Benefits and problems with cloning animals

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Abstract — Animal cloning is becoming a useful technique for producing transgenic farm animals and is likely to be used to produce clones from valuable adults. Other applications will also undoubtedly be discovered in the near future, such as for preserving endangered breeds and species. Although cloning promises great advantages for commerce and research alike, its outcome is not always certain due to high pregnancy losses and high morbidity and mortality during the neonatal period. Research into the mechanisms involved in the reprogramming of the nucleus is being conducted throughout the world in an attempt to better understand the molecular and cellular mechanisms involved in correcting these problems. Although the cause of these anomalies remains mostly unknown, similar phenotypes have been observed in calves derived through in vitro fertilization, suggesting that culture conditions are involved in these phenomena. In the meantime, veterinarians and theriogenologists have an important role to play in improving the efficiency of cloning by finding treatments to assure normal gestation to term and to develop preventative and curative care for cloned neonates.

Résumé — Avantages du clonage des animaux et problèmes. Le clonage des animaux est devenu une technique utile pour la production d'animaux d'élevage transgéniques et il est susceptible de servir à cloner des animaux adultes dont la valeur est élevée. D'autres applications seront sans doute bientôt exploitées comme la préservation des espèces et races menacées. Si le clonage est prometteur pour le commerce et la recherche, ses résultats ne sont pas toujours garantis, en raison des nombreuses interruptions de gestation et des taux élevés de morbidité et de mortalité pendant la période néonatale. Partout dans le monde, des chercheurs étudient les mécanismes en cause dans la reprogrammation du noyau afin de mieux comprendre les mécanismes moléculaires et cellulaires qui permettraient de corriger ces problèmes. Bien que les causes de ces anomalies soient encore quasi inconnues, on a observé des phénotypes semblables chez les veaux obtenus à partir de la fertilisation *in vitro*, ce qui porte à croire que les conditions dans lesquelles la culture est faite sont liées au phénomène. Par ailleurs, les vétérinaires et les thériogénologues ont un rôle important à jouer dans l'amélioration de l'efficacité du clonage, en définissant des traitements qui assurent une gestation normale et en élaborant des soins préventifs et curatifs pour les nouveau-nés issus du clonage.

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Introduction

Although animal cloning has benefitted from intensive research efforts for many decades, the arrival of "Dolly," the cloned sheep, came as a surprise to many scientists and lay people alike (1). Actually, scientists were somewhat "blinded" by the inability to derive live progeny from adult amphibian cells, despite heroic efforts throughout the 50s, 60s, and 70s to understand the fundamental mechanisms involved in nuclear reprogramming (2). With the exception of an unconfirmed successful attempt to clone from embryonic cells in mice (3), early nuclear transfer experiments met with several failures, leading to a generalized feeling that mammals could not be cloned from differentiated cells (4). Renewed hope came when lambs were obtained after nuclear transfers from embryonic blastomeres (5) and inner-cell-mass cells (6). The possibility of producing embryo-derived cloned offspring was later confirmed in several other domestic and laboratory animals, leading to the establishment of private companies aimed at improving and applying the nuclear transfer technology to cattle breeding (7–9). Two factors were directly related to the failure to apply embryo-cloning technology in a commercial setting. First, the efficiency of embryo multiplication was low due to poor development rates up to the blastocyst stage.

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Second, pregnancies derived from nuclear transfer embryos were associated with high embryo mortality throughout gestation, production of oversized calves, extended gestation length, and increased neonatal morbidity and mortality (8,10,11).

The successful cloning of mammals from adult mammalian tissues has renewed interest in the technique of nuclear transfer, both for scientific and applied purposes (1,12,13). Furthermore, a second wave of interest in the application of nuclear transfer has stemmed from the successful use of donor nuclei from fetus-derived cells that had a novel gene incorporated into their chromosomes (transfected) to produce transgenic farm animals (14–16). However, regardless of the potential benefits of producing transgenic farm animals, problems associated with loss during gestation and neonatal mortality have increased substantially with the use of somatic cells in nuclear transfer (17). Therefore, the problems associated with improvements to the technology of somatic cell cloning should be addressed for both economic and ethical reasons, before commercial application is envisaged on a large-scale basis. The purpose of this review is to describe the nuclear transfer technology, as it is currently applied, and to discuss the potential applications and problems associated with producing cloned animals from somatic cells.

Technical procedures

The procedure used to produce cloned animals involves several steps requiring specialized equipment and highly trained personnel (Figure 1). The first step relates to identifying a suitable nuclear donor cell from which to clone. Although donor cells can be obtained directly from an animal, primary cells are normally propagated in vitro beforehand to facilitate manipulation and storage. Skin-derived fibroblasts are often used, due to the ease in producing stable dividing and homogeneous primary lines. In general, fibroblasts have a reasonably long life span and can also be frozen and thawed with limited loss of viability. However, cells from other tissues, such as granulosa (cumulus) and oviduct, and leukocytes have also been used successfully to clone cattle and goats (18–21). Donor nuclei are often treated before transfer to synchronize the cells at the G1/G0 phase of the cell cycle. This is important when oocytes that have been arrested at the metaphase stage at the time of fusion are used as recipients (hosts). The most common procedure to synchronize the cell cycle is to expose cells for several days to culture medium containing low levels of serum (serum starvation). Because many cells stop cycling due to contact inhibition, cell cycle synchrony at the G1 phase can also be achieved without changing the concentration of the serum in the medium, simply by allowing the cells to grow to confluency. It has been proposed that serum starvation is beneficial to donor nuclei, possibly by improving the ability to reset the genetic program of differentiated nuclei to a totipotent state (22). However, somatic nuclei have also been used successfully to produce clones without having been starved of serum, and further controlled studies are required to determine the advantage of serum starvation (15).

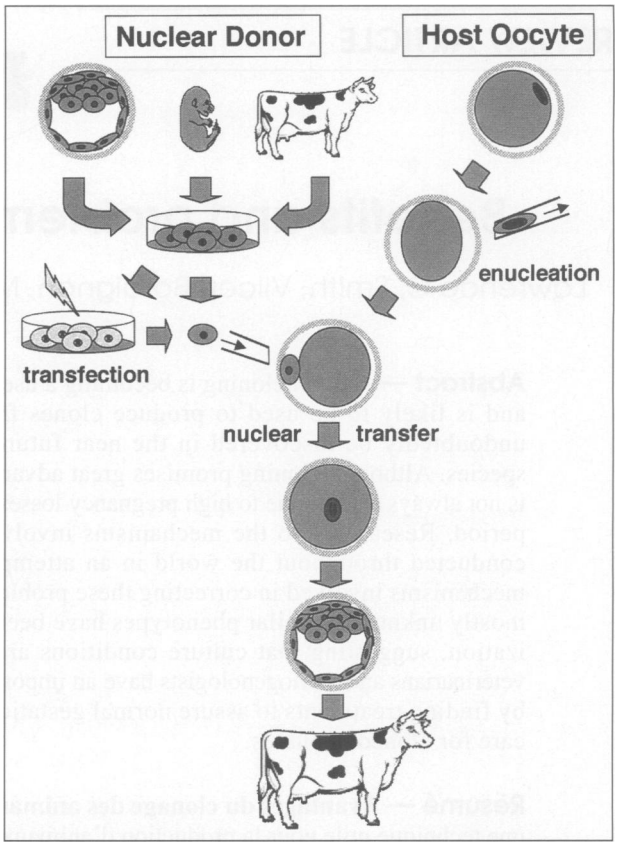

Figure 1. Schematic diagram of the multiple-step procedure used for cloning and producing transgenic mammals by nuclear transfer.

Transfection of nuclear donor cells to produce transgenic animals through cloning can be performed by random integration or, when possible, by introducing the transgene precisely where it is present on the chromosome, a procedure known as gene targeting, through homologous recombination (23). Methods of transfection are similar to those applied to immortalized somatic cell lines; that is, by using the cationic lipid reagent, lipofectamine, which enables the transport of the exogenous DNA (transgene) through the plasma and nuclear membranes. The transgene should be engineered to have the coding region for the gene of interest linked to a promoter that can provide high levels of expression that is directed specifically to the tissues of interest in the cloned animal. The addition (co-transfection) of a selectable marker and/or a reporter gene to the engineered DNA construct will allow the selection of the few cells that successfully integrate the transgene and, therefore, will be useful for choosing properly transfected donor cells for nuclear transfer.

The other important component of nuclear transfer is the provision of a suitable host cytoplasm able to reset the developmental clock of the donor nucleus back to the time of fertilization (chromatin reprogramming). In cattle, oocytes obtained from slaughter houses are often used as the host cell in nuclear transfer, because the current in vitro maturation (IVM) protocols are very effective. Recent improvements in IVM also permit use of the protocols in the goat (21,24). On the other hand, effective in vitro culture systems are not available for other

domestic and laboratory species, requiring the use of in vivo-matured oocytes for nuclear transfer. Chromosome removal (enucleation) is essential to eliminate the genetic contribution of the host oocyte. Enucleation is usually performed by microsurgical aspiration of a portion of the cytoplasm surrounding the 1st polar body and is confirmed by DNA vital staining and ultraviolet (UV) irradiation. Although such staining methods are widely utilized during enucleation, UV exposure should be kept to a minimum to avoid damage to both the cytoplasm and the plasma membrane (25). The use of UV irradiation can be avoided by enucleating after the secondary oocyte has been exposed to an artificial stimulus for activation, at the time of 2nd polar body extrusion (telophase stage). Apart from avoiding the use of UV and DNA vital stains, telophase enucleation reduces the amount of cytoplasm removed and enables selection of oocytes that have responded to activation by extruding a 2nd polar body, a step that cannot be done when enucleation is done with metaphase-arrested oocytes (26).

Nuclear transfer is most commonly performed by introducing a single donor cell into the perivitelline space of an enucleated oocyte, followed by plasma membrane fusion with a direct current electric shock. Donor cells need to be placed in close contact with the enucleated oocyte in order to achieve fusion. Another option when introducing nuclei into oocytes is to perforate the membrane and deliver the nuclei directly into the ooplasm by using a piezo-controlled pipette holder (12). Host oocytes that have been halted in the metaphase of the cell cycle are usually chosen because their mitosis promoting factor (MPF)-kinase activity, which induces donor nuclear membrane breakdown immediately after fusion, is high at this stage of the cell cycle. As long as nuclei are not undergoing DNA replication (S-phase of the cell cycle) at the time of fusion, nuclear membrane breakdown enables a rapid remodeling of chromatin. However, when nuclear donor cells are in the S-phase, the chromatin will undergo premature condensation and pulverization (PCC), which is detrimental to further development.

A problem commonly associated with using somatic cells that have been transfected in vitro before nuclear transfer is that these cells have low cycling activity, which renders cell quiescence protocols, both by confluency and serum starvation, ineffective. When using nonsynchronized donor cells, PCC can be avoided by using host oocytes that have been activated shortly before introducing the donor nucleus. Low MPF levels in telophase-enucleated oocytes will avoid the harmful interactions with nonsynchronized nuclei without affecting their reprogramming properties. Reconstructed oocytes are either cultured in vitro to the blastocyst stage or transferred soon after fusion to a temporary recipient for development in vivo. Finally, selected morula- and blastocyst-stage embryos are transferred to synchronized recipients and allowed to develop to term.

Potential applications

Multiplication of embryos and animals by cloning

Dolly was the first animal to be cloned from an adult-derived cell line (1). Although there was some initial skepticism concerning the cellular origin of Dolly (27), this was later unequivocally confirmed by using microsatellite and fingerprinting analysis (28,29). Furthermore, several other mammalian species have since been cloned from adult-derived tissue, including cows (19), goats (21), and mice (12,30). In cattle, the efficiency of nuclear transfer to produce both embryos and viable offspring varies considerably among different laboratories, possibly due to cell source, conditions for manipulation, and other unknown factors. Most studies have used donor cells arrested at the G0/G1 phase, either artificially or naturally, and have tested various tissue-derived cells, such as fetal and skin fibroblasts, cumulus, granulosa cells, oviductal epithelium, muscle cells, and leukocytes. Development rates from oocyte to blastocyst can vary from 12% to 70% and to full development with production of offspring from 1% to 83%, according to species and laboratory of origin. Further peer-reviewed reports with detailed technical protocols are required to determine the sources of such variability among research groups.

The potential application of adult cloning ranges from multiplying prize-winning animals to producing a large number of genetically identical animals for research purposes. Dissemination of genetically superior animals would be made easier through the production of several copies of top breeding animals and the distribution of clones to production farms, particularly where access to artificial insemination and other assisted reproductive technologies is limited. Genetic improvement programs would benefit from cloning embryos derived from juvenile females of high merit, thereby enabling a shorter generation interval.

Recovery of endangered animals

An excellent example of how cloning has and will help in the recovery of endangered breeds of cattle was reported recently (18). In this study, adult somatic cell nuclear transfer was used to preserve the last surviving cow of the Enderby Island cattle breed from New Zealand. Since a few straws of semen of the Enderby Island breed remain, progeny can now be obtained from the cloned females to recover the breed gradually, using sexual reproduction. Ideally, recovery programs should be initiated with as wide a pool of founder animals as possible to avoid potential problems with inbreeding. Several breeds of domestic animals are currently being extinguished because of lack of market advantage when compared with the most productive dairy and beef breeds. To avoid an irreversible loss of such genetic pools, programs should be devised to store samples of somatic tissue of several individuals from each breed in liquid nitrogen. If and whenever there is a need for such a breed, frozen samples could be harvested and a large number of individuals generated by cloning, thereby reducing the effects of inbreeding in the sexually propagated descendents of the founder population.

Cross-species cloning is another approach to recovering endangered species. This approach is based on using the nucleus from an endangered wild species with the host oocyte derived from a closely related and more abundant domesticated species. This approach was used to clone "Zebulon," a calf derived from an

embryo that was produced by using host oocytes from the European-derived breeds of domestic cattle (*Bos taurus*) and the nucleus of an animal of the Indian-derived Nellore breed (*B. indicus*) (31). *Bos indicus* and *B. taurus* can interbreed and, therefore, are very closely related. The next step will be to examine the developmental potential of clones derived from different species. Cross-species cloning has been proposed and tested with less closely related species with no success. Early stages of pregnancy were achieved after the transfer of cloned embryos produced from the fusion of Argali (*Ovis ammon*) nuclei into enucleated oocytes of domestic sheep (*O. aries*) (32). Others have gone to further extremes in using bovine oocytes with sheep, monkey, and rat nuclei, but with limited developmental success beyond early cleavage (33). Nonetheless, human somatic cells have been cloned into bovine oocytes to produce pluripotent embryonic cell lines (34).

It is proposed that the embryonic cells derived from an individual could be made to differentiate into several tissues. The tissues produced in this way could then be returned to the same individual to replace damaged tissues or organs without causing rejection, a procedure named "human therapeutic cloning."

Production of transgenic animals

Transgenic mammals were first produced through the microinjection of gene constructs into the pronuclei of fertilized mouse zygotes (35). Pronuclear microinjection is an efficient method for species with zygotes showing clear cytoplasm, thus enabling proper visualization of pronuclei. However, zygotes of most domestic animals contain large lipid vesicles that obscure the position of pronuclei, making microinjection a cumbersome approach for introducing foreign DNA. Although protocols have been established to shift lipid granules by centrifugation, other factors have added further constraints to the technology. For instance, only a limited percentage of the zygotes that survive the microinjection procedure integrate the exogenous construct into their chromosomes. Since most embryos that are transferred to synchronized recipients do not carry the transgene, the cost of keeping a large herd of recipients precludes the common use of this technique.

The new, developing solution is to use transfected cells and nuclear transfer to generate transgenic farm animals. This technique was first applied in sheep and cattle (14,15) but, recently, has also been used to produce transgenic mice (36) and goats (37). Fibroblasts are obtained from fetuses and are used to produce a primary cell line, which, once established and checked for chromosomal stability, is transfected by common cell transfection techniques. A selection and reporter gene construct is usually added to the transgene of interest to enable the isolation of suitable cell clones for nuclear transfer, namely, those clones that integrated the transgene and express the reporter gene correctly. The advantage of this procedure over microinjection is that all the embryos derived from the transfected cell line will carry the transgene, which avoids the use of recipients with embryos that are not transgenic. The green fluorescent protein (GFP) reporter gene is a suitable marker for transgene integration and expression, because the screening uses blue wavelength exposure, which does not affect further development.

Another enormous advantage of nuclear transfer over microinjection of DNA into the pronuclei of fertilized zygotes to produce transgenic animals is the possibility of using donor cells that have been transfected by targeting the transgene into exactly the same position where it is found on the chromosome. This has been achieved recently to derive transgenic mice and sheep by using, respectively, embryonic stem or fetal fibroblasts modified by homologous recombination (23,36). Embryonic stem cells have the advantage of being naturally immortal; therefore, they allow for lengthy protocols of targeted mutagenesis. This is not the case for primary somatic cell lines, which reach senescence after a short number of cell doublings in culture. Therefore, more suitable and faster protocols for targeted mutations will be necessary to enable the introduction of transgenes (knock-in) or the functional elimination of endogenous genes (knock-out) in somatic cells that will be later used to derive transgenic animals. These techniques will facilitate the production of farm animals that have been conveniently engineered to produce pharmaceuticals, nutraceuticals, and xenotransplant organs, and to create animal models for several human diseases.

Health hazards

Pregnancy losses

Although the potential economic and social benefits of animal cloning are enormous, many aspects of the production of cloned animals need to be carefully addressed through proper research before the large-scale use of this technology. The most concerning aspect relates to medical problems encountered both during gestation and in the first weeks after birth of the cloned animal. In the former, fetal development seems to be arrested at various stages during gestation, leading to abortions at early and late stages of fetal development (38). Many aspects of embryo and fetal mortality seem to suggest that the placenta does not develop normally, possibly due to an inappropriate transition from yolk sac to allantoic nutrition. Others have observed that the growth of the allantois is severely retarded, or even nonexistent, as characterized by lack of, or reduced, vascularization during early gestation, leading to failure of normal placentome development (39). Although the causes of poor placental development remain unknown, many of the genes that control both placental and fetal growth and differentiation are "imprinted." Loss or modification of these genetic imprints in cloned embryos could be the cause of the observed abnormalities. Fetal losses in the bovine species at later stages of gestation are a consequence of placental dysfunction, leading to hydroallantois and the presence of fewer and enlarged placentomes, enlarged umbilical vessels, and edematous placental membranes (13,40). It is of interest that similar fetal losses have not been seen in goats (37,41).

Neonatal health problems

Increased birth weight and high neonatal mortality are common in cloned calves (11), a phenomenon referred

to as "large offspring syndrome," or LOS. Since LOS also occurs to calves derived from in vitro-fertilized (IVF) embryos, it is not yet clear whether the nuclear transfer itself is the primary cause. Respiratory distress syndromes have been cited frequently in cloned calves (17,42) and lambs (43), which may indicate poor adrenal gland development and function, low fetal cortisol levels, and, hence, insufficient lung surfactant. Corticosteroid treatment of the recipient before delivery to accelerate maturation of the fetal lungs, together with oxygen therapy, may increase survival rates (17). The administration of pulmonary surfactants to cloned calves has been tested with variable success, suggesting that pulmonary hypertension may be the primary problem. Pneumonia is commonly observed in affected calves, requiring immediate antimicrobial therapy. Susceptibility to infection of any kind is also a common trait and may derive from immune dysfunction, as suggested by a sudden decrease in lymphocyte and red cell counts observed in a cloned calf (44).

Conclusion

A comparison of prospects envisaged in a previous review highlight the advancements that have occurred to animal cloning in the last decade (45). Somatic cell cloning is now a reality and the technical and practical uses have been described only briefly above. Due to the potential advantages for pharmaceutical companies, farmers, and research agencies, mammalian cloning is likely to become a common technology towards the end of this decade. Although enormous research efforts are being focused on trying to better understand the source of the problems, theriogenologists and veterinary clinicians need to better understand the medical problems associated with cloned animals in order to develop more effective treatments to reduce pregnancy loss and neonatal morbidity and mortality levels.

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References

1. Wilmut I, Schnleke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult mammalian cells. Nature 1997;385:810–813.
2. McKinnell RG, DiBerardino MA. The biology of cloning: History and rationale. BioScience 1999;49:875–885.
3. Illmensee K, Hoppe PC. Nuclear transplantation in *Mus musculus*: Developmental potential of nuclei from preimplantation embryos. Cell 1981;23:9–18.
4. McGrath J, Solter D. Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development in vitro. Science 1984;226:1317–1319.
5. Willadsen S. Nuclear transplantation in sheep embryos. Nature 1986;320:63–65.
6. Smith LC, Wilmut I. Influence of nuclear and cytoplasmic activity on the development in-vivo of sheep embryos after nuclear transplantation. Biol Reprod 1989;40:1027–1035.
7. Bondioli KR. Commercial Cloning of Cattle by Nuclear Transfer. Fort Collins: Colorado State Univ, 1992:35–38.
8. Willadsen SM, Janzen RE, McAlister RJ, Shea BF, Hamilton G, McDermand D. The viability of late morulae and blastocysts produced by nuclear transplantation in cattle. Theriogenology 1991;35:161–171.
9. Stice SL, First NL. Progress towards efficient commercial embryo cloning. Anim Reprod Sci 1993;33:83–98.
10. Keefer CL, Stice SL, Matthews DL. Bovine inner cell mass cells as donor nuclei in the production of nuclear transfer embryos and calves. Biol Reprod 1994;50:935–939.
11. Wilson JM, Williams JD, Bondioli KR, Looney CR, Westhusin ME, McCalla DF. Comparison of birth weight and growth characteristics of bovine calves produced by nuclear transfer (cloning), embryo transfer and natural mating. Anim Reprod Sci 1995;38: 73–83.
12. Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 1998;394:369–372.
13. Wells DN, Misica PM, Tervit HR. Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. Biol Reprod 1999;60:996–1005.
14. Schnieke AE, Kind AJ, Ritchie WA, et al. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 1997;278:2130–2133.
15. Cibelli JB, Stice SL, Golueke PJ, et al. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 1998;280: 1256–1258.
16. Keefer CL, Baldassarre H, Keyston R, et al. Generation of a transgenic BELE® goat following nuclear transfer of transfected fetal fibroblasts into enucleated, in vitro matured oocytes [abstract]. Transgenic Res 1999;8:483.
17. Hill JR, Roussel AJ, Cibelli JB, et al. Clinical and pathological features of cloned transgenic calves and fetuses (13 case studies). Theriogenology 1999;15:1451–1465.
18. Wells DN, Misica PM, Tervit HR, Vivanco WH. Adult somatic cell nuclear transfer is used to preserve the last surviving cow of the Enderby Island cattle breed. Reprod Fertil Dev 1998;10:369–378.
19. Kato Y, Tani T, Sotomaru Y, et al. Eight calves cloned from somatic cells of a single adult. Science 1998;282:2095.
20. Galli C, Duchi R, Moor RM, Lazzari G. Mammalian leukocytes contain all the genetic information necessary for the development of a new individual. Cloning 1999;1:161–170.
21. Keefer C, Keyston R, Bhatia B, et al. Efficient production of viable goat offspring following nuclear transfer using adult somatic cells [abstract]. Biol Reprod 2000;62 (Suppl.1):218.
22. Campbell KHS, McWhir J, Ritchie WA, Wilmut I. Sheep cloned by nuclear transfer from cultured cell line. Nature 1996;380:64–66.
23. McCreath KJ, Howcroft J, Campbell KHS, Colman A, Schnieke AE, Kind AJ. Production of gene-targeted sheep by nuclear transfer from cultured somatic cells. Nature 2000;405:1066–1069.
24. Reggio BC, James AN, Green HL, et al. Transgenic pregnancies resulting from nuclear transfer in the goat: FSH-stimulated vs. nonstimulated recipient cytoplasts [abstract]. Biol Reprod 2000;62 (Suppl.1):537.
25. Smith LC. Membrane and intracellular effects of ultraviolet irradiation with Hoechst 33342 on bovine secondary oocytes matured *in vitro*. J Reprod Fert 1993;99:39–44.
26. Bordignon V, Smith LC. Telophase enucleation: An improved method to prepare recipient cytoplasts for use in bovine nuclear transfer. Mol Reprod Dev 1998;49:29–36.
27. Sgaramella V, Zinder ND. Dolly confirmation. Science 1998; 279:635–638.
28. Ashworth D, Bishop M, Campbell K, et al. DNA microsatellite analysis of Dolly. Nature 1998;394:329.
29. Signer EN, Dubrova YE, Jeffreys AJ, et al. DNA fingerprinting Dolly. Nature 1998;394:329–330.
30. Wakayama T, Yanagimachi R. Cloning of male mice from adult tail-tip cells. Nat Genet 1999;22:127–128.
31. Smith LC, Bordignon V, Garcia JM, Meirelles FV. Mitochondrial genotype segregation and effects during mammalian development: applications to biotechnology. Theriogenology 2000;53: 35–46.
32. White KL, Bunch TD, Mitalipov S, Reed WA. Establishment of pregnancy after transfer of nuclear transfer embryos produced from the fusion of Argali (*Ovis ammon*) nuclei into domestic sheep (*Ovis aries*) enucleated oocytes. Cloning 1999;1:47–54.
33. Dominko T, Mitalipova M, Haley B, et al. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. Biol Reprod 1999;60:1496–1502.
34. Lanza RP, Cibelli JB, West MD. Human therapeutic cloning. Nat Med 1999;5:975–977.

35. Brinster RL, Chen HY, Trumbauer ME, Yagle MK, Palmiter RD. Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. Proc Natl Acad Sci USA 1985;82:4438–4442.
36. Rideout III WM, Wakayama T, Wutz A, et al. Generation of mice from wild-type and targeted ES cells by nuclear cloning. Nat Genet 2000;24:109–110.
37. Keefer CL, Baldassarre H, Keyston R, et al. Pregnancies following transfer of reconstructed embryos derived from BELE® fetal fibroblasts and in-vitro matured oocytes [abstract]. Biol Reprod 1999;60 (Suppl.1):553.
38. Wells DN. Animal cloning: current progress, challenges and future prospects. Rev Bras Reprod Anim 1999;23:86–97.
39. Stice SL, Strelchenko NS, Keefer CL, Matthews L. Pluripotent bovine embryonic development following nuclear transfer. Biol Reprod 1996;54:100–110.
40. Kruip TAM, den Daas JHG. In vitro produced and cloned embryos: effects on pregnancy, parturition and offspring. Theriogenology 1997;47:43–52.
41. Baguisi A, Behboodi E, Melican DT, et al. Production of goats by somatic cell nuclear transfer. Nat Biotechnol 1999;17:456–461.
42. Garry FB, Adams R, McCann JP, Odde KG. Postnatal characteristics of calves produced by nuclear transfer cloning. Theriogenology 1996;45:141–152.
43. Wells DN, Misica PM, Day TA, Tervit HR. Production of cloned lambs from an established embryonic cell line: a comparison between in vivo- and in vitro-matured cytoplasts. Biol Reprod 1997;57:385–393.
44. Renard JP, Chastant S, Chesne P, et al. Lymphoid hypoplasia and somatic cloning. Lancet 1999;352:1489–1491.
45. Smith LC. Production of multiple genetically identical farm animals by nuclear transplantation. Can Vet J 1991;32:94–98.

BOOK REVIEW

COMPTE RENDU DE LIVRE

Tranquilli WJ, Grimm KA, Lamont LA. ***Pain Management for the Small Animal Practitioner.*** Teton New Media, Jackson, Wyoming, 2000, 125 pp. ISBN 1-893441-07-5. US $32.75 (text). Distributed in Canada by Lifelearn Inc.

This spiral-bound handbook is an excellent quick reference for pain management options for small animal practitioners. The text is divided into 5 sections: physiology of pain, analgesic drugs, analgesic techniques, pain management for specific conditions, and management of chronic pain. A CD-ROM is also available as an accompaniment to the text and includes the complete text, as well as video clips demonstrating the various analgesic techniques in an easy to search format.

Both the book and the CD-ROM provide suggestions and options for providing analgesia in a variety of clinical situations in an easy to use format. The text includes symbols to highlight important points in the text, such as salient features, potential complications, and cautions, as well as techniques, which are demonstrated on the accompanying CD-ROM.

Section 1 provides a fairly complete yet concise overview of the physiology of pain, pain recognition, and the theories of preemptive analgesia and balanced analgesia. This section presents current information that practitioners may not be familiar with, because this is a relatively new area of study.

The 2nd section deals with analgesic drugs, grouping them into categories such as opioids, nonsteroidal anti-inflammatories, and local anesthetics. Under each category of drug are a brief description of the mechanism of action, lists of potential side effects, contraindications, and drug interactions. This section also contains excellent tables for each drug category, listing commonly used drugs, dosage ranges for both cats and dogs, and duration of effect, as well as comments specific to individual drugs. A particularly useful table is that for oral analgesic agents that can be dispensed for ongoing pain control after patients have been discharged from hospital. Although the book is published in the United States, the authors do include drugs that are not licensed in that country but are available in Canada, such as tolfenamic acid.

The 3rd section of the book explains specific analgesic techniques in a clear, easy to follow format. In addition to a detailed explanation of how to perform the technique, there is a list of materials required, drugs and dosages used, potential complications, contraindications, and a comment on the skill level required to perform the technique in question. Clear diagrams demonstrate important relevant anatomic landmarks. Specific techniques described include epidural, dental blocks, brachial plexus blocks, and intravenous constant rate infusion techniques. Many of these techniques are demonstrated in the video clips included on the accompanying CD-ROM. The video clips are clear and easy to follow. The search feature on the CD-ROM makes it possible to quickly review a video prior to performing a given technique.

Section 4 suggests specific analgesic protocols for specific conditions and procedures. The emphasis is on providing balanced analgesia, beginning with preemptive analgesia, following up with suggestions for regional and postoperative analgesia, and even including suggestions for dispensing analgesic agents to provide ongoing pain relief for patients after discharge from the hospital. Specific conditions dealt with range from elective procedures such as ovariohysterectomies and castrations, to dentals, orthopedic procedures, and conditions such as acute pancreatitis.

The final section of the text offers suggestions for managing chronic pain in veterinary patients and includes conditions such as osteoarthritis, osteosarcoma, and chronic otitis.

An additional feature of the text is a comprehensive index, which lists drugs by generic and trade names, and available concentrations as well as supplier names, addresses, and Web sites. There is also a list of recommended readings.

This book and CD-ROM provide a valuable, clear, easy to use guide and reference to assist small animal practitioners in providing analgesia to their patients. The excellent tables, clear diagrams, and easy to read text makes this a handbook that would be useful to keep readily accessible in the treatment room for quick "patient side reference." The video segments on the optional CD-ROM should facilitate the practitioner in mastering new analgesic techniques.

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