would be the exact family lineages, breed, birth weight, body weight, daily gain, feed consumption, and gender for swine or cattle, and milk production, milk composition, milk somatic cell counts, litter size, and drug use for lactating cows. The immune function related monitoring items such as number of lymphocyte, peripheral blood neutrophils, monocytes and spleen and thymus weights should be consider in all animals.

6.4. The first decision

After establishment of a tracking system, standardization of SCNT and the conditions of cloned animals' health testing, the meat and milk derived from a cloned animal go to the next step: "risk assessment."

7. The procedure of risk assessment of meat and milk derived from cloned animals

There are five levels in the procedure of risk assessment of meat and milk derived from cloned animals for guideline establishment and approval; a survey of human consumption experience (or history) for longtime, analysis of similarities of chemical composition, adverse reproductive effects, genotoxicity, and allergic reactions. The 30 days or 90 days rodent feeding studies (adapted from EFSA GMO Panel Working Group on Animal Feeding Trials) are recommend for analysis of similarities of chemical composition, adverse reproductive effects, genotoxicity, and allergic reactions. As mentioned in the Fig. 1, the order of the procedure of risk assessment of meat and milk derived from cloned animal is a survey of human consumption history, analysis of similarities of chemical composition, adverse reproductive effects, genotoxicity, and allergic reactions, respectively. As shown in the Table 3, it is recommended that toxicological evaluations should performed starting from low risk factors to high risk factors, i.e. from the organ weight to morphological changes or from in vitro test to in vivo test.

7.1. Survey of human consumption experience in the world

The extensive survey of consumption experience of meat and

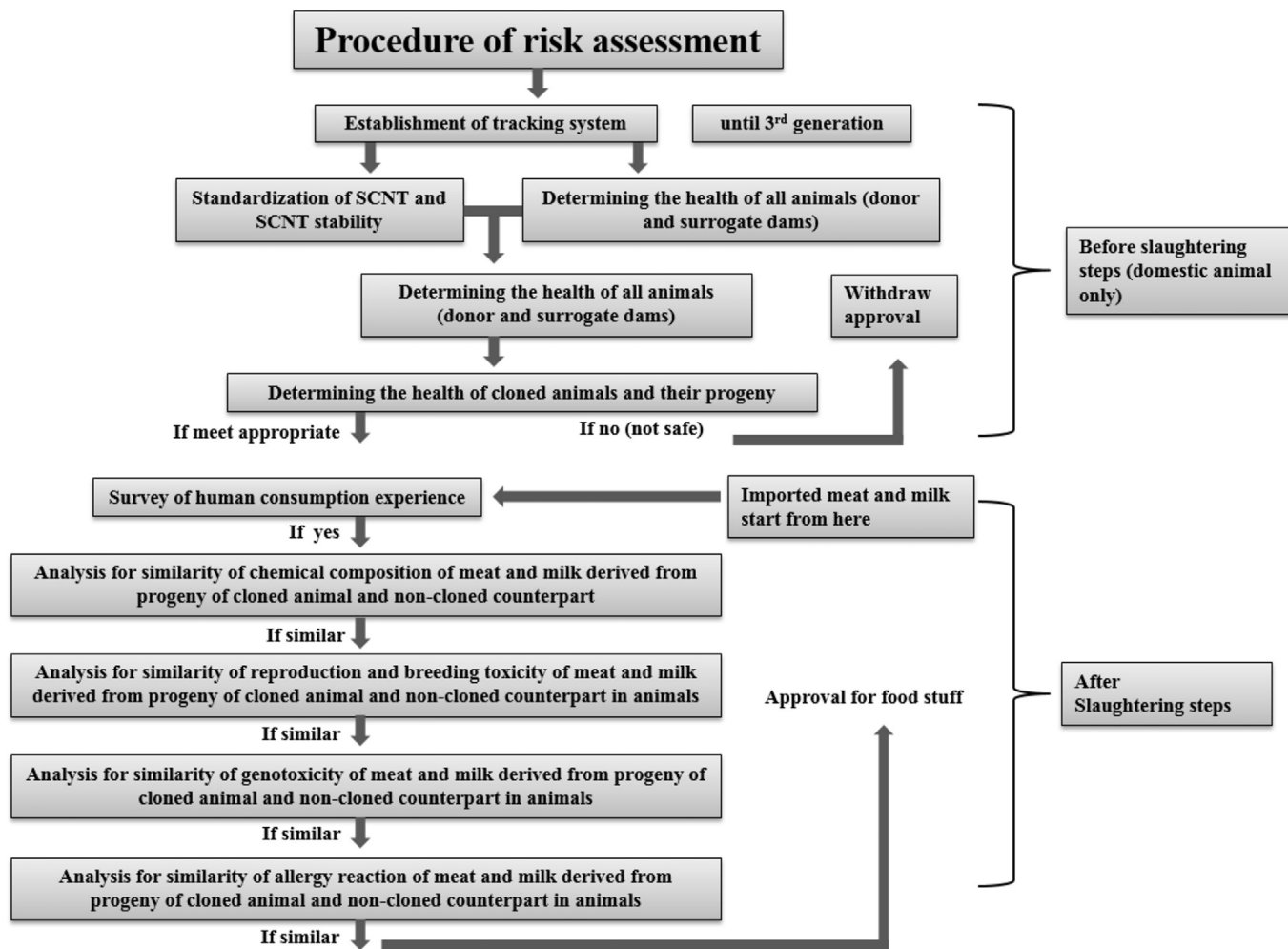


Fig. 1. The procedure of risk assessment for the guidelines on meat and milk derived from cloned animals.

milk derived from cloned animals is the priority for risk assessment. Because long-term consumption without significant issues means that meat and milk derived from cloned animals are as safe as normal foods. The experience of consumption for long periods in some parts of the world will have a positive effect on the use of meat and milk derived from cloned animals. Nonetheless, if there is no consumption experience of meat and milk derived from cloned animals, conditions of approval will be more delicate, and demands are becoming more complex. The objects for the survey of human consumption experience are regions or countries, periods of consumption, types of consumption, amount of consumption, permission, consumer awareness, or data on related diseases.

7.2. Analysis of similarities of chemical composition of meat and milk derived from progeny of cloned animals and from noncloned counterparts

To analyze similarities of chemical composition of meat and milk derived from cloned animals and noncloned counterparts, the experimental items are cited from the above-mentioned studies (Table 1). The experimental items are presented in Table 3. In the meat sample, chemical composition test items are general chemical composition (concentration of water, carbohydrates, protein, fat, ash, and cholesterol), amino acid composition, fatty acid composition, and mineral concentrations. The physicochemical characteristics such as alcohol testing, a specific-gravity test, acid value, pH,

and somatic cell counts are additional assays for milk samples. The comparison conditions such as breed, gender, age, body weight, diet, and experimental methods must be the same between cloned animals and noncloned counterparts.

7.3. Analysis of similarities of reproduction and adverse breeding effects of meat and milk derived from the progeny of cloned animals and noncloned counterparts in rodent models

To analyze the similarities of reproductive and adverse breeding effects of meat and milk derived from cloned animals or from noncloned counterparts, the assays are cited from the above-mentioned studies (Table 1). In general, rodent feeding tests may be recommended for analysis of reproduction and adverse breeding effects. After dietary meat and milk samples derived from cloned animals or from noncloned counterparts were consumed for more than 1 month, health tests related to reproduction, reproductive organ weight, hematological parameters, and urinalysis could be recommended. The experimental items are presented in Table 3.

7.4. Analysis of similarities of genotoxicity of meat and milk derived from the progeny of cloned animals and noncloned counterparts in *in vitro* and rodent models

To analyze the similarity in genotoxicity of meat and milk derived from cloned animals and noncloned counterparts, the

Table 3

Analysis of similarities of chemical composition, adverse reproductive effects, genotoxicity, and allergic reactions under the influence of meat and milk derived from progeny of cloned animals and noncloned counterparts.

Subject	Chemical composition test items	Remarks
Meat	Chemical composition: water, carbohydrate, protein, fat, ash, cholesterol Amino acid composition Fatty acid composition Mineral contents: sodium, calcium, potassium, zinc, iron, phosphorus, magnesium, copper, manganese etc.	Comparison conditions (breed, gender, age, body weight, diet and experiments) between cloned animal and non-cloned counterpart must be the same
Milk	Chemical composition Amino acid composition Fatty acid composition Mineral contents: sodium, calcium, potassium, zinc, iron, phosphorus, magnesium, copper, manganese etc. Physicochemical characteristics: alcohol test, specific gravity test, acid value, pH, somatic cell counts	
Adverse reproduction effects		
Dietary meat and milk	Health test related with reproduction: body weight, feed intake, activity, estrous cycle, rate of pregnancy, delivery rate, litter size, number of mating, monstrosity, etc. Organ weight and conditions: brain, heart, lung, liver, kidney, thyroid gland, adrenal gland, spleen etc. Reproductive organs: testis weight, ovary weight, uterus weight, morphologic change of testis and ovary, morphologic change of sperm and ovum, sperm viability etc. Hematological test: white blood cells, red blood cells, hemoglobin, hematocrit, AST, AST etc. Urinalysis: glucose, pH, ketone, white blood cells, hemoglobin, bilirubin etc.	Feeding test of rodent animals between cloned animals and noncloned counterparts
Genotoxicity		
Meat and milk	In vitro chromosome aberration test In vivo micronucleus test Bacterial reverse mutation assay	
Allergy reactions		
Meat and milk	In vitro digestibility of allergens and nonallergenic proteins Multiple allergen screening test Passive anaphylaxis test Active anaphylaxis test	Human blood Guinea pig Brown Norway rat and Balb/C mice

assays are cited from the above-mentioned studies (Table 1). The most successful and reliable assays of genotoxicity are the in vivo micronucleus test, bacterial reverse mutation assay, and the in vitro chromosome aberration test. The in vivo micronucleus test uses rodent bone marrow or peripheral blood and this test is most widely used for in vivo genotoxicity test guidelines. The Ames test as a bacterial reverse mutation assay is widely used for analysis of mutations in the DNA of the test organism. The Ames test serves as a convenient assay to determine the carcinogenic potential of certain compounds. The in vitro chromosome aberration test is used to identify agents that cause structural chromosomal aberrations in cultured mammalian cells (Galloway et al., 1987). Therefore, the present author recommends these three tests for analysis of genotoxicity.

7.5. Analysis of similarities in the allergic reactions to meat and milk derived from the progeny of cloned animals and noncloned counterparts in in vitro and rodent models

To analyze similarities in allergic reactions to meat and milk derived from cloned animals and noncloned counterparts, the assays are cited from the above-mentioned studies (Table 1). The author found after the survey that the most common tests to determine an allergic reaction is in vitro digestibility of allergens and nonallergenic proteins, the multiple-allergen screening test, and a passive anaphylaxis test. Information on the comparative digestibility of food allergens and nonallergenic proteins is crucial when resistance to digestion is to be used as a criterion to assess the allergenic potential of a novel protein (Fu et al., 2002). Multiple allergen screening tests are referred to as an immunoassay including an enzyme-linked immunosorbent assay and

radioallergosorbent test, and this test detects and measures the amount of allergen-specific antibodies in human blood. A passive anaphylaxis test in a guinea pig model is a successful and reliable assay of allergic reactions to animal products (Ceballos et al., 2009; Bevilacqua et al., 2001; El-Agamy, 2007). The active anaphylaxis test in Brown Norway rat model and Balb/c mice strain are suitable for oral allergy model. Therefore, this author recommends these tests for analysis of allergic reactions.

7.6. Final decision

After a survey of human consumption experience and determination of the similarity of meat and milk derived from cloned animals and noncloned counterparts, if the chemical composition, adverse effects, and allergic reactions to meat and milk are similar between cloned animals and noncloned counterparts, then the meat and milk derived from cloned animals' progeny can be considered a novel food source.

8. Conclusion

In this paper, I extensively surveyed the risk assessment studies of animal products derived from cloned animals, and the assays for establishment of guidelines are cited from various studies. This author found that there is no evidence that meat and milk derived from cloned animals or their progeny pose a food safety risk in terms of genotoxicity, adverse reproductive effects, or allergic reactions. There are no countries in the world that established the guidelines on meat and milk derived from cloned animals at present. Up to the present, determining where animal products came from, i.e., cloned animals produced by SCNT or noncloned animals,

has been difficult because clones and cell donor animals share exactly the same genetic information, and cloning (SCNT) does not involve any genetic modification. Therefore, a tracking system of animals and farms of origin should be set up prior to establishing the guidelines. To establish the guidelines, this author suggests four principles of safety assessment of meat and milk derived from cloned animals. Four main principles for safety assessment are similarities of chemical compositions, adverse reproductive effects, genotoxicity, and allergic reactions to meat and milk from cloned animals and noncloned counterparts. This principle means that meat and milk derived from cloned animals are safe if there are no differences in the four safety assessments of meat and milk between cloned animals or their progeny and noncloned counterparts.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.fct.2017.07.047>.

References

- Aoki, S., Takahashi, R., Nisisouzu, T., Kitamura, S., Dochi, O., Kishi, M., Morita, S., Komiya, M., Tarawaki, Y., Hoyama, H., 2003. A comparative investigation of the characteristics of Holstein coes cloned from colostrum-derived mammary gland epithelial cells in an automatic milking system. *Theriogenology* 59, 324.
- Archer, G.S., Friend, T.H., Piedrahita, J., Nevill, C.H., Walker, S., 2003. Behavioral variation among cloned pigs. *Appl. Anim. Behav. Sci.* 82, 151–161.
- Bernard, L., Richard, C., Gelin, V., Leroux, C., Heyman, Y., 2015. Milk fatty acid composition and mammary lipogenic genes expression in bovine cloned and control cattle. *Livest. Sci.* 176, 188–195.
- Bevilacqua, C., Martin, P., Candali, C., Fauquant, J., 2001. Goats' milk of defective α s1-casein genotype decreases intestinal and systemic sensitization of to β -lactoglobulin in Guinea pigs. *J. Dairy Res.* 68, 217–227.
- Butler, J.E.F., 2009. Cloned animal products in the human food chain: FDA should protect american consumers. *Food Drug Law J.* 64, 473–501.
- Ceballos, L.S., Sampelayo, M.R.S., Extremera, F.G., Osorio, M.R., 2009. Evaluation of the allergenicity of goat milk, cow milk, and their lactosera in a Guinea pig model. *J. Dairy Sci.* 92, 837–846.
- El-Agamy, E.I., 2007. The challenge of cow milk protein allergy. *Small Ruminant Res.* 68, 64–72.
- FDA Center for Veterinary Medicine, 2008. Animal Cloning: a Risk Assessment. Report. <http://www.fda.gov/AnimalVeterinary/SafetyHealth/AnimalCloning/>.
- Fu, T.J., Abbott, U.R., Hatzos, C., 2002. Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid-A comparative study. *J. Agric. Food Chem.* 50, 7154–7160.
- Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., Zeiger, E., 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ. Mol. Mutagen.* 10, 1–35.
- Heyman, Y., Chavatte-Palmer, P., Berthelot, V., Fromentin, G., Hocquette, J.F., Martignat, L., Renard, J.P., 2007. Assessing the quality of products from cloned cattle: an integrative approach. *Theriogenology* 67, 134–141.
- Hwang, S., Lee, N.J., Hwang, J.S., Yang, B.C., Im, G.S., Ko, Y.G., Park, E.W., Park, S.B., Kang, J.K., Seong, H.H., 2010. Effect of cloned-cattle meat on reproductive physiology in rats. *Animal* 4, 218–223.
- Kang, J.K., 2003. Acute and Chronic Safety Studies of Products (Beef, Milk Powder) of Somatogenic Cow. Ministry for Food, Forestry and Fisheries. Report. <http://www.ndsl.kr/ndsl/search/detail/report/reportSearchResultDetail.do?cn=TRKO201400023793>.
- Kang, J.K., 2006. Reproductive and Genetic Toxicity Studies of Products (Beef Powder) of Somatic Cell Clone Cow. Ministry for Food, Forestry and Fisheries. Report. <http://www.ndsl.kr/ndsl/search/detail/report/reportSearchResultDetail.do?cn=TRKO201400023065>.
- Laible, G., Brophy, B., Knighton, D., Wells, D.N., 2007. Compositional analysis of dairy products derived from clones and clones transgenic cattle. *Theriogenology* 67, 166–177.
- Lee, N.J., Yang, B.C., Hwang, J.S., Im, G.S., Ko, Y.G., Park, E.W., Seong, H.H., Park, S.B., Kang, J.K., Hwang, S., 2010. Effects of cloned-cattle meat diet on reproductive parameters in pregnant rabbits. *Food Chem. Toxicol.* 48, 871–876.
- Lee, N.J., Yang, B.C., Im, G.S., Lee, S.S., Seong, H.H., Park, J.K., Chang, W.K., Kang, J.K., Hwang, S., 2013. No long-term feeding toxicities on the health status in rats fed with cloned Korean native cattle (Hanwoo) meat. *Toxicol. Pathol.* 41, 872–879.
- Lee, N.J., Yang, B.C., Jung, Y. r., Lee, J.W., Im, G.S., Seong, H.H., Park, J.K., Kang, J.K., Hwang, S.S., 2011. In vitro and in vivo genotoxic effects of somatic cell nuclear transfer cloned cattle meat. *Food Chem. Toxicol.* 49, 2273–2278.
- NARO Institute of Livestock and Grassland Science, 2011a. Characteristics of Milk/meat Derived from Progeny of Somatic Cell Cloned Cattle. Report. https://www.naro.affrc.go.jp/publicity_report/publication/files/naro-se/mem-nilgs11.pdf.
- NARO Institute of Livestock and Grassland Science, 2011b. Somatic Cell Cloned Cattle and Their Progeny Produced in Japan: a Report for Animal Health and Characteristics of Animal Products. Report. https://www.naro.affrc.go.jp/publicity_report/publication/files/naro-se/mem-nilgs12.pdf.
- Panarace, M., Aguero, J.I., Garrote, M., Jauregui, G., Segovia, A., Cane, L., Gutierrez, J., Marfil, M., Rigali, F., Pugliese, M., Young, S., Lagioia, J., Garnil, C., Forte Pontes, J.E., Ereno Junio, J.C., Mower, S., Medina, M., 2007. How healthy are cloned and their progeny: 5 years of field experience. *Theriogenology* 67, 142–151.
- Rudenko, L., Matheson, J.C., Adams, A.L., Dubbin, E.S., Greenlees, K.J., 2004. Food consumption risks associated with animal clones: what should be investigated? *Cloning Stem Cells* 6, 79–93.
- Takahashi, S., Ito, Y., 2004. Evaluation of meat products from cloned cattle: biological and biochemical properties. *Cloning Stem Cells* 6, 165–171.
- Tian, X.C., Kubota, C., Sakashita, K., Izaike, Y., Okano, R., Tabara, N., Curchoe, C., Jacob, L., Zhang, Y., Smith, S., Bormann, C., Xu, J., Sato, M., Anderw, S., Yang, X., 2005. Meat and milk composition of bovin clones. *PNAS* 102, 6261–6266.
- Tome, D., Dubarry, M., Fromentin, G., 2004. Nutritional value of milk and meat products derived from cloning. *Cloning Stem Cells* 6, 172–177.
- Walker, S.C., Christenson, R.K., Ruiz, R.P., Reeves, D.E., Pratt, S.L., Arenivas, F., Williams, N.E., Bruner, B.L., Polejaeva, I.A., 2007. Comparison of meat composition from offspring of cloned and conventionally produced boars. *Theriogenology* 67, 178–184, 2007.
- Walsh, M.K., Lucey, J.A., Govindasamy-Lucey, S., Pace, M.M., Bishop, M.D., 2003. Comparison of milk produced by cows cloned by nuclear transfer with milk from no-cloned cows. *Cloning Stem Cells* 5, 213–219.
- Wells, D.N., Forsyth, J.T., McMillan, V., Oback, B., 2004. The health of somatic cell cloned cattle and their offspring. *Cloning Stem Cells* 6, 101–110.
- Yamaguchi, M., Ito, Y., Takahashi, S., 2007. Fourteen-week feeding test of meat and milk derived from cloned cattle in the rat. *Theriogenology* 67, 152–165.
- Yang, B.C., Lee, N.J., Seong, G.S., Park, J.K., Kang, J.K., Hwang, S., 2011. A diet of somatic cell nuclear transfer cloned-cattle meat produced no toxic effects on behavioral or reproductive characteristics of f1 rats derived from dams fed on cloned-cattle meat. *Brith Defects Res. B Dev. Reprod. Toxicol.* 92, 224–230.

ICMJE Form for Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

1. Identifying information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Intellectual Property.

This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

5. Relationships not covered above.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.

Grant: A grant from an entity, generally [but not always] paid to your organization

Personal Fees: Monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting, lectures, speakers bureaus, expert testimony, employment, or other affiliations

Non-Financial Support: Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.

Other: Anything not covered under the previous three boxes

Pending: The patent has been filed but not issued

Issued: The patent has been issued by the agency

Licensed: The patent has been licensed to an entity, whether earning royalties or not

Royalties: Funds are coming in to you or your institution due to your patent



ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name)

Sun Jin

2. Surname (Last Name)

Hur

3. Date

25-March-2017

4. Are you the corresponding author?

☒

Yes

☐

No

5. Manuscript Title

A study on current risk assessments and guidelines on the use of animal products derived from cloned animals

6. Manuscript Identifying Number (if you know it)

FCT-S-17-00669

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest?

☐

Yes

☒

No

ADD

Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were **present during the 36 months prior to publication**.

Are there any relevant conflicts of interest?

☐

Yes

☒

No

ADD

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work?

☐

Yes

☒

No



ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 5.

Relationships not covered above

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- ☐ Yes, the following relationships/conditions/circumstances are present (explain below):
- ☒ No other relationships/conditions/circumstances that present a potential conflict of interest

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Section 6.

Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Generate Disclosure Statement

Dr. Hur has nothing to disclose.

Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.