CHALLENGES FOR COMMERCIAL CLONING - PLANNING THE FUTURE.

Meirelles, F.V.[1*]; Providelo, F.D.[1]; Merighe, G.F.K.[1]; Miranda, M.[1]; Traldi, A.[2]; Birgel, E.[2]; Miglino M.A.[2]; Valim, J.R.[2]; Watanabe, Y.F.[3]

[1]Faculdade de Zootecnia e Engenharia de Alimentos (FZEA) Universidade de São Paulo (USP) Pirassununga, SP Brasil; [2]Faculdade de Medicina Veterinária e Zootecnia (FMVZ) Universidade de São Paulo (USP) Pirassununga, SP Brasil; [3]Vitrogem, Pesquisa e Desenvolvimento, Cravinhos SP, Brasil

Corresponding author: Flávio Vieira Meirelles; Departamento de Ciências Básicas, Faculdade de Zootecnia e Engenharia de Alimentos, Rua Duque de Caxias Norte 225, Pirassununga SP, Cep 13635-900, Phone: (19) 35654112, meirellf@usp.br

Abstract.

Somatic nuclei transfer is being commercially exploited in many countries and species. Today hundreds animals have been produced commercially and delivered to owners worldwide. This review discusses the state-of-the-art of this technology and the contribution to the animal genetic improvement and selection especially in cattle. Issues like low efficiency, gestational problems, delivery and epigenetic modifications are addressed aiming to discuss the perspectives of large-scale cloning and possible application in the future.
Key words: commercial cloning, nuclei transfer; somatic cells; clone; oocyte; embryo.

Introduction

Much has been discussed about nuclei transfer advances in the last decade and the early XXI century. The majority of these discussions were trigged by the event of Dolly, the first SCNT (Somatic cell nuclei transfer) derived mammal (adult clone; Wilmut; 1997) and was followed by the many others (Latham, 2004). The possibility to produce identical animals was interesting enough to bring to the laboratory bench many others researchers and technicians. Despite the interest in basic science where since the early days the nuclei transfer experiments brought to light important knowledge as the mechanism of cell cycle control and more recently epigenetics, the technique also seeded technological interest in the animal production field. This allowed an important improvement in the technique and the initial application in the industry.

Cloned individuals are often observed in the agriculture and people often drive in roads neighbored by huge forests of clones (e.g. eucalyptus). Then why not apply in animal production?

In fact, since late 90th many laboratories produced cloned animals of different species and breeds and once again the application of the technique contributed to the advance of the science. Many different cells were capable to reprogram when exposed to the oocyte cytoplasm and to produce individuals showing a very variable gestation rates and viability (Cibelli et al., 1998; Kato et al., 1998, 2000; Baguisi et al., 1999; Hill et al., 2000; Kubota et al., 2000; Ogura et al., 2000; Polejaeva et al., 2000; Kasinathan et al., 2001; Park et al., 2002; Keefer et al., 2002; Galli et al., 2003; Woods et al., 2003). Gestations of cloned animals are generally problematic and delivery and early care of these animals often frustrated (Bertolini and Anderson 2002; Wakayama et al., 2000; Ogura et al., 2002).

Therefore although cloning has shown commercial viability but some important questions

remaining are: why is cloning inefficient? How can to improve cloning efficiency? These questions will provide contribution to embryogenesis, cell biology, and knowledge of mechanisms of gene expression regulation. Advances in these areas may lead to significant feedback and improvements in the cloning technology.

How cloning may help animal production?

Given the importance that nuclei transfer have had to the science, the evolution in the recent years and the potential it has, it is worth to discuss the perspective of the technique at the actual scale and in a large scale (LSC) possibly feasible in the future.

Cloning individuals with high genetic, affective and financial value.

Actually there are many commercial establishments with living clones delivered to client worldwide. In fact, industries are producing clones of bovines, ovine, caprine, swine, and equine and other species not related to animal production with reasonable success rates. These animals are generally superior in certain phenotypic characteristic, with high economical value, with high affective value or with high genetic merit. Obviously, to animal production the last is the major reason to clone a specific animal.

Cattle industry is responsible for the majority of the cloned animals produced commercially. It is very difficult to estimate the number of cows and bulls cloned with commercial purposes but we may certainly indicate that hundreds of farm animals have been cloned in the world.

This cloning process is well justified if these animals are genetically superior in the characteristic they were selected. Bulls with high EPDs, and bull's dams are good example where cloning procedure may help to accelerate the genetic gain in a certain population. But obviously who decides to clone is always the owner of the animal and decision are not always based on genetic merit.

Cloning has allowed the multiplication of famous animals (living or dead), animals that have been castrated because of sports (although no report showed that animals cloned from this animals will be as good as original or the ability will be genetically inherited) and finally animals with demand of gametes higher than production.

In Brazil, 13 animals of Nellore, Hosltain, Simental, "Mocho Nacional" breeds and admixtures were successfully produced by SCNT (3 males and 10 females) and near 30 animals are alive (more than one copy per cloned animal). The older animal ages over 3 years old (Visintin personal communication), some are initiating reproduction or on near puberty and the majority are still suckling.

This scenario demonstrates the competence of the Brazilian research/industry and shows a small contribution to genetic gain and to improvement of our heard. Finally the application is restricted to elite herds and in no way may be transported to the commercial heard unless modifications in efficiency are obtained.

Cloning lineages for production (large scale cloning - LSC).

The SCNT technique may have a grate contribution to animal production once LSC become possible. Clones delivered directly to production may change the paradigm in animal production,

especially in species with large generation intervals and low number of offspring like cattle for example.

Nuclei transfer in large scale may allow the production of lineages, that in similarity with poultry and swine, would bring genetic homogeneity to the herd, by multiplying the best animal available for a specific management. Hence, as mentioned earlier in similarity with the agriculture, the multiplication of hybrid individuals by SCNT allows not only additive genetic gain, but also application of non-additive genetic (Clones enable widespread exploitation of non-additive genetic effects, dominance and epistasis, both within and between breeds).

The figure 1 shows a theoretical study with Monte Carlo simulation of a Nellore herd with high level of genetic selection comparing with a cloned herd. Animals from the normal breeding herd were produced with the best animal available in program (Nellore) for weight at day 550. Cloned animals weights were simulated applying the genetic merit of the bull (though clones and IA are half sib) and the growth curb developed previously (Meirelles et al., 2004). Cloned animals showed an advantage of near 50Kg in average per animal with a more homogeneous distribution in the heard (absence of genetic variation), and could justify an investment of extra 37,4 US$/ animal in the cloning process. Obviously this is a preliminary study and took in account only additive genetics effect, (non-additive effects and other advantages for cloned animals like sex homogeneity, disease resistance and environmental endurance were not evaluated). Therefore LSC has the potential to improve the animal production, however the revenues in agriculture are always low and we must evaluate the cost-benefit effect prior to application.

Barriers to bring LSC to application.

As stated before SCNT is available to producers. However the cloning procedure is very artesian, with low number of individual delivered at the end of the work (Vajta and Gjerris 2006). This morose procedure elevates the cost per individual and therefore justifies only cloning animals with very high economical value (not always the indicated). Automation of the procedure trough eliminating the micromanipulation, or by developing new strategies of producing bovine cloned embryos from stem cells, may modify the actual scenario and together with others improvements allow LSC.

Gestations rates of nuclei transfer embryos are lower in comparison with gestation rates of *in vitro* produced embryos, and the early and late embryo losses higher regardless of species (Wells et al., 2004). In the mouse, investigators have observed high embryo implantation rates (57–71%), but low fetal (5–16%) and very low full-term (2–3% or less) development rates following nuclear transfer using adult somatic cells (Wakayama et al.,1998). Cloned cattle suffer from high mortality during prenatal and perinatal periods as well, limiting the overall efficiency of cloning in this species to 5–6% at best (Chavatte-Palmer et al., 2004; Wells et al., 2004).

Delivery of cloned animals is much more prone for assistance needs, originates animals no rarely with overweight (figure 2) with many placental alterations, higher incidence of hydrallantois, difficulty of parturition (Miglino 2004; Hansen et al., 2005). Significant percentages of calves die within one week of birth due to various health problems. Perinatal and postnatal complications consist of developmental deficiencies like, respiratory distress, abnormal kidney development, difficulties in glycemic regulation, and liver steatosis in cattle (Chavatte-Palmer et al., 2004). Significant postnatal losses within the first six months affect about 30% of clones that develop to term.

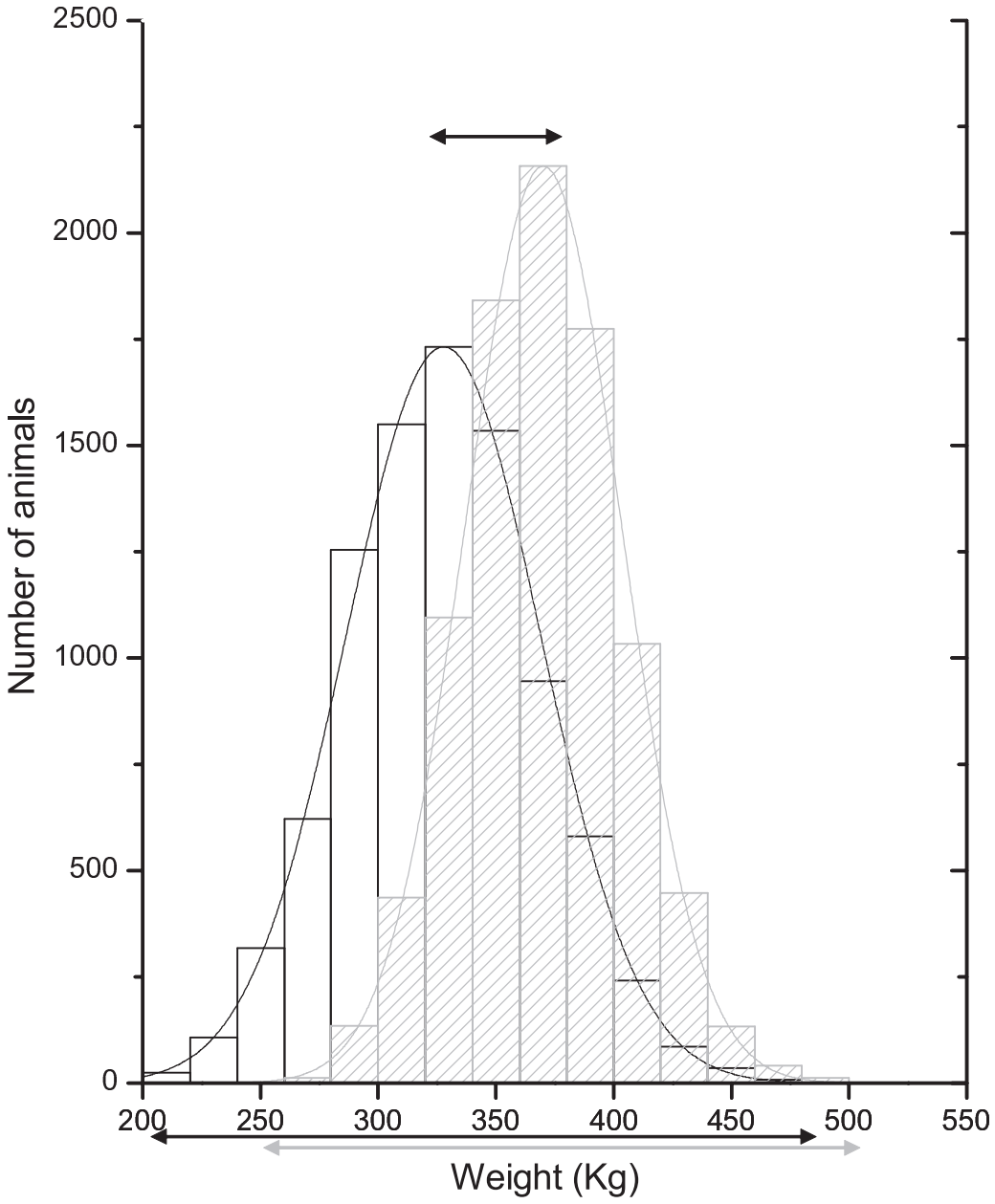

Figure 1. Monte Carlo simulation of Nellore bovine weight at 550 days. White bars represent the distribution of individual weight of a population derived of AI of one bull selected for EPD of weight gain at 405 days. The hachured gray bars represent the distribution of the clone's weight. Note a near 50Kg superiority of the cloned animals average compared with AI derived heard. The arrows under the graphic demonstrate the heard variability. Note a more homogeneous distribution of the cloned animals with a deviation equivalent to 82% of the AI population due to high herdability of the characteristic and absence of genetic variance (Van Vleck., 1998).

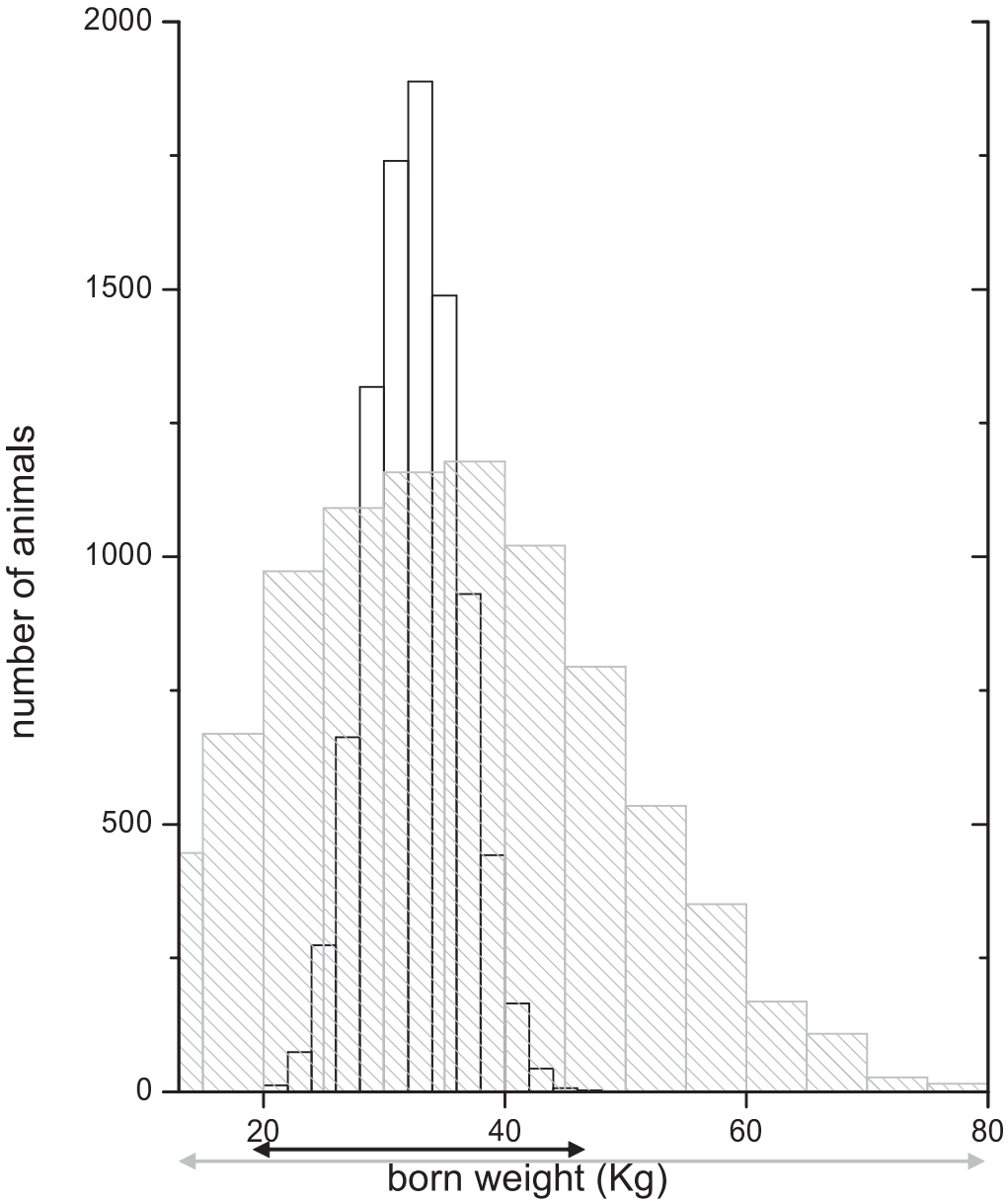

Figure 2. Monte Carlo simulation of bovine birth weight. White bars represent the distribution of individual weight of a population derived of AI of one bull selected for average calve weight EPD. The hachured gray bars represent the distribution of the clone's weight in accordance with the data of birth weight in our experience (Meirelles unpublished data). The arrows under the graphic demonstrate the heard variability. Note a 3-fold variation of the cloned calves weight compared with the AI derived heard.

Nuclear transfer often produces apparently healthy offspring, but some time leads alterations. Although part is evident immediately, few studies showed abnormalities not manifested until adulthood and therefore longitudinal studies of cloned offspring are required. Aberrant patterns of DNA methylation and imprinted gene expression have been reported for cloned embryos and individuals in different species (Humpherys et al., 2001; Cezar et al., 2003; Han et al., 2003; Mann et al., 2003).

One example of adult modifications is cloned mice body composition, showing an increased

body weight associated with increased adipose tissue, hyperinsulinemy and hyperleptinemy (Tamashiro et al., 2002). The mechanism(s) responsible for the development of obesity in cloned mice remains to be determined. Although increased body weight has not been reported in domestic species, postmortem examination of cloned fetuses and newborn calves showed more adipose tissue surrounding the intra-abdominal organs, as compared with controls. Plasma leptin was also elevated, consistent with increased adipose tissue content (Chavatte-Palmer et al., 2002).

Together this problems leads to a lower rate of success in reproduction and higher costs to the procedure. The cost related to the problems mentioned above together with uncertainty about the healthy status of the individual certainly doesn't permit it application of LSC.

Finally although some abnormality may present in cloned animals, clones offspring are normal, indicating that the germinal cycle is enough to reprogram eventual failure in somatic cells clone epigenetic and that cloned individuals produces gametes genetically and epigenetically similar to non-cloned individuals (Tamashiro et al., 2002).

Conclusions and perspectives

As pointed out commercial cloning is being used in the animal production. This cloned animals have a small contribution to the genetic improvement, however contribute to understand the alterations generating a large extend of experience on epigenetics, obstetrics and neonatology aspects of the clone gestation, delivery and early care.

Obviously, the grater contribution of the cloning will occur once it become possible in large scale.

In the future cloning will certainly be applied to produce cloned transgenic animals in the pig, sheep, and cow (Schnieke et al., 1997; Cibelli et al., 1998; Hill et al., 1999; Park et al., 2002; Lee et al., 2003) as successful expression of valuable recombinant protein products in the milk of cloned animals (e.g., a glucosidase, porcine lactoferrin, human factor IX, casein, and immunoglobulin; Schnieke et al., 1997; Eyestone and Campbell, 1999; Lee et al., 2003) provides an important means of producing these proteins in large quantities and at lower costs than some other approaches (Brink et al., 2000; Fan and Watanabe, 2003; van Arendonk and Bijma, 2003). Additionally, expression of transgenes in other tissues opens potential novel avenues for producing materials for xenotransplantation by altering antigenic properties of the tissues or cells (Eyestone and Campbell, 1999; Phelps et al., 2003).

Therefore in the future all reproductive and genetics technologies like artificial insemination, embryo transfer, in vitro fertilization, cloning, transgenics, molecular markers used in combinations will allow the selection of the better animals to multiply.

The animals selected will be multiplied by LSC, modified or not by recombination and finally after evaluation distributed to the farm. The latter will be able to produce animals in a very defined management in similarity to other cultures and sell their products to specialized companies from food or pharmacological chain.

Acknowledgments

The author thanks FAPESP for financial support and FAPESP and CAPES for graduated students scholarship.

Literature

Baguisi A.,et al., Production of goats by somatic cell nuclear transfer. Nat Biotechnol 1999; 17;456–61.

Brink MF, Bishop M.D and Pieper FR. Developing efficient strategies for the generation of transgenic cattle which produce biopharmaceuticals in milk. Theriogenology 2000; 53;139–48.

Bertolini M, Anderson GB. The placenta as a contributor to production of large calves. Theriogenology. 2002;57; 1; 181-7.

Cezar GG, Bartolomei MS, Forsberg EJ, First NL, Bishop MD and Eilertsen KJ. Genome-wide epigenetic alterations in cloned bovine fetuses. Biol Reprod 2003; 68;1009–14.

Chavatte-Palmer P., et al.. Clinical, hormonal, and hematologic characteristics of bovine calves derived from nuclei from somatic cells. Biol Reprod 2002; 66; 1596–603.

Chavatte-Palmer P., et al. Health status of cloned cattle at different ages. Cloning Stem Cells 2004; 6; 94–100.

Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, Ponce de Leon FA and Robl JM. Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. Nat Biotechnol 1998; 16; 642–6.

Eyestone WH and Campbell KH. Nuclear transfer from somatic cells: applications in farm animal species. J Reprod Fertil Suppl 1999; 54; 489–97.

Fan J and Watanabe T. Transgenic rabbits as therapeutic protein bioreactors and human disease models. Pharmacol Ther 2003; 99; 261–82.

Galli C, Lagutina I, Crotti G, Colleoni S, Turini P, Ponderato N, Duchi R and Lazzari G. Pregnancy: a cloned horse born to its dam twin. Nature 2003; 424; 635.

Han YM, Kang YK, Koo DB and Lee KK. Nuclear reprogramming of cloned embryos produced in vitro. Theriogenology 2003; 59; 33–44.

Hansen M et al,. Assisted reproductive technologies and the risk of birth defects - a systematic review. Hum Reprod 2005; 20; 328–38.

Hill JR, Winger QA, Long CR, Looney CR, Thompson JA and Westhusin ME. Development rates of male bovine nuclear transfer embryos derived from adult and fetal cells. Biol Reprod 2000; 62;1135–40.

Kasinathan P, Knott JG, Moreira PN, Burnside AS, Jerry DJ and Robl JM. Effect of fibroblast donor cell age and cell cycle on development of bovine nuclear transfer embryos in vitro. Biol Reprod 2001; 64; 1487–93.

Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, Yasue H. and Tsunoda Y. Eight calves cloned from somatic cells of a single adult. Science 1998: 282; 2095–8.

Keefer CL, Keyston R, Lazaris A, Bhatia B, Begin I, Bilodeau AS, Zhou FJ, Kafidi N, Wang B, Baldassarre H and Karatzas CN. Production of cloned goats after nuclear transfer using adult somatic cells. Biol Reprod 2002; 66; 199–203

Kubota C, Yamakuchi H, Todoroki J, Mizoshita K, Tabara N, Barber M and Yang X. Six cloned calves produced from adult fibroblast cells after long-term culture. Proc Natl Acad Sci USA 2000; 97; 990–5.

Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout WM III, Biniszkiewicz D, Yanagimachi R and Jaenisch R. Epigenetic instability in ES cells and cloned mice. Science 2001; 293; 95–7.

Latham KE. Cloning: questions answered and unsolved Differentiation 2004; 72; 11–22

Lee JW, Wu SC, Tian XC, Barber M, Hoagland T, Riesen J, Lee KH, Tu CF, Cheng WT and Yang X. Production of cloned pigs by whole-cell intracytoplasmic microinjection. Biol Reprod 2003; 69; 995–1001.

Mann MRW, Chung YG, Nolen LD, Verona RI, Latham KE and Bartolomei MS. Disruption of imprinted gene methylation and expression in cloned mouse embryos. Biol Reprod 2003; 69; 902–14.

Meirelles FDP, Costa EJX, Ferraz JBS. Modelo computacional de um rebanho virtual utilizando simulação Monte Carlo. In: Reunião Anual da Sociedade Brasileira de Zootecnia, 2004, Campo Grande. 41ª Reunião Anual da Sociedade Brasileira de Zootecnia.

Miglino, MA. Clonagem Animal e Placentação. Acta Scientiae Veterinariae 2004; 32; 03-7

Ogura A, Inoue K, Takano K, Wakayama T and Yanagimachi R. Birth of mice after nuclear transfer by electrofusion using tail tip cells. Mol Reprod Dev 2000; 57; 55–9.

Park, K.W., et al. Mosaic gene expression in nuclear transfer-derived embryos and the production of cloned transgenic pigs from ear-derived fibroblasts. Biol Reprod 2002; 66; 1001–5.

Phelps, C.J., et al. Production of a 1,3-galactosyltransferase-deficient pigs. Science 2003; 299; 411–4.

Polejaeva IA, Chen SH, Vaught TD, Page RL, Mullins J, Ball S, Dai Y, Boone J, Walker S, Ayares DL, Colman A and Campbell KH. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature 2000; 407; 86–90.

Schnieke AE, Kind AJ, Ritchie WA, Mycock K, Scott AR., Ritchie M, Wilmut I, Colman A and Campbell KH. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 1997; 278; 2130–3.

Vajta G, Gjerris M. Science and technology of farm animal cloning: state of the art. Anim Reprod Sci. 2006 ;92; 3-4; 211-30.

Van Arendonk JA and Bijma P. Factors affecting commercial application of embryo technologies in dairy cattle in Europe — a modelling approach. Theriogenology 2003 59; 635–49.

Van Vleck LD. Implications of cloning for breed improvement strategies. Are traditional methods of animal improvement obsolete? J. Dairy Sci 1998; 77; 111-21.

Wakayama T, et al.. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 1998; 394; 369–74.

Wakayama T, et al. Cloning of mice to six generations. Nature 2000; 407; 318–9.

Wells DN, et al. The health of somatic cell cloned cattle and their offspring. Cloning Stem Cells 2004; 6; 101–10.

Wilmut I, Schnieke AE, McWhir J, Kind AJ and Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature 1997; 385; 810–3.

Woods GL, White KL, Vanderwall DK, Li GP, Aston KI, Bunch TD, Meerdo LN and Pate BJ. A mule cloned from fetal cells by nuclear transfer. Science 2003; 301; 1063.