Advancements in Reproductive Technology in Cattle

D.C. Faber and L.B. Ferré
Trans Ova Genetics, 2938 380th Street, Sioux Center, IA 51250-7075

Abstract

Animal Biotechnology represents an expanding collection of rapidly developing disciplines in science and information technologies. The bovine provides many opportunities to utilize these disciplines and evolving competencies.

Commercialization of biotechnology in cattle is presently taking two pathways. The first application involves the use of animals for biomedical purposes. Very few companies have developed all of the core competencies and intellectual properties to complete the bridge from lab bench to product. The second pathway of application is for the production of animals used for food and fiber.

Artificial insemination, embryo transfer, *in vitro* fertilization, cloning, transgenics, and genomics all are components of the tool box for present and future applications. Individually, these are powerful tools capable of providing significant improvements in productivity. Combinations of these technologies coupled with information systems and data analysis, will provide even more significant changes in the next decade.

Any strategies for the commercial application of animal biotechnology must include a careful review of regulatory and social concerns. Careful review of industry infrastructure is also important. Our colleagues in plant biotechnology have helped highlight some of these pitfalls and provide us with a retrospective review.

In summary, today we have core competencies which provide a wealth of opportunities for the members of society, commercial companies, and cattle producers. Successful commercialization will benefit all of the above stakeholders, and provide a safe and efficient supply of food and pharmaceuticals.

Introduction

Reproductive technology on cattle has made significant strides over the past fifty years. This is a continuum which began with artificial insemination. The utilization of AI was greatly enhanced with cryopreservation of semen and the ability to synchronize estrus by utilizing prostaglandins. The beef and dairy industry has focused on developing elite sires and selection through pregnancy testing.

Genetic progress was further enhanced via embryo transfer technology. Non-surgical collection and transfer, cryopreservation of embryos, improved synchronization methods, and "direct transfer" embryos have improved efficiency, decreased costs, and increased the utilization of embryo transfer by both beef and dairy producers.

The continuum of reproductive technology continues with techniques such as *in vitro* fertilization, separated semen, and nuclear transfer or cloning. Each of these areas will be discussed in greater depth in this paper.

In Vitro Fertilization

By the middle of the 1990's, several commercial IVF laboratories were developed in the United States, Canada and Europe (mainly in Germany, Italy, France and Holland). Years later, they were accompanied by other laboratories in South America (i.e. Brazil and Argentina) and Oceania (i.e. Australia and New Zealand). The adoption of the transvaginal ovum pick-up guided by ultrasonography (OPU), facilitated IVF use in live females (11). The initial purpose of commercial IVF was to obtain viable embryos from females that may not be able to produce progeny through conventional techniques. At present, IVF is a complement to an ET program. Its application could be for females that will not respond to superstimulatory treatments, fail to produce transferable embryos, or possess abnormalities in their reproductive tracts (i.e. ovarian adhesions or blocked fallopian tubes). IVF is also used for females that are terminal (age, accident, disease, etc.), or that are pregnant heifers and cows during the first trimester of gestation, and for heifers and cows with and without calf during the first one, two or three months after calving (post-partum period). It also has applications for normal cyclic heifers and cows, and pre-puberal calves.

IVF allows an improvement in efficiency of utilization of sperm. While Intracytoplasmic Sperm Injection (ICSI) has not been widely implemented in commercial bovine IVF programs, IVF still provides opportunities to use relatively low numbers of sperm to produce viable embryos. This allows for the utilization of high value semen and may provide significant opportunities when coupled with gender separated semen.

Commercial and research centers have used OPU-IVF in diverse categories of females (pre-puberal calves, heifers, cows), age (pre-puberal, post-puberal, aged cows), breeds, reproductive status (cyclic, pregnant, post-partum), aspiration frequency (once weekly, twice weekly, twice per month), use of hormones (FSH, rBST) and IVF protocols (co-culture BRL cells, chemically defined media, serum) with different degree of success (4, 5, 6, 16, 23, 26, 30, 31, 33, 42, 43, 53, 67, 74, 77). Overall results with problem cows are presented in Table 1. A summary of results with and without

superstimulation is presented in Table 2. Oocyte quality aspirated is presented in Table 3, and breed performance is presented in Table 4. Data was compared by "T" Student and Chi-square analysis. During the period from 1992 to 2000, a TCM-199 and then Menezo B2 with BRL cells co-culture system (with 10% FCS) was used to produce embryos. At the beginning of 2001, the culture system was changed to SOF citrate semi-defined culture media with 5% FCS (36) to avoid or diminish the risk of large syndrome calves. In the SOF system, the petri dish is not observed until day 6.5 of culture and the incubator atmosphere condition is 5% O_2, 6% CO_2 and 89% N_2 with high humidity.

All of these embryos were transferred fresh due to the poor results obtained with frozen *in vitro* embryos. This higher sensibility (48, 59, 66, 71) would be due to the culture conditions or fertilization protocol and would produce modifications in the *in vitro* embryo (13, 25, 29, 37, 38, 52, 57, 68, 70, 76, 82, 85, 86, 88, 102).

Table 1. Overall OPU-IVF results with problem cows.

Years	No. Donors	FSH Treatment	OPU Sessions	Oocytes	Oocytes/ Session	Embryos/ Session	Embryos (%)	Pregnancy Rates (%)
1992	47	-	331	1769	5.34	0.98	323 (18.3)	117 (36.2)
	4	+	4	22	5.50	1.75	7 (31.8)	3 (42.9)
1993	152	-	795	5775	7.26	1.20	952 (16.5)	414 (43.5)
	48	+	75	738	9.84	1.53	115 (15.8)	56 (48.7)
1994	153	-	846	7238	8.56	1.37	1162 (16.0)	591 (50.9)
	89	+	155	2185	14.10	2.01	312 (14.3)	182 (58.3)
1995	160	-	853	5769	6.76	0.70	595 (10.3)	326 (54.8)
	173	+	569	7544	13.26	1.27	721 (9.6)	390 (54.1)
1996	107	-	595	4010	6.74	1.01	603 (15.0)	294 (48.8)
	111	+	315	3599	11.43	1.45	457 (12.7)	249 (54.5)
1997	72	-	375	2189	5.84	1.15	430 (19.6)	175 (40.7)
	48	+	80	773	9.66	2.83	226 (29.2)	105 (46.5)
1998	52	-	344	1869	5.43	0.98	338 (18.1)	139 (41.1)
	40	+	65	678	10.43	2.46	160 (23.6)	80 (50.0)
1999	62	-	376	1704	4.53	0.86	322 (18.9)	157 (48.8)
	43	+	68	615	9.04	2.12	144 (23.4)	77 (53.5)
2000	45	-	222	881	3.97	0.65	144 (16.3)	65 (45.1)
	51	+	103	878	8.52	2.11	217 (24.7)	111 (51.1)
2001	37	-	187	829	4.43	0.65	121 (14.6)	49 (40.5)
	37	+	69	509	7.38	1.55	107 (21.0)	40 (37.4)
2002	36	-	151	699	4.63	0.99	150 (21.5)	44 (29.3)
	17	+	28	156	5.57	1.50	42 (27.0)	16 (38.1)
Total	1584		6606	50429	7.63	1.16	7648 (15.2)	3680 (48.1)

Table 2. Summary OPU-IVF results with problem cows with and without superstimulation.

Treatment	No. Donors	OPU Sessions	Oocytes	Oocytes/ Session	Embryos/ Session	Embryos (%)	Pregnancy Rates (%)
No-FSH	923	5075	32732	6.4	1.0	5140 (15.7)[a]	2371 (46.1)[a]
FSH	661	1531	17697	11.6	1.6	2508 (14.2)[b]	1309 (52.2)[b]

[a,b]Values with different superscripts in the same column differ ($P < 0.05$).

Table 3. Oocyte quality in OPU-IVF problem cows.

Treatment	Oocyte quality. No. (%)				
	A	B	C	D	E
No-FSH	295 (7.75)[a]	643 (17.0)[a]	1947 (51.1)[a]	601 (15.8)[a]	322 (8.4)[a]
FSH	360 (17.0)[b]	495 (23.3)[b]	885 (41.7)[b]	254 (12.0)[b]	128 (6.0)[b]

Grade A: many layers of cumulus cells, B: 3 to 4 layers of cumulus, C: 1 to 2 layers of cumulus, D: denuded, E: expanded cumulus.
[a,b]Values with different superscripts in the same column differ ($P < 0.05$).

Many factors influence the efficiency of IVF technology, but the main factors could be the status of the donor, oocyte quality and the technique used to culture the embryos from the zygote to blastocyst stage. Although there has been enormous progress in IVF since the beginning of its implementation in animal breeding, particular areas need to improve. These include improving the freezability of oocytes and embryos, minimizing the culture effect on calf size, improving oocyte quality, successful use of sexed semen, ICSI and preantral follicle culture.

Commercial Semen, Embryo and Fetus Sexing

The possibility of sex pre-selection always had sparked great interest among livestock producers and the cattle industry. Sexed semen could contribute to increasing the profitability desired by the dairy and beef industries through desired sex offspring production, thus taking advantage of specific marketing or commercial production demands (like herd replacement, herd expansion, or increasing the male sales to slaughter). The clearest examples could be the production of females for dairy or replacement and males for meat production. Other applications would be for cattle breeders and AI semen companies to test elite bulls on a small number of females (35). Several methods have been used to reach this objective which is presented in Table 4.The result and accuracy of most of these techniques are satisfactory, and according to the established objective, it is convenient to opt for a pre-selection (sexing semen or embryo) as opposed to post-selection (fetus) methods of sex. In the case of sexing embryos, the only method used routinely on a commercial scale is to biopsy embryos and amplify Y-chromosome-specific DNA using polymerase chain reaction. This method is effective for more than 90% of embryos and is > 95% accurate (81).

Table 4. Different methods of sexing.

Sexing	Method	References
Semen	DNA content	(3, 34, 40, 62, 79, 87)
Embryo	Biopsy and PCR, fluorescence in situ hybridization,	(9, 51, 78)
Fetus	Ultrasonography at 60-90 days of gestation	(14)

However, determination of embryo sex by PCR is inefficient. All embryos are biopsied, tested, and then approximately 50% of the undesired sex are discarded. Costs of donor board, superovulation and collection have to be carried by a small number of embryos. The determination of fetal gender can be identified at 55 – 90 days of gestation. While this provides management opportunities, it fails to alter the sex ratio unless one elects to terminate unwanted pregnancies. Such methods of altering the resulting sex ratio are both cumbersome and expensive.

A commercial embryo sexing program was initiated at Trans Ova Genetics with AB Technology methodology (Pullman, WA). The procedure takes 5 minutes to perform each embryo biopsy and 2 hours for the PCR process. With some embryos, primers Ampli-Y (Finnzymes, Finland) were used. The results between the years 1994 and 2002 are presented in Table 5.

Table 5. Trans Ova Genetics results of sexed embryos using embryo biopsy and PCR technique.

		Fresh	Frozen
No. biopsies		716	144
No. indeterminate tubes following PCR (%)	AB Technology primers	57/665 (8.6)	4 (2.8)
	Finnzymes primers	14/51 (27.4)	
No. transfers		389	67
Pregnancy rates (%)		184 (47.3)[a]	20 (29.8)[b]
Sex confirmations by ultrasound - Accuracy (%)	AB Technology primers	30/33 (91)	
	Finnzymes primers	10/15 (66.6)	

[a,b]Values with different superscripts in the same row differ ($P < 0.05$).

At present, one company (XY, Inc., Fort Collins, Colorado) has technology that has been documented to be successful in the separation of X and Y bearing spermatozoa. The sex pre-selection is based on identifying differences in DNA content between X- and Y-bearing sperm. The X chromosome contains about 4% more DNA in cattle and horses than the Y chromosome. The high-speed cell sorting machine employed can separate 6 million X or Y sperm per hour with 90% purity (40).

Sexed semen appears to be an interesting tool that can be implemented in AI, ET, and IVF programs. The results published currently indicate that AI of heifers results in a similar pregnancy rate (around 50%) between low (1-1.5 $x10^6$ sperm) and high dose (3 $x10^6$ sperm) units of frozen sexed semen deposited in the uterine body (79). Similar results were obtained by Goyaike in Argentina (10). In IVF, it is feasible to reach 18% - 26% of embryo development with frozen sexed semen (54, 56).

The commercial application for Artificial Insemination will depend on separation efficiency (cost), and resulting pregnancy rates. This application has the potential to revolutionize cattle breeding strategies in both beef and dairy. Presently, the efficiencies obtained with separated semen are on the verge of commercial application. At first glance, application of sexed semen technology would appear to fit well when coupled with embryo transfer programs. However, super-stimulated beef and dairy donors may fail to transport sperm efficiently to the site of fertilization in the oviduct. (76) This may delay the widespread application of sexed semen in commercial embryo transfer programs.

The commercial application of separated semen coupled with IVF appears to provide the most logical and first commercial application for separated semen. The inherent cost of separated sperm fits well into commercial IVF schemes, where small quantities of sperm are needed to achieve fertilization. The potential to separate frozen-thawed sperm would provide additional advantages to applications with IVF production of embryos.

Trans Ova Genetics is currently harvesting ovaries from Holstein cows. Oocytes are recovered and fertilized with X bearing, or female, sperm. The resulting embryos are then implanted into dairy cows. Improved conception rates, sustainable cross breeding, and approximately 90% heifer progeny are all potential value propositions.

Somatic Cell Cloning

Cloning is the colloquial term used to describe the process of somatic cell nuclear transfer (SCNT), and falls on a continuum of assisted reproductive technologies (ARTs) currently used in agriculture.

The most acclaimed example of animal cloning is, of course, the report by Wilmut et al. in 1997 (97), the first to demonstrate that cloning of adult mammals was possible. While animal cloning by nuclear transplantation is inefficient, the fact that cloned animals representing various species have not been produced by a number of different laboratory groups has spawned great interest in reproducing (cloning) specific genotypes (1, 12, 84, 94). Economics and genetic improvement are not always the sole purpose of cloning. Cattle may be cloned for show purposes, "insurance" purposes, and sentimental value.

Presently cloning applications are limited to high value bio-medical or seedstock production. In the future, cloning technology could play an important role in commercial beef and dairy production. Cloning could speed the dissemination of genetic progress generated in the nucleus population(s) to the commercial populations. Embryo cloning could have a large impact in dairy cattle. Instead of inseminating commercial cows with high-merit semen, embryos of the best available clone in the nucleus population could be used. Having been selected as the best of the clones being produced in the nucleus, the genetic merit will be greater than the average merit of the commercial population (14, 16, 17, 64, 100). Clones enable widespread exploitation of non-additive genetic effects, dominance and epistasis, both within and between breeds.

Using cloning in commercial farms to produce replacement animals reduces the percentage of cows that are required to produce replacement heifers. This advantage could also be captured by the use of sexed semen or embryos. The use of sexed semen or embryos also offers an opportunity for the farmer to reduce calving difficulties and thereby improve animal welfare. The remainder could be used for the production of animals for beef production or to gestate nucleus herd embryos (99).

The efficiency of cloning cattle by nuclear transplantation is extremely variable (94). The sources of variation which likely affect the outcome of nuclear transplantation include not only genotype, but the type of nuclei donor cell utilized, treatment of donor cells prior to nuclear transfer, and source of recipient ova. Dermal fibroblasts are the most common source for donor cells. These cells are easily harvested from either sex and cultured using standard tissue culture conditions.

In our facility, we have worked with various laboratories. In addition, cloning attempts have been made from unmodified fetal cells, genetically manipulated cells, second generation clonal lines, and unmodified adult cells. Attempts have also been made with endangered species where donor cells are fused with bovine cytoplasts.

Significant percentages of calves die within one week of birth due to various health problems. In our facility, 24% of cloned calves born failed to survive the first week. The leading causes of mortality include respiratory distress, birth defects, non-viable calves, and enteritis (Clostridium sp)

The commercial application of cloning in cattle is dependent on societal and regulatory acceptance, coupled with favorable cost vs. return economics.

Economics can be evaluated with the following parameters:

a. Cost of implementation
b. Genetic gain / Improved productivity
c. Uniformity of clones
d. Cost of cloning.

Certainly the primary driver in all assisted reproductive technology is economic return versus cost. With the extreme variability and relative inefficiency reported with cloning, its primary application was for bio-medical applications and for the elite agricultural animals. Bovine cloning holds great promise to be used in wide scale applications. This stems from the fact that cloned embryos can be made efficiently, and acceptable pregnancy rates are already being achieved. Pregnancy maintenance and calf livability are the major hurdles to widespread application of the technology (2, 27, 98).

Potential reduction of the cost in producing cloned animals can be divided into three primary areas: embryo production, gestation, and improved calf survival.

Cloned embryo production has essentially three cost drivers: output of volume produced, embryo development efficiency, and the number or percentage of embryos transferred.

Cloning Laboratories are expensive to equip and operate. Calculated cost can range from $100 to $200 per blastocyst. Production must forecast a conservative rate of development to assure an adequate number of embryos are available to implant into available recipients. This means that excess embryos are often created and wasted from days or cell lines where development rates are high.

The best way to reduce embryo cost per pregnancy would be to transfer one embryo per recipient. This would result in a 50% reduction in embryo cost, assuming comparable calving rates. The second best way to reduce costs would be to have consistent development so that output per fixed cost could be maximized. This could also result in a 50% reduction in cost. The third would be to improve blastocyst development rates. This potential would represent only a 5 to 10% reduction in cost.

Maintenance of large open recipient populations, embryo transfer, gestation, and calving contribute the majority of cost in producing cloned cattle. The majority of clones are presently harvested by caesarian section due to calf value and LOS.

While input costs will vary widely depending on geography, and resources, the basic physiology of estrus synchronization and gestation are relatively consistent around the world (Table 6). Table 7 is included to reveal calving rate as the primary driver in reducing the cost of producing cloned animals. Improved neonatal survival represents the second largest opportunity. Reduction in the cost of cloned embryo(s) that are implanted into recipients is also important.

Combinations of the above could significantly reduce the cost of clones, and allow for significant market penetration (Fig. 1).

Table 6. Cloning cost inputs.

Recipient Needs	
Pre Implant days	45
Implant to Preg. Check, days	45
Post Implant/calving, days	45
Total Head Days	135
Recipient Head Day Cost/day	$3.25
Gestation cost/day	$3.25
Total Head Day Cost	$438.75
Embryo Implant Cost	$50.00
Health Tests & Vaccines	$50.00
Synchronization	$14.00
Recipient Interest & Depreciation	$75.00
	$627.75
Cloned Embryo Cost	$102.58
C-Section cost % Recipient cow depreciation	$400.00

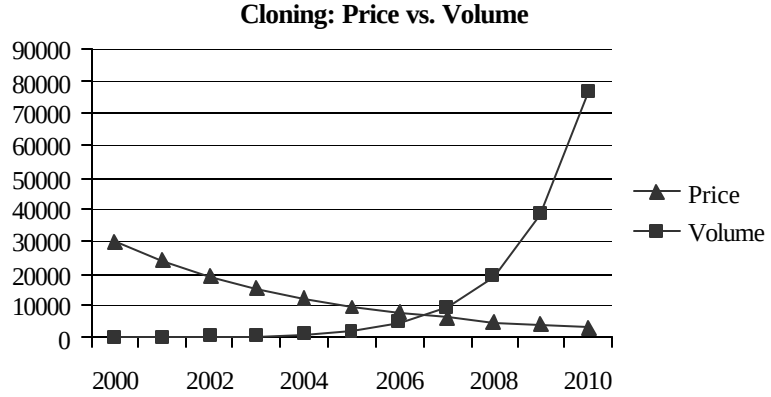

FIG. 1. Cost and marketing estimates of cloned cattle. (Trans Ova Genetics, 2002).

Table 7. Cost of producing a cloned calf.

Assumptions								
# embryos implanted/recipient	2							
40 days pregnancy rate		30%	35%	40%	45%	50%	55%	60%
% of 40 day pregnancies carried to term		25%	30%	35%	40%	45%	50%	55%
% calving rate		7.5%	10.5%	14%	18%	22.5%	27.5%	33%
% survival rate		50%	55%	60%	65%	70%	75%	80%
% live calves		3.75%	5.78%	8.4%	11.7%	15.75%	20.63%	24.6%
Cost per calf produced								
Recipient costs	$628	$16,740	$10,870	$7,473	$5,365	$3,986	$3,044	$2,378
Cloned embryo cost (2 embryos)	$205	$5,471	$3,552	$2,442	$1,753	$1,303	$995	$777
Gestation cost of pregnancy loss	$390	$1,560	$1,300	$1,114	$975	$867	$780	$709
Gestation cost of live born calves	$780	$1,560	$1,418	$1,300	$1,200	$1,114	$1,040	$975
C-section cost & recipient cow depreciation	$400	$800	$727	$667	$615	$571	$533	$500
Total Cost Cloned Calf		$26,131	$17,868	$12,996	$9,909	$7,841	$6,392	$5,339

Genetic Gain /Improved Productivity

Breed improvement is accomplished through two objectives. The first is the generation of genetic improvement by selecting animals based on their estimated breeding value (EBV). In most livestock improvement schemes, selection is based on breeding values that are estimated using "best linear unbiased prediction" (BLUP). BLUP utilizes the phenotypic information on all traits and relatives to predict the EBV.

Secondly, genetic superiority must be distributed from the nucleus to the commercial population. The nucleus animals usually represent a small fraction of the population. In pigs and poultry, closed nucleus schemes are generally used in which nucleus animals are kept on a small number of farms and only animals from these nucleus farms contribute to genetic improvement of the nucleus population. In beef and dairy cattle, nucleus animals are identified from open seed stock and commercial herds. These animals are used for artificial insemination and MOET programs for both current commercial production and generation of the next nucleus animals.

By the creation of large numbers of identical individuals, embryo cloning has the potential to greatly increase accuracy of selection. Each clonal line can be evaluated on the average phenotypic performance of many copies of itself. Cloning offers the opportunity to test candidates under different environments, to subject them to a disease challenge that would not ordinarily be applied in other breeding schemes, or to measure carcass and meat quality traits directly on selection candidates. Testing clones instead of half-sibs or full-sibs provides more information in these cases because the clones share Mendelian sampling (dominance and epistasis) with the selection candidate. Clones can be tested under various conditions. Conversely, modern agricultural producers would have the opportunity to manage, refine, and optimize the environment for specific clonal lines. This may allow for more uniformity than predicted by heritability of a particular trait (90). The use of crossbred clones in dairy cattle offers a unique opportunity to protect the breeding stock of individual companies, while producing the opportunity for a sustainable crossbred dairy cow strategy.

A key element in the dissemination of genetic material is the genetic lag, i.e. the difference in genetic merit between the nucleus and the commercial populations. Cloning can be used to improve the dissemination of genetic gain generated in the nucleus population to the commercial population. Van Vleck (90) and Villanueva and Simm (91) described that cloning could lead to the removal of one or two tiers in the pig breeding pyramid. van Arendonk and Bijma (88), for example, concluded that the main advantage of cloning is faster dissemination of superior genetics to commercial farmers using cloned embryos from desirable genotypes. In beef and dairy cattle, elite seedstock genetics could be rapidly distributed resulting in a short term genetic gain. Since beef and dairy cattle breeding has been an open system where genetics are sampled and selected from an open population, this "quick" gain could also improve the availability and accuracy of future selection candidates.

Uniformity of clones

Ideally, cloning individuals with outstanding performance would guarantee that all mates of the clone are genetically superior to other animals and that the clones would be uniform and predictable.

The usual, but perhaps incorrect, perception would be that an animal with a high record or other desirable attributes could be safely selected to be the origin of a family of clone mates. There is no sure way to identify superior animals except by testing many clone mates or by testing multiple progeny of a bull. The situation would also change when molecular information is available to assist in prediction of the phenotype.

Phenotype (P) equals Genotype (G) plus Environment (E). P = G + E.

For most traits, additive genetic variance accounts for 10 – 50% of total variance, a fraction denoted as heritability (h^2). (Table 8 and 9) Only if heritability is 100% will clone mates have complete uniformity. For example, with h^2 of .50, which is larger than for most traits, this measure of uniformity is only 30% better than for unrelated animals. If heritability is 25%, then the standard deviation among clones would be 87% of that of uncloned animals (90).

Table 8. Heritability estimates for Holsteins from the USDA-AIPL website (www.aipl.arsusda.gov).

Trait	*Heritability %*	*Trait*	*Heritability %*
Milk Yield	30	Feet & Legs	15
Productive Life	8.5	Daughter Pregnancy Rate	4
Somatic Cell Score	10	Direct Calving Ease	9
Size	40	Maternal Calving Ease	6
Udder	27		

Table 9. Heritability estimates for Angus cattle from the American Angus Association website (www.angus.org).

Trait	*Heritability %*	*Trait*	*Heritability %*
Birth weight	33	Scanning weight	57
Weaning direct	20	Intra muscular fat	31
Weaning (milk)	14	Rib eye Area	38
Post weaning gain	20	12-13th Rib Fat Thickness	39
Yearling height	50	Retail Product	39
Yearling weight	37	Scrotal circumference	43
Mature Height	87	Mature Weight	53
Carcass weight	30	% Retail product	25
Rib eye area	28	Marbling Score	36
Fat Thickness	25		

Currently, most cloned embryos are gestated by non-lactating beef cows. Low conception and calving rates coupled with dystocia associated with Large Offspring Syndrome (LOS), prohibit the use of lactating dairy cows as recipients (2).

However, cloning a genetically superior animal also could capture optimum dominance and epistatic genetic effects that are otherwise difficult to select for. Capturing this effect could allow producers to manage the environment to maximize agro-economic traits of the clones.

Societal Values and Regulatory Impact on Commercialization

Historically, most technology introductions have been met with some skepticism. The birth of Dolly has tended to polarize public opinion on the application of biotechnology in agriculture. In agriculture, Artificial Insemination was greeted with questions and concerns about the normality of the resulting calves. The birth of the first human baby by IVF created a lot of public debate on the morality and ethics of technology. Over twenty years have passed and 100,000 assisted reproductive technology babies have now been born.

Regarding agriculture, the ultimate test for most consumers is the level of assurance that can be credibly provided that the application of these technologies does not inversely impact food safety. These risks may be real or perceived. Our fellow researchers in transgenic plants have helped illustrate the consumer concerns.

Society is placing animal welfare as an increasingly important part of food production. The public and regulatory officials are increasingly seeking assurances and demands to

ensure that advances in biotechnology will not result in an increase in animal suffering (22, Table 8).

Environmental concerns included numbers or population density of specific genotypes, and the lack of genetic diversity. In addition, some species such as transgenic salmon must provide assurances that the escape of transgenic salmon will not upset indigenous feral populations and ecosystems. Livestock have an advantage in containment and trace ability when compared to plants and species such as fish. However, in many countries inadequate systems for cattle identification and traceability are in place to provide for conception to consumer tracking of product.

In the United States, the FDA commissioned the National Academy of Sciences (NAS) to identify and prioritize any safety concerns that bioengineered and cloned animals might present to food, animals and the environment.

After consulting with pioneers in the field of cloning and holding a public workshop, the NAS published its report entitled "Animal Biotechnology: Science-Based Concerns" in August 2002. According to the report, "there is no current evidence that food products derived from adult somatic cell clones or their progeny present a food safety concern." The report recommends collecting additional information about food composition to confirm that these food products are, in fact, safe. The NAS's job was to identify the potential risks of cloning. Now the FDA is studying those risks to determine how to manage them. The FDA is developing risk assessments describing the potential risks, if any, of consuming food products from animal clones and their offspring, and describing health risks to animal clones and their offspring. The FDA will use these assessments to develop an appropriate science-based regulatory approach, in the form of policy or guidance for industry, to manage any food and animal health risks.

Summary

Commercialization of bovine reproductive technology for food and bio-medical applications represents significant opportunities. Artificial insemination, embryo transfer, *in vitro* fertilization, cloning, transgenics, and genomics all are components of the tool box for present and future applications. Individually, these are powerful tools capable of providing significant improvements. However the greatest gain will come from the application of combinations of these technologies.

References

1. Baguisi A, Behboodi E, Melican DT, Pollock JS, Destrempes MM, Cammuso C, Williams JL, Nims SD, Meade HM, Godke RA, Gavin WG, Overstrom EW, Echelard Y. Production of goats by somatic cell nuclear transfer. Nat Biotechnol 1999; 17:456-461
2. Behboodi E, Anderson GB, BonDurant RH, Cargill SL, Kreuscher BR, Medrano JF, Murray JD. Birth of large calves that developed from *in vitro*-derived bovine embryos. Theriogenology 1995; 44:227-232.
3. Blecher SR, Howie R, Li S, Detmar J, Blahut, LM. A new approach to immunological sexing of sperm. Theriogenology 1999; 52:1309-1321.
4. Blondin P, Bousquet D, Twagiramungu H, Barnes F, Sirad MA. Manipulation of follicular development to produce developmentally competent bovine oocytes. Biol Reprod 2002; 66:38-43.
5. Bols PEJ. Transvaginal ovum pick-up in the cow: Technical and biological modifications. PhD Thesis, Faculty of Veterinary Medicine, University of Gent, 1997, Salisburylaan 133, 9820 Merelbeke, Belgium.
6. Bousquet D, Twagiramungu H, Morin N, Brisson C, Carboneau G, Durocher J. *In vitro* embryo production in the cow: An effective alternative to the conventional embryo production approach. Theriogenology 1999; 51: 59-70.
7. Brackett BG, Bousquet D, Boice ML, Donawick WJ, Evans JF, Dressel MA. Normal development following *in vitro* fertilization in the cow. Biol Reprod 1982; 27:147-158.
8. Brackett BG. A review of *in vitro* fertilization. Theriogenology 1983; 19:1-15.
9. Bredbacka P, Kankaanpää A, Peippo J. PCR-Sexing of bovine embryos: A simplified protocol. Theriogenology 1995; 44:167-176.
10. Brogliatti G, Cerrate H, Caballero J, Cattaneo L, Cantarelli A, Feula P, Dalla Lasta M, Ferré L, Panarace M, Medina M. Pregnancy rates and first born calves by artificial insemination using sexed semen in Argentina. Theriogenology 2002; 57:369.
11. Callensen H, Greve T, Christensen F. Ultrasonically guided aspiration of bovine follicular oocytes. Theriogenology 1987; 27:217.
12. Campbell KH, McWhir J, Ritchie WA, Wilmut I. Sheep cloned by nuclear transfer from a cultured cell line. Nature 1996; 380:64-66.
13. Carolan C, Lonergan P, Van Langendonckt A, Mermillod P. Factors affecting bovine embryo development in synthetic oviduct fluid following oocyte maturation and fertilization *in vitro*. Theriogenology 1995; 43:1115-1128.
14. Colleau JJ. Combining use of embryo sexing and cloning within mixed MOETS for selection of dairy cattle. Genet. Sel. Evol. 1992; 24:345-361.
15. Curran S, Ginther OJ. Ultrasonic determination of fetal gender in horses and cattle under farm conditions. Theriogenology 1991; 36:809-814.
16. De Boer IJM. The use of clones in dairy cattle breeding. Ph.D. dissertation. Wageningen Agricultural University. The Netherlands. 1994.

17. De Boer IJM, van Arendonk JAM. Market share for semen and cloned embryos in dairy herds. J. Dairy Sci. 1994; 77:3691-3703.
18. De Boer IJM, Meuwissen THE, van Arendonk JAM. Combining the genetic and clonal responses in a closed dairy cattle nucleus scheme. Anim. Prod. 1994; 59:345-358.
19. Eikelmann E, Frank KU, Schindler L, Niemann H. Repeated ultrasound-guided follicular aspiration in pregnant heifers and cows. Theriogenology 2000; 53:351.
20. Elsden RP, Hasler JF, Seidel Jr. GE. Non surgical recovery of bovine eggs. Theriogenology 1976; 6:523-532.
21. Eppig JJ, Schroeder AC. Culture systems for mammalian oocyte development: Progress and prospects. Theriogenology 1986; 25:97-106.
22. Evans BR. The prospects for international regulatory interventions in embryo transfer and reproductive technologies in the next century. Theriogenology 1999; 51:71-80.
23. Ferré LB, Dalla Lasta M, Medina M, Brogliatti G. *In vitro* embryo production and pregnancy rates from problem, pregnant and cyclic cows by transvaginal ovum pick-up. Theriogenology 2002; 57:664.
24. Fukuda Y, Ichikawa M, Naito K, Toyoda Y. Birth of normal calves resulting from bovine oocytes matured, fertilized, and cultured with cumulus cells *in vitro* up to the blastocyst stage. Biol Reprod 1990; 42:114-119.
25. Fukui Y, McGowan LT, James RW, Pugh PA, Tervit HR. Factors affecting the in-vitro development to blastocysts of bovine oocytes matured and fertilized *in vitro*. J Reprod Fert 1991; 92:125-131.
26. Galli C, Lazzari G. Practical aspects of IVM/IVF in cattle. Anim Reprod Sci 1996; 42:371-379
27. Garry FB, Adams R, McCann JP, Odde KG. Postnatal characteristics of calves produced by nuclear transfer cloning. Theriogenology 1996; 45:141-152.
28. Goto K, Kajihara Y, Kosaka S, Koba M, Nakanishi Y, Ogawa K. Pregnancies after co-culture of cumulus cells with bovine embryos derived from in-vitro fertilization of in-vitro matured follicular oocytes. J Reprod Fert 1988; 83:753-758.
29. Greve T, Avery B, Callesen, H. Viablity of *in vivo* and *in vitro* produced bovine embryos. Reprod Domestic Anim 1993; 28:164-169.
30. Guyader Joly C, Ponchon S, Thuard JM, Durand M, Nibart M, Marguant-LeGuienne B, Humblot P. Effects of superovulation on repeated ultrasound guided oocytes collection and *in vitro* embryo production in pregnant heifers. Theriogenology 1997; 47:157.
31. Guyader-Joly C, Durand M, Morel A, Ponchon S, Marquant-Leguienne B, Guérin B, Humblot P. Sources of variation of blastocyst production in a commercial ovum pick-up, *in vitro* embryo production program in dairy cattle. Theriogenology 2000; 53:355.
32. Hammer RE, Pursel VG, Rexroad CE Jr, Wall RJ, Bolt DJ, Ebert KM, Palmiter RD, Brinster RL. Production of transgenic rabbits, sheep and pigs by microinjection. Nature 1985; 315:680-683.
33. Hasler JF, Henderson WB, Hurtgen PJ, Jin ZQ, McCauley AD, Mower SA, Neely B, Shuey LS, Stokes JE, Trimmer SA. Production, freezing and transfer of bovine IVF embryos and subsequent calving results. Theriogenology 1995; 43:141-152.
34. Hendriksen PJM. Do X and Y spermatozoa differ in proteins?. Theriogenology 1999; 52:1295-1307.
35. Hohenboken WD. Applications of sexed semen in cattle production. Theriogenology 1999; 52:1421-1433.
36. Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H. High Bovine Blastocyst Development in a Static *in Vitro* Production System Using SOFaa Medium Supplemented with Sodium Citrate and Myo-Inositol With or Without Serum-Proteins. Theriogenology 1999; 52:683-700.
37. Holm P, Callensen H. *In vivo* versus *in vitro* produced bovine ova: similarities and differences relevant for practical application. Reprod Nutr Development 1998; 38:579-594.
38. Hyttel P, Callesen H, Greve T. A comparative ultrastructural study of *in vivo* versus *in vitro* fertilization of bovine oocytes. Anat Embryol 1989; 179:435-442.
39. Hyttinen JM, Peura T, Tolvanen M, Aalto J, Alhonen L, Sinervita R, Halmekyto M, Myohanen S, Janne J. Generation of transgenic cattle from transgene analysed and sexed embryos produced *in vitro*. Biotechnology (NY) 1994; 12:606-608.
40. Johnson LA, Welch GR. Sex preselection: High-speed flow cytometric sorting of X and Y sperm for maximum efficiency. Theriogenology 1999; 52:1323-1341.
41. Kane MT. Culture media and culture of early embryos. Theriogenology 1987; 27:49-57.
42. Kruip ThAM, Boni R, Wurth YA, Roelofsen MWM, Pieterse MC. Potential use of ovum pick-up for embryo production and breeding in cattle. Theriogenology 1994; 42:675-684.
43. Larsson B. *In vitro* fertilization using different sources of oocytes. Acta Agric Scand Sect A, Animal Sci 1998; 29:30-36.
44. Lehn-Jensen H. Deep freezing of cattle embryos. 10th Inter Cong Anim Reprod and AI, Illinois, USA 1984; 4:1-12.
45. Leibfried L, First NL. Characterization of bovine follicular oocytes and their ability to mature *in vitro*. J Anim Sci 1979; 48:76-86.
46. Leibfried-Rutledge ML, Critser ES, Eyestone WH, Northey DL, First, NL. Development potential of bovine oocytes matured *in vitro* or *in vivo*. Biol Reprod 1987; 36:376-383.

47. Leibfried-Rutledge ML, Critser ES, Parrish JJ, First NL. *In vitro* maturation and fertilization of bovine oocytes. Theriogenology 1989; 31:61-74.
48. Leibo SP, Loskutoff, NM. Cryobiology of *in vitro*-derived bovine embryos. Theriogenology 1993; 39:81-94.
49. Leibo SP. A one-step method for direct nonsurgical transfer of frozen-thawed bovine embryos. Theriogenology 1984; 21:767-790.
50. Leibo SP. Cryopreservation of embryos. 11th Inter Cong Anim Reprod and AI, Dublin, Ireland 1988; 5:370-377.
51. Lonergan P, Gutierrez-Adan A, Rizos D, Ward FA, Boland MP, Pintado B, de la Fuente J. Effect of the *in vitro* culture system on the kinetics of development and sex ratio of bovine blastocysts. Theriogenology 2001; 55:430.
52. Lonergan P, Rizos D, Ward F, Boland MP. Factors influencing oocyte and embryo quality in cattle. Reprod Nutr Dev 2001; 41:427-437.
53. Looney CR, Lindsay BR, Gonseth, CL, Johnson DL. Commercial aspects of oocyte retrieval and *in vitro* fertilization (IVF) for embryo production in problem cows. Theriogenology 1994; 41:67-72.
54. Lu KH, Cran DG, Seidel Jr. GE. *In vitro* fertilization with flow-cytometrically-sorted bovine sperm. Theriogenology 1999; 52:1393-1405.
55. Lu KH, Gordon I, Gallagher M, McGovern H. Pregnancy established in cattle by transfer embryo derived from *in vitro* fertilization of follicular oocytes matured *in vitro*. Vet Rec 1987; 121:159-160.
56. Lu KH, Suh TK, Seidel Jr. GE. *In vitro* fertilization of bovine oocytes with flow-cytometrically sorted and unsorted sperm from different bulls. Theriogenology 2001; 55:431.
57. Massip A, Mermillod P, Dinnyes, A. Morphology and biochemestry of in-vitro produced bovine embryos: implications for their cryopreservation. Human Reproduction 1995; 10:3004-3011.
58. Massip A, Van Der Zwalmen P, Ectors F. Recent progress in cryopreservation of cattle embryos. Theriogenology 1987; 27:69-79.
59. Massip A. Cryopreservation of embryos of farm animals. Reprod Dom Anim 2001; 36:49-55.
60. Maurer RR. Freezing mammalian embryos: A review of the techniques. Theriogenology 1978; 9:45-68.
61. Mazur P. Fundamental aspects of the freezing of cells, with emphasis on mammalian ova and embryos. 9th Inter Cong Anim Reprod and AI 1980; 1:99-114.
62. Morrell JM. Applications of flow cytometry to artificial insemination: a review. Vet Rec 1991; 129:375-378.
63. Nagase H, Graham EF, Niwa T, Yamashita S. Deep freezing bull semen in concentrated pellet-form. 5th Inter Cong Anim Reprod and AI 1964; 6:387-391.
64. Nicholas FW. Genetic improvement through reproductive technology. Anim. Reprod. Sci. 1997; 42:205-214.
65. Niemann H. Cryopreservation of ova and embryos from livestock: current status and research needs. Theriogenology 1991; 35:109-124.
66. Palasz AT, Mapletoft RJ. Cryopreservation of mammalian embryos and oocytes: Recent advances. Biotechnology Advances 1996; 14:127-149.
67. Perez O, Richard R, Green HL, Youngs CR, Godke RA. Ultrasound-guided transvaginal oocyte recovery from FSH-treated post-partum beef cows. Theriogenology 2000; 53:364.
68. Pinyopummintr T, Bavister BD. Development of bovine embryos in a cell-free culture medium: effects of type of serum, timing of its inclusion and heat inactivation. Theriogenology 1994; 41:1241-1249.
69. Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 1949; 164:666-668.
70. Pollard JW, Leibo SP. Chilling sensitivity of mammalian embryos. Theriogenology 1994; 41:101-106.
71. Pollard JW, Leibo SP. Comparative cryobiology of *in vitro* and *in vivo* derived bovine embryos. Theriogenology 1993; 39:287.
72. Prather RS, Barnes FL, Sims MM, Robl JM, Eyestone WH, First NL. Nuclear transplantation in the bovine embryo: assessment of donor nuclei and recipient oocyte. Biol Reprod 1987; 37:859-866.
73. Rall WF. Cryopreservation of oocytes and embryos: methods and implications. Anim Reprod Sci 1992; 28:237-245.
74. Reis A, Staines ME, Watt RG, Dolman DF, McEvoy TG. Embryo production using defined oocyte maturation and zygote culture media following repeated ovum pick-up (OPU) from FSH-stimulated Simmental heifers. Anim Reprod Sci 2002; 72:137-151.
75. Renard JP, Zhou QI, LeBourhis D, Chavatte-Palmer P, Hue I, Heyman Y, Vignon X. Nuclear transfer technologies: Between successes and doubts. Theriogenology 2002; 57:203-222.
76. Rizos D, Ward F, Duffy P, Boland MP, Lonergan P. Consequences of bovine oocyte maturation, fertilization or embryo development *in vitro* versus *in vivo*: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev 2002; 61:234-248.
77. Schmidt M, Greve T, Avery B, Beckers JF, Sulon J, Hansen HB. Pregnancies, calves and calf viability after transfer of *in vitro* produced bovine embryos. Theriogenology 1996; 46:527-539.
78. Seidel GE Jr. Commercializing reproductive biotechnology - the approach used by XY, Inc. Theriogenology 1999; 51:5.

79. Seidel Jr. GE, Johnson LA. Sexing mammalian sperm - Overview. Theriogenology 1999; 52:1267-1272.
80. Seidel Jr. GE, Schenk JL, Herickhoff LA, Doyle SP, Brink Z, Green RD, Cran DG. Insemination of heifers with sexed semen. Theriogenology 1999; 52:1407-1420.
81. Seidel Jr. GE. Sexing mammalian spermatozoa and embryos - State of the art. J Reprod Fert 1999; 54:477-87.
82. Shamsuddin M, Larsson B, Gustafsson H, Gustari S, Bartolome J, Rodriguez-Martinez H. Comparative morphological evaluation of *in vivo* and *in vitro* produced bovine embryos. Proc. 12th Inter Cong Anim Reprod and AI, Hague, The Netherland 1992; 1333-1335.
83. Smith AU, Polge C. Survival of spermatozoa at low temperatures. Nature 1950, 166:669-769.
84. Stice SL, Robl JM, Ponce de Leon FA, Jerry J, Golueke PF, Cibelli JB, Kane JJ. Cloning: new breakthroughs leading to commercial opportunities. Theriogenology 1998; 49:129-138.
85. Suzuki T, Yamamoto M, Ooe M, Nishikata Y, Okamoto K, Tsukihara T. Effect of media on fertilization and development rates of *in vitro* fertilized embryos, and of age and freezing of embryos on pregnancy rates. Theriogenology 1991; 35:278.
86. Trounson A, Pushett D, Maclellan LJ, Lewis I, Gardner KI. Current status of IVM/IVF and embryo culture in humans and farm animals. Theriogenology, 1994; 41:57-66.
87. van Munster EB, Stap J, Hoebe RA, te Meerman GJ, Aten JA. Difference in sperm head volume as a theoretical basis for sorting X and Y bearing spermatozoa: Potentials and limitations. Theriogenology 1999; 52:1281-1293.
88. van Arendonk JAM, Bijma P. Factors affecting commercial application of embryo technologies in dairy cattle in Europe-a modeling approach. Theriogenology 2003; 59:635-649.
89. Van Soom A, de Kruif, A. A comparative study of *in vivo* and *in vitro* derived bovine embryos. Proc. 12th Inter Cong Anim Reprod and AI, Hague, The Netherland 1992; 1363-1365.
90. Van Vleck LD. Implications of cloning for breed improvement strategies. Are traditional methods of animal improvement obsolete? J. Dairy Sci. 1998; 77:111-121.
91. Villaneuva B., Simm G. The use and value of embryo manipulation techniques in animal breeding. Proc. 5th World Congr. Genet. Appl. Livest. Prod. 1994; 20:200-207.
92. Voelkel SA, Hu YX. Use of ethylene glycol as a cryoprotectant for bovine embryos allowing direct transfer of frozen-thawed embryos to recipient females. Theriogenology 1992; 37:687-697.
93. Wall RJ. New gene transfer methods. Theriogenology 2002; 57:189-201.
94. Wells DN, Misica PM, Tervit HR. Production of cloned calves following nuclear transfer with cultured adult mural granulose cells. Biol Reprod 1999; 60:996-100.
95. Westhusin ME, Long CR, Shin T, Hill JR, Looney CR, Pryor JH, Piedrahita JA. Cloning to reproduce desired genotypes. Theriogenology 2001; 55:35-49.
96. Wilmut I, Rowson LEA. Experiments on the low-temperature preservation of cow embryos. Vet Rec 1973; 92:686-690.
97. Wilmut I, Schniecke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult mammalian cells. Nature 1997; 385:810-813.
98. Wilson JM, Williams JD, Bondioli KR, Looney CR, Westhusin ME, McCalla DF. Comparison of birthweight and growth characteristics of bovine calves produced by nuclear transfer (cloning), embryo transfer and natural mating. Anim Reprod Sci 1995; 38:73-83.
99. Woolliams JA. Modifications to MOET nucleus breeding schemes to improve rates of genetic progress and decrease rates of inbreeding in dairy cattle. Anim. Prod. 1989a; 49:1-4.
100. Woolliams JA. The value of cloning in MOET nucleus breeding schemes for dairy cattle. Anim. Prod. 1989b; 48:31-35.
101. Wright G, Carver A, Cottom D, Reeves D, Scott A, Simons P, Wilmut I, Garner I, Colman A. High level expression of active human alpha-1-antitrypsin in the milk of transgenic sheep. Biotechnology (NY) 1991; 9:830-834.
102. Wright RW, Ellington J. Morphological and physiological differences between *in vivo* and *in vitro*-produced preimplantation embryos from livestock species. Theriogenology 1995, 44:1167-1189.
103. Xu KP, Greve T, Callesen H, Hyttel O. Pregnancy resulting from cattle oocytes matured and fertilized *in vitro*. J Reprod Fert 1987; 81:501-504.
104. Ziomek CA. Commercialization of proteins produced in the mammary gland. Theriogenology 1998; 49:139-144