



Assisted Reproductive Technologies in Cattle: Applications in Livestock Production, Biomedical Research and Conservation Biology*

Miguel A Velazquez[†]

Escuela Superior de Ciencias Agropecuarias, Universidad Autónoma de Campeche, MEXICO

Received: 24 October 2007; accepted 16 March 2008
Online on 26 March 2008

Abstract

Velazquez MA. Assisted Reproductive Technologies in Cattle: Applications in Livestock Production, Biomedical Research and Conservation Biology. *ARBS Annu Rev Biomed Sci* 2008;10:36-62. In cattle, assisted reproductive technologies (ART) can be defined as techniques that manipulate reproductive-related events and/or structures to achieve pregnancy with the final goal of producing healthy offspring in bovine females. The present review includes manipulation of female reproductive tract physiology, artificial insemination, multiple ovulation and embryo transfer, *in vitro* production of embryos, *in vitro* assisted fertilization, cloning, transgenesis, xenografting-germ cell transplantation, preimplantation genetic diagnosis and sperm sexing. This review shows that several ART are being currently applied commercially in the cattle industry with acceptable results. On the other hand, others have low efficiency in producing cattle offspring and are predominantly applied in experimental settings. Several of these ART can cause detrimental effects at the prenatal and postnatal period and therefore they need to be improved. However, even if these bovine-related biotechnologies are properly improved, they might be more useful in the conservation of endangered ungulates, production of pharmaceuticals, or as experimental models for human reproduction.
© by São Paulo State University – ISSN 1806-8774

Keywords: assisted reproduction, cattle, conservation biology, biomedicine

Table of Contents

1. Introduction
 2. Historical Background
 - 2.1. Manipulation of female reproductive tract physiology
 - 2.2. Artificial insemination
 - 2.3. Multiple ovulation and embryo transfer
-

*Part of this manuscript was developed while the author was undertaking his MSc in Animal Reproduction at the University of Liverpool, UK.

[†]Correspondence

Miguel A Velazquez. Escuela Superior de Ciencias Agropecuarias, Universidad Autónoma de Campeche, Calle 53 s/n, C.P. 24350, Escárcega, Campeche, México. E-mail: lestaurus_18@hotmail.com

- 2.4. *In vitro* production of embryos
 - 2.5. Cloning
 - 2.6. *In vitro* assisted fertilization
 - 2.7. Transgenesis
 - 2.8. Xenografting-germ cell transplantation
 - 2.9. Preimplantation genetic diagnosis
 - 2.10. Sperm sexing
 - 3. Concluding Remarks
 - 4. Acknowledgments
 - 5. References
-

1. Introduction

The way domestic cattle are used for meat and milk production has been changing during the last decades. New biotechnologies have been created and applied to the cattle industry to increase efficiency in both beef and dairy production systems. Among these technologies are those involved in assisted reproduction. The ultimate aim of assisted reproductive technologies (ART) is the birth of healthy offspring. The techniques that are considered ART are usually the ones related to gamete and embryo manipulation (Galli *et al.*, 2003; McEvoy *et al.*, 2003; Mapletoft & Hasler, 2005). However, for the sake of this review, ART will be defined as any technique that interferes with the normal biological pathways of reproductive-related events and/or structures in order to contribute to the establishment of pregnancy with the final goal of producing healthy offspring in a female bovine. In general, ART manipulate events and/or structures related to ovulation, fertilization and embryo development. The ART considered in this review includes manipulation of female reproductive tract physiology, artificial insemination (AI), multiple ovulation and embryo transfer (MOET), *in vitro* production of embryos, *in vitro* assisted fertilization, cloning, transgenesis, xenografting-germ cell transplantation, preimplantation genetic diagnosis, and sperm sexing. Cryopreservation of gametes and embryos (Curry, 2000; Massip, 2003; Tominaga, 2004; Moore & Bonilla, 2006; Seidel, 2006b) will not be addressed in this review.

Besides its use in livestock production, bovine ART are important for studying reproductive processes. This is reflected by the exponential increase in literature on this subject in recent years (Seidel, 2006a). At present, commercial application of some ART is being compromised by the low production of offspring achieved. The aim of this review is to give a current view on how efficient and useful are the ART in cattle. Besides describing their use in the cattle industry, applied uses and possible applications in the field of biomedical research and conservation biology will be given.

2. Historical Background

2.1. Manipulation of female reproductive tract physiology

In order to achieve pregnancy, the ovarian activity can be controlled by mechanical (*i.e.* ultrasound-guided transvaginal follicular ablation) and/or exogenous hormonal interventions on luteal and follicular development (Diskin *et al.*, 2002; Bo *et al.*, 2003). These approaches are used for the treatment of conditions such as postpartum acyclicity, repeat breeding and ovarian cysts (Mwaanga & Janowski, 2000; Yavas & Walton, 2000; Wiltbank *et al.*, 2002; Bo *et al.*, 2003; Macmillan *et al.*, 2003; Rhodes *et al.*, 2003). Another use of these approaches is the synchronization of the estrous cycle. Synchronization is used in animal production systems where calving patterns are important and as a complement to other ART such as AI and MOET programs (Lowman *et al.*, 1994; De Rensis & Peters 1999; Bo *et al.*, 2002; Cavalieri *et al.*, 2006). Hormonal treatments have been also used to try and improve embryo survival after AI or embryo transfer in an effort to achieve acceptable or high pregnancy rates (Thatcher *et al.*, 2001, 2002, 2003). The most common pharmacological approaches include the use of gonadotropin release hormone (GnRH), progestagens (*e.g.*, progesterone releasing intravaginal device [PRID], controlled internal drug release [CIDR]), prostaglandin F_{2α} (PGF_{2α}), gonadotropins (*e.g.*, human chorionic gonadotropin [hCG], equine chorionic gonadotropin [eCG]), estrogens (*e.g.*, estradiol benzoate, estradiol cypionate) and growth factors (*e.g.*, bovine somatotropin, insulin). These hormonal regimes

are applied alone or in combination, depending on the production system, husbandry facilities (especially in developing countries) and cost.

From hundreds of literature about these methods it can be observed that there is variability in the response of such approaches. For example, the protocol known as “Ovsynch” and its several combinations, which allows a timed insemination without the necessity of estrus detection, has been claimed to be the most successful with pregnancy rates ranging from 50 to 75% (Mapletoft *et al.*, 2003; Thatcher *et al.*, 2004; Johnson, 2005; Thatcher *et al.*, 2006). However, synchronization treatments can increase the incidence of abnormal estrus and the risk of embryo loss (Macmillan *et al.*, 2003). No response and variation in the intervals from treatment to estrus and ovulation are in part related to the follicular status at the time of treatment (De Rensis & Peters, 1999). The response to treatment for anovulatory conditions is also affected by age and body condition. In the case of postpartum anestrus, the interval from calving to treatment is important as well (Rhodes *et al.*, 2003). Ultrasonography and estrus detection devices are helpful tools in the improvement of the efficiency of these treatments (Ribadu & Nakao, 1999; Diskin & Sreenan, 2000; Fricke, 2002). Treatments with growth factors such as bovine somatotropin appear to be more useful for solving subfertility problems than for improving reproductive efficiency in herds with good fertility (Morales-Roura *et al.*, 2001; Selvaraju *et al.*, 2002; Oropeza *et al.*, 2004; Thatcher *et al.*, 2006). Furthermore, in some cases these treatments can infringe detrimental effects on embryo survival with the concurrent negative effect on pregnancy rates (Bilby *et al.*, 2004).

In most areas of Europe, because of both year-round calving and ethical or consumer concerns, hormones are mainly used to treat reproductive diseases and not for pharmaceutical control of breeding (van Arendonk & Liinamo, 2003). However, research done in the UK has shown that dairy farmers could have obtained benefits from a planned breeding program, especially in farms with low estrus detection and moderate pregnancy rates (Esslemont & Mawhinney, 1996). Nevertheless, careful analysis regarding cost of labor and hormone administration should be taken into account before considering the application of any reproductive hormonal regime (Rabiee *et al.*, 2005).

To obtain precise control of the estrous cycle, it is necessary to understand the hormonal control of ovarian physiology. Knowledge on embryo-maternal interactions is also of pivotal importance for the application of hormonal treatments aimed at correcting situations of subfertility or for the improvement of reproductive efficiency. Detailed characterization of the complex processes of bovine reproductive physiology will be of great importance in the cattle industry, and the knowledge gained will be helpful in understanding clinical reproductive conditions in humans (Campbell *et al.*, 2003).

2.2 Artificial insemination

Worldwide, AI has been the main vehicle for the improvement of genetic quality herds. Risks associated with natural mating such as disease transmission and libido problems can be highly controlled with the use of AI (Vishmanath, 2003; Thibier, 2005). The reproductive potential of valuable males has been maximized by combining AI with semen cryopreservation without limitations by time or distance in such a way that a single bull can produce approximately 50,000 offspring in one year (Funk, 2006). AI also plays an important role in MOET programs (Saacke *et al.*, 2000; Kanitz *et al.*, 2002; Funk, 2006). Deposition of semen in the uterine body is the conventional form of AI, giving pregnancy rates of 55-60% (Verberckmoes *et al.*, 2004). However, other insemination techniques have been developed in an effort to improve pregnancy rates, especially when valuable semen (*i.e.* sexed sperm) is used (Hunter, 2003; Kurikyn *et al.*, 2003; Verberckmoes *et al.*, 2004). The additional skills needed for such techniques limits their use to special cases (Hunter, 2003).

Variability in fertility among bulls is still one of the problems influencing this biotechnology and an accurate test to predict bull fertility has not been developed (Garner, 1997; Tanghe *et al.*, 2002; Flint *et al.*, 2003; Rodríguez-Martínez, 2006, 2007). Nevertheless, the main factor affecting the success of AI is the efficiency of estrus detection (Barth, 1993). Radiotelemetric devices and camera systems are becoming useful tools in the accurate prediction of estrus behavior, however visual observation cannot be ruled out completely (Dransfield *et al.*, 1998; Peralta *et al.*, 2005; Alawneh *et al.*, 2006). Timed insemination protocols have been developed with the aim of performing insemination without

necessity of estrus detection, but sometimes when they are applied under field conditions, AI with natural heat detection can be more efficient (Tóth *et al.*, 2006). Therefore, besides improvements in estrus detection or hormonal treatments, more important would be to develop a tool capable of predicting the process of ovulation. Several approaches have been tested, including measurement of progesterone concentrations, analysis of estrus behavioral signs and pedometer readings (Velasco-Garcia & Mottram, 2001; Roelofs *et al.*, 2005a,b, 2006). However, it is not yet possible to accurately predict ovulation in cattle.

2.3. Multiple ovulation and embryo transfer

The first calf produced by embryo transfer was born more than 50 years ago (see Willet *et al.*, 1951). Since then, embryo transfer programs have been implemented with acceptable results into livestock production. The aim of MOET programs in the cattle industry is the production of calves from cattle of high genetic merit (Merton *et al.*, 2003). *In vivo* production of embryos by superovulation also offers a safe (*e.g.*, prevention of disease transmission) and economic (*e.g.*, complete herds transported as frozen embryos) way of trading genetic material through cryopreservation (Le Tallec *et al.*, 2001). However the variability in the embryo production of donors (Mapletoft *et al.*, 2002) and low pregnancy rates (Peterson & Lee, 2003) after transfer are limiting factors affecting MOET programs. In well-organized embryo transfer teams the general mean production of viable embryos is 4 to 8 and 1 to 3 in cows and young heifers, respectively. Approximately 20% of donors do not respond to the superovulatory treatment and do not produce any embryos. Expected pregnancy rates after transfer are between 50-60%, with best results from unfrozen embryos, and heifers are the best recipients (Thibier, 2005; Velazquez *et al.*, 2005; Hasler, 2006; Stroud & Hasler, 2006). Intrinsic factors related to the donor (Kafi & McGowan, 1997; Stroud & Hasler, 2006) and the recipient (Broadbent *et al.*, 1991; Stroud & Hasler, 2006) need to be taken into account when applying such a technology. In addition, environmental factors also play a pivotal role in the success of this biotechnology (Kafi & McGowan 1997), especially under tropical conditions (Benyei *et al.*, 2006).

Despite these problems, most of the embryos produced worldwide for commercial purposes are obtained by this biotechnology (Thibier, 2001, 2004). In recent years, considerable progress has been made in the improvement of the outcome in MOET programs (Kanitz *et al.*, 2002; Mapletoft *et al.*, 2002; Peterson & Lee, 2003; Baruselli *et al.*, 2006; Bo *et al.*, 2006; Looney *et al.*, 2006; Vasconcelos *et al.*, 2006). However, reliable parameters for the prediction of the outcome in terms of embryo viability and pregnancy results are not yet available (Velazquez *et al.*, 2005). If this biotechnology is to gain more acceptance in the livestock industry, strategies to identify superior recipients and to improve response and reduce variability in donors have to be developed (Hasler, 2003). Identification of superior recipients is even more important when handling extremely valuable transgenic or cloned embryos.

Another problem affecting MOET efficiency is the evaluation of embryo quality. Embryo transfer teams rely on visual morphological observation for this purpose, which is very subjective; as shown by Aguilar *et al.* (2002), great proportion of embryos classified as good by stereoscopic evaluation showed characteristics of cells in degenerative stage when evaluated by light microscopy and electron microscopy. Given the importance of embryo quality for successful implantation (Mann & Lamming, 2001), it is still necessary to improve the evaluation of embryo competence under field conditions.

Besides the role in livestock production, *Bos taurus* recipients have been used in interspecies embryo transfer for the preservation of endangered species. One example is the live offspring obtained by transferring gaur embryos (*Bos gaurus*) into a dairy cow (Pope *et al.*, 1988). In a later study, *in vitro* produced gaur embryos transferred into cattle recipients achieved pregnancy. However, offspring obtained were either stillborn or died soon after birth. Although the authors could not differentiate between problems resulting from *in vitro* procedures and those from interspecies incompatibilities, the model was strongly discouraged (Hammer *et al.*, 2001). Nevertheless, with the increasing understanding of the embryo-maternal interactions, interspecies embryo transfer bovine models might achieve acceptable results in the conservation of endangered ungulate species.

Superovulated cattle could also be used as a model for the study of human clinical problems related to the response to gonadotropin stimulation during assisted reproduction cycles. This is supported

by the fact that cattle share similarities with humans in terms of ovarian and embryo physiology (Ménézo & Hérubel, 2002; Campbell *et al.*, 2003). Accordingly, patterns of superovulation ranging from low to high response have been reported in cows (De Roover *et al.*, 2005; Durocher *et al.*, 2006), which is similar to the situation reported in human assisted reproduction, regarded as “low responders” and “the ovarian hyperstimulation syndrome” (Karande & Gleicher, 1999; Whelan & Vlahos, 2000).

2.4. *In vitro* production of embryos

The birth of “Virgil”, the first calf produced by *in vitro* fertilization (IVF) (Brackett *et al.*, 1982), marked the beginning of IVF as a tool for production in the cattle industry. In the last few years there has been an increment in the *in vitro* production of embryos (IVPE) worldwide (Thibier, 2001, 2004). In some countries it is more expensive to produce embryos with this method than with conventional embryo transfer programs (Hasler, 2003). Despite financial concerns, mass production of *in vitro* embryos has been carried out in some countries (*e.g.*, Japan and Italy) for the commercial production of calves for beef production (Galli & Lazzari, 1996, 2003; Hamano *et al.*, 2006). Although the transmission of infection to recipients or offspring has not been demonstrated with *in vitro* embryos, the sanitary risk for IVPE is less conclusive than for *in vivo* embryos and generation of data is required (Le Tallec *et al.*, 2001; Hansen, 2006). *In vitro* production of embryos consists of three steps: oocyte *in vitro* maturation (IVM), IVF, and embryo culture. A method for the *in vivo* culture of IVM/IVF embryos has been developed; however, the technical skills required for such a procedure might not popularize its use (Havlicek *et al.*, 2005; Wetscher *et al.*, 2005). Oocytes for IVPE can be recovered from the ovaries of slaughtered donors or from live animals by ultrasound-guided transvaginal follicular aspiration (ovum pick-up) (Galli & Lazzari, 2003). *In vitro* fertilization in conjunction with ovum pick-up (OPU) has become important for the production of embryos from superstimulated donors (Galli *et al.*, 2001). It is important to recognize that superstimulatory protocols used for the production of embryos *in vivo* are different than those used to produce embryos *in vitro* with oocytes obtained via OPU. The objective of superovulation in MOET programs is to maximize the number of ovulations without compromising embryo quality, whereas superstimulatory treatments prior to OPU are aimed at increasing the number of follicles suitable for puncture (van Wagendonk-de Leeuw, 2006), preferentially with a diameter between 5 to 10 mm (Pieterse *et al.*, 1988). The efficiency of OPU sessions is affected by several factors, but operator skill is the most single factor influencing efficient oocyte retrieval (Merton *et al.*, 2003). Currently, the proportion of presumptive zygotes that become transferable blastocysts during the culture period is 15-40% (Hansen & Block, 2004; Lonergan, 2007). Although high rates of blastocyst production (up to 80%) have been reported using superstimulation protocols in dairy cattle subjected to OPU/IVF programs (Blondin *et al.*, 2002), in *Bos indicus* cattle these results could not be reproduced (Barros *et al.*, 2005). From the welfare point of view, the general consensus is that donors can tolerate current oocyte collection regimes and resume regular estrous cyclicity shortly after the OPU sessions have ceased (McEvoy *et al.*, 2006).

Ovum pick-up is a practical way to obtain oocytes for IVPE in countries where oocyte collection from abattoir material is not possible for religious reasons (Manik *et al.*, 2003). Reproductive programs working with OPU/IVF can also be used to produce embryos and calves from valuable cows that are infertile to AI (*i.e.* repeat breeding), that do not respond to superovulation in MOET programs or from animals with blocked oviducts (Galli *et al.*, 2001; Faber *et al.*, 2003; Hasler, 2003; Imai *et al.*, 2006; van Wagendonk-de Leeuw, 2006). Moreover, OPU/IVP programs cannot only obtain offspring from non-pregnant adult cows but also from prepubertal and pubertal animals and from pregnant cows in the first three months of pregnancy (Armstrong *et al.*, 1997; Galli *et al.*, 2001; Imai *et al.*, 2006). However juvenile embryo production is not used widely, as embryo yield is low compared to adult cows and sometimes involves more invasive procedures (*e.g.*, in calves aged three months) than OPU in adult animals. This can generate animal welfare issues (Armstrong *et al.*, 1997; van Wagendonk-de Leeuw, 2006). Nevertheless, OPU devices have been developed for the collection of oocytes from prepubertal animals as early as six months of age (Oropeza *et al.*, 2004). In addition, blastocyst production from young animals can be enhanced to levels found in adult cows with the use of hormonal treatments (Oropeza *et al.*, 2004). The use of juvenile animals in OPU/IVF programs has the potential of reducing

the generation interval, but a decrease in selection accuracy might occur as the information on parent performance might not be available at the time of selection (van Arendonk & Bijma, 2003). Although speculative, this could be bypassed by the use of animals cloned from already proved high genetic merit animals. In fact, healthy offspring have been obtained with *in vitro* produced blastocysts from oocytes collected by OPU in cloned heifers (Lucas-Hahn *et al.*, 2005).

Another suggested use of IVPE is the production of embryos from dairy cattle of average genetics for developing countries (Galli & Lazzari, 2003), although this might not be entirely applicable in hot climates. Production of hybrid genotypes (*i.e.* *Bos taurus* x *Bos indicus*) with the potential for better productive performance as compared with local breeds would be a better option for the tropics. This can be achieved in a faster way with IVF than with traditional genetic schemes. Embryo transfer protocols using embryos either produced by IVF or superovulation can also serve as a tool to bypass the effect of heat-stress in lactating dairy cows (Hansen & Block, 2004). Interesting is also the suggestion that the bovine model could be useful for the study of human IVF. This is because the cow and the human share some similarities regarding the final stages of oocyte maturation and the biochemical and intrinsic paternal and maternal regulatory processes in preimplantation embryos (Ménézo & Hérubel, 2002). This has especial relevance in patients undergoing assisted reproductive cycles displaying hormonal imbalances that can be mimicked in cattle (*e.g.*, hyperinsulinemia) (Adamiak *et al.*, 2005).

However, abnormalities in embryonic, fetal and postnatal (*i.e.* large calf syndrome) development have been associated with *in vitro* procedures (Lonergan *et al.*, 2003; McEvoy, 2003). These abnormalities are related to the aberrant expression of developmentally important genes imposed by culture conditions (Niemann & Wrenzycki, 2000). For most cattle farmers this technology is an advantage only for extremely valuable cows that are infertile or fail to respond to superovulation. This is likely to change only when the efficiency of *in vitro* production improves significantly and the problems with pregnancies and calves are reduced (Hasler, 2003).

2.5. Cloning

The word clone comes from the Greek, *klon*, meaning a twig or a small branch, and the cloning technique as a scientific procedure might have begun with bacterial cloning (Weiss, 2005). Nowadays, the word cloning is mainly associated with reproductive cloning. Individual separation of embryonic blastomeres up to the fourth cell stage, embryo bisection at the morula or blastocyst stage (embryo splitting), and nuclear transfer (NT) are the three methods carried out so far to get genetically identical individuals in bovine species (Wells, 2003). Live offspring have been obtained in the three methods (Williams *et al.*, 1984; Johnson *et al.*, 1995; Cibelli *et al.*, 1998a). However the first two cloning methods rely on very early embryonic cells and this limits the number of viable embryos and offspring that can be obtained (Wells, 2003). Embryo splitting has been applied to MOET programs, playing an important role in beef production (Gearheart *et al.*, 1989). The major commercial advantage of demi-embryos is that more calves result per embryo. This is especially valuable when only one or a few embryos are obtained from high genetic merit donors (Seidel, 1984).

There are approximately 160 NT cloning laboratories, across 37 countries, of which 75% are working with livestock (cattle, pig, sheep, goat and buffalo) cloning (Obach & Wells, 2007). Nearly 50% of these livestock cloning organizations are involved in bovine NT cloning (Obach & Wells, 2007). Somatic cell NT (SCNT) has been suggested as the most efficient technique for obtaining large numbers of genetically identical individuals in farm animals (Kato *et al.*, 1998). Although embryonic and fetal cells are also useful for NT cloning, the economic potential of the donor is unknown at the time of the procedure. In contrast, adult somatic cells can be selected from animals already proven to be good milk or meat producers (Kato *et al.*, 1998; Bousquet & Blondin, 2004). For instance, SCNT could be used to multiply identical animals of high genetic merit, whether they are founder dams of important families, show cows or progeny-tested sires (Galli *et al.*, 2003; Wells, 2003). This could be advantageous for the propagation of valuable F_1 cattle (Obach & Wells, 2007), especially in tropical areas. Moreover, valuable bulls could be cloned to increase the availability of semen for the market (Galli *et al.*, 2003). In addition, somatic cell storage from bulls and dams affords the possibility of replacing injured or dead individuals with new identical animals (Galli *et al.*, 2003). In theory, SCNT technology could modify

the normal progeny testing schemes by reducing the cost involved in management and feeding of all the bulls included in the test. After the first semen collections, all bulls could be slaughtered and after a few years when enough cows will be in lactation, the best genotypes could be rescued from the frozen stocks of somatic cells (Galli *et al.*, 2003). Another practical application in the cattle industry has been reported recently where a bull with a inherent resistance to bovine brucellosis was cloned using cryopreserved fibroblast from a bull that died 10 years ago (Westhusin *et al.*, 2007). Another advantage of cloning would be the generation of more reliable and interpretable data in the field of reproductive biology by reducing the genetic variation in experimental trials (Sreenan, 1983). This suggestion has been proved recently in an OPU/IVF program in monozygotic twin cows (Machado *et al.*, 2006).

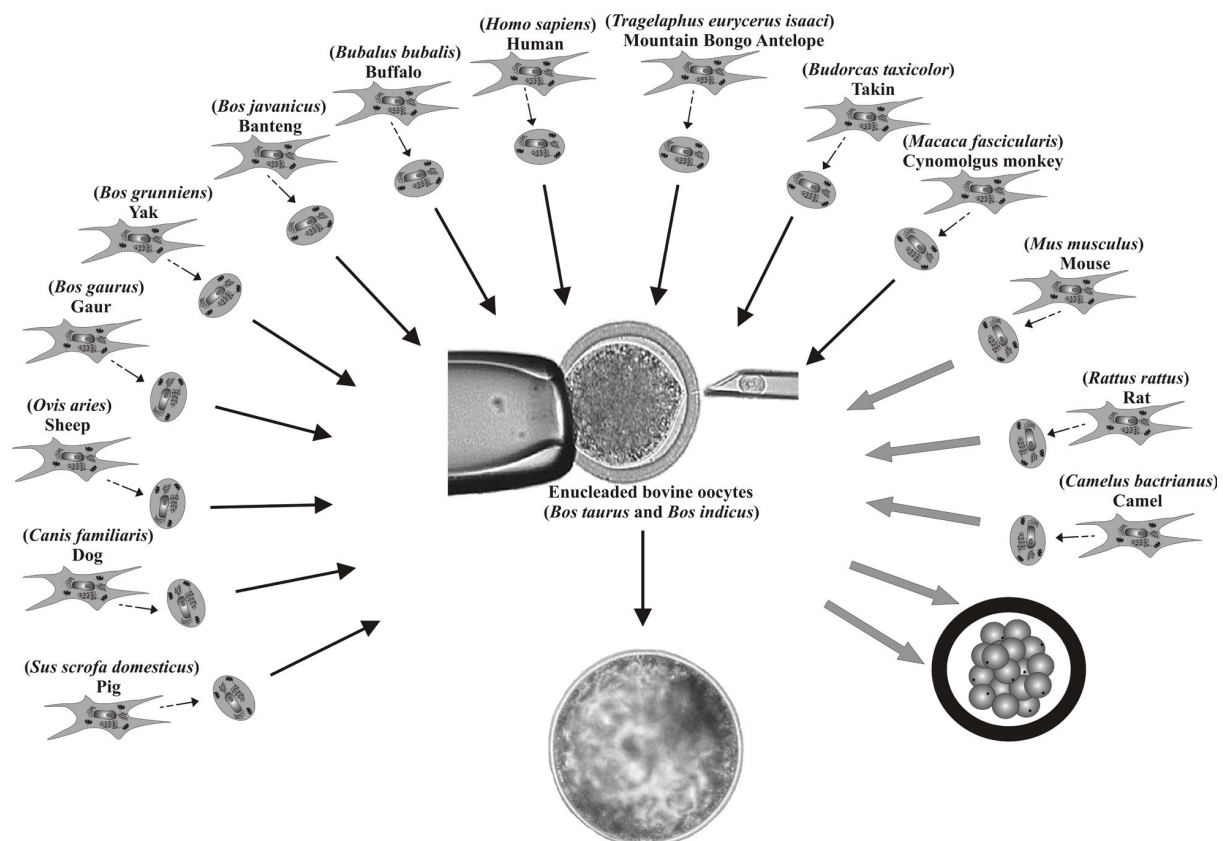


Figure 1. Blastocyst production with interspecies cloning by nuclear transfer using bovine oocytes as recipients. So far only early stage embryos have been produced in the mouse, rat and camel. Based on Dominko *et al.*, 1999; Lanza *et al.*, 2000; Kitiyanant *et al.*, 2001; Saikhun *et al.*, 2002; Chang *et al.*, 2003, 2004; Lee *et al.*, 2003; Atabay *et al.*, 2004; Dindot *et al.*, 2004; Lu *et al.*, 2005; Murakami *et al.*, 2005; Sansinena *et al.*, 2005; Illmensee *et al.*, 2006; Li *et al.*, 2006; Zavos & Illmensee, 2006; Zhou & Guo, 2006.

Somatic cell NT may also be used to preserve endangered cattle breeds, especially when no fertile males are available (Wells *et al.*, 1998; Cseh & Solti, 2000; McEvoy *et al.*, 2003). In addition to the application to animal production and conservation biology, the production of interspecies nuclear transfer embryos using cattle oocytes as recipients can be used as an experimental model to investigate epigenetic modifications and genomic imprinting (Dindot *et al.*, 2004). Also, NT studies in cattle might

be helpful to understand the fundamental mechanisms of differentiation, and aging (Tsunoda & Kato, 2000). Moreover, although ethic and legal concerns have to be taken into account, bovine reconstructed oocytes with human fibroblast cells can be useful in the improvement of cloning technology to bypass human infertility (Zavos & Illmensee, 2006) and in the production of embryonic stem cells for therapeutic purposes (Reproductive BioMedicine Online news, 2007). Production of xenogenic nuclear transfer embryonic stem cells using the equine-cow model has been highlighted recently as a putative method to produce material for cell-replacement therapy in horses (Tecirlioglu & Trounson, 2007). Production of pig-bovine blastocysts has been reported in some (Dominko *et al.*, 1999) but not all studies (Lagutina *et al.*, 2005). Cattle oocytes have been also reconstructed with mouse embryonic fibroblasts, and adult fibroblasts from rat and camel; however, most of the embryos were arrested at early embryonic stages and no blastocyst production was achieved (Dominko *et al.*, 1999; Arat *et al.*, 2003; Zhou & Guo, 2006). Nevertheless, the above-mentioned ideas are becoming very feasible, as several interspecies blastocysts have been already produced using bovine oocytes as recipients (Fig. 1).

Nevertheless, the current efficiency of NT is low (Wells, 2003; Heyman, 2005). For example, some pregnancies have been achieved with interspecies nuclear transfer embryos, but usually they are lost between days 30 to 90 after transfer (Sansinena *et al.*, 2005). In addition, although pregnancy rates for intraspecies cloned embryos can be similar to embryos produced *in vitro* and artificially inseminated up to day 50, there are more continual losses throughout gestation compared to IVF and AI (Wells, 2003). Surviving animals represent only 5-15% of cloned embryos transferred (Obach & Wells, 2003; Wells, 2003; Obach & Wells 2007). Most of the remaining 85-95% die at various stages of development due to placental and fetal abnormalities collectively referred to as the “cloning syndrome” (Tsunoda & Kato, 2002; Obach & Wells, 2003). For large-scale commercial application pregnancy rates of at least 50% per recipient will be required (Lewis *et al.*, 1998). To achieve this, efficiency has to be increased and the frequency of abnormalities reduced (Wilmut, 2003). Its degree of utilization in the cattle industry, however, will depend also on social acceptance (Faber *et al.*, 2004). In this regard is worthy to mention that current data indicate that there are no major differences in milk and muscle characteristics between cloned and non-cloned cattle (Heyman *et al.*, 2007; Yang *et al.*, 2007).

2.6. *In vitro* assisted fertilization

In vitro fertilization is normally accomplished by incubating oocytes and sperm cells together in fertilization medium. However, microinsemination techniques have been developed to bypass the hurdles imposed by the zona pellucida during fertilization, which is especially useful in situations of infertility (Gwatkin, 1993). These techniques have a major relevance in treating human infertility, but its use has been explored also in productive animals, including cattle. Bovine oocytes have been fertilized using zona pellucida drilling (ZD), partial zona pellucida dissection (PZD), subzonal injection (SUZI) and intracytoplasmic sperm injection (ICSI) (Figs. 2 and 3).

Schutze *et al.* (1994) attempted to fertilize cattle oocytes by drilling the zona pellucida with an ultraviolet-laser microbeam and by inserting, directly through the laser drilled hole, one sperm with optical tweezers into the perivitelline space (PVE). The tweezer trap consisted of a single strongly focused laser beam that could be used to capture, move, and position a wide variety of cells. In that study no fertilization was observed, but the same group in a later experiment achieved fertilization by inserting in the PVE three to five sperms instead of one (Clement-Sengewald *et al.*, 1996). Laser-drilled openings have been also used in an effort to increase pregnancy rates after transfer of embryos produced by standard IVF (Schmoll *et al.*, 2003).

Fertilization by means of PZD has been also reported in cattle (Basovskii, 1999). Zona pellucida dissection is carried out usually with a fine needle and sometimes acidified solutions (partial zona digestion) can also be used for this purpose (Gwatkin, 1993), but this latter procedure has not been reported in bovine species. Subzonal injection has been reported in cattle using both bovine and equine spermatozoa (Heuwieser *et al.*, 1991; Li *et al.*, 2003). In the bovine-equine SUZI model the maximum development of embryos was only to the 8-cell stage (Li *et al.*, 2003). Although its use in bovine oocyte microfertilization might not be practical, SUZI is important for other ART such as transgenesis and cloning by NT (Liu *et al.*, 2000; Hofmann *et al.*, 2003). The only microinsemination technique that has

produced live offspring in cattle is ICSI (Goto *et al.*, 1990). In bovine oocytes, this technique is usually accomplished by inserting a needle carrying a single male gamete through the ZP into the oocyte cytoplasm. Although laser-assisted ICSI in which part of the ZP is removed to introduce the needle has been carried out in humans and other mammals, no reports in cattle were found in the present work. Despite several works reporting production of calves with this technique, its effectiveness remains unsatisfactory for commercial application (Horiuchi & Numabe, 1999; Horiuchi, 2006). The main underlying cause of the reduced efficiency of ICSI is the lack of protocols able to induce proper oocyte activation and decondensation of the sperm nucleus (Horiuchi & Numabe, 1999). ICSI may be used in circumstances in which natural mating and conventional IVF is not an option for the production of calves (McEvoy *et al.*, 2003).

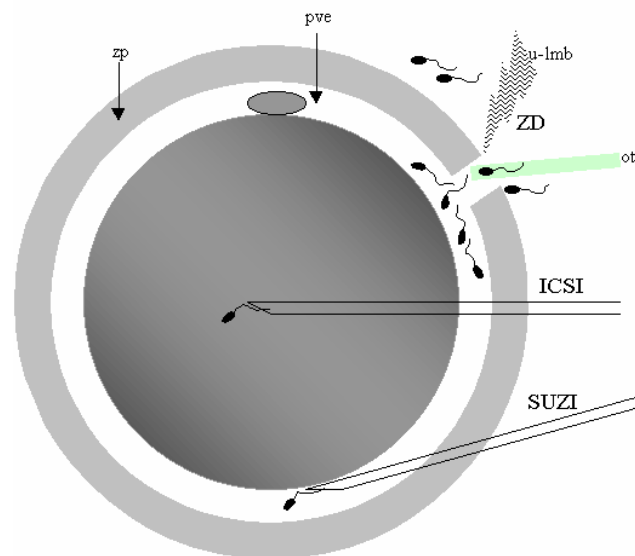


Figure 2. Microinsemination procedures carried out in cattle. Fertilisation was achieved by drilling (zona drilling [ZD]) a hole in the zona pellucida (zp) with an ultraviolet-laser microbeam (u-lmb) and introducing 3-5 sperms into the perivitelline space (pve) with optical tweezers (ot). Bovine oocytes have been fertilized by subzonal injection (SUZI); however, live offspring have been obtained only with intracytoplasmic sperm injection (ICSI). Data based on Goto *et al.* (1990), Heuwieser *et al.* (1991), and Clement-Sengewald *et al.* (1996).

For McEvoy *et al.* (2003), ICSI is unlikely to be used for commercial purposes in the cattle industry, as it is a technically demanding and costly procedure. Instead, its use for conservation biology of non-productive ungulates might be more applicable (Cseh & Solti, 2000; McEvoy *et al.*, 2003). This seems very promising especially when bovine embryos have been already produced with ICSI using freeze-dried spermatozoa, heat-dried sperm heads or frozen-thawed oocytes (Keskinetepe *et al.*, 2002, Rho *et al.*, 2004; Lee & Niwa, 2006). Interspecies microfertilization using bovine oocytes can also be used to study key processes during sperm-oocyte fusion, oocyte activation and fertilization (Li *et al.*, 2003; Kobayashi *et al.*, 2006). This has a special relevance in human fertility, as bovine ICSI has been suggested to be an appropriate model to assess human sperm oocyte activation ability (Terada *et al.*, 2004). Considering animal production, Horiuchi *et al.* (2002) suggested that ICSI could be used to maximize the use of costly semen. Because sperm motility is not essential in this technique, if cheaper and efficient procedures capable of maintaining the nuclear integrity of the spermatozoa are developed, ICSI might be acceptable (McEvoy *et al.*, 2003). However, its effectiveness has to be improved and acceptable before this can be considered a viable proposition.

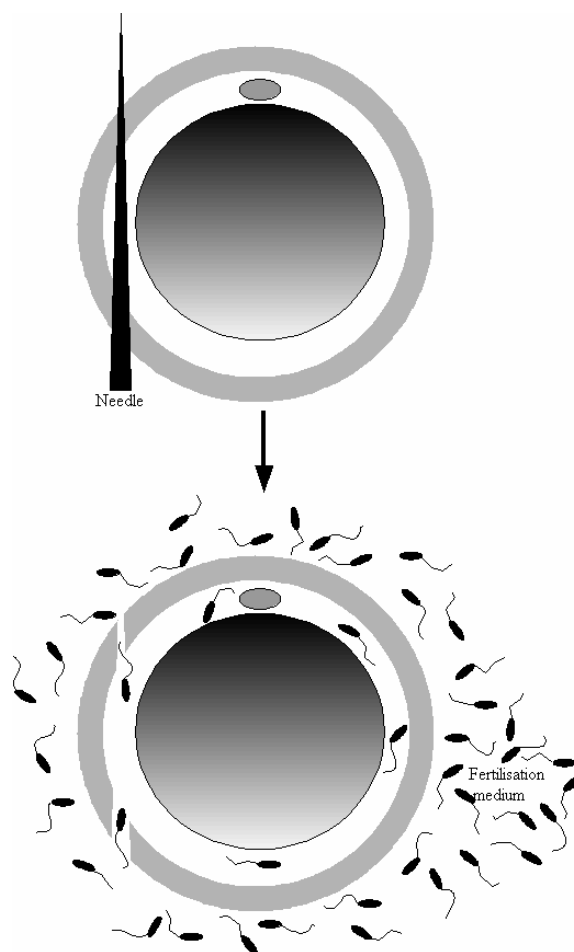


Figure 3. Fertilization has been achieved in cattle with partial zona dissection (PZD). Based on Basovskii (1999).

2.7. Transgenesis

Transgenic technology provides a method to rapidly introduce “new” or modified genes and DNA fragments into cattle and other livestock species without crossbreeding (Wheeler, 2003, 2007). Approximately 25 animal species are currently involved in the developing of transgenic lines for basic biomedical research and applied purposes (Houdebine, 2005). The first transgenic farm animals (*i.e.* rabbits, pigs and sheep) were produced by Hammer *et al.* (1985). Roschlau *et al.* (1989) were the first to report a successful production of transgenic cattle. Since then, steady progress has been made with cattle transgenesis and several research groups have managed to produce transgenic bovine offspring (Table 1). Possible applications of gene transfer in cattle include the production of valuable proteins in milk and serum for therapeutic purposes in humans (“biopharming”), which is currently the most advance state of bovine transgenesis (Piedrahita, 2000; Kuroiwa *et al.*, 2002; van Berkel *et al.*, 2002;; Keefer, 2004; Robl *et al.*, 2006). In fact, several therapeutic proteins (*i.e.* growth hormone, albumin, fibrinogen, collagen, and lactoferrin) produced in the milk of transgenic cows are currently under preclinical trial for future commercialization (Niemann & Kues, 2007). Research in New Zealand is also being carried

Table 1. Examples of production of transgenic bovine offspring.

Gene inserted	Gene transfer method	Possible application	Reference
• <i>hLF</i>	Microinjection	Therapy for infectious and inflammatory diseases, and production of infant formulas	•Krimpenfort <i>et al.</i> , 1991
• <i>Chicken c-ski</i>	Microinjection	No therapeutic or productive purpose ¹	•Bowen <i>et al.</i> , 1994
• <i>hEpo</i>	Microinjection	No therapeutic or productive purpose ¹	•Hytinen <i>et al.</i> , 1994
• <i>HbsAg</i>	Retroviral infection	No therapeutic or productive purpose ¹	•Chang <i>et al.</i> , 1998
• <i>β-galactosidase</i>	Microinjection and nuclear transfer	No therapeutic or productive purpose ¹	•Cibelli <i>et al.</i> , 1998b
• <i>hα-LA</i>	Microinjection	Therapy for PKU ² and production of infant formulas	•Eyestone <i>et al.</i> , 1998
• <i>hSA</i>	Microinjection	Therapy for restoration and maintenance of blood volume	•Behboodi <i>et al.</i> , 2001
• <i>Prochymosin</i>	Nuclear transfer	No therapeutic or productive purpose ¹	•Zakhartchenko <i>et al.</i> , 2001
• <i>BSSL</i>	Nuclear transfer	Therapy for pancreatic insufficiency and production of infant formulas	•Chen <i>et al.</i> , 2002
• <i>hIg</i>	Nuclear transfer	Therapy for immuno-related diseases	•Kuroiwa <i>et al.</i> , 2002
• <i>β- and κ-casein</i>	Nuclear transfer	Improvement of nutritional and processing properties of milk	•Brophy <i>et al.</i> , 2003
• <i>EGFP</i>	SMGT	No therapeutic or productive purpose ¹	•Shemesh <i>et al.</i> , 2000
	Nuclear transfer		..Bordignon <i>et al.</i> , 2003
	Nuclear transfer		..Gong <i>et al.</i> , 2004
	Lentiviral infection		..Hofmann <i>et al.</i> , 2004
• <i>r28M*</i>	Nuclear transfer	Tumor therapy	•Grosse-Hovest <i>et al.</i> , 2004
• <i>Lysostaphin</i>	Nuclear transfer	Resistance to mastitis ³	•Wall <i>et al.</i> , 2005
• <i>hGH</i>	Nuclear transfer	Therapy for growth-related disorders	•Salamone <i>et al.</i> , 2006

hLF= Human lactoferrin*hEpo*= Human erythropoietin*HbsAg*= hepatitis B surface antigen gene*hα-LA*= Human α-lactalbumin*hSA*= Human serum albumin*BSSL*= Bile salt-stimulated lipase*hIg*= Human immunoglobulin*EGFP*= Enhanced green fluorescent protein*hGH*= Human growth hormone

SMGT= Sperm-mediated gene transfer

*A recombinant bispecific single-chain antibody directed against T-cell surface-associated costimulatory molecule CD28 and a melanoma-associated proteoglycan (MAPG)

¹Studies were conducted to increase the efficiency of transgenic animal production²Phenylketonuria³In mastitis caused by *Staphylococcus aureus*

out with transgenic cows that can produce human myelin basic protein with the goal of extracting and purifying this protein for the treatment of multiple sclerosis (Rutter, 2006).

Improvement of milk composition and disease resistance are examples of agricultural applications of this biotechnology in cattle (Paape *et al.*, 2002). Transgenic dairy cows capable of producing high levels of casein (Brophy *et al.*, 2003) and resistant to mastitis caused by *Staphylococcus aureus* (Wall *et al.*, 2005) are now available. Other economically important traits in livestock production like growth rate and feed conversion have not yet been explored in cattle using transgenic technology.

Due to inefficient outcomes obtained from pronuclear DNA microinjection, NT became the most feasible technique to generate transgenic livestock (McEvoy *et al.*, 2003; Niemann & Kues, 2003; Thomson *et al.*, 2003; Niemann *et al.*, 2005). Other approaches to generate transgenic cattle include the production of chimeric embryos with DNA-modified stem-like cells and through microinsemination (notably ICSI) of oocytes with DNA integrated into bovine spermatozoa (sperm-mediated gene transfer) (Cibelli *et al.*, 1998a,b; Gandolfi, 1998; Shemesh *et al.*, 2000; Celebi *et al.*, 2003; Lavitrano *et al.*, 2006). In addition, transgenic cattle embryos have been produced delivering genes into the oocyte and embryo with recombinant viruses (viral transgenesis). However, only lentiviral vectors injected to the oocyte generated viable offspring (Hofmann *et al.*, 2003, 2004). Nevertheless, abnormalities have been reported in transgenic calves during pregnancy and only 1-10% of transgenic embryos produced resulted in the birth of healthy offspring. These abnormalities have been suggested to be more related to the cloning techniques and *in vitro* culture conditions than to the gene targeting *per se* (Cibelli *et al.*, 1998a,b; Hill *et al.*, 1999; Zakhartchenko *et al.*, 2001; McEvoy *et al.*, 2003; Thomson *et al.*, 2003). Animal transgenesis relies heavily in some ART used to generate non-transgenic offspring. Therefore, improvements in reproductive biotechnologies, such as cloning and ICSI, would give major benefits to the production of transgenic cattle.

2.8. Xenografting-germ cell transplantation

Xenografting in bovine species is being carried out mainly with ovarian and testis tissue. Germ cell transplantation is also being actively investigated in cattle, but mainly in bulls. The bovine model has been used for the implementation of protocols for fertility preservation of cancer patients. For example, spermatogonial proliferation has been observed with bovine male germ cells transplanted into mice recipients (Dobrynski *et al.*, 2000). Although no further differentiation was accomplished in this model, the results have encouraged more research into the understanding of testis function in order to preserve male fertility, including humans. Cross-species ovarian tissue transplantation has been also achieved using cattle-mice models. Herrera *et al.* (2002) transplanted fresh or frozen-thawed bovine ovarian cortex grafts into mice under the kidney capsule and subcutaneously. They observed follicular development up to the antral stage in subcutaneously transplanted ovarian tissue but not in tissue transplanted under the kidney capsule (Herrera *et al.*, 2002). In other studies, newborn and adult bovine ovarian cortical pieces were transplanted into male severe combined immunodeficient (SCID) mice, and after treatment with gonadotropins recovery of oocytes was possible (Hernandez-Fonseca *et al.*, 2004, 2005). Furthermore, some experiments have demonstrated that xenografting of bovine ovarian follicles under the kidney capsules of female SCID mice can develop until the antral stage. These follicles contained oocytes that were capable of resuming meiosis, achieving fertilization, cleavage and develop until the 5- to 8-cell embryonic stage, (Senbon *et al.*, 2003, 2004, 2005). Due to the similarities between humans and cattle in terms of ovarian physiology (Campbell *et al.*, 2003), bovine models for xenografting could be very valuable in biomedical research.

Male germ cell transplantation in the same species has been carried out in cattle to elucidate basic biological aspects of testis function. For instance, experimental models have been developed in bulls to study the possibility of restoring spermatogenesis in individuals with azoospermia (Schlatt *et al.*, 1999; Dobrynski, 2005a). In fact, regeneration of spermatogenesis has been achieved in cattle by autologous and heterologous male germ cell transplantation (Izadyar *et al.*, 2003; Herrid *et al.*, 2006). It has been suggested that transplantation of germ cells could be also used to restore bull fertility after an insult to the testis or to preserve genetic material from valuable bovine males that are lost before reaching puberty (Dobrynski, 2005b). Another option includes studies regarding spermatogenesis *in vitro* with

male bovine animals in conjunction with germ cell transplantation in order to get new insights concerning male gamete biology (Parks *et al.*, 2003). Since germ cell transplantation protocols are relatively well established in cattle, the propagation of endangered ungulates could be achieved through bovine surrogate recipients (Dobrinski & Travis, 2007). Besides germ cells, testis xenografts from several species (including cattle) could also be used to study toxicants or drugs with the potential to reduce or improve male fertility without the necessity of performing experiments in the target species (Dobrinski, 2005b). In addition, application of testicular xenografting and germ cell transplantation techniques can be useful in shortening the interval to produce transgenic bulls (Dobrinski, 2006, 2007). Accordingly, transgenic spermatogonia were obtained with bovine testicular tissue transduced with β -galactosidase and subsequently grafted onto the backs of castrated immunodeficient nude mice (Oatley *et al.*, 2004). At present, offspring have been obtained only in goats with germ cell transfer protocols (Hill & Dobrinski, 2006).

Grafting of ovarian and testicular tissue and male germ cell transplantation are unlikely to be used for breeding purposes in the cattle industry. This is because other ART, such as cloning, might offer a more practical approach. Instead, its usefulness will be more applicable in experimental models to develop strategies aimed at restoring fertility in human patients subjected to gonadotoxic therapy and for the conservation of endangered bovid species.

2.9. Preimplantation genetic diagnosis

Currently, the most applicable use of preimplantation genetic diagnosis (PGD) in cattle is the sex determination of embryos. In several countries embryo sexing is being applied at commercial level in companies and farms working with embryo transfer technology. Knowing the sex of embryos produced for the use in an embryo transfer program can assist the producer in managing resources more effectively by choosing future replacement heifers and sires (Shea, 1999). Several attempts have been carried out to sex embryos; however, the polymerase chain reaction (PCR) technique seems to be the more efficacious with an accuracy of 90 to 100% even under field conditions. Pregnancy rates with sexed embryos produced *in vivo* are comparable to those achieved with intact embryos in MOET programs. On the contrary, sexed IVF embryos have a reduced capability to attain pregnancy than *in vivo*-produced embryos (van Vliet *et al.*, 1989; Thibier & Nibart, 1995; Shea, 1999; Lopes *et al.*, 2001; Hasler *et al.*, 2002; Alves *et al.*, 2003). Still, normal calves have been born using vitrified-thawed sexed embryos produced *in vitro* (Agca *et al.*, 1998). Embryo sexing with PCR implies embryo biopsies, with possible concurrent damage that can influence the probability of pregnancy. Nevertheless, non-invasive techniques are available, such as the method based on the detection of the H-Y sex-specific male antigen with 80% accuracy and with similar pregnancy rates to sexing using PCR (Ramalho *et al.*, 2004).

Embryo sexing is only one of the advantages of PGD in cattle. Identification of genetic abnormalities in preimplantation embryos prior to embryo transfer may improve the likelihood of a successful pregnancy and/or viable offspring. Preimplantation GD had played a pivotal role in improving the outcome of assisted reproduction technologies in humans (Kuliev & Verlinski, 2005). In cattle, assays have been developed for the simultaneous detection of embryo sex and genes of importance for the bovine industry, including some relevant diseases and production traits (Table 2).

This biotechnology will be pivotal in the identification of genes of reproductive importance, as shown recently by El-Sayed *et al.* (2006). Using microarrays to analyse bovine embryo biopsies, these authors revealed differential gene expression between biopsies derived from embryos that resulted in no pregnancy, resorption or calf delivery, thus providing candidate genes of embryo developmental competence (El-Sayed *et al.*, 2006). PGD has been also used to assess transgenic integration in bovine embryos (Bowen *et al.*, 1994; Chen *et al.*, 2002; Forsyth *et al.*, 2005). Besides its usefulness in animal breeding, PGD in bovine species has been used to develop a training protocol aimed at improving the performance of professionals working with human embryos (Almodin *et al.*, 2005). Another major commercial application of PGD may be the analysis of selected genetic markers. Such marker-assisted selection (MAS) can be applied at the pre-elongation embryo stage. Selection of embryos carrying genes of economic importance would revolutionise the cattle industry (Bodo *et al.*, 2001; Mapletoft & Hasler, 2005; Moore & Thatcher, 2006). Cloning procedures could be advantageous in the multiplication

of these embryos (Oback & Wells, 2007). However, this will not be possible until sufficiently valuable markers are identified.

Table 2. Detection of genes related to productive traits and genetic diseases via PGD in cattle.

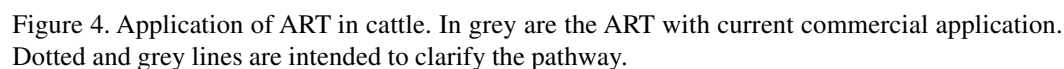
Gene	Reference
Kappa-casein	Schellander <i>et al.</i> , 1993
Growth Hormone (GH)	Chrenek <i>et al.</i> , 2001
Prolacting (PRL)	Chrenek <i>et al.</i> , 2001
Growth Hormone Receptor (GHR)	Peippo <i>et al.</i> , 2007
Prolacting Receptor (PRLR)	Peippo <i>et al.</i> , 2007
Bovine Leukocyte Adhesion Deficiency (BLAD)	Hochman <i>et al.</i> , 1996
Claudin-16 Deficiency	Hirayama <i>et al.</i> , 2004
Band 3 deficiency	Kageyama <i>et al.</i> , 2006

2.10. Sperm sexing

Embryo sexing is identification rather than selection of sex (Seidel & Johnson, 1999). Hence, sex pre-selection is more advantageous for productive purposes than embryo sexing. Hundreds of thousands of calves have been born from sexed sperm (SS). Most of these calves were produced in USA, UK, Argentina, Brazil, and Mexico, with lesser numbers in several other countries. Currently, sexed bovine sperm can be purchased from companies in the UK, Canada, USA, Mexico, Argentina, Brazil and China. In several countries licensing and commercialization of this biotechnology is in various phases of development (Garner, 2006). Companies have to decide exactly what product might be provided (*e.g.*, fresh and/or frozen sperm, number of sperms/dose, which class of bull, etc.) (Seidel, 2003a). Predetermination of sex of offspring with SS could increase efficiency of producing meat and milk and improve cattle welfare. An example of the influence on welfare of SS is a decrease of calving difficulty in primiparous heifers by selecting for female calves (Seidel, 2003b).

Several approaches to sexing sperm have been proposed (Seidel & Garner, 2002). However, the sorting of sperm by flow cytometric has been found to be the more efficacious so far (Johnson, 2000). Accuracy of the process is about 90% for either sex, and resulting calves appear to be no different from non-sexed controls in birth weight, mortality, rate of body weight gain, and incidence of abnormalities (Seidel, 2003a). Sorted sperm can also be used for *in vitro* production of embryos and large-scale production is on the way to practical application (Wheeler *et al.*, 2006). Encouraging results have been obtained in China, where healthy offspring was achieved with sexed IVF embryos. Moreover, there were no differences in pregnancy rates when compared to nonsexed IVF and *in vivo*-derived embryos (Xu *et al.*, 2006). Sexed sperm has been also used in ICSI procedures where normal calves have been obtained (Hamano *et al.*, 1999). Although SS tends to degenerate faster than normal sperm, cryopreservation overcomes this problem allowing its use anywhere in the world. Furthermore, pregnancies have been reported with sperm that was frozen-thawed before being sorted and re-frozen-thawed after sexing (Underwood *et al.*, 2007). All applications of SS required strict management practices and the use of AI or IVF, which is a limitation. However, sorting speed is the primary limitation to the technology. In addition, when superovulated animals are inseminated with SS, the production of transferable embryos is low compared with non-sexed controls (Schenk *et al.*, 2006). Another disadvantage is that when SS is used in lactating cows (Seidel, 2003a,b; Garner, 2006) and in heifers subjected to timed AI (Seidel, 2007), pregnancy rates are usually low. Despite these constraints, sorted sperm is already at commercial level and as efficiency improves and cost declines sperm sexing will be more widely used (Seidel & Garner, 2002).

Several ART have been applied in the cattle industry (Fig. 4). Some of them have provided acceptable results; but others have low efficiency, which limits their use for cattle breeding. This is because they do not always achieve pregnancy and in some cases have prejudicial affects in the prenatal and postnatal period. Hormonal treatments to correct reproductive problems or to improve reproductive performance have produced variable results and sometimes might infringe negative effects. Currently, the most cost-effective way to disseminate genes is with conventional AI. Among the ET technologies, MOET is the most efficient so far. Improvements in estrus detection and prediction of superior recipients will benefit AI (especially with SS) and embryo transfer-related technologies. Improvements in OPU/IVF programs would have a great economical impact in the cattle industry and could overtake the traditional MOET programs. Embryo and sperm sexing are already at commercial level with acceptable results. Improvements in the sorting of sperm will make this biotechnology more acceptable and might overtake embryo sexing.



ARBS Annu Rev Biomed Sci 2008;10:36-62

addition, it is important to take into account the repercussions that can occur using ART to overcome fertility problems, as there is a risk of disseminating infertile genotypes. Although generation of information is of pivotal importance, it is worthy to consider the possibility that a concise understanding of the current knowledge on reproductive physiology would give us palpable improvements. As highlighted by Lucy (2005) and Seidel (2006a), our current capacity to generate information is great, however our skill to understand it properly is low. It is curious how information generated more than 20 years ago (Linares *et al.*, 1982) is being just recently (Wathes *et al.*, 2003) put into perspective (*i.e.* the early “window” of progesterone rise is very important for preimplantation embryo development). Perhaps it is time to recapitulate a little bit.

4. Acknowledgments

The author is very grateful to Professor George Seidel Jr., Animal Reproduction and Biotechnology Laboratory, Colorado State University, USA, and Professor Heiner Niemann, Department of Biotechnology, Institute for Animal Breeding (FAL), Germany, for their valuable comments and advices on the manuscript.

5. References

- Adamiak SJ, Mackie K, Watt RG, Webb R, Sinclair KD. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. *Biol Repro* 2005;73:918-26.
- Agca Y, Monson RL, Northey DL, Peschel DE, Schaefer DM, Rutledge JJ. Normal calves from transfer of biopsied, sexed and vitrified IVP bovine embryos. *Theriogenology* 1998;50:129-45.
- Aguilar MM, Galina CS, Merchant H, Montiel F, Canseco R, Márquez YC. Comparison of stereoscopy, light microscopy and ultrastructural methods for evaluation of bovine embryos. *Reprod Domest Anim* 2002;37:341-6.
- Alawneh JI, Williamson NB, Bailey D. Comparison of a camera-software system and typical farm management for detecting oestrus in dairy cattle at pasture. *N Z Vet J* 2006;54:73-7.
- Almodin CG, Moron AG, Kulay L Jr., Minguetti-Câmara VC, Moraes AC, Torloni MR. A bovine protocol for training professionals in preimplantation genetic diagnosis using polymerase chain reaction. *Fertil Steril* 2005;84:895-9.
- Alves BC, Hossepian de Lima VF, Teixeira CM, Moreira-Filho CA. Use of primers derived from a new sequence of the bovine Y chromosome for sexing *Bos taurus* and *Bos indicus* embryos. *Theriogenology* 2003;59:1415-9.
- Arat S, Rzuclido S, Stice SL. Gene expression and *in vitro* development of inter-species nuclear transfer embryos. *Mol Reprod Dev* 2003;66:334-42.
- Armstrong DT, Kotaras PJ, Earl CR. Advances in *in vitro* production from juvenile and prepubertal calf and lamb oocyte donors. *Reprod Fertil Dev* 1997;9:333-40.
- Atabay EC, Takahashi Y, Katagiri S, Nagano M, Koga A, Kanai Y. Vitrification of bovine oocytes and its application to intergeneric somatic cell nucleus transfer. *Theriogenology* 2004;61:15-23.
- Barros CM, Ferreira MMG, Potiens JR, Eberhardt BG, Melo DS, Monteiro FM. Influence of superstimulation and hormonal deprivation protocols on *in vitro* production of Nelore embryos (*Bos taurus indicus*). *Reprod Fertil Dev* 2005;18: 291.
- Barth AD. Factors affecting fertility with artificial insemination. *Vet Clin North Am Food Anim Pract* 1993;9:275-89.
- Baruselli PS, de Sá Filho MF, Martins CM, Nasser LF, Nogueira MFG, Barros CM, Bo GA. Superovulation and embryo transfer in *Bos indicus* cattle. *Theriogenology* 2006;65:77-88.
- Basovskii DN. The effect of partial dissection of the zona pellucida on the fertilization of bovine oocytes *in vitro* and on the subsequent development of the embryos outside the body. *TSitol Genet (English summary)* 1999;33:58-64.
- Behboodi E, Groen W, Destempes MM, Williams JL, Ohlrichs C, Gavin WG, Broek DM, Ziomek CA, Faber DC, Meade HM, Echelard Y. Transgenic production from *in vivo*-derived embryos: effect on calf birth weight and sex ratio. *Mol Reprod Dev* 2001;60:27-37.

- Benyei B, Komlosi I, Pecsai A, Pollott G, Marcos CH, de Oliveira Campos A, Lemes MP. The effect of internal and external factors on bovine embryo transfer results in a tropical environment. *Anim Reprod Sci* 2006;93:268-79.
- Bilby TR, Guzeloglu A, Kamimura S, Pancarci SM, Michel F, Head HH, Thatcher WW. Pregnancy and bovine somatotropin in nonlactating dairy cows: I. Ovarian, conceptus, and insulin-like growth factor system responses. *J Dairy Sci* 2004;87:3256-67.
- Blondin P, Bousquet D, Twagiramungu H, Barnes F, Sirard M-A. Manipulation of follicular development to produce developmentally competent bovine oocytes. *Biol Reprod* 2002;66:38-43.
- Bo GA, Baruselli PS, Moreno D, Cutaia L, Caccia M, Tribulo R, Tribulo H, Mapletoft RJ. The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology* 2002;57:53-72.
- Bo GA, Baruselli PS, Martinez MF. Pattern and manipulation of follicular development in *Bos indicus* cattle. *Anim Reprod Sci* 2003;78:307-26.
- Bo GA, Baruselli PS, Chesta PM, Martins CM. The timing of ovulation and insemination schedules in superstimulated cattle. *Theriogenology* 2006;65:89-101.
- Bodo S, Baranyai B, Gocza E, Dohy J, Makkula M. Preimplantation genetic diagnosis in cattle: a review. *Acta Vet Hung*, 2001;49:99-109.
- Bordignon V, Keystone R, Lazaris A, Bilodeau AS, Pontes JHF, Arnold D, Fecteau G, Keefer C, Smith LC. Transgene expression of green fluorescent protein and germ line transmission in cloned calves derived from *in vitro*-transfected somatic cells. *Biol Reprod* 2003;68:2013-23.
- Bousquet D, Blondin P. Potential uses of cloning in breeding schemes: dairy cattle. *Cloning Stem Cells* 2004;6:190-7.
- Bowen RA, Reed ML, Schnieke A, Seidel GE Jr, Stacey A, Thomas WK, Kajikawa O. Transgenic cattle resulting from biopsied embryos: expression of c-ski in a transgenic calf. *Biol Reprod* 1994;50:664-8.
- Brackett BG, Bousquet D, Boice ML, Donawick WJ, Evans JF, Dressel MA. Normal development following *in vitro* fertilization in the cow. *Biol Reprod* 1982;27:147-58.
- Broadbent PJ, Steward M, Dolman DF. Recipient management and embryo transfer. *Theriogenology* 1991;35:125-39.
- Brophy B, Smolenski G, Wheeler T, Wells D, L'Huillier P, Laible G. Cloned transgenic cattle produce milk with higher levels of β -casein and κ -casein. *Nat Biotechnol*, 2003;21:157-62.
- Campbell BK, Souza C, Gong J, Webb R, Kendall N, Marsters P, Robinson G, Mitchell A, Telfer EE, Baird DT. Domestic ruminants as models for the elucidation of the mechanisms controlling ovarian follicle development in humans. *Reproduction* 2003;61(Sup):429-43.
- Cavalieri J, Hepworth G, Fitzpatrick LA, Shephard RW, Macmillan KL. Manipulation and control of the oestrus cycle in pastured-based dairy cows. *Theriogenology* 2006;65:45-64.
- Celebi C, Guillaudaux T, Auvray P, Vallet-Erdtmann V, Jégou B. The making of "transgenic spermatozoa". *Biol Reprod* 2003;68:1477-83.
- Chang AWS, Homan EJ, Ballou LU, Burns JC, Bremel RD. Transgenic cattle produced by reverse-transcribed gene transfer in oocytes. *Proc Natl Acad Sci USA* 1998;95:14028-33.
- Chang KH, Lim JM, Kang SK, Lee BC, Moon SY, Hwang WS. Blastocyst formation, karyotype, and mitochondrial DNA of interspecies embryos derived from nuclear transfer of human cord fibroblast into enucleated bovine oocytes. *Fertil Steril* 2003;80:1380-7.
- Chang KH, Lim JM, Kang SK, Lee BC, Moon SY, Hwang WS. An optimized protocol of a human-to-cattle interspecies somatic cell nuclear transfer. *Fertil Steril* 2004;82:960-2.
- Chen *et al.* Efficient production of transgenic cloned calves using preimplantation screening. *Biol Reprod* 2002;67:1488-92.
- Chrenek P, Boulanger L, Heyman Y, Uhrin P, Laurincik J, Bulla J, Renard JP. Sexing and multiple genotype analysis from a single cell of bovine embryo. *Theriogenology* 2001;55:1071-81.
- Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, Ponce de Leon FA, Robl JM. Cloned transgenic calves produced from nonquiescent fetal fibroblast. *Science* 1998a;280:1256-8.

- Cibelli JB, Stice SL, Golueke PJ, Kane JJ, Jerry J, Blackwell C, Ponce de Leon FA, Robl JM. Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. *Nat Biotechnol* 1998b;16:642-6.
- Clement-Sengewald A, Schutze K, Ashkin A, Palma GA, Kerlen G, Brem G. Fertilization of bovine oocytes induced solely with combined laser microbeam and optical tweezers. *J Assist Reprod Genet* 1996;13:259-65.
- Cseh S, Solti L. Importance of assisted reproductive technologies in the conservation of wild rare or indigenous ungulates: review article. *Acta Vet Hung* 2000;48:313-23.
- Curry MR. Cryopreservation of semen from domestic livestock. *Rev Reprod* 2000;5:46-52.
- De Rensis F, Peters AR. The control of follicular dynamics by PGF₂₄, GnRH, hCG, and oestrus synchronization in cattle. *Reprod Domest Anim* 1999;34:49-59.
- De Roover R, Bols PEJ, Genicot G, Hanzen Ch. Characteristics of low, medium and high responders following FSH stimulation prior to ultrasound-guided transvaginal oocyte retrieval in cows. *Theriogenology* 2005;63:1902-13.
- Dindot SV, Farin PW, Farin CE, Romano J, Walker S, Long C, Piedrahita JA. Epigenetic and genomic imprinting analysis in nuclear transfer derived *Bos gaurus/Bos taurus* hybrid fetuses. *Biol Reprod* 2004;71:470-8.
- Diskin MG, Sreenan JM. Expression and detection of oestrus in cattle. *Reprod Nutr Dev* 2000;40:481-91.
- Diskin MG, Austin EJ, Roche JF. Exogenous hormonal manipulation of ovarian activity in cattle. *Domest Anim Endocrinol* 2002;23:211-28.
- Dobrinski I. Germ cell transplantation. *Semin Reprod Med* 2005a;23:257-65.
- Dobrinski I. Germ cell transplantation and testis tissue xenografting in domestic animals. *Anim Reprod Sci* 2005b;89:137-45.
- Dobrinski I. Transplantation of germ cells and testis tissue to study mammalian spermatogenesis. *Anim Reprod* 2006;3:135-45.
- Dobrinski I. Transplantation of germ cells and testis tissue for the study and preservation of fertility. *Soc Reprod Fertil Suppl* 2007;65:447-58.
- Dobrinski I, Travis AJ. Germ cell transplantation for the propagation of companion animals, non-domestic and endangered species. *Reprod Fertil Dev* 2007;19:732-9.
- Dobrinski I, Avarbock MR, Brinster RL. Germ cell transplantation from large domestic animals into mouse testes. *Mol Reprod Dev* 2000;57:270-9.
- Dominko T, Mitalipova M, Haley B, Beyhan Z, Memili E, McKusick B, First NL. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. *Biol Reprod* 1999;60:1469-502.
- Dransfield MBG, Nebel RL, Pearson RE, Warnick LD. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. *J Dairy Sci* 1998;81:1874-82.
- Durocher J, Morin N, Blondin P. Effect of hormonal stimulation on bovine follicular response and oocyte developmental competence in a commercial operation. *Theriogenology* 2006;65:102-15.
- El-Sayed A, Hoelker M, Rings F, Salilew D, Jennen D, Tholen E, Sirard M-A, Schellander K, Tesfaye D. Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. *Physiol Genomics* 2006;28:84-96.
- Esslemont RJ, Mawhinney I. The cost benefits of a planned breeding routine for dairy cows (Ovsynch/Intercept). *Cattle Pract* 1996;4:293-300.
- Eyestone WH, Gowallis M, Monohan J, Sink T, Ball SF, Cooper JD. Production of transgenic cattle expressing human α -lactalbumin in milk. *Theriogenology* 1998;49:386.
- Faber DC, Molina JA, Ohlrichs CL, Vander Zwaag DF, Ferre LB. Commercialization of animal biotechnology. *Theriogenology* 2003;59:125-38.
- Faber DC, Ferre LB, Metzger J, Robl JM, Kasinathan P. Agro-economic impact of cattle cloning. *Cloning Stem Cells* 2004;6:198-207.
- Flint AF, Chapman PL, Seidel GE Jr. Fertility assessment through heterospermic insemination of flow-sorted sperm-cattle. *J Anim Sci* 2003;81:1814-22.

- Forsyth JT, Troskie HE, Pugh PA, Brophy B, Wells DN, Laible G. Utilizing pre-implantation genetic diagnosis and OPU-IVP-ET to generate multiple progeny of predetermined genotype from cloned transgenic heifers. *Reprod Fertil Dev* 2005;17:316.
- Fricke PM. Scanning the future—ultrasonography as a reproductive management tool for dairy cattle. *J Dairy Sci* 2002;85:1918-26.
- Funk DA. Major advances in globalization and consolidation of the artificial insemination industry. *J Dairy Sci* 2006;89:1362-8.
- Galli C, Lazzari G. Practical aspects of IVM/IVF in cattle. *Anim Reprod Sci* 1996;42:371-9.
- Galli C, Lazzari G. *In vitro* production of embryos in farm animals. *In: Proceeding of the 19th Scientific Meeting of the European Embryo Transfer Association*. Rostock: Germany, 12-13 Sept, 2003. p.93-101.
- Galli C, Crotti G, Notari C, Turini P, Duchi R, Lazzari G. Embryo production by ovum pick up from live donors. *Theriogenology* 2001;55:1341-57.
- Galli C, Duchi R, Crotti G, Turini P, Ponderato N, Colleoni S, Lagutina I, Lazzari G. Bovine embryo technologies. *Theriogenology* 2003;59:599-616.
- Gandolfi F. Spermatozoa, DNA binding and transgenic animals. *Transgenic Res* 1998;7:147-155.
- Garner DL. Ancillary test of bull semen quality. *Vet Clin North Am Food Anim Pract* 1997;13:313-30.
- Garner DL. Flow cytometric sexing of mammalian sperm. *Theriogenology* 2006;65:943-57.
- Gearheart WW, Smith C, Teeper G. Multiple ovulation and embryo manipulation in the improvement of beef cattle: relative theoretical rates of genetic change. *J Anim Sci* 1989;67:2863-71.
- Gong G, Dai Y, Fan B, Zhu H, Zhu S, Wang H, Wang L, Tang B, Li R, Wan R, Liu Y, Huang Y, Zhang L, Sun X, Li N. Birth of calves expressing the enhanced green fluorescent protein after transfer of fresh or vitrified/thawed blastocysts produced by somatic cell nuclear transfer. *Mol Reprod Dev* 2004;69:278-88.
- Goto K, Kinoshita A, Takuma G, Ogawa K. Fertilisation of bovine oocytes by the injection of immobilised, killed spermatozoa. *Vet Rec* 1990;127:517-20.
- Grosse-Hovest L, Müller S, Minoia R, Wolf E, Zakhartchenko V, Wenigerkind H, Lassnig C, Besenfelder U, Müller M, Lytton SD, Jung G, Brem G. Cloned transgenic farm animals produce a bispecific antibody for T-cell mediated tumor cell killing. *Proc Natl Acad Sci USA* 2004;101:6858-63.
- Gwatkin RBL. Micromanipulation-assisted fertilization. *Mol Reprod Dev* 1993;36:285-7.
- Hamano K-I, Li X, Qian X-Q, Funauchi K, Furudate M, Minato Y. Gender preselection in cattle with intracytoplasmically injected, flow cytometrically sorted sperm heads. *Biol Reprod* 1999;60:1194-7.
- Hamano S, Miyamura M, Tuchiya H, Watanabe Y, Sato A, Harasawa M, Hamawaki A, Yoshikawa M. Mass production of cattle from IVM, IVF, and IVP embryos in Japan. *J Reprod Fertil* 2006;52(Sup):S77-S85.
- Hammer RE, Pursel VG, Rexroad CE, Wall RJ Jr, Bolt DJ, Ebert KM, Palmiter RD, Brinster RL. Production of transgenic rabbits, sheep and pigs by microinjection. *Nature* 1985;315:680-3.
- Hammer CJ, Tyler HD, Loskutoff NM, Armstrong DL, Funk DJ, Lindsey BR, Simmons LG. Compromised development of calves (*Bos gaurus*) derived from *in vitro*-generated embryos and transferred interspecifically into domestic cattle (*Bos taurus*). *Theriogenology* 2001;55:1447-55.
- Hansen PJ. Realizing the promise of IVF in cattle - an overview. *Theriogenology* 2006;65:119-25.
- Hansen PJ, Block J. Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. *Repro Fertil Dev* 2004;16:1-14.
- Hasler JF. The current status and future of commercial embryo transfer in cattle. *Anim Reprod Sci* 2003;79:245-64.
- Hasler JF. The Holstein cow embryo transfer today as compared to 20 years ago. *Theriogenology* 2006;65:4-16.
- Hasler JF, Cardney E, Stokes JE, Bredbacka P. Nonelectrophoretic PCR-sexing of bovine embryos in a commercial environment. *Theriogenology* 2002;58:1457-69.
- Havlicek V, Wetscher F, Huber T, Brem G, Mueller M, Besenfelder U. *In vivo* culture of IVF/IVM embryos in bovine oviducts by transvaginal endoscopy. *J Med Vet A* 2005;52:94-8.

- Hernandez-Fonseca HJ, Bosch P, Wininger D, Massey JB, Brackett, BG. Recovery of oocytes from bovine ovarian tissue transplanted in Nod Scid mice. *J Anim Vet Adv* 2004;3:597-603.
- Hernandez-Fonseca HJ, Bosch P, Miller DM, Wininger JD, Massey JB, Brackett BG. Time course follicular development after bovine ovarian tissue transplantation in male non-obese diabetic severe combined immunodeficient mice. *Fertil Steril* 2005;84(Sup 1):1180-7.
- Herrera C, Conde P, Donaldson M, Quintans C, Cortvrindt R, de Matos DG, Pasqualini RS. Bovine follicular development up to antral stages after frozen-thawed ovarian tissue transplantation into nude mice. *Theriogenology* 2002;57:608.
- Herrid M, Vignarajan S, Davey R, Dobrinski I, Hill JR. Successful transplantation of bovine testicular cells to heterologous recipients. *Reproduction* 2006; 132:617-24.
- Heuwieser W, Yang X, Jiang S, Foote RH. Fertilization of bovine oocytes by microinjection of spermatozoa into the perivitelline space. *Theriogenology* 1991;35:213.
- Heyman Y. Nuclear transfer: a new tool for reproductive biotechnology in cattle. *Reprod Nutr Dev* 2005;45:353-61.
- Heyman Y, Chavatte-Palmer P, Berthelot V, Fromentin G, Hocquette JF, Martignat L, Renard JP. Assessing the quality of cloned cattle: An integrative approach. *Theriogenology* 2007;67:134-41.
- Hill JR, Dobrinsky I. Male germ cell transplantation in livestock. *Reprod Fertil Dev* 2006;18:13-18.
- Hill JR, Roussel AJ, Cibelli JB, Edwards JF, Hooper NL, Miller MW, Thompson JA, Looney CR, Westhusin ME, Robl JM, Stice SL. Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology* 1999;51:1451-65.
- Hirayama H, Kageyama S, Moriyasu S, Hirano T, Sugimoto Y, Kobayashi N, Inaba M, Sawai K, Onoe S, Minamihashi A. Genetic diagnosis of Claudin-16 deficiency and sex determination in bovine preimplantation embryos. *J Reprod Dev* 2004;50:613-18.
- Hochman D, Zaron Y, Dekel I, Feldmesser E, Medrano JF, Shani M, Ron M. Multiple genotype analysis and sexing of IVF bovine embryos. *Theriogenology* 1996;46:1063-75.
- Hofmann A, Kessler B, Ewerling S, Weppert M, Vogg B, Ludwig H, Stojkovic M, Boelhauve M, Brem G, Wolf E, Pfeifer A. Efficient transgenesis in farm animals by lentiviral vectors. *EMBO Rep* 2003;4:1054-60.
- Hofmann A, Zakhartchenko V, Weppert M, Sebald H, Wenigerkind H, Brem G, Wolf E, Pfeifer A. Generation of transgenic cattle by lentiviral gene transfer into oocytes. *Biol Reprod* 2004;71:405-9.
- Horiuchi T. Application study of intracytoplasmic sperm injection for golden hamster and cattle production. *J Reprod Dev* 2006;52:13-21.
- Horiuchi T, Numabe T. Intracytoplasmic sperm injection (ICSI) in cattle and other domestic animals: problems and improvements in practical use. *J Mamm Ova Res* 1999;16:1-9.
- Horiuchi T, Emuta C, Yamauchi Y, Oikawa T, Numabe T, Yanagimachi R. Birth of normal calves after intracytoplasmic sperm injection of bovine oocytes: a methodological approach. *Theriogenology* 2002;57:1013-24.
- Houdebine L-M. Relationships between animal transgenesis and reproduction. *Reprod Nutr Dev* 2005;45:363-76.
- Hunter RH. Advances in deep uterine insemination: a fruitful way forward to exploit new sperm technologies in cattle. *Anim Reprod Sci* 2003;79:157-170.
- Hyttinen J-M, Peura T, Tolvanen M, Aalto J, Alhonen L, Sinervirta R, Halmekytö M, Myöhänen S, Jänne J. Generation of transgenic dairy cattle from transgene-analyzed and sexed embryos produced *in vitro*. *Bio/Technology* 1994;12:606-8.
- Illmensee K, Levanduski M, Zavos PM. Evaluation of the embryonic preimplantation potential of human adult somatic cells via an embryo interspecies bioassay using bovine oocytes. *Fertil Steril* 2006;85(Sup 1):1248-60.
- Imai K, Tagawa M, Yoshioka H, Matoba S, Narita M, Inaba Y, Aikawa Y, Ohtake M, Kobayashi S. The efficiency of embryo production by ovum pick-up and *in vitro* fertilization in cattle. *J Reprod Dev* 2006;52(Sup):S19-S29.

- Izadyar F, *et al.* Autologous and homologous transplantation of bovine spermatogonial stem cells. *Reproduction* 2003;126:765-74.
- Johnson LA. Sexing mammalian sperm for production of offspring: the state-of the-art. *Anim Reprod Sci* 2000;60-61:93-107.
- Johnson SK. Possibilities with today's reproductive technologies. *Theriogenology* 2005;64:639-56.
- Johnson WH, Loskutoff NM, Plante Y, Betteridge KJ. The production of four identical calves by the separation of blastomeres from an *in vitro* derived four cell embryo. *Vet Rec* 1995;137:15-6.
- Kafi M, McGowan MR. Factors associated with variation in the superovulatory response of cattle. *Anim Reprod Sci* 1997;48:137-57.
- Kageyama S, Hirayama H, Moriyasu S, Inaba M, Ito D, Ohta H, Sawaik K, Minamihashi A, Onoe S. Genetic diagnosis of band 3 deficiency and sexing in bovine preimplantation embryos. *J Vet Med Sci* 2006;68:319-23.
- Kanitz W, Becker F, Schneider F, Kanitz E, Leidig C, Nohner HP, Pohland R. Superovulation in cattle: practical aspects of gonadotropin treatment and insemination. *Reprod Nutr Dev* 2002;42:587-99.
- Karande V, Gleicher N. A rational approach to the management of low responders in in-vitro fertilization. *Hum Reprod* 1999;14:1744-8.
- Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, Yasue H, Tsunoda Y. Eight calves cloned from somatic cells of a single adult. *Science* 1998;282:2095-8.
- Keefer CL. Production of bioproducts through the use of transgenic animals models. *Anim Reprod Sci* 2004;82-83:5-12.
- Keskintepe L, Pacholczyk G, Machnicka A, Norris K, Curuk MA, Khan I, Brackett BG. Bovine blastocyst development from oocytes injected with freeze-dried spermatozoa. *Biol Reprod* 2002;67:409-15.
- Kitiyanant Y, Saikhun J, Chaisalee B, White KL, Pavasuthipaisit K. Somatic cell cloning in Buffalo (*Bubalus bubalis*): effect of interspecies cytoplasmic recipients and activation procedures. *Cloning Stem Cells* 2001;3:97-104.
- Kobayashi T, Amemiya K, Takeuchi K, Tsujioka T, Tominaga K, Hirabayashi M, Ishikawa H, Fukui Y, Hochi S. Contribution of spermatozoal centrosomes to the microtubule-organizing centre in Antarctic minke whale (*Balaenoptera bonaerensis*). *Zygote* 2006;14:45-51.
- Krimpenfort P, Rademakers A, Eyestone W, van der Schans A, van den Broek S, Kooiman P, Kootwijk E, Platenburg G, Pieper F, Strijker R, de Boer H. Generation of transgenic dairy cattle using '*in vitro*' embryo production. *Bio/Technology* 1991;9:844-7.
- Kuliev A, Verlinski Y. Preimplantation diagnosis: a realistic option for assisted reproduction and genetic practice. *Curr Opin Obstet Gynecol* 2005;17:179-83.
- Kurikyn J, Jaakma U, Majas L, Jalakas M, Aidnik M, Waldmann A, Padrik P. Fixed time deep intracornual insemination of heifers at synchronized estrus. *Theriogenology* 2003;60:1261-8.
- Kuroiwa Y, Kasinathan P, Choi YJ, Naeem R, Tomizuka K, Sullivan EJ, Knott JG, Duteau A, Goldsby RA, Osborne BA, Ishida I, Robl JM. Cloned transchromosomal calves producing human immunoglobulin. *Nat Biotechnol* 2002;20:889-94.
- Lagutina I, Brunetti D, Lazzari G, Galli C. Preliminary data on pig-bovine interspecies nuclear transfer embryo development. *Reprod Fertil Dev* 2005;18:134-5.
- Lanza RP, Cibelli JB, Diaz F, Moraes CT, Farin PW, Farin CE, Hammer CJ, West MD, Damiani P. Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. *Cloning* 2000;2:79-90.
- Lavitrano M, Busnelli M, Cerrito MG, Giovannoni R, Manzini S, Vargiolu A. Sperm-mediated gene transfer. *Reprod Fertil Dev* 2006;18:19-23.
- Lee KB, Niwa K. Fertilization and development *in vitro* of bovine oocytes following intracytoplasmic injection of heat-dried sperm heads. *Biol Reprod* 2006;74:146-52.
- Lee B, Wirtu GG, Damiani P, Pope E, Dresser BL, Hwang W, Bavister BD. Blastocyst development after intergeneric nuclear transfer of mountain bongo antelope somatic cells into bovine oocytes. *Cloning Stem Cells* 2003;5:25-33.
- Le Tallec B, Ponsart C, Marquant-Le Guienne B, Guérin B. Risk of transmissible diseases in relation to embryo transfer. *Reprod Nutr Dev* 2001;41:439-50.

- Lewis IM, Peura TT, Trounson AO. Large-scale applications of cloning technologies for agriculture: an industry perspective. *Reprod Fertil Dev* 1998;10:677-81.
- Li G-P, Seidel GE Jr, Squires EL. Intracytoplasmic sperm injection of bovine oocytes with stallion spermatozoa. *Theriogenology* 2003;59:1143-55.
- Li Y, Dai Y, Du W, Zhao C, Wang H, Wang L, Li R, Liu Y, Wan R, Li N. Cloned endangered species takin (*Budorcas taxicolor*) by inter-species nuclear transfer and comparison of the blastocyst development with yak (*Bos grunniens*) and bovine. *Mol Reprod Dev* 2006;73:189-95.
- Linares T, Larsson K, Edqvist L-E. Plasma progesterone levels from oestrus through day 7 after AI in heifers carrying embryos with normal or deviating morphology. *Theriogenology* 1982;17:115-230.
- Liu JL, Wang MK, Sun QY, Xu Z, Chen DY. Effect of telophase enucleation on bovine somatic nuclear transfer. *Theriogenology* 2000;54:989-98.
- Loneragan P. State-of-the-art embryo technologies in cattle. *Soc Reprod Fertil Suppl* 2007;64:315-25.
- Loneragan P, Rizos D, Gutierrez-Adan A, Fair T, Boland MP. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. *Reprod Domest Anim* 2003;38:259-67.
- Looney CR, Nelson JS, Schneider HJ, Forrest DW. Improving fertility in beef cow recipients. *Theriogenology* 2006;65:201-9.
- Lopes RF, Forell F, Oliveira AT, Rodrigues JL. Splitting and biopsy for bovine embryo sexing under field conditions. *Theriogenology* 2001;56:1383-92.
- Lowman BG, Scott NA, Scott PR. An evaluation of some breeding management options in beef herds in the United Kingdom. *Vet Rec* 1994;135:9-12.
- Lu F, Shi D, Wei J, Yang S, Wei Y. Development of embryos reconstructed by interspecies nuclear transfer of adult fibroblast between buffalo (*Bubalus bubalis*) and cattle (*Bos indicus*). *Theriogenology* 2005;64:1309-19.
- Lucas-Hahn A, Lemme E, Hadeler K-G, Sander H-G, Niemann H. Efficiency of ovum pickup and embryo production *in vitro* in cloned cattle. *Reprod Fertil Dev* 2005;18:137.
- Lucy MC. Non-lactational traits of importance in dairy cows and applications for emerging biotechnologies. *N Z Vet J* 2005;53:406-15.
- Machado SA, Reichenbach HD, Weppert M, Wolf E, Goncalves PB. The variability of ovum-pick response and *in vitro* production from monozygotic twin cows. *Theriogenology* 2006;65:573-83.
- Macmillan KL, Segwagwe BE, Pino CS. Associations between the manipulation of patterns of follicular development and fertility in cattle. *Anim Reprod Sci* 2003;78:327-44.
- Manik RS, Singla SK, Palta P. Collection of oocytes through transvaginal ultrasound-guided aspiration of follicles in an Indian breed of cattle. *Anim Reprod Sci* 2003;76:155-61.
- Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001; 121:175-80.
- Mapletoft RJ, Steward KB, Adams GP. Recent advances in the superovulation in cattle. *Reprod Nutr Dev* 2002;42:601-11.
- Mapletoft RJ, Martínez MF, Colazo MG, Kastelic JP. The use of controlled internal drug release devices for the regulation of bovine reproduction. *J Anim Sci* 2003;81(E Sup):E28-E36.
- Mapletoft RJ, Hasler JF. Assisted reproductive technologies in cattle: a review. *Rev Sci Tech* 2005;24:393-403.
- Massip A. Crypreservation of bovine oocytes: current status and recent developments. *Reprod Nutr Dev* 2003;43:325-30.
- McEvoy TG. Manipulation of domestic animal embryos and implications for development. *Reprod Domest Anim* 2003;38:268-75.
- McEvoy TG, Ashworth CJ, Rooke JA, Sinclair KD. Consequences of manipulating gametes and embryos of ruminant species. *Reproduction* 2003;61(Sup):167-82.
- McEvoy TG, Alink FM, Moreira VC, Watt RG, Powell KA. Embryo technologies and animal health-consequences for the animal following ovum pick-up, *in vitro* embryo production and somatic cell nuclear transfer. *Theriogenology* 2006;65:926-42.
- Ménézo YVR, Hérubel F. Mouse and bovine models for human IVF. *Reprod Biomed Online* 2002;4:170-5.

- Merton JS, de Roos APW, Mullaart E, de Ruigh L, Kaal L, Vos PLAM, Dieleman SJ. Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. *Theriogenology* 2003;59:651-74.
- Moore K, Bonilla AQ. Cryopreservation of mammalian embryos: the state of the art. *Annu Rev Biomed Sci* 2006;8:19-32.
- Moore K, Thatcher WW. Major advances associated with reproduction in dairy cattle. *J Dairy Sci* 2006;89:1254-66.
- Morales-Roura JS, Zarco L, Hernández-Cerón J, Rodríguez G. Effect of short-term treatment with bovine somatotropin at estrus on conception rate and luteal function of repeat-breeding dairy cows. *Theriogenology* 2001;55:1831-41.
- Murakami M, Otoi T, Wongsrikeao P, Agung B, Sambuu R, Suzuki T. Development of interspecies cloned embryos in yak and dog. *Cloning Stem Cells* 2005;7:77-81.
- Mwaanga ES, Janowski T. Anoestrus in dairy cows: causes, prevalence and clinical forms. *Reprod Domest Anim* 2000;35:193-200.
- Niemann H, Kues WA. Application of transgenesis in livestock for agriculture and biomedicine. *Anim Reprod Sci* 2003;79:291-317.
- Niemann H, Kues W, Carnwath JM. Transgenic farm animals: present and future. *Rev Sci Tech* 2005;24:285-98.
- Niemann H, Kues WA. Transgenic farm animals: an update. *Reprod Fertil Dev* 2007;19:762-70.
- Niemann H, Wrenzycki C. Alterations of expression of developmentally important genes in preimplantation bovine embryos by in culture conditions: implications for subsequent development. *Theriogenology* 2000;53:21-34.
- Oatley JM, de Avila DM, Reeves JJ, McLean DJ. Spermatogenesis and germ cell transgene expression in xenografted bovine testicular tissue. *Biol Reprod* 2004;71:494-501.
- Oback B, Wells DN. Cloning cattle. *Cloning Stem Cells* 2003;5:243-56.
- Oback B, Wells DN. Cloning cattle: the methods in the madness. *Adv Exp Med Biol* 2007;591:30-57.
- Oropeza A, Wrenzycki C, Herrmann D, Haderl K-G, Niemann H. Improvement of the developmental capacity of oocytes from prepubertal cattle by intraovarian insulin-like growth factor-1 application. *Biol Reprod* 2004;70:1634-43.
- Paape M, Mehrzad J, Zhao X, Detilleux J, Burvenich C. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. *J Mammary Gland Biol Neoplasia* 2002;7:109-21.
- Parks JE, Lee DR, Huang S, Kaproth MT. Prospects for spermatogenesis *in vitro*. *Theriogenology* 2003;59:73-86.
- Peippo J, Viitala S, Virta J, Rätty M, Tammiranta N, Lamminen T, Aro J, Myllymäki H, Vilkkilä J. Birth of correctly genotyped calves after multiplex marker detection from bovine embryo microblade biopsies. *Mol Reprod Dev* 2007;74:1373-8.
- Peralta OA, Pearson RE, Nebel RL. Comparison of three estrus detection systems during summer in a large commercial dairy herd. *Anim Reprod Sci* 2005;87:59-72.
- Peterson AJ, Lee RS-F. Improving successful pregnancies after embryo transfer. *Theriogenology* 2003;59:687-97.
- Piedrahita JA. Targeted modification of the domestic animal genome. *Theriogenology* 2000;53:105-16.
- Pieterse MC, Kappen KA, Kruip ThAM, Taverne MAM. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. *Theriogenology* 1988;30:751-62.
- Pope CE, Dresser BL, Kuehn G, Kramer L, Gillespie D. Live birth of a gaur (*Bos gaurus*) calf following nonsurgical embryo transfer to a Holstein (*Bos taurus*) recipient. *Theriogenology* 1988;29:289.
- Rabiee AR, Lean IJ, Stevenson MA. Efficacy of Ovsynch program on reproductive performance in dairy cattle: a meta analysis. *J Dairy Sci* 2005;88:2754-70.
- Ramalho MF, Garcia JM, Esper CR, Vantini R, Alves BC, Almeida Junior IL, Hossepian de Lima VF, Moreira-Filho C.A. Sexing of murine and bovine embryos by developmental arrest induced by high-titer H-Y antisera. *Theriogenology* 2004;62:1569-76.
- Reproductive BioMedicine Online News. Stem cells from human/cow hybrid embryos? *Reprod Biomed Online* 2007;14:109.

- Rho G-J, Lee S-L, Kim Y-S, Yeo H-J, Ock S-A, Balasubramanian S, Choe S-Y. Intracytoplasmic sperm injection of frozen-thawed bovine oocytes and subsequent embryo development. *Mol Reprod Dev* 2004;68:449-55.
- Rhodes FM, McDougall S, Burke CR, Verkerk GA, Macmillan KL. Treatment of cows with extended postpartum anestrus interval. *J Dairy Sci* 2003;86:1876-94.
- Ribadu AY, Nakao T. Bovine reproductive ultrasonography: a review. *J Reprod Dev* 1999;45:13-28.
- Robl JM, Wang Z, Kasinathan P, Kuroiwa Y. Transgenic animal production and animal biotechnology. *Theriogenology* 2006;67:127-33.
- Rodríguez-Martínez H. Can we increase the estimate value of semen assessment? *Reprod Domest Anim* 2006;41(Suppl 2):2-10.
- Rodríguez-Martínez H. State of the art in farm animal sperm evaluation. *Reprod Fertil Dev* 2007;19:91-101.
- Roelofs JB, van Eerdenburg FJ, Soede NM, Kemp B. Various behavioral signs of oestrus and their relationship with time of ovulation in dairy cattle. *Theriogenology* 2005a;63:1366-77.
- Roelofs JB, van Eerdenburg FJ, Soede NM, Kemp B. Pedometers readings for estrous detection and as predictor for time of ovulation in dairy cattle. *Theriogenology* 2005b;64:1690-703.
- Roelofs JB, Van Eerdenburg FJ, Hazeleger W, Soede NM, Kemp B. Relationship between progesterone concentrations in milk and blood and time of ovulation in dairy cattle. *Anim Reprod Sci* 2006;91:337-43.
- Roschlau K, Rommel P, Andreewa L, Zackel M, Roschlau D, Zackel B, Schwerin M, Hühn R, Gazarjan KG. Gene transfer experiments in cattle. *J Reprod Fertil* 1989;38(Sup):153-60.
- Rutter ERF. Multiple sclerosis and milk: to drink or not to drink? *Int J Dairy Technol* 2006;59:223-228.
- Saacke RG, Dalton JC, Nadir S, Nebel RL, Bame JH. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. *Anim Reprod Sci* 2000;60-61:663-77.
- Saikhun J, Pavasuthipaisit K, Jaruansuwa M, Kitiyanant Y. Xenonuclear transplantation of buffalo (*Bubalus bubalis*) fetal and adult somatic cell nuclei into bovine (*Bos indicus*) oocyte cytoplasm and their subsequent development. *Theriogenology* 2002;57:1829-37.
- Salamone D *et al.* High level expression of bioactive recombinant human growth hormone in the milk of a cloned transgenic cow. *J Biotechnol* 2006;124:469-72.
- Sansinena MJ, Hylan D, Hebert K, Denniston RS, Godke RA. Banteng (*Bos javanicus*) embryos and pregnancies produced by interspecies nuclear transfer. *Theriogenology* 2005;63:1081-91.
- Schellander K, Mayr B, Ertl K, Peli J. Simultaneous genotyping of sex and kappa-casein of bovine *in vitro* fertilized embryos by PCR technique. *Zentralbl Veterinarmed A* 1993;40:307-9.
- Schenk, J.L., Suh, T.K. and Seidel, G.E. Jr., 2006. Embryo production from superovulated cattle following insemination of sexed sperm. *Theriogenology* 2006;65:299-307.
- Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF, Nieschlag E. Germ cell transfer into rat, bovine, monkey and human testes. *Hum Reprod* 1999;14:144-50.
- Schmoll F, Schneider H, Montaq M, Wimmers K, Rink K, Schellander K. Effects of different laser-drilled openings in the zona pellucida on hatching of *in vitro*-produced cattle blastocyst. *Fertil Steril* 2003;80(Sup 2):714-9.
- Schutze K, Clement-Sengewald A, Ashkin A. Zona drilling and sperm insertion with combined laser microbeam and optical tweezers. *Fertil Steril* 1994;61:783-6.
- Seidel GE Jr. Application of embryo transfer and related technologies to cattle. *J Dairy Sci* 1984;67:2786-96.
- Seidel GE Jr. Economics of selecting for sex: the most important genetic trait. *Theriogenology* 2003a;59:585-98.
- Seidel GE Jr. Sexing mammalian sperm - Intertwining of commerce, technology, and biology. *Anim Reprod Sci* 2003b;79:145-56.
- Seidel GE Jr. On the usefulness of an update on assisted reproductive technologies in cattle. *Theriogenology* 2006a;65:1-3.
- Seidel GE Jr. Modifying oocytes and embryos to improve their cryopreservation. *Theriogenology* 2006b;65:228-35.

- Seidel GE Jr. Overview of sexing sperm. *Theriogenology* 2007;68:443-6.
- Seidel GE Jr, Garner DL. Current status of sexing mammalian spermatozoa. *Reproduction* 2002;124:733-43.
- Seidel GE Jr, Johnson LA. Sexing mammalian sperm—Overview. *Theriogenology* 1999;52:1267-72.
- Selvaraju S, Agarwal SK, Karche SD, Srivastava SK, Majumdar AC, Shanker U. Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. *Theriogenology* 2002;73:141-9.
- Senbon S, Ota A, Tachibana M, Miyano T. Bovine oocytes in secondary follicles grow and acquire meiotic competence in severe combined immunodeficient mice. *Zygote* 2003;11:139-49.
- Senbon S, Ota A, Tachibana M, Miyano T. Xenografting of bovine secondary follicles into ovariectomized female severe combined immunodeficient mice. *J Reprod Dev* 2004;50:439-44.
- Senbon S, Ishii K, Fukumi Y, Miyano T. Fertilization and development of bovine oocytes grown in female SCID mice. *Zygote* 2005;13:309-15.
- Shea BF. Determining the sex of bovine embryos using polymerase chain reaction results: a six-year retrospective study. *Theriogenology* 1999;51:841-54.
- Shemesh M, Gurevich M, Harel-Markowitz E, Benvenisti L, Shore LS, Stram Y. Gene integration into bovine sperm genome and its expression in transgenic offspring. *Mol Reprod Dev* 2000;56:306-8.
- Sreenan JM. Embryo transfer procedure and its use as a research technique. *Vet Rec* 1983;112:494-500.
- Stroud B, Hasler JF. Dissecting why superovulation and embryo transfer usually work on some farms but not in others. *Theriogenology* 2006;65:65-76.
- Tanghe S, Van Soom A, Sterckx V, Maes D, de Kruif, A. Assessment of different sperm quality parameters to predict *in vitro* fertility of bulls. *Reprod Domest Anim* 2002;37:127-32.
- Tecirlioglu RT, Trounson AO. Embryonic stem cells in companion animals (horses, dogs and cats): present status and future prospects. *Reprod Fertil Dev* 2007;19:740-7.
- Terada Y, Nakamura S, Morita J, Tachibana M, Morito Y, Ito K, Murakami T, Yaegashi N, Okamura K. Use of mammalian eggs for assessment of human sperm function: molecular and cellular analyses of fertilization by intracytoplasmic sperm injection. *Am J Reprod Immunol* 2004;51:290-3.
- Thatcher WW, Moreira F, Santos JEP, Mattos RC, Lopes FL, Pancarci SM, Risco CA. Effects of hormonal treatments on reproductive performance and embryo production. *Theriogenology* 2001;55:75-89.
- Thatcher WW, Moreira F, Pancarci SM, Bartolome JA, Santos JEP. Strategies to optimize reproductive efficiency by regulation of ovarian function. *Domest Animal Endocrinol* 2002;23:243-54.
- Thatcher WW, Guzeloglu A, Meikle A, Kamimura S, Bilby T, Kowalski AA, Badinga L, Pershing R, Bartolome J, Santos JEP. Regulation of embryo survival in cattle. *Reproduction* 2003;61(Sup):253-66.
- Thatcher WW, Bartolome JA, Sozzi A, Silvestre F, Santos JEP. Manipulation of ovarian function for the reproductive management of dairy cows. *Vet Res Commun* 2004;28(Sup 1):111-9.
- Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JEP. Strategies for improving fertility in the modern dairy cow. *Theriogenology* 2006;65:30-44.
- Thibier M. The animal embryo transfer industry figures a report from the IETS data retrieval committee. *IETS Newslett* 2001;19:16-22.
- Thibier M. Stabilization of numbers of *in vivo* collected embryos in cattle but significant increases of *in vitro* bovine produced embryos in some parts of the world. *IETS Newslett* 2004;22:12-19.
- Thibier M. The zootechnical applications of biotechnology in animal reproduction: current methods and perspectives. *Reprod Nutr Dev* 2005;45:235-42.
- Thibier M, Nibart M. The sexing of bovine embryos in the field. *Theriogenology* 1995;43:71-80.
- Thomson AJ, Marques MM, McWhir J. Gene targeting in livestock. *Reproduction* 2003;61(Sup):495-508.
- Tominaga K. Cryopreservation and sexing of *in vivo*- and *in vitro*-produced bovine embryos for their practical use. *J Reprod Dev* 2004;50:29-38.
- Tóth F, Gábor J, Mézes M, Váradi É, Ózsván L, Sasser RG, Abonyi-Tóth Zs. Improving the reproductive efficiency by zoo-technical methods at a dairy farm. *Reprod Domest Anim* 2006;41:184-88.
- Tsunoda Y, Kato Y. The recent progress on nuclear transfer in mammals. *Zool Sci* 2000;17:1177-84.

- Tsunoda Y, Kato Y. Recent progress and problems in animal cloning. *Differentiation* 2002;69:158-61.
- Underwood SL, Bathgate R, Maxwell WMC, O'Donnell M, Evans G. Pregnancies after artificial insemination of frozen-thawed, sex-sorted, re-frozen-thawed dairy bull sperm. *Reprod Domest Anim* 2007;42(Sup 2):78.
- van Arendonk JAM, Bijma P. Factors affecting commercial application of embryo technologies in dairy cattle in Europe-a modelling approach. *Theriogenology* 2003;59:635-49.
- van Arendonk JAM, Liinamo A-E. Dairy cattle production in Europe. *Theriogenology* 2003;59:563-9.
- van Berkel PH, Welling MM, Geerts M, van Veen HA, Ravensbergen B, Salaheddine M, Pauwels EK, Pieper F, Nuijens JH, Nibbering PH. Large scale production of recombinant human lactoferrin in the milk of transgenic cows. *Nat Biotechnol* 2002;20:484-7.
- van Vliet RA, Verrinder Gibbins AM, Walton JS. Livestock embryo sexing: A review of current methods, with emphasis on Y-specific DNA probes. *Theriogenology* 1989;32:421-38.
- van Wagtendonk-de Leeuw AM. Ovum pick up and *in vitro* production in the bovine after use in several generations: a 2005 status. *Theriogenology* 2006;65:914-25.
- Vasconcelos JLM, Demétrio DGB, Santos RM, Chiari JR, Rodrigues CA, Sá Filho OG. Factors potentially affecting fertility of lactating dairy recipients. *Theriogenology* 2006;65:192-200.
- Velasco-Garcia MN, Mottram T. Biosensors in the livestock industry: an automated ovulation prediction system for dairy cows. *Trends in Biotechnol* 2001;19:433-4.
- Velazquez MA, Newman M, Christie MF, Cripps PJ, Crowe MA, Smith RF, Dobson H. The usefulness of a single measurement of insulin-like growth factor-1 as a predictor of embryo yield and pregnancy rates in a bovine MOET program. *Theriogenology* 2005;64:1977-94.
- Verberckmoes S, Van Soom A, De Pauw I, Dewulf J, Vervaeke C, de Kruif A. Assessment of a new utero-tubal junction insemination device in dairy cattle. *Theriogenology* 2004;61:103-15.
- Vishmanath R. Artificial insemination: the state of the art. *Theriogenology* 2003;59:571-84.
- Wall RJ, Powell AM, Paape MJ, Kerr DE, Bannerman DD, Pursel VG, Wells KD, Talbot N, Hawk HW. Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. *Nat Biotechnol* 2005;23:445-51.
- Wathes DC, Taylor VJ, Cheng Z, Mann GE. Follicle growth, *corpus luteum* function and their effects on embryo development in postpartum dairy cows. *Reproduction* 2003;61(Sup):219-37.
- Weiss RA. Robert Koch: the grandfather of cloning? *Cell* 2005;123:539-42.
- Wells DN. Cloning in livestock agriculture. *Reproduction* 2003;61(Sup):131-50.
- 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;10:369-78.
- Westhusin ME, Shin T, Templeton JW, Burghardt RC, Adams LG. Rescuing valuable genomes by animal cloning: a case for natural disease resistance in cattle. *J Anim Sci* 2007;85:138-42.
- Wetscher F, Havlicek V, Huber T, Gilles M, Tesfaye D, Griesse J, Wimmers K, Schellander K, Muller M, Brem G, Besenfelder U. Intrafallopian transfer of gametes and early stage embryos for *in vivo* culture in cattle. *Theriogenology* 2005;64:30-40.
- Wheeler MB. Production of transgenic livestock: promise fulfilled. *J Anim Sci* 2003;81(Sup 3):32-7.
- Wheeler MB. Agricultural applications for transgenic livestock. *Trends Biotechnol* 2007;25:204-10.
- Wheeler MB, Rutledge JJ, Fischer-Brown A, VanEtten T, Malusky S, Beebe DJ. Application of sexed semen technology to *in vitro* embryo production in cattle. *Theriogenology* 2006;65:219-27.
- Whelan JG III, Vlahos NF. The ovarian hyperstimulation syndrome. *Fertil Steril* 2000;73:883-96.
- Wiltbank MC, Gumen A, Sartori R. Physiological classification of anovulatory conditions in cattle. *Theriogenology* 2002;57:21-52.
- Willet EL, Black WG, Casida LE, Stone WH, Buckner PJ. Successful transplantation of a fertilized bovine ovum. *Science* 1951;113:247.
- Williams TJ, Elsdon RP, Seidel GE Jr. Pregnancy rates with bisected bovine embryos. *Theriogenology* 1984;22:521-31.
- Wilmot I. Livestock cloning: Past, present, and future. *In: Proceeding of the 19th Scientific Meeting of the European Embryo Transfer Association*. Rostock: Germany, 12-13 Sept, 2003. p.15-21.

- Xu J, Guo Z, Su L, Nedambale TL, Zhang J, Schenk J, Moreno JF, Dinnyés A, Ji W, Tian XC, Yang X, Du F. Developmental potential of vitrified Holstein cattle embryos fertilized *in vitro* with sex-sorted sperm. *J Dairy Sci* 2006;89:2510-8.
- Yang X, Tian XC, Kubota C, Page R, Xu J, Cibelli J, Seidel G Jr. Risk assessment of meat and milk from cloned animals. *Nat Biotechnol* 2007;25:77-83.
- Yavas Y, Walton JS. Induction of ovulation in postpartum suckled beef cows: a review. *Theriogenology* 2000;54:1-23.
- Zakhartchenko V, Mueller S, Alberio R, Schernthaner W, Stojkovic M, Wenigerkind H, Wanke R, Lassnig C, Mueller M, Wolf E, Brem G. Nuclear transfer in cattle with non-transfected and transfected fetal or cloned transgenic fetal and postnatal fibroblasts. *Mol Reprod Dev* 2001;60:362-9.
- Zavos PM, Illmensee K. Possible therapy of male infertility by reproductive cloning: one cloned human 4-cell embryo. *Arch Androl* 2006;52:243-54.
- Zhou H, Guo Z. Heterogeneous nuclear transfer embryos reconstructed by bovine oocytes and camel (*Camelus bactrianus*) skin fibroblast and their subsequent development. *In Vitro Cell Dev Biol Anim* 2006;42:16-9.