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Animal cloning drawbacks an-overview

Abstract

Cloning is powerful tool for production of genetically identical copies of desired donor animal but its success is still questionable. Due to number of factors, many scientist and common people are against cloning. Up to now it's consider as inefficient technique due to high failure of cloned animal growth from gestation to adulthood. Mostly losses in cloned animals are due to placental abnormalities, cardiovascular and respiratory problems. These anomalies are most likely due to incorrect epigenetic reprogramming of the donor genome, leading to inappropriate patterns of gene expression during the development of clone. "Large offspring syndrome" is an example of phenotypic anomalies in cloned animals. Including this animals' welfare, health and the significant consequence on food safety are reason behind its insufficiency. That's why still more work need to understand exact cause of failure during pre- and postnatal development of cloned. This article generally focused the problems, due to which cloning is considered as an inefficient technique.

Keywords: animal, cloning, drawbacks, food safety, pregnancy

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Introduction

A sexually Production of genetically identical organisms is knows as cloning it involves division of a single embryo, either nuclear genes and the small number of mitochondrial genes would be "identical", or it may involve nuclear transfer, in which case only the nuclear genes would be "identical". After struggle of many year in 1995 scientists cloned two lambs "Megan and Morag" at "Roslin Institute" in Scotland and they were cloned from cells from an early embryo,[1] Although amphibians was already successfully cloned in the 1950s. After that it was reported that a number of lambs had been born by cloning. In February 1997 after 277 attempts a lamb was cloned named as "Dolly "the life span of that lamb was quite more than other cloned lambs.[2] Cloning of Dolly heralded the commencement of a new period in agriculture with the possibility for the protection and rapid multiplication of selected genotypes.[3] Up to now many animal animals have been cloned by nuclear transfer in mammals like sheep, goat, cattle, rabbits, mice.[4] Cloning has many application and it make easy to rapidly propagate desirable animal stocks, propagating transgenic livestock, targeted genetic alterations in domestic animals and conservation of endangered species.[1] But some of question are still un solved like, Ethical and moral issues, and the low success rates with somatic cell nuclear transfer due to epigenetic errors as a result of inaccurate nuclear programming,[5] all the factors have restricted the satisfactoriness and applicability of cloning in agriculture.

Cloning limitation is due to complex interaction of many reasons like animal welfare and lack of confidence in long term health of cloned animal.[6] During the use of cloning technique mostly apparently good quality embryos fail to flourish during pregnancy or following birth.[7] Failure in reprogramming of donated nucleus is main cause of flop because of its importance in correct pattern of gene expression to occur during subsequent embryogenesis.[8] This reprogramming must occur within a short timeframe, in a different cellular context compared with normal development, and is prone to error. There are increasing amounts of data documenting deviations in epigenetic reprogramming.[9]

Failure rate of cloning is very high,[10] many cloned embryos may develop to preimplantation stage, but the vast majority will not result in a viable pregnancy.[11] Despite the use of healthy, fertile synchronous females as recipients it was reported that 50% loss occurred during early first trimester and near about 80% miscarry by second trimester mostly due to placental abnormalities.[12] According to studies in cows and sheep most dramatic period of fetal loss is at the time of placental attachment.[4] After completing gestation cloned animals face greater difficulties adjusting to extra uterine life,[13] they look like normal but they are genetically different and that differences are due to epigenetic abnormalities acquired during nuclear reprogramming.[10] Many Gestational and neonatal abnormalities are found in cloned animal,[12] that is may be due irregular expression and likely incomplete reprogramming of imprinted genes.[14] According to some other reports the difference is due abnormalities in telomere length, gene expression or methylation patterns.[4]

Another problem that is found in Cloned animal is placental abnormalities. According to many scientists early death of embryo is due to poor development of placenta. In cloned animal abnormal development of allontoic membrane and reduced development of placental blood vessel has been reported, number of placentome are quit less in cloned animal[15] and it appears that placental gas exchange capacity is significantly reduced as late gestation cloned foetuses have been found to be hypoxic.[16]

Post-natal viability of cloned offspring is lower in cloned animal but it depends on animal.[17] In post-natal mostly calves showed respiratory problems[18] cardiovascular, skeletal and central nervous systems problem.[7] The potential for epigenetic errors effects on the composition of cloned animals that's why safety of food products derived had major obstacle in gaining regulatory approval for entry into the food chain.

Epigenetic anomalies

Epigenetics means ''the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes

in DNA sequence.[19] According to theory clone and the donor animal should have same genome but it's not likely that cloned mammals occasionally show developmental anomalies due to epigenetic errors.[20] Sometime overgrowth of placenta and foetus termed as "large offspring syndrome"[15] other anomalies include respiratory distress, major cardiovascular abnormalities, and enlargement of organs are commonly seen in cloned ruminants[21] that's why applicability of cloning in agriculture is very limited.[22]

The practical outcome is that there are many cloned animals that behave and appear normal, while closer investigations have revealed that even some of these apparently normal animals are subtly different from the naturally produced population.[23] In cloning, when somatic cell is transferred from donor to recipient at that time somatic cell nucleus must be speedily reprogrammed to presume its new life of an embryonic cell.[24] In any case if cell unable to reprogram or in complete reprogramming lead to abortive development and possibly non-lethal abnormalities in surviving clones.[4] Some genes are correctly expressed in cloned embryos, such as important metabolic enzymes but expression of some other gene mostly abnormal that affect the future life of clone. In such condition mostly clone apparently experienced a normal pregnancy and neonatal period, the outlook for a normal life appears good, but placental development and the intrauterine environment for many clones is suboptimal and this alone may impact on their health in later life.[25]

DNA methylation play a important role in gene expression which control animal development.[5] It is reported that epigenetic errors primarily in the patterns of DNA methylation and chromatin organization in cloned embryo.[19] Frequently, the donor cell pattern of DNA methylation is maintained during pre implantation development[14] which lead to effect reprogramming and that cause abnormal placentation.[26] Secondary that's an important reason for the low birth rate.[24]

Placental abnormalities

Placenta is main organ of materno-fetal contact, it plays a vital role in maintaining pregnancy, and it maintains the fetus by satisfying positive nutrient partitionning and critical endocrine functions. Fetal survival and development during pregnancy depends on appropriate morphological and functional development of the placenta. Cloning is always associated with placental abnormalities and Indications of placental failure in nuclear transfer pregnancies include anomalies such as large offspring syndrome, altered placental and fetal membrane proteins, increased placental weight, and placentome enlargement and edema in cattle.[27] Hydroallantois has been classified by Farin as a Type II abnormal offspring syndrome condition in This pathologic placental condition fetal fluid increases and create difficulties in locating the fetus within the uterine horn.[27]

In early pregnancy of cloned animal fetal death rate is near about 80%[26] that is mostly due to malfunction and underdevelopment of placenta.[15] Typically, in cattle, 50-70% of pregnancies at day 50 are lost throughout the remainder of gestation and up to term due to lack of placental vascularisation and attachment sites which are important for nutrient exchange and prevent foetus from hypoxic condition.[7] In third trimester of cows pregnant with cloned foetuses; some placental abnormalities, such as edema and hydroallantois with occurring chance near about 45%, have been also reported.[28] The hydroallantois condition most commonly observe between Days 150 and 180 of cloned pregnancies.[28] According to research the dysfunction and enlargement of placenta can be due to placenta proteins that showed an over expression of TIMP-2[29] but according to some other it can be due to alteration gene expression in trophoblast of cloned placentas.[30]

Difficulties in parturition

At present, cloning generally has low efficiency, as of quite a lot of factors, like reprogramming of nucleus, differentiation of donor cells, and situation of oocyte.[31] Reprogramming period is the key stage for optimizing cloning.[32] At that time if reprogramming not take place properly then it lead to epigenetic abnormalities and increase the death rate. and somatic cloned calves are apparently heavier than embryonic clones[33] that is also a example of epigenetic abnormalities which increase the birth complication.[13] Large sized fetus including all neonatal abnormalities due to epigenetic error termed as "large calf syndrome".[34] It is common that gestation length in nuclear transfer pregnancies is quit prolong, that is due to failure of placenta to fetal cortisol near term or to a lack of ACTH release from the fetus.[26] Hormonal imbalance and large size of fetus are the step toward dystocia, in addition to related post-partum problems can also have serious negative effects on the dam's health as well as her future production and reproductive performance in the herd.[7]

Pre and postnatal viability

Embryo is considered a good quality if number of cell are quite good because it is an important criterion that verify the feasibility of the embryo after transfer into a surrogate mother so the low number of cell in the cloned embryo will be linked with a low percentage of survivability after embryo transfer in several species.[35] Cloned bovine embryos with a higher percentage of apoptotic blastomeres showed lower pregnancy rate after 90days of embryo transfer and subsequently lower calving rate.[36]

Postnatal viability is markedly lower for many cloned.[37] The proportion of cloned calves born that are longer-term survivors ranges between 47% and 80%.[38] At birth, cloned calves and lambs commonly show signs of a stressful uterine environment; Placental reserve capacity is most likely limited due to inadequate development.[7] It is apparent that fetal viability in cloned animals varies between experiments and between species, with cloned mice and goats displaying better post natal viability that is may be due to technique, animal strain, or to placental type.[26] Some other cases are also reported about sallow cord of cloned calves which can be a potential route of death in postnatal.[39] In cloned calves enlarged umbilical veins and arteries are also important cause of death in post natal due to sepsis in umbilical structures.[39]

According to new research vascular problems are also a cause of post-natal death like pulmonary hypertension, lesions, edema and pleural effusions, in addition to capillary congestion of the alveolar septa and pulmonary thrombosis causing hemodynamic disturbances. These alterations likely inhibited complete alveolar expansion and explained the pulmonary insufficiency that contributed to the low post natal survival rate of cloned calves.[40] These vascular developmental problems resulted as the primary or key alterations due to epigenetic modifications caused by cloning.

Although these particular epigenetic aberrations may be minor and not a welfare issue for the animal, they may limit some practical applications of the technology because they decrease the potential uniformity of cloned livestock.[41] A clone phenotype well-known diagonally species is confirmation of compromised immune systems,

with thymic aplasia in cloned cattle and lower antibody production in cloned mice.[26] Compromised immune systems may increase their propensity to infection and disease.[9]

In postpartum of Blood samples collected from the cloned lambs after birth revealed a wide range of abnormalities indicative of kidney and liver dysfunction.[32] Further losses throughout the post-natal period are mostly due to abnormalities of the cardiovascular, skeletal and central nervous systems, umbilical and lung infections, along with digestive and kidney disorders.[7] Post natal losses in sheep are greater than cattle.[26]

Phenotypes of adult clones

If expression patterns genes is abnormal at time of pre-implantation and in early implantation stages then morphology of clone will be also abnormal.[42] In most of cases clone look like normal and have normal physiology but still many report are present that show the abnormalities associated with phenotype,[41] like "large offspring syndrome".[9] That is due to error in gene expression of fetal growth and development.[43] The incidence of these anomalies may vary according to species, genotype, sex, type of cell or specific aspects of the Nuclear transfer protocol.[4]

High failure rate

One barrier to the practical use of techniques involving somatic cell nuclear transfer is the low possibility of cloned embryos; only a few percent of reconstructed oocyte are always able to develop.[43] Up-to now success rate if cloning of intra species is 1%[44] despite use of healthy and synchronize recipient in addition to it good quality of embryo used. In most mammalian species, assessment of embryo morphology remains the method of selection for variety of practicable embryos earlier to transfer. It is the most realistic and clinically useful way to assess of embryo viability.[14] But still most of cases significant loss of pregnancy is reported in early developmental stages approximately at the age of 30-90days of gestation[45] due to failure of placental membrane and reduce placental vessel development,[39] it is reported that Early first trimester pregnancy rates are less than 1/2 that normally expected, embryonic loss is very high and near about 80% miscarry by second trimester in addition late gestation high level of chances to develop placental and fatal abnormalities then normal.[7] The main cause of third trimester losses are hydroallantois and fetal hydrops, usually attributed to inadequate placentation.[16] It is also present in literature that loss of clone foetus is also due to hypoxic condition because in cloned animal number of placentome is less which able to exchange less nutrient from dam to foetus and make a unfavourable condition for development of foetus.[15]

The high rate of pregnancy loss and neonatal death of cloned calves to is due to incomplete nuclear reprogramming; several authors have persuasively confirmed that the donor somatic cell is reprogrammed such that expression pattern at the blastocyst stage is significantly different from that of the somatic cell prior to nuclear transfer.[46] According to Arnold et al.,[47] expression of genes critical to normal placental development is malformed in cloned bovine embryos, and this is likely to cause abnormal trophoblast differentiation and add to pregnancy loss. Even the ongoing offspring have large placentas and increased birth weights, known as large offspring syndrome[14] and some with a apparently healthy appearance undergo from immune dysfunction, leading to increased mortality.[24]

According to one report of Marfil et al.[48] in cloned calves Respiratory distress 19% and in that condition calves died without any other sign of abnormality. An enlarged umbilical cord 37%, hyper/hypothermia 17% and depressed/prolonged recumbency 20% are the most common causes of death between 24h and 60days after birth. Problems that may lower the survival of these calves at or around the time of birth include increased length of gestation, severe dystocia, insufficient placental development and function, and failures in metabolic pathways necessary for extra-uterine life and some congenital problems such as cerebellar hypoplasia, respiratory distress and heart enlargement.[4]

It may be normal that clones have a different sensitivity to stress compared to conservative animals due to it they are more prone to pathogens which can also increase the death rate of clones.[23] To prevail over the low efficiency of cloning, various different method have been tried such as using different types of donor cell lines, a variety of culture systems, different fusion methods, and chemicals.[35] Some of these research efforts have led to minor upgrading in the quality of cloned embryos, which is closely related with embryo development and offspring productivity.

Ethnics

Cloning of animal is the key of development in field of agriculture and medical but it is acceptable only when the aims and methods are ethically justified and when it is carried out under ethical conditions. Scientists are doing extensive research in animal cloning but now many ethical issues are raised. The remarkable inefficiency of cloning poses serious threats to animal welfare.[49] Often, less than one percent of cloning attempts will result in a successful birth, and of those that are born, only a relatively small percentage are healthy enough to live for more than a few days or weeks.[35] According to one survey 64% of USA people are against the cloning they think that it is against the animal welfare that's why it is morally wrong.[50]

During cloning most serious ethical outcome is the pain that animal suffer during that process in addition to it cloning also effect on other population of animal. Due to it Human beings may be also badly affected by animal cloning through compromising the safety of the livestock used in food production.[51] Cloning badly effect both the donor and the recipient because in cloning firstly surgery is done to remove the egg from donor and then again surgery of recipient to implant the egg with least chances to get a goal in addition to it if animal successfully complete its gestation length the due to some unknown reason high weight of offspring[17] for it mostly need C-section due to which animal again suffer from pain.

Some other problem are also reported in which animal suffer from pain and that is against animal welfare like Hydroallantois, the typically fatal condition in which the pregnant animal swells with fluid to the point of looking like she is about to burst.[48] It is clear that embryos produced by nuclear transfer can lead in some cases to unstable foetal development, and to increased incidences of Dystocia, Sectio caesarea and perinatal death, which can have negative effects on recipient and offspring all these thing cumulatively raises moral difficulties.[52] Biodiversity is a safety net that protects against the spread of diseases in animal but cloning is effort to fix with one set of desirable genes, and create exact copies of the source animal which is opposite to diversity also raises a host of ethical issues.[53]

Food safety

Every country has special department to deal with the food

product produced by a biotechnology. However animal biotechnology products have to pass through safety assessments that is perform performed before public and governmental acceptance of their use for human consumption.[23] The possible for epigenetic errors ability to change the composition of food that's why food products derived from cloned livestock animals has been a major obstacle in gaining regulatory approval for entry of cloned products into the food chain. Additionally, a feeding trial in rats confirmed that the utilization of meat from cloned animals had no effect on body growth, food intake, general condition, locomotors activity, reflexes, sexual cycle, urinalysis, haematology, blood biochemistry, or histology.[23] Given the technology is still considered new and unfamiliar, plus the lack of any products with direct benefits for today's consumer, it is not shocking that current acceptance is low. However, the maturation of the technology and the availability of such products in combination with aging populations in the developed western world, with a strong desire to stay healthy for longer, could eventually increase acceptance.[54]

Conclusion

Advancement in cloning technology promise new possibilities, but many ethical challenges have emerged with it. Decreasing the disease susceptibility of animal will benefit animal welfare and agricultural productivity so for that more research is needed for the understanding of the process involving the failures in pre- and postnatal development.

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Conflict of interest

Author declares that there is no conflict of interest.

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