

Assisted reproductive technologies in cattle: a review

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Summary

Over a period of approximately thirty years, commercial bovine embryo transfer has become a large international business. The technology is well established, and more than 500,000 embryos are produced annually from superovulated cows world wide. Since bovine embryos with intact *zonae pellucidae* can be specified pathogen-free through washing procedures, thousands of frozen embryos are routinely sold and transferred between countries. Throughout the world, approximately 15% of bovine embryos are produced by *in vitro* technology. Polymerase chain reaction technology is currently being used for sexing embryos on a small scale, and it is likely that this technology will be used for 'embryo diagnostics' in the future. Semen sexing is an established technology and is likely to be used on a small scale in the near future, especially in *in vitro* embryo production systems. The cloning of adult cattle through nuclear transfer and the production of cloned, transgenic cattle has been technically achieved. However, this is an expensive and inefficient technology, which is being used primarily by the pharmaceutical industry. Benefits in agriculture are likely to be minimal in the near future.

Keywords

Assisted reproductive technologies – Cattle – Cloning – Embryo diagnostics – Embryo sexing – Embryo transfer – In vitro technology – Semen sexing.

Introduction

For an historical perspective on assisted reproduction, the reader is referred to a recent, comprehensive review of farm animal embryo transfer and its associated technologies (4). The period before 1970 will not be covered in any great detail in this paper, and reviews will be referenced as often as possible to conserve space. In brief, the first successful transfer of mammalian embryos was performed by Walter Heape in 1890. Heape transferred two four-cell Angora rabbit embryos into an inseminated Belgian doe, which subsequently gave birth to four Belgian and two Angora young (3, 4, 30). There appear to be no reports of further success in mammalian embryo transfer until the 1920s, when several investigators again described embryo transfer in rabbits (3). Warwick and colleagues did considerable work on embryo transfer in sheep and goats in the 1930s

and 1940s (4), but it was Umbaugh (73) who reported on the first successful embryo transfers in cattle in 1949. He produced four pregnancies from the transfer of cattle embryos, but all the recipients aborted before the pregnancies reached full term. In 1951, the first embryo transfer calf was born following the surgical transfer of an abattoir-derived day-5 embryo (82). However, it was Rowson and colleagues at Cambridge who developed much of the technology that later found commercial use (4).

The bovine embryo transfer industry as it is known today arose in North America in the early 1970s (3, 4). Continental breeds of cattle imported into Canada were very valuable and relatively scarce because of international health and trade restrictions. Embryo transfer offered a means by which their numbers could be multiplied rapidly. For several years, the most common use of embryo

transfer in animal production programmes was the proliferation of so-called desirable phenotypes. However, in 1987, Smith (59, 60) at the University of Guelph introduced the concept of multiple ovulation and embryo transfer (MOET). He showed how well-designed MOET programmes could lead to increased selection intensity and reduced generation intervals, resulting in improved genetic gains. The establishment of nucleus herds and subjecting heifer offspring to 'juvenile MOET' could result in genetic gains that approached twice those achieved with traditional progeny test programmes. It is noteworthy that, prior to the work of Smith, most embryo transfers conducted in Canada occurred in beef cattle, whereas approximately 75% of the embryo transfer work in Canada in 2002 involved dairy cattle. Approximately 65% of embryo transfer work in the United States of America (USA) continues to involve beef cattle (70).

Embryo transfer is now commonly used to produce artificial insemination (AI) sires from highly proven cows and bulls (10, 68). Although technical costs would seem to preclude the use of embryo transfer techniques for anything but seed-stock production at this time, the commercial cattle industry can benefit by the use of bulls produced through well-designed MOET programmes (15, 53). The success of MOET programmes has now led to the use of this technology to test AI sires genetically (40). Selected cows are superstimulated and inseminated to highly proven bulls. Male offspring are placed in waiting while female offspring are placed into production. Bulls are then proven by production records from siblings rather than offspring (61). With this approach, it is possible to test a bull genetically in three-and-a-half years, as opposed to five-and-a-half years using traditional progeny testing schemes. Although some accuracy may have been sacrificed, the shorter generation intervals result in a greater overall genetic gain. Although results supported the theory, physiology was a limiting factor. Superovulatory results made it difficult to produce the desired number of offspring for genetic testing.

Although the applications (42) and techniques (43) associated with bovine embryo transfer have previously been reviewed, a brief historical perspective may be useful. Early investigators described non-surgical embryo recovery techniques (52), but these were not very successful, and so all embryo recoveries and transfers performed in the early 1970s were conducted surgically. As a consequence, the first commercial embryo transfer programmes relied on mid-ventral surgical exposure of the uterus and ovaries with the donor under general anaesthesia. This required surgical facilities and limited use of the technology in the dairy industry because the udder of dairy cows hindered mid-ventral access to the reproductive tract. It was not until the mid-1970s that non-surgical embryo recovery became sufficiently developed to be used in commercial practice (17, 19, 50).

In the early 1980s, non-surgical embryo transfer techniques (51, 86) were adopted, allowing embryo transfer to be practised on the farm and making it especially attractive to dairy farmers.

The embryo transfer industry grew rapidly in the late 1970s, both in terms of the number of practitioners and the number of donors flushed. Seidel (54) reported that, in 1979, more than 17,000 pregnancies resulting from the transfer of bovine embryos were recorded in North America. More recently, Thibier (70) reported that, in the year 2002, 538,312 bovine embryos were transferred world wide, of which 52% were transferred after on-farm freezing and thawing and 15% were produced by *in vitro* techniques. North America has continued to be the centre of commercial embryo transfer activity, with more than 42,000 donor cows superstimulated and more than 190,000 embryos transferred (35% of all reported embryo transfers in the world). However, commercial embryo transfer in North America is static or declining. In South America, by comparison, commercial embryo transfer is expanding, accounting for 22% of embryo transfers throughout the world in 2002. Europe and Asia each reported about 17% of the total number of bovine embryo transfers in 2002 (70).

The International Embryo Transfer Society (IETS) was founded in 1974, with 82 Charter Members, representing researchers, academics and veterinary practitioners (69). However, with the founding of regional embryo transfer organisations, a growing number of commercial embryo transfer practitioners have discontinued membership in the IETS in favour of their regional organisations. It is also clear that a growing number of the IETS membership are basic researchers representing government, industrial or academic institutions, including human medicine (30). However, the IETS has played a very important role in the dissemination of basic and applied information, assisting in the rapid growth of the embryo transfer industry in the 1980s and 1990s. In particular, the Import/Export Committee of the IETS, now referred to as the Health and Safety Advisory Committee (HASAC), has been instrumental in gathering and disseminating scientific information on the potential for disease control through the use of bovine embryo transfer (69). The following contributions are of note:

- the 'round table' meeting on disease control issues and embryo transfer, organised by the IETS and the World Organisation for Animal Health (OIE) in 1985 (46)
- the formulation of disease control procedures for the international movement of embryos, as established in the OIE *Terrestrial Animal Health Code* (47)
- the organisation of the International Embryo Movement Symposium, sponsored by the IETS at the XXXIII World Veterinary Congress in Montreal in 1987.

These landmark events, in addition to continued close collaboration between the IETS and the OIE, have made the international movement of cattle embryos possible. In this regard, the *Manual of the IETS* (64) has become the reference source for disease control procedures used in export protocols (64).

In 1982, the American Embryo Transfer Association was formed to unite and organise the commercial embryo transfer industry in the USA. In 1984, the Canadian Embryo Transfer Association was formed. The objectives of both organisations include the following:

- to establish standards for performance and conduct
- to liaise with Federal agencies for both domestic and international embryo transfer.

These associations also co-operate directly with breed associations, producer groups and international groups, such as the IETS. Their purpose is to establish standards of practice which ensure confidence in the use of embryo transfer technology for disease control, in the USA and Canada and throughout the world. Their certification programmes are vital in ensuring that embryo transfer practitioners are technically and ethically competent to handle embryos for international trade.

There has been no appreciable increase in the number of embryos produced per superovulated donor over the past twenty years. However, recognising the importance of follicle wave dynamics (1, 7) and devising methods for the synchronisation of follicular wave emergence (7, 8) have simplified the way in which superovulation is achieved, resulting in increased embryo production per unit of time. Donor cows are being superstimulated more frequently than in the past, and more embryos are being produced per year with no change in the actual superstimulation protocol. Applying similar procedures to the recipients has made oestrus detection, and the need to wait for animals to 'come into heat', unnecessary, facilitating the management of commercial embryo transfer programmes (8).

Disease control

Several large studies have now shown that the bovine embryo does not transmit infectious diseases. In fact, the Research Subcommittee of HASAC, within the IETS, has categorised disease agents based on the risk of transmission with a bovine embryo (66). Category 1 comprises diseases or disease agents for which sufficient evidence has accrued to show that the risk of transmission is negligible, provided that embryos are properly handled between collection and transfer. Proper handling includes the following:

- microscopic inspection of the *zona pellucida* at a magnification of at least 50× to ensure that it is intact and free of adherent material
- ten washes of the embryo with at least 100-fold dilution of each wash
- on occasion, two trypsin treatments to dissociate viruses that tend to stick to the *zona pellucida*.

Category 1 diseases include, as follows:

- enzootic bovine leukosis
- foot and mouth disease (cattle)
- bluetongue (cattle)
- *Brucella abortus* (cattle)
- infectious bovine rhinotracheitis (trypsin treatment required)
- Aujeszky's disease (pseudorabies) in swine (trypsin treatment required)
- bovine spongiform encephalopathy.

Category 2, 3 and 4 diseases are those for which less research information has been generated. However, it should be noted that none of the infectious diseases studied has been transmitted by *in vivo*-produced bovine embryos, provided embryo handling procedures were followed correctly (58). Consequently, it has been suggested that embryo transfer be used to salvage genetic material in the event of a disease outbreak (85), which could be a useful alternative in establishing disease-free herds.

Embryo export and import

The intercontinental transportation of live animals costs several thousands of dollars, whereas an entire herd can be transported, in the form of frozen embryos, for less than the price of a single plane fare. Additional benefits of frozen embryos in comparison to live animals include, as follows:

- a reduced risk of disease transmission
- reduced quarantine costs
- the ability to select animals from a wider genetic base
- the ability to retain the genes of the selected animals within the exporting country
- the ability of the animals to adapt.

Adaptation is particularly important in tropical and subtropical environments, where the resulting calf would have the opportunity to adapt first while in the uterus and

then while suckling a recipient cow indigenous to the area. However, the reduced risk of infectious disease transmission is the overwhelming benefit of using embryos in international trade.

In 1961, the successful long-distance transportation of sheep embryos in the oviducts of rabbits was reported (30). Although there are no published records of cattle embryos being transported in a similar way, the advent of reliable cryopreservation techniques has aided the movement of cattle embryos across international borders. Over the last ten years, embryo import regulations for many countries have been simplified to such a degree that embryo exporters are now able to operate in a predictable and routine manner. In 2002, approximately 30,000 embryos were frozen in North America for export. Obviously, the growth of embryo exports is closely linked to the existence of realistic health regulations in the importing countries. However, changes in these regulations are often unpredictable, especially when relatively new disease problems arise.

Although handling procedures recommended by the IETS make it possible to safely export *in vivo*-derived embryos originating from donors which are sero-positive to certain pathogens (63), the case is very different for embryos produced with *in vitro* techniques (44). The structure of the *zona pellucida* of *in vitro*-produced (IVP) bovine embryos differs from that in *in vivo*-derived embryos (79). In a recent review, it was shown that a number of pathogens are more likely to remain associated with *in vitro*-derived embryos following washing than with *in vivo*-derived embryos (65). This has potentially serious ramifications for the international movement of IVP embryos. Serological testing for the microbes in question could be performed on donor cows that produce oocytes through transvaginal ultrasound-guided ovum pick-up (OPU). However, there may be a serious health risk when oocytes are recovered from ovaries derived from abattoirs (44).

Cryopreservation: direct transfer of frozen/thawed embryos and vitrification

The development of effective methods of freezing embryos (38, 83) has made embryo transfer a much more efficient technology, which no longer depends on the immediate availability of suitable recipients. Freezing bovine embryos is now common and pregnancy rates are only slightly less than those achieved with fresh embryos (39). Recently, the use of highly permeating cryoprotectants, such as ethylene glycol, has allowed the direct transfer of bovine embryos

(32, 75). In this approach, the embryo straw is thawed in a water bath, much like semen, and its contents are deposited directly into the uterus of the recipient, as occurs in AI. There is no need for a microscope or complicated dilution procedures. The cryoprotectant leaves the embryo in the uterus, without causing osmotic stress. In a recent study of the North American embryo transfer industry, pregnancy rates from direct-transfer embryos were comparable to those achieved with glycerol (39). During 2002, more than half the embryos collected in North America were frozen, and most were frozen in ethylene glycol for direct transfer (70). Although the level of skill required to transfer these embryos is the same as that needed for conventionally frozen embryos, no embryologist is required at the time of thawing. Consequently, a growing number of direct-transfer embryos are now being transferred by technicians with experience in AI.

Freezing and thawing procedures are time-consuming and require the use of biological freezers and a microscope. Complicated embryo freezing procedures may soon be replaced by a relatively simple procedure called vitrification (48). With vitrification, high concentrations of cryoprotectants are used and the embryo in its cryoprotectant solution is placed directly into liquid nitrogen. As a result of the high concentration of cryoprotectants and the ultra-rapid method of freezing, ice crystals do not form; instead the frozen solution forms a 'glass'. Since ice crystal formation is one of the most damaging processes in freezing, vitrification has much to offer in the cryopreservation of oocytes and IVP embryos. However, its greatest advantage is its simplicity. Vitrification is now widely used experimentally and recent results suggest that bovine embryos can be vitrified in 0.25 ml straws for direct transfer (76).

Embryo production *in vitro*

Although each ovary contains hundreds of thousands of oocytes at birth, most are lost through atresia. This process starts even before birth. This tremendous loss of genetic material could be reduced by harvesting oocytes from the ovary and using IVP techniques (12, 29). Bovine IVP is now a well-established and reasonably efficient procedure. Moreover, OPU at frequent intervals, in combination with *in vitro* fertilisation, has proved its worth in improving or increasing the yield of embryos from designated donors. In addition, IVP can be used to salvage irreplaceable genetic material following slaughter for infectious disease control or culling for other reasons (30). *In vitro* fertilisation has also been used to produce the thousands of embryos needed for scientific research (26), including efforts to produce embryonic stem cells. The constituent oocyte

maturation and embryo culture techniques are also integral parts of the procedures for cloning by somatic cell nuclear transfer and generating transgenic cattle which produce valuable pharmaceutical proteins in their milk (45). *In vitro* fertilisation by intracytoplasmic sperm injection, so prominent in assisted human reproduction, is feasible in cattle, even with freeze-dried sperm (36), but not yet widely applied.

A few laboratories have reported very modest successes in producing pregnancies from IVP of embryos from calves (18, 22, 67), which offers the potential for increased genetic gain by decreasing generation intervals (5). In addition, OPU has proven to be safe and very successful in pregnant cattle and is often used when there is high demand for offspring from a particular donor cow, or MOET programmes require additional offspring. Oocytes with good viability have been collected once or twice weekly, or after pre-treatment with follicle-stimulating hormone, as late into gestation as 90 to 150 days, with very few abortions (23, 25).

Several authors have directly addressed the question of using IVP as a substitute for *in vivo* embryo production by conventional embryo transfer procedures (11, 29, 57). It is clear that pregnancies can be produced by IVP from donor females that are infertile both to AI and conventional embryo transfer technology (20, 31, 41). However, it is unclear whether IVP is a realistic alternative to conventional superovulation and embryo transfer for producing embryos from reproductively healthy cattle. Data for 2002 show that, on a worldwide basis, more than 80,000 IVP embryos (both fresh and frozen) were transferred (70). This is nearly double the number reported for 2001, but is accounted for, almost entirely, by the increase of activity in Brazil.

One commercial embryo transfer unit in North America has provided data comparing the efficacy of conventional embryo transfer to that of IVP in cattle (11). Success rates for IVP (4.7 embryos per OPU session, 48% blastocysts from oocytes recovered) greatly exceeded the published results of other commercial programmes. The authors directly compared the results of IVP and the conventional *in vivo* programmes and concluded that IVP would produce about 3.4 times more embryos and 3.2 more pregnancies in a 60-day period, assuming only one superovulation per donor. This rate is somewhat higher than that reported by other commercial embryo transfer practitioners (see above). At present, under commercial conditions in North America, it appears to be more expensive to produce pregnancies by IVP than with conventional superovulation and embryo transfer. For most breeders, this technology is an advantage only for extremely valuable cows which are infertile or fail to produce embryos after superstimulation.

Prenatal determination of sex

Determining the sex of bovine embryos before implantation, using polymerase chain reaction (PCR), is a service offered by a moderate number of embryo transfer businesses (71). However, removing the biopsy from the embryo requires a high level of operator skill, and embryo biopsy is an invasive technique that results in disruption of the integrity of the *zona pellucida* and some reduction in the viability of the embryo. Both this procedure and a successful PCR programme also require a higher level of hygiene and care than is often practised with 'on farm' embryo transfer. Although a modest number of livestock breeders readily accept embryo sexing, it is not a technology that has found widespread use in the embryo transfer industry. During 2002, almost 3,800 sexed embryos were transferred in Canada, one-third of them after freezing and thawing (70).

In the near future, PCR assays to identify other traits of economic importance will no doubt become available (6). The extent of the market for this technology will depend on the value of the genes in question to cattle breeders. Marker-assisted selection (MAS), based on identifying genetic markers for unknown alleles of valuable traits, probably has a similar future (24). Like genotyping of specific alleles, MAS can potentially be applied to embryo biopsies if sufficiently valuable markers can be identified. A PCR assay currently exists for simultaneous detection of the bovine leucocyte adhesion deficiency gene and the sex of embryo biopsies (30). It is probable that PCR techniques will be developed that permit the analysis of a large number of markers from one biopsy simultaneously, leading to the concept of 'embryo diagnostics'.

The flow cytometric technology used to separate X- and Y-bearing sperm into live fractions has been improved over the last ten years (34, 35). Approximately 10 million live sperm of each sex can be sorted per hour (55), with a resulting purity rate of 90%. In AI field trials involving approximately 1,000 heifers, pregnancy rates following insemination with one million sexed, frozen sperm were reported to be 70% to 90% the rate of unsexed controls inseminated with 20 to 40 million sperm (56). A recent study which compared 574 calves produced from sex-sorted sperm with 385 control calves concluded that there were no differences in gestation, neonatal deaths, ease of calving, birth weight or survival rate to weaning (72). The disadvantages of flow cytometry are the slow speed of sorting, the decreased sperm viability (pregnancy rates), especially in superovulated donor cows, the cost of the semen, and the availability of semen from specific bulls (2). It is likely that sexed semen will have the greatest use in IVP of bovine embryos in the near future.

Production of identical offspring

Embryos can be split before transfer to produce identical twins. Pregnancy rates of 50% or more per demi-embryo have been reported, resulting in a net pregnancy rate of more than 100% per original bovine embryo (27). Cloning can also be used to produce identical offspring (9). Embryonic cloning was first reported by Willadsen (81). He showed that the developmental programme for eight- and sixteen-cell nuclei could be reset back to fertilisation by the oocyte cytoplasm. The births of lambs cloned from cultured embryonic cells in 1996 (14), and of 'Dolly', cloned from a mammary cell taken from an adult ewe (84), have resulted in a great deal of research on cloning in a number of mammals. The results have contradicted two long-held models in developmental biology, as follows:

- a) that differentiated embryonic cells are irreversibly modified
- b) that cells (in this case, somatic cells) from adult mammals could not be re-programmed to develop into embryos.

Following the success of cloning in sheep, bovine clones have also been produced, using the following cell types (reviewed in 30):

- foetal fibroblasts
- oviductal and cumulus cells
- granulosa cells
- skin fibroblast cells
- muscle cells.

Adult somatic cell nuclear transfer also has been used to preserve the last surviving cow in a rare breed (80), and fibroblasts from a 21-year-old bull were successfully used to produce a cloned calf (33). Progress has been hindered by very poor rates of cloning efficiency, low pregnancy rates, high abortion rates and poor calf survival (21, 37, 77, 87). Consequently, the use of this technology for multiplying elite cattle on a large scale depends on improving the efficiency of the procedures (28). However, it has been reported recently that more than 2,600 cloned cattle embryos have been transferred into recipients in one programme in the USA (20). Moreover, very recently, it was reported that it was possible to clone cattle with nothing more than regular embryo transfer equipment (74).

Transgenics

The use of cultured somatic cells to produce clones allows workers to genetically modify the cells through gene transfer (16). Unfortunately, very poor rates of cloning

efficiency, low pregnancy rates, high abortion rates and poor calf survival are common. Transfection has largely replaced the inefficient technique of pro-nuclear micro-injection, which was used during the early years of transgenic animal production. Transfection has proved very successful in producing transgenic cells with relatively short deoxyribonucleic acid (DNA) sequences. However, longer DNA sequences, which incorporate large and complex genes, have been successfully incorporated into human artificial chromosomes, which were then introduced into bovine fibroblasts and, ultimately, into bovine clones (49). This work involved a number of steps, including the production of intermediate foetuses, which were genetically tested and then used to produce the desired cloned cattle. Robl *et al.* (49) reported that 21 calves carrying the human artificial chromosomes were produced and that at least some of these offspring produced human polyclonal antibodies.

Transgenic technology could also be used to produce clonal lines of embryos that have been genetically modified (13, 45, 49, 62, 78) to:

- improve the efficiency of meat or milk production
- modify milk composition
- improve disease resistance.

However, the use of this technology to multiply elite or genetically modified cattle on a large scale depends on major improvements in the efficiency of the procedures. It is highly likely that cloned transgenic embryos will be used by the pharmaceutical industry well before they can be produced at a cost and with an efficiency that is acceptable to the cattle industry. Thus, the availability of transgenic clones for the cattle industry will probably be quite limited for some years to come.

Conclusion

Commercial embryo transfer in cattle has become a well-established industry in many parts of the world, with more than 500,000 embryos being transferred on an annual basis. Although this results in a very small number of offspring, considering the total numbers of calves born throughout the world each year, the impact is large because of the quality of animals being produced. Multiple ovulation and embryo transfer are now being used for real genetic improvement, especially in the dairy industry, and most semen used today comes from bulls produced by embryo transfer. However, the real benefit of embryo transfer is that *in vivo*-produced bovine embryos can be specified pathogen-free through washing protocols, making this an ideal procedure for disease control programmes or in the international movement of animal

genetic material. Techniques have improved over the past thirty years so that frozen-thawed embryos can be transferred to suitable recipients as easily and simply as in AI. *In vitro* embryo production and embryo and semen sexing are also successfully performed, but time and cost limit their widespread use. Somatic cell cloning and the production of transgenic, cloned embryos have also been shown to be possible, but the high cost and inefficiency of these procedures preclude their use in cattle improvement

programmes at this time. A combination of embryo transfer, using highly proven cows inseminated with semen from highly proven bulls, and industry-wide artificial insemination would appear to be the most likely use for bovine embryo transfer in the near future.

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Le point sur les techniques de reproduction assistée chez les bovins

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Résumé

Depuis une trentaine d'années, le transfert d'embryons de bovins à l'échelle industrielle est devenu un secteur d'activité considérable au plan international. La technologie est bien établie et plus de 500 000 embryons sont produits chaque année dans le monde à partir de vaches traitées par des techniques de superovulation. Étant donné que les embryons de bovins dont la zone pellucide est intacte peuvent être rendus exempts d'agents pathogènes par des procédures de lavage, des milliers d'embryons congelés sont régulièrement vendus et transférés entre pays. Environ 15 % des embryons de bovins dans le monde sont produits par une technologie *in vitro*. L'amplification en chaîne par polymérase (PCR) est actuellement utilisée pour le sexage des embryons à petite échelle, et il est probable que cette technologie sera utilisée à l'avenir à des fins diagnostiques chez les embryons. Le sexage de la semence est une technologie établie qui sera probablement utilisée à petite échelle dans un futur proche, notamment dans les systèmes de production d'embryons *in vitro*. Il existe actuellement des techniques pour cloner des bovins adultes par transfert nucléaire et produire des bovins transgéniques clonés. Il s'agit cependant d'une technologie onéreuse et peu rentable, utilisée avant tout par l'industrie pharmaceutique et présentant dans l'immédiat un intérêt limité pour l'élevage.

Mots-clés

Bovin – Clonage – Diagnostic chez l'embryon – Sexage de la semence – Sexage des embryons – Technique de reproduction assistée – Technologie *in vitro* – Transfert d'embryons.

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Panorámica de las técnicas de reproducción asistida en bovinos

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Resumen

La transferencia de embriones bovinos con fines comerciales ha llegado a convertirse, en espacio de unos treinta años, en un importante negocio a escala internacional. Las técnicas están consolidadas, y anualmente se generan en el mundo más de 500.000 embriones a partir de vacas superovuladas. Desde que es posible certificar mediante procedimientos de lavado que un embrión bovino con la zona pelúcida intacta está libre de patógenos, miles de embriones congelados se venden y transfieren de un país a otro con toda normalidad. Aproximadamente un 15% de los embriones bovinos generados en el mundo se obtienen empleando técnicas *in vitro*. Hoy en día, a pequeña escala, se utiliza la reacción en cadena de la polimerasa (PCR) para elegir el sexo de los embriones, y es probable que en el futuro se extienda este procedimiento al 'diagnóstico de embriones'. La elección del sexo en muestras de semen es una técnica bien descrita que seguramente va a aplicarse a pequeña escala en un futuro próximo, sobre todo en sistemas de producción de embriones *in vitro*. Técnicamente ya es posible clonar bovinos adultos por transferencia nuclear y generar animales clonados transgénicos. Se trata sin embargo de un procedimiento caro y poco eficiente, utilizado sobre todo por la industria farmacéutica. No cabe prever que resulte de gran utilidad para la producción agropecuaria en un futuro próximo.

Palabras clave

Bovino – Clonación – Diagnóstico de embrión – Sexaje de embriones – Sexaje de semen – Técnica de reproducción asistida – Técnica *in vitro* – Transferencia de embrión.



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